UNIVERSIDADE DE LISBOA

Faculdade de Medicina

Morphofunctional analysis of temporomandibular joint after bilateral discectomy and discopexy. Preclinical study

David Serrano Faustino Ângelo

Orientadores: Prof. Doutor Francisco João Salvado e Silva
Prof. Doutor Florencio Monje Gil
Prof. Doutor Raúl González-Garcia

Tese especialmente elaborada para a obtenção do grau de Doutor em Medicina, Especialidade de Cirurgia Maxilofacial

2018

UNIVERSIDADE DE LISBOA

Faculdade de Medicina

Morphofunctional analysis of temporomandibular joint after bilateral discectomy and discopexy. Preclinical study

David Serrano Faustino Ângelo

Orientadores: Prof. Doutor Francisco João Salvado e Silva
Prof. Doutor Florencio Monje Gil
Prof. Doutor Raúl González-Garcia

Tese especialmente elaborada para a obtenção do grau de Doutor em Medicina, Especialidade de Cirurgia Maxilofacial

Júri:

Presidente:
- Doutor José Augusto Gamito Melo Cristino, Professor Catedrático, Presidente do Conselho Científico da Faculdade de Medicina da Universidade de Lisboa.

Vogais:
- Doutor Josep Maria Ustrell, Professor Titular da Universidade de Barcelona, Espanha;
- Doutor José Pedro Henriques Figueiredo, Professor Auxiliar Convidado da Faculdade de Medicina da Universidade de Coimbra;
- Doutor Raul José de Castro Antunes Duarte, Professor Coordenador da Escola Superior de Saúde Ribeiro Sanches, Lisboa;
- Doutor Jacinto Manuel de Melo Oliveira Monteiro, Professor Catedrático da Faculdade de Medicina da Universidade de Lisboa;
- Doutor João Eurico Cortez Cabral Fonseca, Professor Catedrático da Faculdade de Medicina da Universidade de Lisboa;
- Doutor Francisco João Salvado e Silva, Professor Auxiliar Convidado da Faculdade de Medicina da Universidade de Lisboa (orientador).

2018

As opiniões expressas nesta dissertação são da exclusiva responsabilidade do seu autor

ÍNDICE

LISTA DE ABREVIATURAS

Português

3D – Tridimensional

ATM – Articulação Temporomandibular

DTM – Disfunção Temporomandibular

RM – Ressonância Magnética

RX – Raio X

TC – Tomografia Computorizada

Inglês

AC – Articular Cartilage

APT – Anteroposterior Tests

ARRIVE – Animal Research: Reporting of In Vivo Experiments

CT – Compression Tests CT

H&E – Hematoxylin and Eosin

MDT – Mediolateral Tests

OA – Osteoarthrosis

PCL – Polycaprolactone

PCL – Polycaprolactone

PDS – Poly-p-Dioxanone

PDS – Poly- p-Dioxanone

PGS – Poly(Glycerol Sebacate)

PGS – Poly(Glycerol Sebacate)

PGs – Proteoglycans

RDC/TD – Research Diagnostic Criteria for Temporomandibular Disorders

SPIRIT – Standard Protocol Items: Recommendations for Interventional Trials

TEMPOJIMS – Temporomandibular Joint Interposal Material Study

THF – Tetrahydrofuran

TII – Temporomandibular Interposal Implant TII

TMD – Temporomandibular Dysfunction

TMJ – Temporomandibular Joint

RESUMO

INTRODUÇÃO

A articulação temporomandibular (ATM) é a mais usada no corpo humano, realizando cerca de 2000 movimentos por dia, sendo essencial para as funções básicas do dia a dia como: mastigar, falar, sorrir ou bocejar. A ATM relaciona o côndilo mandibular com a cavidade glenóide do osso temporal. Na interposição destas superfícies articulares encontra-se o disco intra-articular que permite absorver e distribuir as forças mastigatórias, diminuir as incongruências ósseas, contribuindo no movimento normal desta articulação. As disfunções da ATM (DTM) têm uma prevalência de cerca de 34%, e representam a principal causa de dor orofacial de origem não dentária, podendo estar associadas a morbilidade elevada nos doentes com patologia mais avançada (categoria 3-5 na classificação de *Dimitroulis).* Nestes casos, o tratamento recomendado é predominantemente cirúrgico, sendo maioritariamente realizado através de discopexia ou discectomia. Os resultados destas intervenções constituem um atual tema de debate, principalmente no caso da discectomia, uma vez que a ATM fica sem o disco articular. Pela literatura científica existente, é possível verificar que não existe nenhum estudo randomizado, oculto, com grupo controlo, seja no homem ou no animal, que avalie o efeito destas intervenções na ATM.

OBJETIVOS

Assim, o objetivo principal desta dissertação foi avaliar o efeito da discectomia e discopexia na ATM em modelo animal (ovelha *Black Merino*), analisando o seu impacto histológico, radiológico, na mastigação e na cinemática ruminatória.

Objetivos específicos:

1. Definir o modelo animal apropriado para estudos pré-clínicos na área da ATM.
2. Caracterizar com rigor a anatomia e biomecânica do disco nativo da ovelha *Black Merino*.

3. Elaborar um desenho de estudo de investigação pré-clínico orientado à área da ATM, respeitando as ARRIVE *guidelines*.
4. Aplicar o modelo animal da ovelha *Black Merino* no desenho de estudo proposto.
5. Propor e testar variáveis piloto na área da cinemática ruminatória e tempo de mastigação para estudos pré-clínicos na ATM e avaliar o seu impacto após discectomia bilateral e discopexia bilateral na ovelha *Black Merino*.
6. Avaliar o impacto histológico e imagiológico da discectomia bilateral e discopexia bilateral na ovelha *Black Merino*.

MÉTODOS

Para alcançar os objetivos definidos, foram propostas e desenvolvidas as seguintes atividades:

1) Definição do modelo animal apropriado para estudos pré-clínicos na área da ATM: foram utilizadas 15 cabeças de ovelha *Black Merino* fêmeas, com 40 a 50 Kg, saudáveis. Na fase 1 foi efetuada uma descrição anatómica nos domínios cirúrgico, topográfico e histológico da ATM, comparando à articulação humana. Na fase 2 procedeu-se à análise histológica e biomecânica do disco articular após dissecção microcirúrgica dos discos. Para a análise histológica, as ATM foram removidas em bloco, descalcificadas em ácido fórmico, incluídas em parafina e coradas com hematoxilina & eosina e orceína. Para a avaliação biomecânica do disco articular, foram randomizados 9 discos, dos quais 3 testados à compressão, 3 à tensão anteroposterior e 3 à tensão mesiolateral.

2) Caracterizar com precisão a anatomia e biomecânica do disco nativo da ovelha *Black Merino*: foi realizada a remoção microcirúrgica de 6 discos articulares. Após submersão do disco numa solução impregnante, foi realizado um scanner do mesmo com um sistema de luz branca, reproduzindo-o com elevada precisão num modelo virtual em 3D.

3) Elaborar um desenho de estudo de investigação pré-clínico orientado à área da ATM, respeitando as ARRIVE *guidelines*: foi proposto um desenho de

estudo randomizado com 10 ovelhas e receptiva alocação em 3 intervenções: discectomia bilateral, discopexia bilateral e cirurgia placebo. Uma ovelha foi alocada a um grupo de reserva. Foi planeado um registo pré-operatório do peso, do tempo de mastigação de 150gr de ração e da cinemática ruminatória. Foi planeada também uma tomografia computorizada (TC) pré-cirúrgica. Os animais seriam intervencionados cirurgicamente e avaliados mensalmente, num total de 6 meses, sendo posteriormente sacrificados. Após sacrifício seria realizada nova TC, para avaliação imagiológica e preparada a avaliação histológica das articulações em ocultação para a intervenção.

4) Aplicar o modelo animal da ovelha *Black Merino* no desenho de estudo proposto: o estudo teve início em dezembro de 2015, o registo pré-operatório teve início em janeiro de 2016, as intervenções cirúrgicas foram realizadas durante o mês de fevereiro. Os animais foram acompanhados durante 6 meses com registos periódicos predefinidos. Os animais foram sacrificados em agosto de 2016.

5) Testar variáveis piloto na área da mastigação e da cinemática ruminatória para estudos pré-clínicos na ATM: diariamente, às 08:30h, os animais foram colocados em divisória própria com abertura frontal, previamente desenvolvida para avaliação do tempo de mastigação e da cinemática ruminatória em ovelha. Seguiu-se uma cronometragem do tempo para comer uma dose controlada de 150gr de ração Rico Gado A3®, registando o respetivo tempo de mastigação. Os animais foram colocados no campo, ao ar livre, e às 13:00h voltaram a ser instalados nas divisórias para serem filmados 15 ciclos ruminatórios. Com recurso a software apropriado, foi efetuado um mapa do trajeto da mandíbula durante a ruminação e obtido o tempo de cada ciclo ruminatório, a forma geométrica e a área de ruminação. Após este registo os animais foram pesados numa balança devidamente calibrada.

6) Avaliar o impacto histológico e imagiológico da discectomia bilateral e discopexia bilateral na ovelha *Black Merino*: após o sacrífico das ovelhas, foi realizada TC ao crânio, separados os blocos de cada ATM, e preparados para

análise histológica. Em ocultação, os diferentes avaliadores pontuaram, respetivamente, as articulações com escalas apropriadas.

RESULTADOS

1) O acesso cirúrgico à ATM da ovelha *Black Merino* realiza-se através de uma incisão pré-auricular, dissecção por planos até à cápsula sinovial. As dimensões das estruturas anatómicas envolvidas (*e.g.* fossa temporal, côndilo mandibular, espaço interarticular e disco articular) são aproximadas às do humano, tal como a histologia e a biomecânica do disco que apresentam idênticas semelhanças. No entanto, registou-se um perfil ligeiramente côncavo do côndilo mandibular da ovelha, que na raça humana é convexo.

2) Foi possível, através do método anteriormente descrito, replicar com rigor a morfologia do disco nativo da ovelha *Black Merino*. O disco apresentou em média 20.93±1.33mm no sentido mesiolateral e 11.2±0.78mm no sentido anteroposterior. A média da espessura da banda anterior foi 1.03±0.06mm, a zona central 0.76±0.08mm e a banda posterior 1.23±0.07mm. A morfologia em fresco evidenciou uma ligeira convexidade da superfície inferior, correspondendo a um perfil adaptativo ao côndilo mandibular.

3) O desenho do estudo randomizado, oculto com alocação em 3 intervenções bilaterais, respeitando as ARRIVE *guidelines,* foi executado com rigor e provou ser exequível na ovelha *Black Merino*. A avaliação do tempo para mastigar uma dose fixa de 150gr de ração mostrou ser adequada para avaliar o tempo de mastigação inicial. A captação vídeo de 15 ciclos ruminatórios foi reprodutível, tendo o animal demonstrado capacidade de se manter estático no processo de ruminação, nas divisões desenvolvidas para este efeito.

4) Foram detetadas diversas alterações histológicas nos grupos da discectomia e da discopexia. O grupo controlo apresentou as características da ATM normal previamente descrita. No grupo da discectomia verificou-se fibrilhação e perda da

camada superficial laminar, com aumento marcado da população de proteoglicanos e invasão vascular da zona intermédia. Em alguns casos observou-se remodelação subcondral. A avaliação em ocultação mostrou diferenças estatisticamente significativas entre este grupo e o grupo de controlo. Na discopexia verificou-se ligeira fibrilhação sem perda da continuidade da superfície laminar, havendo aumento do número de proteoglicanos e da densidade celular. Na avaliação em ocultação não se encontraram diferenças estatisticamente significativas entre o grupo da discopexia e o de controlo.

5) A avaliação em ocultação das imagens de TC mostrou diferenças estatisticamente significativas apenas para a discectomia (R^2 correspondendo a 92,9% de degeneração na avaliação global). Na discopexia foram encontradas ligeiras diferenças sem significado estatístico.

6) Em ambas as técnicas cirúrgicas houve alterações estatisticamente significativas do tempo de mastigação, no primeiro mês após a cirurgia, que foram normalizando e, após 2 meses, deixaram de ser significativas. Na avaliação da ruminação no grupo da discopexia, foi encontrada uma diferença estatisticamente significativa, com aumento do ciclo de ruminação no 5º mês pós cirurgia. Não se encontraram outras alterações significativas, ao longo dos meses, na avaliação do tempo de cada ciclo de ruminação e respetiva área.

7) Após a intervenção cirúrgica, os animais do grupo da discectomia e discopexia perderam peso até ao final do 1º mês. No decorrer do estudo, o grupo da discectomia apenas conseguiu recuperar o peso inicial, enquanto o grupo da discopexia continuou em progressão de peso, acompanhando o perfil do grupo de controlo. No presente estudo, não houve diferenças estatisticamente significativas no peso dos animais dos diferentes grupos estudados.

CONCLUSÕES

A ovelha *Black Merino* é um animal com características apropriadas para ser utilizado em estudos pré-clínicos da ATM, por apresentar uma anatomia cirúrgica e histológica semelhante ao humano, e o disco apresentar morfologia e comportamento biomecânico idêntico. Por ser um animal ruminante, com consequente aumento do número de movimentos da ATM, poderá contribuir favoravelmente em estudos posteriores para testar potenciais biomateriais ou próteses da ATM (*e.g.* testar fadiga do material). O desenho de estudo proposto randomizado, oculto, com intervenções cirúrgicas bilaterais verificou-se exequível e reprodutível, para investigação na área da ATM em ovelha *Black Merino*. A discectomia promoveu um processo degenerativo severo, na análise histológica e radiológica, com uma repercussão significativa no tempo inicial de mastigação apenas no 1º mês pós cirurgia. A discopexia não induziu um dano degenerativo significativo na articulação. A bioengenharia de tecidos e a medicina regenerativa poderão desempenhar um papel significativo na regeneração do disco articular, contribuindo para uma melhoria das técnicas cirúrgicas atuais, nomeadamente a discectomia.

ABSTRACT

INTRODUCTION

The temporomandibular joint (TMJ) is the most used joint in the human body, with over 2000 movements *per* day, being essential for everyday functions (*e.g.* mastication, speech, deglution and yawning). The TMJ is responsible for the relation between the mandibular condyle and glenoid fossa of temporal bone. This joint contains an articular disc, an important functional unit interposed between the bony structures, contributing for a congruent movement of this joint. TMJ disorders (TMD) have a prevalence of 34% and represent the main non-tooth origin cause of orofacial pain, leading to high morbidity in severe cases (category 3-5 *Dimitroulis* classification). In those cases, the treatment is mostly surgical, being the common surgical options discopexy or discectomy. The outcomes of these interventions are a topic of debate, namely in discectomy, once TMJ is left without the TMJ disc. Moreover, in the available scientific literature it is not possible to state any randomized study, blind and with control group, both in human or animals, evaluating the effect of those interventions in TMJ.

OBJECTIVES

The main goal of this dissertation was evaluating the effect of TMJ discectomy and discopexy in animal model (*Black Merino* sheep), examining their impact in histologic, imaging and kinematic outcomes.

Specific goals:

1. Characterisation of the adequate animal model for preclinical studies in the TMJ.
2. Characterize the anatomy and biomechanics of the native TMJ disc of *Black Merino* sheep.
3. Develop a study design for preclinical investigation in the TMJ, according to the ARRIVE guidelines.

4. Application of the proposed study design on the *Black Merino* animal model.
5. Propose and test pilot outcomes on the kinematic mastication for preclinical studies in the TMJ and evaluating their impact on bilateral discectomy and bilateral discopexy in *Black Merino* sheep.
6. Evaluating the histologic and imaging impact of bilateral discectomy and bilateral discopexy in *Black Merino* sheep.

METHODS

To achieve the define goals, the following activities were developed and proposed:

1) Characterisation of the adequate animal model for preclinical studies in the TMJ: 15 female *Black Merino* sheep heads of healthy animals with 40-50 Kg were used to describe the surgical, topographic and histologic anatomy of the TMJ, comparing with the human joint. In a second phase, histologic and biomechanical analysis of the disc were performed. For the anatomical characterisation, a surgical dissection was performed exposing and identifying the TMJ structures. To analyse the TMJ disc, a microsurgical dissection was performed and the TMJ discs were submersed in an impregnated solution (Colorbond), allowing a 3D scanning preserving the morphology of the native disc. To the histologic analysis, the TMJ was removed in block, decalcified in formic acid, included in paraffin and stained with hematoxilin & eosin and orcein. For the biomechanical evaluation, 9 discs were randomized (3 were tested to compression, 3 to anteroposterior strain and 3 to mesiolateral strain).

2) Characterize the anatomy and biomechanics of the native TMJ disc of *Black Merino* sheep: a microsurgical extraction of 6 discs was completed, removing all the muscular attachments. The discs were submersed in an impregnate solution to preserve their native morphology. A white light system scanner was used, replicating a 3D virtual model with high precision.

3) Develop a study design for preclinical investigation in the TMJ, according to the ARRIVE guidelines: a randomized preclinical trial with 10 sheep was proposed with allocation in 3 intervention groups: bilateral discectomy, bilateral discopexy, sham surgery and one reserve sheep was allocated in a reserve group. A baseline pre-intervention record of body mass, mastication time for 150gr of pellets and rumination kinetics was included. In addition, imaging by computed tomography (CT) was suggested before surgical intervention. It was proposed that the animals would be submitted to surgical intervention and evaluated every 6 months, being posteriorly sacrificed. After sacrifice, a new CT should be done for imaging scoring and discs ought to be prepared for the histologic evaluation.

4) Application of the proposed study design on the *Black Merino* animal model: the study started in December 2015, the baseline records occurred in January 2016, and the surgical interventions took place in February 2016. According to the established protocol, the animals were monitored for 6 months (with various assessments being performed in specific time points) and were sacrificed in August 2016.

5) Propose and test pilot outcomes on the kinematic mastication for preclinical studies in the TMJ and evaluating their impact on bilateral discectomy and bilateral discopexy in *Black Merino* sheep: nine specific cages were constructed with a frontal window where all the animals were placed at 08:30 am. The time to eat 150gr of dry pellets Rico Gado A3® was assessed by a chronometer. Following, the animals were placed in their natural habitat and around 01:00 pm they returned to the boxes to record the kinetic rumination of 15 cycles. With adequate software, the trajectory of the jaw was designed during rumination and the time for each cycle was calculated, as well as the ruminant area. Next, the body mass was obtained in a certified balance.

6) Evaluating the histologic and imaging impact of bilateral discectomy and bilateral discopexy in *Black Merino* sheep: after sacrifice, a cranium CT was performed and the TMJ block was removed. Each block was prepared for histology. In occultation, the different evaluators scored the joint using appropriate scales.

RESULTS

1) The obtained surgical anatomy was similar to the human, with a direct access to TMJ through a pre-auricular incision. The size of the anatomic structures (*e.g.* temporal fossa, mandibular condyle, inter-articular space and TMJ disc) were also similar to the human. In addition, high similarity was also obtained in the disc histology and biomechanics. A slight concave mandibular condyle was noticed, which is convex in humans.

2) With the proposed method, it was possible to accurately replicate the morphology of the native TMJ disc of *Black Merino* sheep. The average size was 20.93±1.33mm in the mesiolateral orientation and 11.2±0.78mm in anteroposterior orientation. The thickness of the anterior band was 1.03±0.06mm, the central zone 0.76±0.08mm and the posterior band 1.23±0.07mm. The morphology of the fresh disc showed a slight convexity of the inferior surface, consistent to an adaptive outline to the mandibular condyle.

3) The randomized and blinded study design, with allocation in 3 different bilateral interventions, respecting the ARRIVE guidelines was executed with accuracy and proved to be feasible in *Black Merino* sheep. The video caption of 15 rumination cycles was achievable, showing this animal the ability to stand still during the rumination.

4) Various histologic changes were noticed in the discectomy and discopexy groups. As expected, the control group presented the main characteristics of the normal TMJ. In the discectomy group, fibrillation and loss of typical laminar structure, with an increased population of proteoglycan stain in all layers and vascular invasion, were observed. In some cases, osteochondral changes were also noticed. The blinded scoring showed statistical significance for the discectomy group only. While no statistical significance, with no loss of the laminar surface, was detected in the discopexy group, a marked proteoglycan increase was noticed.

5) The blinded evaluation of CT images showed statistical significance only for the discectomy group (R^2 corresponding to 92.9% of degeneration for global appreciation). In the discopexy group, slight differences were noticed without reaching statistical significance.

6) For both surgical techniques, significant changes were noticed for mastication time in the first month post-operative. Those changes gradually returned to normal and 6 months after surgery no changes were observed. No rumination movements were detected in T1 a T2 after surgery in the discectomy group. In rumination evaluation, a significant difference was stated in T5 for discopexy group with an increased time *per* cycle. No other changes occurred, neither for rumination time or area.

7) In the first month after surgery, discectomy and discopexy groups loss body mass. However, with the progress of the study, those animals were capable to increase body mass; discectomy group was only capable to return to original weight, and discopexy groups could follow the control group increasing 8% and 8.2% respectively. No significant changes were noticed in the different groups.

CONCLUSIONS

The *Black Merino* sheep has proven to be an adequate animal model to conduct TMJ preclinical trials, considering the surgical anatomy, biomechanics and histology like human. Because it is a ruminant animal, it may have an increased value in further studies, examining interposal biomaterials or TMJ prosthesis, and allowing to accurately test the material stress. The proposed randomized, blinded, with bilateral interventions study was feasible and reproducible for TMJ investigation in Black Merino sheep. Discectomy promoted a severe degenerative process in histologic and radiologic analysis, with a repercussion in mastication (only in the first month post-surgery). Discopexy did not induce significant degenerative changes in the TMJ. With a significant role in tissue regeneration, bioengineering and regenerative medicine will be critical to increase and optimize the current surgical techniques, mostly discectomy.

LISTA DE PUBLICAÇÕES

Durante esta investigação foram publicados diversos artigos científicos tendo como objetivo partilhar, com a comunidade científica, o conhecimento obtido ao longo deste estudo.

- **Ângelo, David F;** Morouço, Pedro; Alves, Nuno; Viana, Tânia; Santos, Fábio; González, Raúl; Monje, Florencio; Macias, Domingos; Carrapiço, Belmira; Sousa, Rita; Gonçalves, Sandra; Salvado, Francisco. Animal model for temporomandibular joint research: morphological, histological and biomechanical characterization of the Black Merino sheep joint disc. Morphologie. 2016 Dec;100(331):223-233.
- **Ângelo, David F;** Morouço, Pedro; Alves, Nuno; Carvalho, Tânia; Bonaparte Dolores; Carrapiço, Belmira; Monje, Florencio; Furtado, Ivo; Salvado, Francisco. Novel approach for 3D virtual model of TMJ disc morphology. Preliminary results. Surg Radiol Anat. 2016 Jan;38(1):5-47.
- **Ângelo, David F;** Monje, Florencio; González, Raúl; Little, Christopher B; Mónico, Lisete; Pinho, Mário; Santos, Fábio; Carrapiço, Belmira; Cavaco, Sandra; Morouço, Pedro; Alves, Nuno; Moura, Carla; Wang, Yadong; Jeffries, Eric; Gao, Jin; Sousa, Rita; Lucas, Lia; Caldeira, Daniel; Salvado, Francisco. Bioengineered Temporomandibular Joint Disk Implants: Study Protocol for a Two-Phase Exploratory Randomized Preclinical Pilot Trial in 18 Black Merino Sheep (TEMPOJIMS). JMIR Res Protoc. 2017 Mar 2;6(3):e37.
- **Ângelo, David F;** Monje Florencio; González, Raúl; Mónico, Lisete; Moura, Carla; Francisco, Luís; Sanz, David; Alves, Nuno; Salvado, Francisco; Morouço, Pedro. Effects of bilateral discectomy and bilateral discopexy on Black Merino Sheep rumination kinematics: TEMPOJIMS – a randomized preclinical trial. Accepted in Journal of Cranio and Maxillofacial Surgery.
- **Ângelo, David F**; Monje Florencio; González-García Raúl, Morouço Pedro; Sousa Rita; Neto Lia; Caldeira Inês; M. Smith Margaret; M. Smith Susan; Sanz David; Carvalho Fábio; Carrapiço Belmira; Cavaco Sandra, Pinho Mário; Wang Yadong; Jeffries Eric; Gao Jin; Moura Carla; Alves Nuno; Mónico Lisete; Salvado Francisco; Little Christopher B. A Pilot Preclinical Randomized Controlled Trial

of Bilateral Discectomy versus Bilateral Discopexy in Black Merino Sheep Temporomandibular Joint: TEMPOJIMS Phase 1 - Histologic, Imaging and Body Weight results. Accepted in JCMFS.

LISTA DE COMUNICAÇÕES EM CONGRESSOS

Durante a investigação foi possível obter diversos resultados preliminares, que permitiram ir divulgando os resultados em congressos, assim como discutir várias ideias com conferencistas, contribuindo para a melhoria contínua do projeto.

- **Ângelo, David F**, *et al.* Bioengineering and Regenerative Medicine in Temporomandibular Joint. CDRSP Seminar. Marinha Grande. 12 de julho de 2017
- **Ângelo, David F**, *et al*. Temporomandibular joint interposal material study - TEMPOJIMS. 24 Congreso nacional de cirurgia oral y maxilofacial. Málaga - Espanha. 10 de junho de 2017
- **Ângelo, David F,** *et al*. Novas tecnologias no tratamento das DTM. XXV Jornadas Internacionais de Medicina Dentária. Grande Auditório da Faculdade de Medicina Dentária Egas Moniz. Almada. Portugal. 24 de março de 2017
- **Ângelo, David F**, *et al.* Bioengenieria de tejidos en articulacion temporomandibular. 4º curso de regeneración y reconstruction óssea de los maxilares - Sociedade espanõla de cirurgia oral y maxilofacial. Madrid - Espanha. 11 de novembro de 2016
- **Ângelo, David F**, et al. Study protocol bioengineered disc implants in temporomandibular joint (tmj): protocol for a two-phase exploratory randomized preclinical trial in 18 black merino sheep (TEMPOJIMS). TMJ bioengineering conference. Barcelona - Espanha. 13 de setembro de 2016
- **Ângelo, David F**, et al. Animal model for temporomandibular joint research: morphological, histological and biomechanical characterization of the black merino sheep joint disc. tmj bioengineering conference. Barcelona - Espanha. 12 de setembro de 2016
- **Ângelo, David F**, *et al*. Articular changes after tmj discectomy. Preliminary results. tempojims study. Terceras jornadas internacionales de diagnostico y tratamento de las patologias de la atm. Puerto Madryn - Argentina. 2 de setembro de 2016

- **Ângelo, David F**, *et al*. Investigação na área da Articulação Temporomandibular. Congresso de Dor Orofacial e Disfunção Temporomandibular: O Futuro. Campus Universitário Egas Moniz - Monte da Caparica. Lisboa. Portugal. 18 e 19 de março de 2016
- **Ângelo, David F**, Morouço P, *et al.* Novel approach for 3d virtual model of tmj disc morphology. Preliminary results. International congress of clinical anatomy Rouen - França. 24- 27 de junho de 2015

CAPÍTULO 1. INTRODUÇÃO

A articulação temporomandibular (ATM) é responsável pela relação do côndilo mandibular com a cavidade glenóide do osso temporal [1]. É uma articulação sinovial classificada como bicondilo-meniscartrose, sendo a única articulação do corpo humano que conjuga movimentos de rotação e translação (Figura 1) [2].

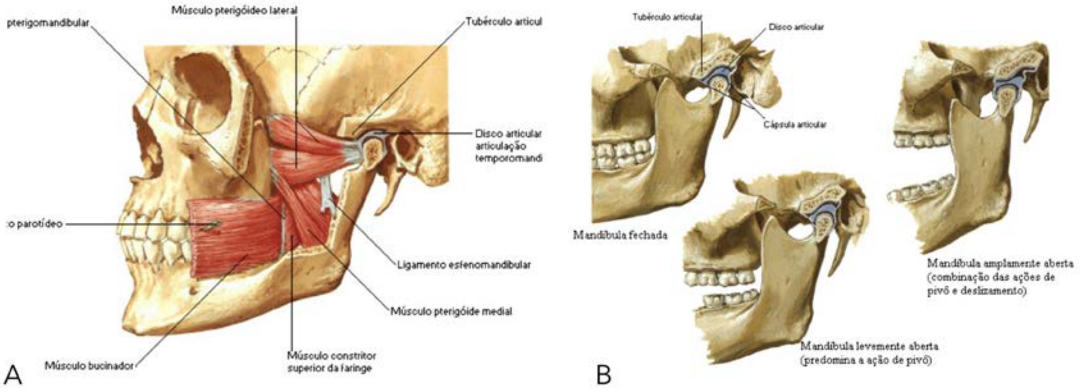


Figura 1. (A) Vista lateral da ATM. (B) Movimento de abertura da boca com detalhe para a posição do disco articular [3].

A par de outras articulações móveis, a ATM é caracterizada por ter uma membrana sinovial que reveste a cápsula articular e engloba as superfícies articulares. A articulação é dividida em compartimento superior e inferior pelo disco articular fibrocartilaginoso, permitindo um deslizamento suave destas estruturas ósseas. O disco articular é composto por uma população mista de fibroblastos e condrócitos que produzem maioritariamente colagénio tipo 1 [4]. O disco é, na sua grande maioria, avascular, aneural e alinfático [4]. As características da ATM e do disco articular são únicas e não devem ser comparadas, por exemplo, ao menisco do joelho [5]. O disco articular tem, como principais funções: lubrificar a ATM, absorver/distribuir as forças mastigatórias e estabilizar o movimento desta articulação [6]. Em média, no adulto, o disco tem 19mm no sentido mesiolateral e 13mm no sentido anteroposterior [6]. A superfície inferior do disco apresenta uma excelente adaptação à forma do côndilo articular, e reduz assim os níveis de atrito nos movimentos de rotação e translação [7]. A membrana sinovial desempenha um papel importante na função da articulação, nomeadamente na produção de líquido sinovial, principal fonte de nutrientes para o disco e cartilagem articular. A cartilagem que reveste a superfície óssea é não hialina, tornando esta articulação atípica [8]. Os condrócitos que caracterizam a cartilagem hialina podem ser identificados em 4 zonas na cartilagem: superficial, média, profunda e calcificada. Em contraste, a cartilagem da ATM tem zonas de fibrocartilagem e de cartilagem tipo hialina, representadas por uma zona fibrosa superficial fibroproliferativa e uma zona inferior com características hialinas. Esta zona inferior apresenta sobretudo colagénio tipo 1 e fibroblastos. Na camada inferior são encontradas células mesenquimais indiferenciadas, que constituem uma zona proliferativa de reserva para a camada fibrosa superficial [8]. Esta articulação desempenha uma função importante no crescimento da mandíbula, pois o côndilo tem uma área de atividade de crescimento [1]. No recém-nascido, a fossa mandibular é aplanada e não existe eminência articular. Só após a erupção dos primeiros dentes definitivos, por volta dos 6-7 anos de idade, a eminência articular começa a ser mais proeminente e a fossa temporal a ser moldada pelo côndilo [9]. A ATM é a articulação mais utilizada do corpo humano, contabilizando cerca de 2000 movimentos por dia e é essencial para manter as funções básicas oromaxilares do dia a dia (*e.g.* mastigar, falar, deglutir e bocejar), e está dependente do movimento indissociável das duas articulações (direita e esquerda), particularidade exclusiva da ATM [10].

As disfunções temporomandibulares (DTM) podem resultar de diversos fatores etiológicos: trauma [11], perda de dentes [12], parafunções [13], infeção [14], autoimunidade [15], sobrecarga articular [16] e diminuição da respetiva lubrificação [17]. As DTM representam a principal causa de dor orofacial de origem não dentária no humano [18,19]. Estas disfunções têm assumido uma importância crescente na comunidade médica, pela relevante prevalência na população (5-32,5%) [19–29], pelo grande impacto na qualidade de vida dos doentes [30–32] e custos importantes para os sistemas de saúde [33]. Prevê-se que no ano de 2030 dupliquem as intervenções cirúrgicas com próteses totais da ATM nos Estados Unidos [34]. Concomitantemente, a comunidade científica tem-se debruçado exaustivamente sobre este tema, contribuindo para um aumento evidente do número de trabalhos publicados na *Web of Science®* (Figura 2).

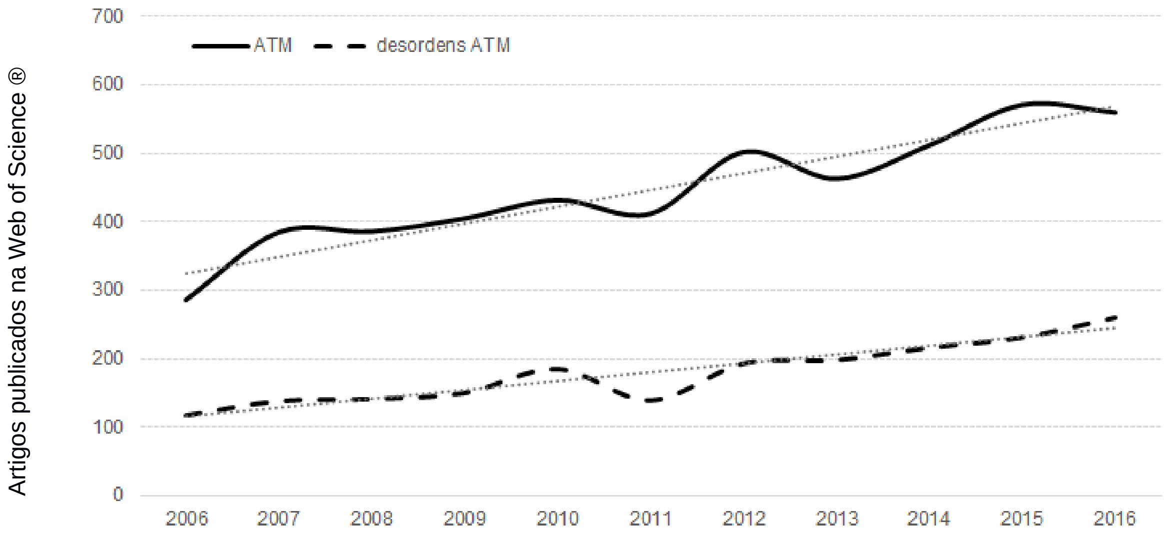


Figura 2. Ilustração representativa do número e linha de tendência de trabalhos publicados na Web of Science® no domínio da ATM e das disfunções da ATM.

Existem diversas classificações para as DTM. A classificação *Research Diagnostic Criteria for Temporomandibular Disorders* (RDC/TMD), publicada em 1992, continua a ser a mais aceite. Avalia os doentes em dois eixos [35]: o eixo 1 está orientado para a patologia funcional e o eixo 2 consiste numa avaliação psicossocial do doente. A avaliação RDC/TMD foi elaborada principalmente para doentes não cirúrgicos e, embora a sua utilização na prática clínica seja reduzida, ela mantém um papel importante na área da investigação [36]. Na tabela 1 é apresentado o eixo 1 da RDC/TMD. O eixo 2, por ser orientado para a avaliação psicossocial, não foi apresentado. Esta classificação foi revista em 2014 [37], mas não se encontra ainda validada para a língua portuguesa pelo que se consideramos a versão de 1992.

Grupo RDC/TMD		Diagnóstico	Definição	Critério diagnóstico
I	Ia	Dor miofascial	Dor de origem muscular, incluindo zonas de contratura muscular.	1.Dor mandibular, têmporas, face, área pré auricular ou ouvido, em repouso ou função; mais 2.Dor após palpação em 3 dos seguintes 20 locais (lado direito e esquerdo contam como áreas separadas): músculo temporal zona anterior, músculo temporal zona média, músculo temporal zona posterior, corpo do masséter, inserção do masséter, região posterior da mandíbula, região submandibular, região do pterigoideu lateral, tendão do músculo temporal.
	Ib	Dor miofascial com limitação da abertura da boca	Dor de origem muscular, incluindo zonas de contratura muscular e limitação da abertura da boca	1. Dor miofascial como definida em Ia; mais 2. Abertura oral espontânea, sem dor inferior a 40mm; mais

				3. Abertura forçada de mais 5mm do que abertura espontânea sem dor.
II	IIa	Deslocamento do disco com redução	O disco está deslocado da sua posição entre o côndilo e a eminência. O deslocamento pode ser anterior, posterior, medial ou lateral, mas reduz em abertura total, normalmente havendo um estalido. Se este diagnóstico for acompanhado de dor na articulação, o diagnóstico será de artralgia (IIIa) ou de osteoartrite (IIIb). O disco está deslocado da sua posição entre o côndilo e a eminência, associado a limitação da abertura da boca.	1. (a) estalido recíproco que deve ocorrer pelo menos após 5mm da abertura máxima e no movimento de fechar a boca. Este estalido deve ser eliminado no movimento de protusão. Este processo deve ser reprodutível em pelo menos 3 aberturas; ou (b) estalido na abertura ou fecho da boca, em pelo menos 2 aberturas consecutivas, ou estalido em movimento lateral e protusão reprodutível, em pelo menos 2 aberturas.
	IIb	Deslocamento do disco sem redução com limitação da abertura da boca	O disco está deslocado da sua posição entre o côndilo e a eminência, sem associação a limitação da abertura da boca.	1. História de limitação progressiva da abertura da boca; mais 2. Abertura espontânea < 35mm; mais 3. Abertura oral forçada melhora < 4mm; mais 4. movimento lateral < 7mm; mais 5. Abertura com desvio sem correção ipsilateral; mais 6. (a) sem presença de estalidos ou (b) estalidos que não se incluam nos critérios de Iia.
	IIc	Deslocamento do disco sem redução e sem limitação da abertura da boca		1. História de limitação progressiva da abertura da boca; mais 2. Abertura espontânea > 35mm; mais 3. Abertura oral forçada melhora > 5mm; mais 4. movimento lateral >7mm; mais 5. (a) presença de estalidos que não se incluam nos critérios de IIa.
III	IIIa	Artralgia	Dor ou tumefação da cápsula sinovial da ATM	1.Dor à palpação do polo lateral ou zona retrodiscal; mais 2. Uma ou mais das seguintes situações: (a) dor na zona da ATM, (b) dor articular na abertura espontânea, (c) dor na abertura forçada, (d) dor no movimentos laterais. 3. Para artralgia simples não pode haver crepitação.
	IIIb	Osteoartrite da ATM	Inflamação articular que resulta em processo degenerativo das estruturas articulares	1.Artralgia; mais 2.crepitação.
	IIIc	Osteoartrose da ATM	Alteração degenerativa onde a morfologia articular está alterada	1. Inexistência dos sintomas de artralgia, inexistência de dor articular ou de dor na abertura espontânea, ou nos movimentos de lateralidade. 2. Crepitação

Tabela 1. Eixo I da classificação RDC/TMD [35].

Em 1989, *Wilkes et al* propôs outra classificação (Tabela 2), que se veio a tornar amplamente aceite para a classificação das DTM intra-articulares [38]. O sistema de *Wilkes et al* engloba uma avaliação clínica, radiológica e cirúrgica. No entanto, esta classificação está limitada aos processos de osteoartrite e aos deslocamentos do disco articular, não sendo mencionados processos mais severos (*e.g.* anquiloses da ATM, tumores da ATM) ou as DTM ligeiras com envolvimento muscular. Para estes casos é necessário recorrer a outras subclassificações [39,40] que, por não serem objetos de estudo do presente trabalho, não serão abordadas nesta dissertação.

Estádio	Sinais e sintomas	Achados imagiológicos	Achados cirúrgicos
I	Estalido articular indolor. Sem bloqueio. Sem limitação da abertura oral.	Ligeiro deslocamento do disco, com redução na abertura. Contorno ósseo preservado.	Disco com morfologia normal. Ligeiro deslocamento anterior do disco.
II	Estalido articular, ocasionalmente doloroso. Bloqueio articular intermitente. Cefaleias.	Ligeiro deslocamento do disco, com redução na abertura. Deformação do disco ligeira. Contorno ósseo preservado.	Disco delgado. Deslocamento anterior do disco.
III	Dor frequente. Sensibilidade articular. Cefaleias. Bloqueios articulares.	Deslocamento anterior do disco sem redução. Deformação do disco moderada. Contorno ósseo preservado.	Disco deformado e deslocado. Aderências intra-articulares. Osso preservado.
IV	Dor crónica. Cefaleias. Limitação da abertura da boca com crepitação.	Deslocamento anterior do disco sem redução. Deformação do disco importante Degeneração óssea.	Perfuração do disco, deslocamento e adesões. Alterações degenerativas do côndilo e/ou fossa temporal.
V	Dor variável. Crepitação.	Deslocamento anterior do disco sem redução. Deformação do disco importante Degeneração óssea.	Perfuração do disco, deslocamento e adesões. Alterações degenerativas do côndilo e/ou fossa temporal.

Tabela 2. Classificação de *Wilkes et al* [38].

Mais recentemente, *Dimitroulis* publicou uma classificação simples, atualizada e abrangente das disfunções temporomandibulares [36], que se encontra dividida em cinco categorias (Tabela 3). Esta classificação engloba os diferentes tipos de DTM de um modo organizado, expondo as diversas propostas terapêuticas em relação à categoria. Assim, na prossecução dos objetivos da presente tese, foi adotada esta classificação como referência.

Categoria 1	ATM Normal Não é necessário procedimento cirúrgico.
Categoria 2	ATM com danos minor Todos os componentes estruturais estão preservados. Indicação para lavagem articular com artrocentese / artroscopia
Categoria 3	ATM com danos moderados A maioria dos componentes estruturais está preservada. Indicação para artroscopia cirúrgica / artroplastia ou discectomia.
Categoria 4	ATM com danos severos Poucos componentes estruturais estão preservados. Indicação para artroplastia, discectomia ou condilectomia.
Categoria 5	ATM com danos catastróficos Sem componentes preservados. Indicação para ressecção da ATM ou substituição por prótese total.

Tabela 3. Classificação das disfunções da ATM por *Dimitroulis* [36].

Na categoria 1, o doente pode apresentar dor na ATM, mas sem história de estalidos ou bloqueios articulares. O doente apresenta uma boa abertura oral e mastiga sem alterações. Nesta categoria, os exames complementares de imagem (ressonância magnética (RM), tomografia computorizada (TC) ou raio-x (RX)) não apresentam alterações. Os diagnósticos podem incluir dor miofascial, contusão da ATM pós trauma ou otalgia. Pode ainda coexistir dor psicossomática. Os tratamentos indicados nesta categoria são conservadores: medicação / goteira / educação / fisioterapia. Na categoria 2, o doente pode apresentar ocasionalmente dor, estalidos e bloqueios articulares. Os exames complementares de imagem podem evidenciar alterações ligeiras, como deslocamento do disco com redução parcial ou excesso de líquido articular. A primeira opção deve ser um tratamento conservador com medicação / goteira / educação / fisioterapia. No entanto, em doentes na categoria 2, pode haver um benefício no tratamento com artrocentese de lise e lavagem com viscosuplementação, especialmente em casos de deslocamento do disco sem redução aguda. A artroscopia pode mostrar inflamação sinovial com algumas aderências intra-articulares. Ambos os procedimentos podem ser úteis, mas o tratamento inicial deve ser, essencialmente, conservador. Na categoria 3, existe história de deslocamento sem redução prolongada (>2 meses), edema pré-articular e pode haver história de luxação recorrente. O doente poderá ter dificuldade em mastigar e apresentar níveis de dor intensos exacerbados pela mastigação. A abertura oral está normalmente diminuída por receio de abrir e deslocar a mandíbula ou devido à dor intensa. Na abertura oral verifica-se um desvio para o lado afetado, o que poderá significar um deslocamento anterior do disco sem redução. Pela limitação da abertura da boca, normalmente, não existem estalidos. Os exames complementares de imagem podem não evidenciar alterações. Na RM há normalmente deslocamento do disco sem redução, associada ou não, a deformação do disco e/ou hipertrofia da eminência articular. Estes doentes podem beneficiar de artroscopia cirúrgica, condilectomia funcional, artroplastia da ATM com reposicionamento do disco, discectomia, eminectomia ou redução aberta de fratura do côndilo com osteosíntese rígida.

Na categoria 4, existem alterações severas da ATM onde poucas estruturas articulares podem ser preservadas. Nesta categoria, os doentes apresentam dor constante com crepitação dolorosa da ATM e limitação moderada da abertura da boca. Mastigar é difícil e bocejar desencadeia frequentemente dor intensa. Os exames complementares de imagem mostram sinais de alterações morfológicas do côndilo, tais como aplanamento ou afilamento. Estas alterações são melhor visualizadas na TC. A RM mostra danos degenerativos severos, o disco deformado e/ou deslocado, podendo haver em vários casos perfuração. Alterações degenerativas da ATM como a presença de osteófitos, de pequenos quistos subcondrais com perda ou diminuição da fibrocartilagem podem ser observados na TC. O doente apresenta normalmente um quadro típico de disfunção intra-articular com osteoartrite recente. Nesta categoria podem estar incluídos casos de doenças metabólicas, auto-imunes ou inflamatórias da ATM. As opções terapêuticas recomendadas são discopexia, discectomia da ATM com ou sem desbridamento do espaço intra-articular, ou condilectomia funcional. Na categoria 5 estão os casos com alterações muito graves da ATM. Estes doentes apresentam dor incapacitante, crepitação e incapacidade de mastigar sólidos. Os exames complementares imagiológicos evidenciam alterações degenerativas óbvias, com superfície irregular e quistos subcondrais. A RM mostra alterações severas do disco, que, por vezes, não se visualizam corretamente pelo sinal hipointenso do côndilo deformado. Estes doentes apresentam osteoartrite ou patologia degenerativa severa que pode estar associada a cirurgias prévias à ATM. Quando não existe dor, ou quando esta é tolerável, o doente pode ter osteoartrose, anquilose óssea ou patologia tumoral na

ATM. Estes doentes podem beneficiar de discectomia, condilectomia ou prótese total da ATM.

Tendo como referência a referida classificação, o principal objetivo desta investigação consistiu em alcançar um progresso clínico e científico na compreensão do efeito da discectomia e discopexia na ATM.

Historicamente, a discectomia da ATM (Figura 3) é a técnica cirúrgica mais realizada para tratamento de patologia intra-articular [41].

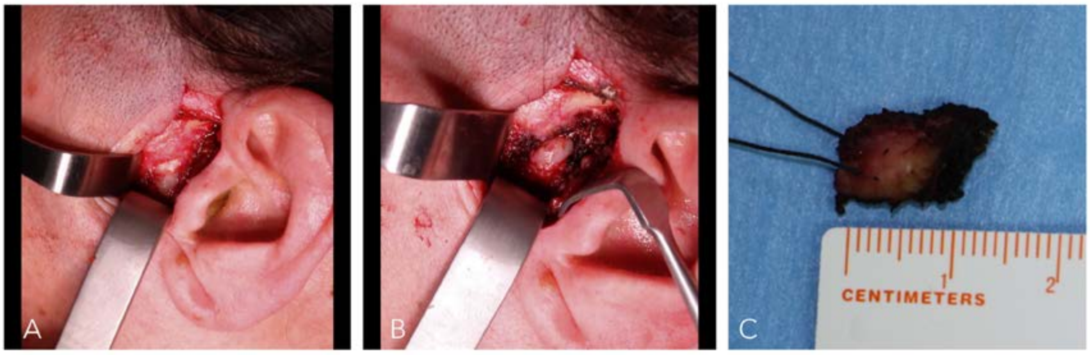


Figura 3. Doente na categoria 4 da classificação de *Dimitroulis* com fragmentação do segmento lateral do disco. O tratamento cirúrgico proposto foi discectomia da ATM esquerda. (A) acesso à ATM via pré auricular; (B) ATM após ser removido o disco (discectomia) e (C) elemento principal do disco. Não foi fotografado o fragmento lateral do disco.

Com vários estudos a evidenciar resultados satisfatórios pós-discectomia [42–46], esta técnica continua a ser utilizada com frequência, sendo, no entanto, controversa por eliminar da articulação um elemento estrutural importante, o disco articular. Vários grupos tentaram, através de estudos pré-clínicos [47–52], compreender o efeito histológico da discectomia, mas os resultados foram díspares, tornando-os inconclusivos. A heterogeneidade dos resultados pode estar associada a: (1) uso de diferentes modelos de animais [53–56], (2) desenhos de estudo pouco rigorosos [48,56,57], (3) uso da articulação contralateral como controlo [48,56], (4) limitação das variáveis estudadas à radiologia e histologia [48,54,56]. Assim, decorrente das referidas limitações, foi objetivo inicial do presente trabalho definir: o modelo animal ideal para conduzir investigações na área da ATM, desenhar um estudo pré-clínico randomizado, oculto, com grupo controlo *sham* e, respeitando as ARRIVE *guidelines* [58] que possa contribuir como modelo para futuras investigações nesta área, e estudar o efeito da discectomia e discopexia com intervenções bilaterais.

Os estudos pré-clínicos assumem um papel importante para o desenvolvimento da medicina, representando uma indispensável fronteira entre a investigação *in vitro* e os ensaios clínicos em humanos. Um fármaco ou dispositivo médico inicia o seu desenvolvimento com múltiplos testes e ensaios *in vitro*. Após verificados os perfis de toxicidade e/ou viabilidade celular, seguem-se estudos em animais para avaliar o comportamento do fármaco ou dispositivo (*e.g.* metabólico, farmacocinético, farmacodinâmico e perfis de segurança). Normalmente, estes testes são realizados em dois tipos diferentes de animais: roedores e não-roedores, existindo vários tipos de modelo animal para realizar investigação pré-clínica. No entanto, recorrendo ao princípio de *Krogh* [59], existem animais que são mais indicados no estudo de determinado tipo de problemas, tornando muitas vezes a sua escolha complexa. A reprodução de patologia articular em animais deve ter em consideração a anatomia, biomecânica e comportamento animal para reduzir possíveis erros nos estudos. Assim, a escolha do animal revela-se determinante para o desenvolvimento rigoroso de estudos que visem a futura aplicabilidade em ensaios clínicos.

O rigor do desenho experimental é essencial para reduzir a probabilidade de erro nas conclusões. Existem potenciais origens para erros nos estudos desenhados, podendo esses erros ser diminuídos adotando métodos como a randomização, ocultação e descrição rigorosa da metodologia. Numa revisão recente, observou-se que cerca de 70% dos estudos em animais não eram randomizados, cerca de 85% não tinham um método definido para alocação e em cerca de 65% a análise das variáveis não era realizada em ocultação [60]. Uma das estratégias para melhorar o rigor dos estudos pré-clínicos foi a introdução das ARRIVE *guidelines* [58]. Estas *guidelines* consistem numa *check-list* de 20 itens, contendo a informação considerada mínima e obrigatória para qualquer estudo em animais. Esta informação deve especificar para cada estudo: espécie, sexo, idade e detalhes sobre o tipo de alimentação dos animais, métodos experimentais e estatísticos. Deve incluir também uma secção orientada para o desenho do estudo (randomização, alocação e ocultação). Todos estes itens têm como objetivo aumentar o rigor, a transparência e a compreensão do estudo e dos resultados. Por sua vez, para melhorar a qualidade dos ensaios clínicos em humanos, existem *guidelines* desde 1996 com modelos como o SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) [61] e o CONSORT (Consolidated Standards of Reporting Trials) [62].

A utilização de um grupo controlo deve fazer parte do desenho experimental a adotar. A ATM, sendo uma articulação bilateral dependente, onde vários estudos mostraram que uma intervenção influencia o lado contralateral [47,63], merece reflexão sobre como introduzir o controlo no estudo. Com efeito, vários estudos utilizaram o lado contralateral como controlo [53,64–66], introduzindo uma possível interpretação errada dos resultados. Assim, outras estratégias deverão ser adotadas, a fim de remover as referidas incertezas. Por último, a seleção das variáveis a estudar é, frequentemente, tema de debate. Para além das variáveis convencionalmente utilizadas, histológica [56,67,68] e radiológica [69], foi desígnio do presente trabalho propor a análise do tempo de mastigação e da cinemática ruminatória do modelo animal proposto. Não tendo conhecimento de estudos anteriores, que tenham avaliado o impacto de alterações na ATM na cinemática ruminatória e na mastigação, será importante perceber se esta avaliação é exequível e reprodutível em estudos futuros.

CAPÍTULO 2. OBJETIVOS DO ESTUDO

O principal objetivo desta dissertação foi avaliar o efeito da discectomia e discopexia da ATM, e analisar o seu impacto histológico, radiológico, no tempo de mastigação e na cinemática ruminatória da ovelha *Black Merino*.

Assim, foram definidos como objetivos específicos:

1. Definir o modelo animal apropriado para estudos pré-clínicos na área da ATM.
2. Caracterizar com precisão a anatomia e biomecânica do disco nativo da ovelha *Black Merino*.
3. Elaborar um desenho de estudo de investigação pré-clínico orientado à área da ATM, respeitando as ARRIVE *guidelines*.
4. Aplicar o modelo animal da ovelha *Black Merino* no desenho de estudo proposto.
5. Propor e testar variáveis piloto na área da cinemática ruminatória e tempo de mastigação e avaliar o seu impacto após discectomia bilateral e discopexia bilateral na ovelha *Black Merino*.
6. Avaliar o impacto histológico e imagiológico da discectomia bilateral e discopexia bilateral na ovelha *Black Merino*.

CAPÍTULO 3. DEFINIR MODELO ANIMAL PARA ESTUDOS PRÉ-CLÍNICOS NA ÁREA DA ARTICULAÇÃO TEMPOROMANDIBULAR

Trabalho 1

Animal model for temporomandibular joint research: morphological, histological and biomechanical characterization of the Black Merino sheep joint disc

David Faustino Ângelo, Pedro Morouço, Nuno Alves, Tânia Viana, Fábio Santos, Raúl González, Florencio Monje, Domingos Macias, Belmira Carrapiço, Rita Sousa, Sandra Cavaco Gonçalves, Francisco Salvado, Conceição Peleteiro, Mário Pinho

Morphologie. 2016 Dec;100(331):223-233.

Animal model for temporomandibular joint research: morphological, histological and biomechanical characterization of the Black Merino sheep joint disc

D.F. Angelo[a], P. Morouço[b], N. Alves[b], T. Viana[b], F. Santos[c], R. González[d], F. Monje[d], D. Macias[e], B. Carrapiço[c], R. Sousa[f], S. Cavaco-Gonçalves[g], F. Salvado[f], C. Peleteiro[c], M. Pinho[h]

[a] Hospital de Santa Maria, Faculty of Medicine, Lisbon, Portugal

[b] Centre for Rapid and Sustainable Product Development, Polytechnic Institute of Leiria, Leiria, Portugal

[c] CIISA, Interdisciplinary Centre of Research in Animal Health, Faculty of Veterinary Medicine, Lisbon, Portugal

[d] Infanta Cristina Hospital, Faculty of Medicina, University of Extremadura, Badajoz, Spain

[e] Department of Anatomy, Faculty of Medicina, University of Extremadura, Badajoz, Spain

[f] Hospital de Santa Maria, Faculty of Medicina, Lisbon, Portugal

[g] National Institute of Agrarian and Veterinary Research, Santarém, Portugal

[h] Interdisciplinary Centre of Research in Animal Health, Faculty of Veterinary Medicine, Lisbon, Portugal

Corresponding author:

E-mail address: david.serrano.angelo@gmail.com (D.F. Angelo).

KEYWORDS

Sheep; Temporomandibular joint; Anatomy; Histology; Biomechanical characterization

SUMMARY

Preclinical trials are essential to the development of scientific technologies. Remarkable molecular and cellular research has been done using small animal models. However, significant differences exist regarding the articular behavior between these models and humans. Thus, large animal models may be more appropriate to perform trials involving the temporomandibular joint (TMJ). The aim of this work was to make a morphological (anatomic dissection and white light 3D scanning system), histological (TMJ in bloc was removed for histologic analysis) and biomechanical characterization (tension and compression tests) of sheep TMJ comparing the obtained results with human data. Results showed that sheep processus condylaris and fossa mandibularis are anatomically similar to the same human structures. TMJ disc has an elliptical perimeter, thinner in the center than in periphery. Peripheral area acts as a ring structure supporting the central zone. The disc cells display both fibroblast and chondrocyte-like morphology. Marginal area is formed by loose connective tissue, with some chondrocyte-like cells and collagen fibers in diverse orientations. Discs obtained a tensile modulus of 3.97 ± 0.73 MPa and 9.39 ± 1.67 MPa, for anteroposterior and mediolateral assessment. The TMJ discs presented a compressive modulus (E) of 446.41 ± 5.16 MPa and their maximum stress value (σ max) was 18.87 ± 1.33 MPa. Obtained results suggest that these animals should be considered as a prime model for TMJ research and procedural training. Further investigations in the field of oromaxillofacial surgery involving TMJ should consider sheep as a good animal model due to its resemblance of the same joint in humans.

INTRODUCTION

To improve human health, scientific discoveries and technologies must be translated into practical applications. Such advances classically begin with basic research and then progress to the clinical level. Inherent to the development of new technologies is the role of preclinical trials using animal models. Although no animal model can fully replicate human conditions, animal models are key for the evaluation of mechanisms of disease, testing new technologies and applying new procedures. Temporomandibular joint (TMJ) is the most frequently used joint in the human body. TMJ opens and closes 1500-2000 times daily and is essential for everyday functions of the mouth such as mastication, speech, deglutition, yawning and snoring involving special mandatory synergy of both articular sides [10]. Joint surfaces are convex and, therefore, smooth joint movements are only possible due to an intra-articular disc between them. TMJ disc is an essential component in the normal TMJ and has the following functions: it distributes the intra-articular load, stabilizes the joints during translation and decreases the wear of the articular surface [70,71]. TMJ disc displaced, malformed or damaged, can induce pathologic processes of internal derangement and/or osteoarthritis [72,73]. Currently patients suffering from severe temporomandibular dysfunction (TMD) have few treatment options. Without safe, effective TMJ disc implants, many patients undergo discectomy: a surgical procedure that removes the injured TMJ disc aiming to reduce severe TMD symptoms. This procedure may not be the ideal as the TMJ is left without an important functional structure. Since the previous problems associated with alloplastic materials used to substitute TMJ disc such as silicone and Proplast-Teflon (PTIPI, Vitek, Inc, Houston, Texas, USA) [74,75], many groups discarded investigation in this field. However, the potential impact of a synthetic temporomandibular interposal implant (TII) is immense. Failures of the synthetic TII have generally been attributed to the lack of knowledge concerning the TMJ biomechanical and biochemical aspects. The development of new technologies for scaffolds engineering regarding TMJ disc is growing [65,76–78] and the ideal animal model for TMJ research should be well characterized. The choice of an animal for experimental design is not straightforward. Due to physiological and anatomical differences between the human TMJ and that of experimental animals, there is no animal model that is valid *per se*. TMJ is a cardinal feature that defines the class *Mammalia* and separates mammals from other vertebrates [79]. TMJ shows remarkable morphological and functional variation between different species, reflecting not only the great mammalian adaptation to feeding mechanisms but also different biomechanical behavior [80]. The morphological variations are either correlates of loading (e.g. size of articular surfaces) or movement (e.g. orientation of the joint), or both. Loading of the TMJ is a reaction force arising from the contraction of masticatory muscles; its magnitude depends strongly on the position of the bite point relative to the muscle action line [81]. Many commonly used laboratory animals, especially rodents, fall in the category of minimal TMJ loading, especially during chewing. In contrast, carnivores such as dogs sustain TMJ loads that are higher than those of primates [82]. Opening of the jaw usually involves a combination of rotation and forward sliding (translation), but some carnivores have lost the ability to slide and some specialized anteaters instead use a rotation around the long axis of the curved mandible [80]. The most extreme evolutionary variants include:

- loss of the synovial cavity in some baleen whales;
- loss (or possibly primitive absence) of the disc in monotremes, some marsupials, and some edentates (anteaters and sloths);
- variations in the orientation of the joint cavity from sagittal (many rodents) to transverse (many carnivores);
- reversal of the usual convex/concave relationship so that the processus condylaris becomes the female element (many artiodactyl ungulates such as sheep and cattle).

In addition, the relative size of the joint is exceedingly variable. Sheep, rabbit and monkey have been used as TMJ disc defect models in many studies [83–90]. Monkey model is barely used in recent years, considering the high cost, difficult surgical operation and ethical approval. Rabbit is an excellent option for TMJ disc anterior dislocation studies but the small size of TMJ increases the difficulty for surgical approach and disc manipulation. The authors agree with others studies considering sheep is a valid option for TMJ studies due to TMJ size, processus condylaris and fossa mandibularis shape, disc size, morphology and attachments [79]. However, a deep biochemical and biomechanical characterization of the sheep TMJ is lacking in the available literature. Hence, the aim of the present study was to examine the morphological, histological and biomechanical properties of TMJ discs extracted from sheep (*Ovis aries*). It was hypothesized that these discs would present high similarity with available data on human TMJ.

Materials and methods

The material used for this study was obtained from sheep slaughtered for meat consumption. A total of 15 heads from *Black Merino* female sheep, 40 to 50kg, were used: 6 for morphological characterization, 4 for histological characterization and 5 for biomechanical testing. One of the major requirements for this study was to use fresh TMJ discs; for that reason a team of certified surgeons was available 5 days weekly to collect fresh TMJ up to a maximum of 5 hours after death. Regarding the animal ethical considerations, the present study design was approved by the Portuguese National Authority for Animal Health.

Morphological characterization

For morphological characterization 12 fresh TMJ discs were collected from six sheep heads. A surgical discectomy was performed exposing and identifying TMJ anatomical structures. All muscular attachments were removed to obtain clean TMJ discs. Discs were submersed for 5 minutes in a *ColorBond* solution, an extremely fast curing infiltrant, designed to rapidly strengthen 3D-printed parts. This submersion was essential to maintain the correct morphology for the 3D scanning. A white light 3D scanning system (Steinbichler — COMET 5®) and the appropriate software were used to replicate the discs in a 3D virtual model. Once the discs removed, two of the skulls were boiled in water (120 ºC) for 2 h to allow the procurement of complete clean crania.

Histological characterization

Four sheep heads were used to conduct the histological investigation. The TMJ were removed using a necropsy bone oscillatory saw according to the following anatomic references: cranial — cranial aspect of processus coronoideus in the section of the arcus zygomaticus; caudal — external to the meatus acusticus. The dorsal reference was established to the squamous temporal bone. The ventral reference was 2 cm ventral to the meatus acusticus in the zone of angulus stylohyoideus. The joints were fixed in 10% buffered formalin for ten days. Decalcification was obtained by immersion in 10% formic acid for three weeks, after which the articulations were cut sagittally and transversally through the whole processus condylaris. After intensive washing the fragments were submitted to routine tissue processing with paraffin embedding. Four-micron sections were stained with hematoxylin and eosin (H&E) and with Orcein to show elastic fibers in the disc. Digital images were obtained with an Olympus DP21 camera.

Biomechanical testing

Five sheep heads were used for biomechanical studies. TMJ discs were removed and immersed in a saline solution for transport up to the bioengineering facilities (1 hour maximum). All muscular attachments and ligaments were removed to obtain a clean fibrocartilaginous disc. Ten clean discs were obtained but one was excluded due to surgical damaging. Consequently, 9 discs were randomized in 3 groups and tested in different mechanical tests: Tensile modulus (E), tensile strength and elongation were tested in: anteroposterior tests (APT) and mediolateral tests (MDT). Compression tests (CT) were performed using a stress-strain tests. In case of anteroposterior tensile test, during loading, the TMJ discs were stretched in the direction represented on Fig. 1A, while in mediolateral tensile test the direction of stretching was as shown on Fig. 1B.

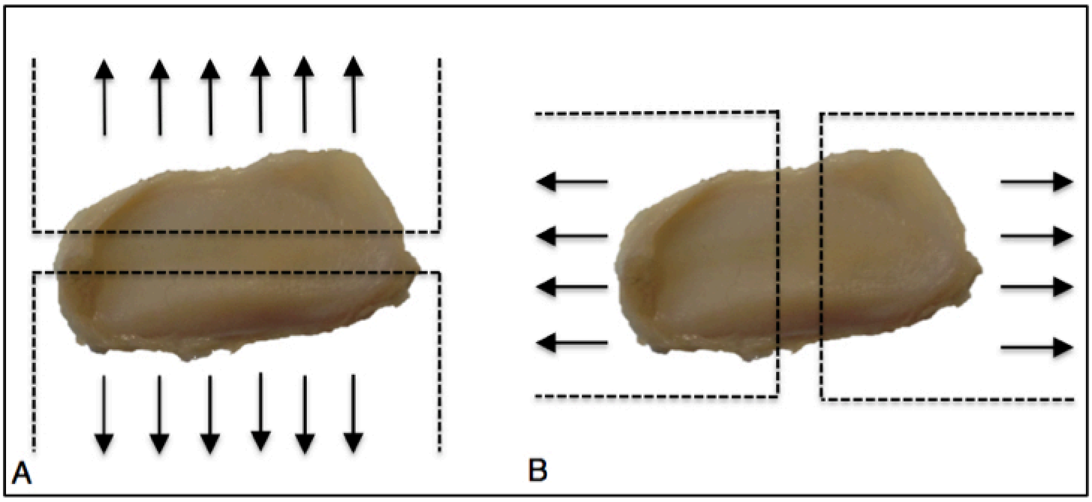


Figure 1. Direction of loading on: (A) anteroposterior and (B) mediolateral tensile tests. The dotted line represents the limit used to fix temporomandibular joint (TMJ) discs in grips. Tension tests used a strain rate of 0.5 mm/min with an initial distance between grips (L0) of 2 mm. All tests were conducted on a Zwick Z100 strength-testing machine (Zwick GmbH & Co. kg, Germany) equipped with a 10 kN load cell. For the compression tests the same rate was applied.

Results

Morphological characterization

In the sheep heads studied, the TMJ was located, as expected, in the posterior segment of the side of the face, cranioventral to the external meatus acusticus, being a diarthrodial, bicondylar joint that allows normal opening and closing of the mandible. It comprised the superior articulating face, the fossa mandibularis of temporal bone, and the processus condylaris, as the inferior articulating surface (Figs. 2 and 6). A protruding processus coronoideus was noted (Fig. 2).

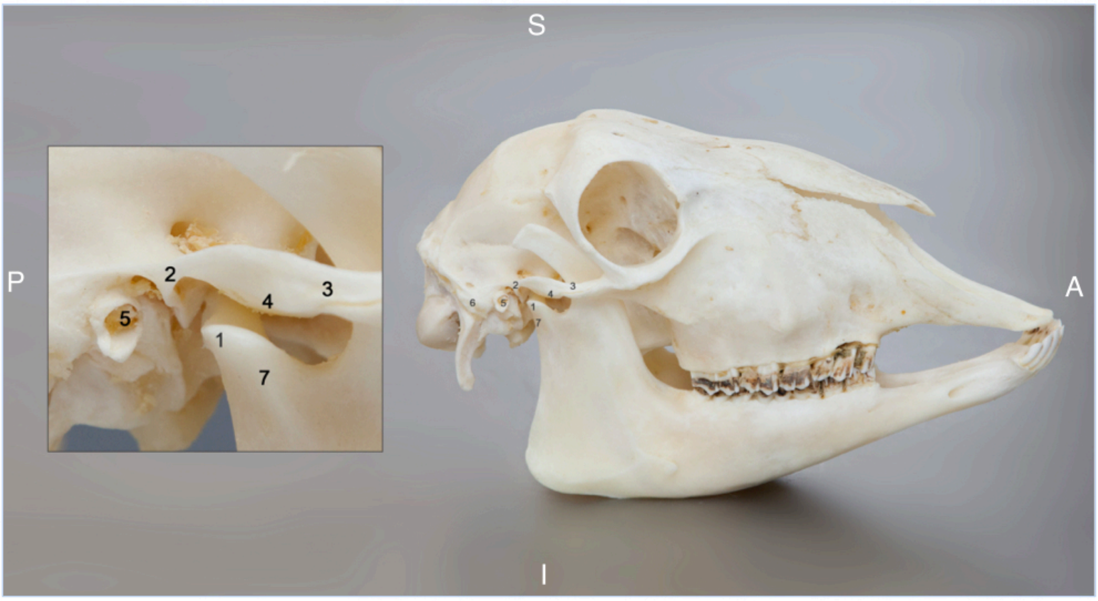


Figure 2. Right view of a sheep skull used in the present study. (1) Processus condylaris, (2) fossa mandibularis, (3) arcus zygomaticus, (4) eminentia articularis, (5) external meatus acusticus, (6) processus mastoideus, (7) collum mandibulae. P: posterior; A: anterior; S: superior; I: inferior.

The superior articulating surface (fossa mandibularis) was located in the inferior zone of temporal bone, lateral of foramen ovale and anterior to the external meatus acusticus. The fossa mandibularis was anteroposterior larger than mediolateral with a convexity downwards. The inferior articulating surface (Fig. 3) is represented by the processus condylaris, with ellipsoidal shape with the longer axis in the mediolateral position, the mean measures being 23.47 mm long (σ=0.87) and 8.32mm wide (σ=1.54). The processus condylaris was mediolateral concave. The fossa mandibularis receives the processus condylaris. With an easy surgical approach the authors located the fibrocartilaginous joint disc interposed between the fossa mandibularis and the processus condylaris (Fig. 4).

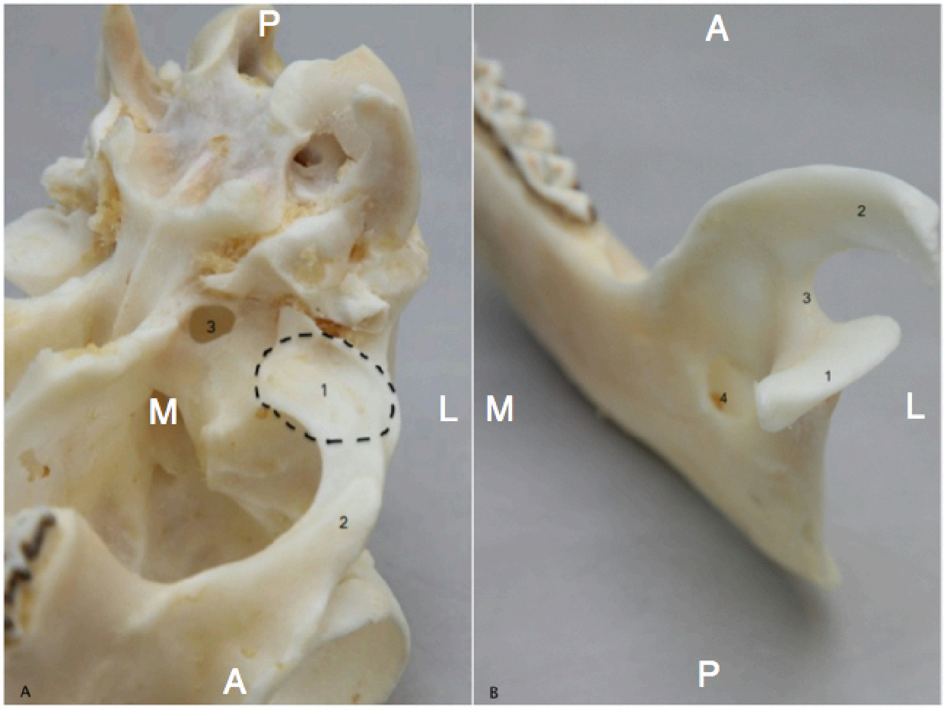


Figure 3. Articular surfaces of the temporomandibular joint (TMJ). A. Superior articular surface: (1) fossa mandibularis, (2) arcus zygomaticus, (3) foramen ovale. B. Inferior articular surface: (1) processus condylaris, (2) processus coronoideus, (3) incisura mandibulae, (4) foramen mandibulae. P: posterior; A: anterior; M: medial; L: lateral.

Figure 4. View of the right temporomandibular joint (TMJ). To improve visualization the authors pulled down the processus condylaris. (1) Cartilage surface of fossa mandibularis in the upper joint compartment, (2) temporomandibular joint disc, (3) retrodiscal tissue, (4) muscle pterygoideus lateralis (5) car- tilage surface of the processus condylaris (6) external meatus acusticus. P: posterior; A: anterior; M: medial; L: lateral.

This disc separates an upper joint cavity from a lower one. The first was consistently larger than the second. The bony structures were coated with cartilage more evident in the processus condylaris. In the ewes studied, the joint disc had an elliptical shape, being substantially thinner in the center than at the periphery. TMJ disc regions are commonly classified as anterior band, posterior band, and intermediate zone (Fig. 5). The intermediate zone exhibits differences from its lateral to medial aspects, being often subdivided into lateral, medial and central region. The bands discs are thicker than the intermediate zone. The mean length and width of the 12 analyzed fresh TMJ discs were 21.23 mm (σ = 1.53) and 11.49 mm (σ = 0.62), respectively. Anterior and posterior band thicknesses were 1.05mm (σ = 0.07) and 1.27mm (σ = 0.04), respectively. Mean central thickness was 0.76 mm (σ = 0.09). The same measures obtained from the 3D virtual models were totally similar to the ones registered in the fresh discs. An important report and consistent with all TMJ was the presence of viscous fluid in upper and lower compartment. This fluid was not analyzed.

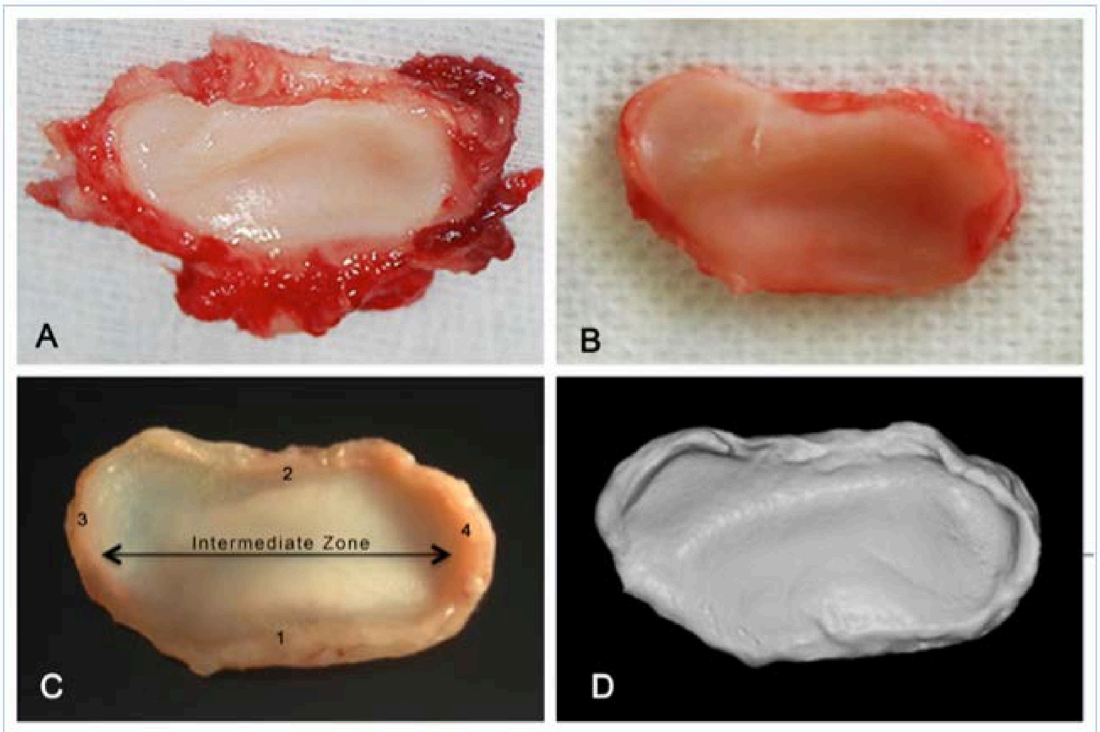


Figure 5. Temporomandibular joint (TMJ) disc. A. Fresh disc with attachments. B. Fresh disc without attachments. C. TMJ disc submitted to ColorBond treatment: (1) anterior band, (2) posterior band, (3) medial band, (4) lateral band. D. TMJ disc 3D virtual model.

Histological characterization

The histological study of the sheep TMJ revealed that the articular disc was attached anteriorly and posteriorly to the articular capsule composed by fibrous tissue. Both the fossa mandibularis and the processus condylaris surfaces were covered by a fibrocartilaginous layer. However, the fibrocartilaginous layer covering the processus condylaris was considerably thicker than the layer covering the fossa mandibularis (Fig. 6). The central thin part of the disc consisted of scattered fibroblasts and densely packed, thick collagen fiber bundles arranged mainly in an anteroposterior direction. The collagen fibers were not straight but showed evidence of a wavy outline. The anterior and posterior disc portions were in turn occupied by collagen fiber bundles with diverse orientations (Fig. 7). In some areas, these two portions showed chondrocyte-like cells residing in lacunae distributed among less compact collagen fibers (Fig. 7). Each lacuna was surrounded by minimal amount of amorphous matrix. The posterior band blended, in the retrodiscal space, with loose connective tissue with profuse blood and nerve supply. A few small caliber blood vessels, surrounded by loose connective tissue, were observed in all parts of the disc (Fig. 7). Also occasional unilocular adipocytes were present at both the anterior and posterior attachments of the disc. Orcein-positive elastic fibers were found throughout the disc, being apparently more abundant in the thinnest central portion. In this disc area, elastic fibers were arranged mostly in parallel to the collagen bundles (Fig. 8). Instead, in the anterior and posterior disc portions, elastic fibers showed a reticular distribution among collagen fibers and chondrocyte-like cells (Fig. 8).

Figure 6. Microscopic overview of a sagittal section of the temporomandibular joint (TMJ) stained with haematoxylin- eosin. T: temporal bone; D: central region of the intermediate area of the joint disc; M: processus condylaris (bar = 10 μm).

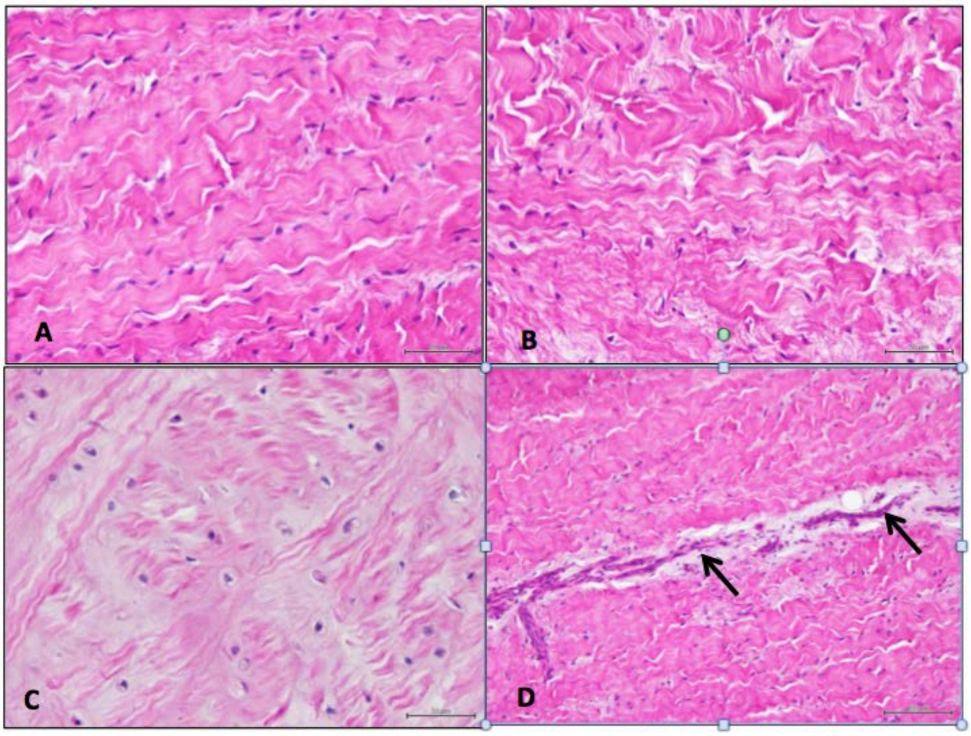


Figure 7. Photomicrographs of various regions of the sheep temporomandibular joint (TMJ) disc stained with haematoxylin-eosin. A. Tightly packed collagen fibers with parallel arrangement interspersed by fibroblasts in the central portion of the TMJ disc (bar = 50 μm). B. Haphazardly arranged collagen fiber bundles in the posterior band of the TMJ disc (bar = 50 μm). C. Chondrocyte-like cells in the anterior band of the TMJ disc (×200, bar = 50 μm). D. Small caliber blood vessels (arrows) in the TMJ disc (×100, bar = 100 μm).

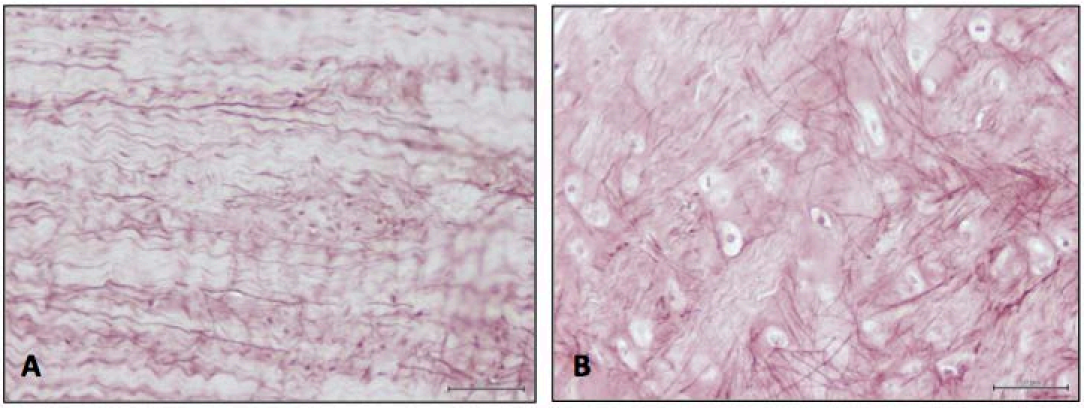


Figure 8. Photomicrographs of the central zone (A) and (B) anterior band of sheep temporomandibular joint (TMJ) disc stained with orcein for detection of elastic fibers (bar = 50 μm). A. Longitudinal elastic fibers follow the wavy structure of collagen bundles. B. Loose mesh elastic fibers distributed between chondrocyte-like cells.

Biomechanical characterization

In Table 1, the measures of the discs used in the mechanical tests are presented. Tensile tests performed revealed that TMJ discs presented different behaviors for anteroposterior and mediolateral directions (Fig. 9). The obtained results demonstrated that the tensile modulus of mediolateral tensile tests is higher than anteroposterior tensile tests, as well as the tensile strength and elongation at break (Figs. 10 and 11). In Table 2 the results obtained for the tested discs for tensile modulus, tensile strength and elongation at break are summarized.

Sample	Dimensions (mm)					Test
	Length	Width	Thickness			
			Posterior band	Central	Anterior band	
APT1	22.71	11.06	1.23	0.55	1.14	Anteroposterior
APT2	23.89	10.69	1.12	0.77	0.98	
APT3	20.43	11.29	1.23	0.79	1.12	
MDT1	19.60	12.63	1.24	0.82	0.94	Mediolateral
MDT2	20.57	10.56	1.36	0.72	1.02	
MDT3	20.05	11.39	1.26	0.85	1.10	
CT1	20.75	10.07	1.12	0.81	1.02	Compression
CT2	20.49	11.93	1.25	0.79	0.97	
CT3	19.94	10.44	1.29	0.80	1.03	

APT: anteroposterior tests; MDT: mediolateral tests; CT: compression tests.

Table 1. Length, with and thickness of 9 discs tested.

Mechanical testing under compression was performed to evaluate the macro-mechanical performances of the TMJ discs. Fig. 12 demonstrates the compressive stress-strain curves of the tested discs. The TMJ discs presented a compressive modulus (E) of 446.41±5.16MPa and their maximum stress value (σ max) was 18.87 ± 1.33 MPa.

Tensile test	Tensile modulus E (MPa)	Tensile strength (MPa)	Elongation at break (%)
Anteroposterior	3.97 ± 0.73	4.34 ± 1.22	170.92 ± 47.87
Mediolateral	9.39 ± 1.67	13.21 ± 0.85	195.23 ± 20.44

TMJ: temporomandibular joint. Tensile modulus (E), tensile strength and elongation at break are reported as mean value ± standard deviation.

Table 2. Mechanical tensile properties of TMJ discs.

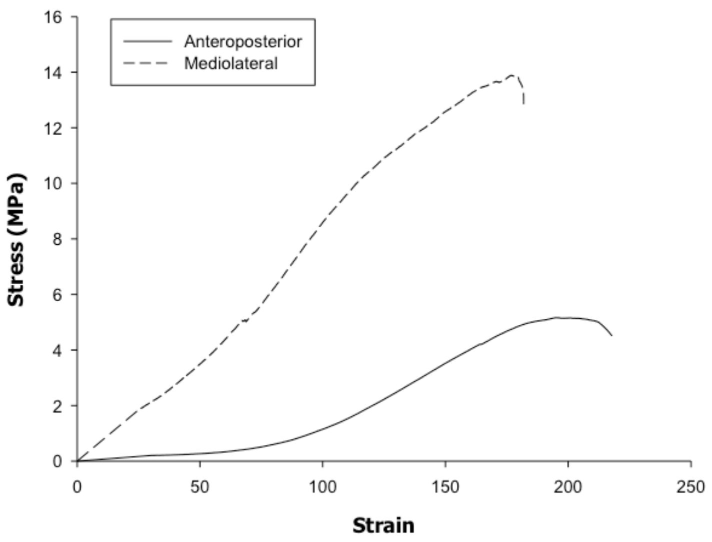


Figure 9. Tensile mechanical performance of temporomandibular joint (TMJ) discs in anteroposterior and mediolateral directions.

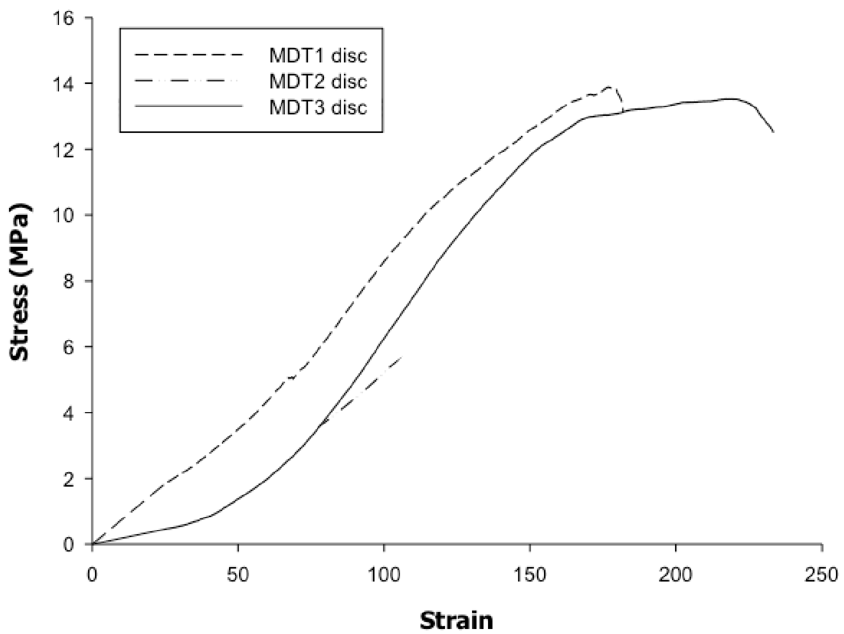


Figure 10. Mediolateral tensile mechanical performance of temporomandibular joint (TMJ) discs. Tension tests used a strain rate of 0.5 mm/min with an initial distance between grips (L0) of 2 mm. All tests were conducted on a Zwick Z100 strength-testing machine equipped with a 10 kN load cell.

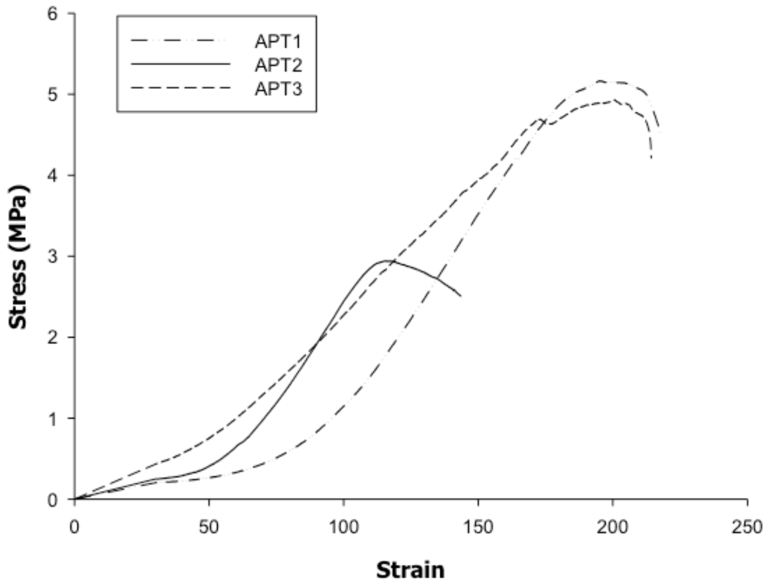


Figure 11. Anteroposterior tensile mechanical performance of temporomandibular joint (TMJ) discs. Tension tests used a strain rate of 0.5 mm/min with an initial distance between grips (L0) of 2 mm. All tests were conducted on a Zwick Z100 strength- testing machine equipped with a 10 kN load cell

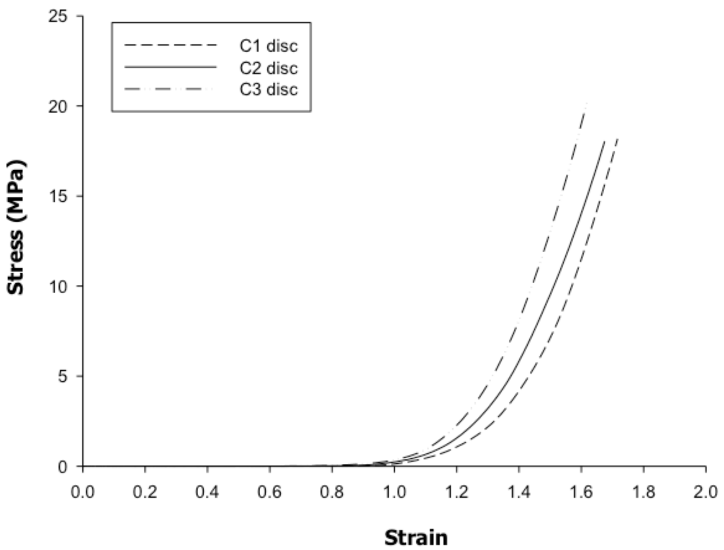


Figure 12. Compressive mechanical performance of temporomandibular joint (TMJ) discs. Compression tests used a compression rate of 0.5 mm/min.

Discussion

TMJ disc is a specialized fibrocartilaginous tissue, located between the processus condylaris and the fossa mandibularis [70,75,91] as shown in our sheep morphologic characterization. In humans TMJ disc has an elliptical perimeter, thinner in the center than on periphery. Disc periphery acts like a ring structure supporting the central zone. The same was observed in sheep disc morphology. The functions of the TMJ disc are:

• to improve the fit between bony surfaces;
• to provide stability during mandibular movements;
• to distribute masticatory forces [92].

This capacity is due to the high concentration of collagen fibers. This ring structure around the disc is an important structural aspect to support disc connections. The connection area is rich in elastic fibers, which is essential to disc mobility in the joint. As it was shown in the morphological characterization of the sheep TMJ, this anatomical structure revealed several similar characteristics with the TMJ in humans, including the mediolateral diameter being longer than the anteroposterior, the long axis of the processus condylaris directing backwards, and larger anterior condylar slope. One of the main differences is the concave form of the mediolateral processus condylaris that is convex in humans. The processus condylaris forms a small anteroposterior and mediolateral depression to fit exactly in the fossa mandibularis, unlike the human processus condylaris, which is rounded anteroposterior and mediolateral. The fossa mandibularis is anteroposterior larger than mediolateral with a convexity downwards contrarily to the fossa mandibularis in humans that is concave upwards. The fossa mandibularis allows for the free mediolateral movement of the processus condylaris for rumination. The articular tubercle, a special feature in humans, is rudimentary in the sheep, since the path of the processus condylaris movement is mediolateral, contrarily to the one in humans, which is mostly anteroposterior. Comparatively, the fossa and processus condylaris of the sheep are much like edentulous human TMJ, much flatter. Architecturally, the processus condylaris in both species also has a thin external cortex that surrounds the medullary bone that is made up of trabecular bone. There is also a thin layer of fibrocartilage covering the condylar surface and entire fossa mandibularis, indicating parts of the temporomandibular joint that are subject to highest loading. TMJ relation with the external acusticus meatus, foramen ovale and the joint disc position interposing processus condylaris and fossa mandibularis are similar to human TMJ anatomy. TMJ disc morphology is very similar to human TMJ disc. The choice of sheep as an animal model for TMJ studies has been used for several years [83–90]. TMJ disc implants can be an efficacious complement in bioengineered joint reconstruction and animal models may offer the possibility to conduct informative preclinical studies. One of the most important problems to create an effective TII is to replicate the biomechanics characteristics of the native disc. Therefore, information on the biomechanical properties of the substitute material is indispensable for further investigation in TMJ disc tissue engineering. During mandibular movements the TMJ disc is subject to a multitude of different loading regimens. TMJ disc behaves as a viscoelastic structure acting as a stress absorber and a stress distributor [92–94]. Elastic fibers play an important role providing the disc with the necessary viscoelastic structure. During every type of loading, the disc undergoes a deformation, while internal forces are produced within the tissue [91]. The internal forces are quantified by the amount of stress, which is defined as force per unit area in Pa (1 Pa = 1 N/m2). There are only two studies available on bovine TMJ disc in which tensile and compressive modulus have been compared using the same experimental protocol and material [91,95]. In these studies tensile modulus ranged between 22 and 26MPa, and compressive modulus between 14 and 17 MPa. Data on the porcine, canine and human TMJ discs are available in the literature but methods used for disc obtainment and processing are not always clear. Reported tensile modulus are approximately 0.5—80MPa, 20—25MPa and 40—100MPa, respectively, for the referred above animal models [91,96,97]. In order to evaluate the mechanical behavior of the sheep TMJ discs, the authors report, for the first time as it was possible to estimate, anteroposterior and mediolateral tensile modulus and compressive modulus. The use of fresh TMJ discs have contributed for the results to be representative of reality. Sheep mandibular movements are mostly mediolateral explaining the better performance of TMJ disc supporting tension in the mediolateral direction. In conclusion, sheep seems to be an excellent experimental model for TMJ studies, being a large species with many anatomical similarities to the human structure in relation to surgical approach, anatomical structures size, shape and position of the processus condylaris. TMJ disc seems to be very similar human TMJ disc concerning morphology, histology and biomechanics. It is the author's purpose that the present work will help further research in the field of oromaxillofacial conducted in sheep as an excellent alternative to other more conventional experimental animal species, also more suitable for procedural surgical training.

CAPÍTULO 4. RECONSTRUÇÃO EM 3 DIMENSÕES DO DISCO DA ARTICULAÇÃO TEMPOROMANDIBULAR DA OVELHA *BLACK MERINO*

Trabalho 2

Novel approach for 3D virtual model of TMJ disc morphology. Preliminary results

D. F. Ângelo, P. Morouço, N. Alves, T. Carvalho, D. Bonaparte, B. Carrapiço, F. Monje, I. A. Furtado, F. Salvado

Surg Radiol Anat. 2016 Jan;38(1):5-47.

Novel approach for 3D virtual model of TMJ disc morphology. Preliminary results

D. F. Angelo[1], P. Morouco[2], N. Alves[2], T. Carvalho[3], D. Bonaparte[3], B. Carrapiço[4], F. Monje[3], I. A. Furtado[1], F. Salvado[1]

[1] Centro Hospitalar Lisboa Norte
[2] Centre for Rapid and Sustainable Product Development
[3] Instituto de Medicina Molecular
[4] Faculdade de Medicina Veterinária,

Corresponding author:
E-mail address: david.serrano.angelo.phd@gmail.com (Angelo DF)

ABSTRACT

Introduction: Regenerative medicine is an immense field with extreme and challenging obstacles. The first challenge is to understand the morphological, histological, biochemical and biomechanical characteristics of the structure to reproduce. This multitask and multidisciplinary approach is essential to determine the success of regenerative medicine. The authors present a new method to reproduce 3D morphology of anatomical structures with sheep TMJ disc as an example.

Objective: The main objective of the authors was to reproduce a 3D virtual model of six sheep TMJ disc.

Methods: A medical surgeon performed surgical discectomy of TMJ disc in fresh sheep cadaver with microscope. The second step was related to remove all disc muscular attachments to obtain a clean cartilage disc. The disc was submersed in a solution to maintain the correct morphology. With a white light 3D scanning system and appropriate software we reproduce the morphology of six sheep TMJ disc.

Conclusion: 3D virtual model of TMJ disc were success- fully reproduced using a white light 3D scanning system. This technique has economic and time related advantages. For precision and detailed results we need to conduct more studies.

CAPÍTULO 5. PROTOCOLO PARA MODELO DE INVESTIGAÇÃO PRÉ-CLÍNICA EM ARTICULAÇÃO TEMPOROMANDIBULAR RESPEITANDO AS *ARRIVE GUIDELINES*

Trabalho 3

Bioengineered Temporomandibular Joint Disk Implants: Study Protocol for a Two-Phase Exploratory Randomized Preclinical Pilot Trial in 18 Black Merino Sheep (TEMPOJIMS)

David Faustino Ângelo, Florencio Gil Monje, Raúl González-García, Christopher B Little, Lisete Mónico, Mário Pinho, Fábio Abade Santos, Belmira Carrapiço, Sandra Cavaco Gonçalves, Pedro Morouço, Nuno Alves, Carla Moura, Yadong Wang, Eric Jeffries, Jin Gao. Rita Sousa, Lia Lucas Neto, Daniel Caldeira, Francisco Salvado

JMIR Res Protoc. 2017 Mar 2;6(3):e37.

Bioengineered Temporomandibular Joint Disk Implants: Study Protocol for a Two-Phase Exploratory Randomized Preclinical Pilot Trial in 18 Black Merino Sheep (TEMPOJIMS)

David Faustino Ângelo[1,2,3]; Florencio Gil Monje[4]; Raúl González-García[4]; Christopher B Little[5]; Lisete Mónico[6]; Mário Pinho[7]; Fábio Abade Santos[7]; Belmira Carrapiço[8]; Sandra Cavaco Gonçalves[9]; Pedro Morouço[2]; Nuno Alves[2]; Carla Moura[2]; Yadong Wang[10]; Eric Jeffries[10]; Jin Gao[10]; Rita Sousa[3]; Lia Lucas Neto[3]; Daniel Caldeira[3]; Francisco Salvado[3]

[1]Centro Hospitalar de Setúbal, EPE, Stomatology Department, Setúbal, Portugal

[2]Instituto Politécnico de Leiria, Centre for Rapid and Sustainable Product Development (CDRSP), Leiria, Portugal

[3]Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal

[4]University Hospital Infanta Cristina, Badajoz, Spain, Department of Oral and Maxillofacial Surgery, Badajoz, Spain

[5]Kolling Institute, Institute of Bone and Joint Research, University of Sydney, Sydney, Australia

[6]Coimbra University, Coimbra, Portugal

[7]Faculdade de Medicina Veterinaria, Interdisciplinary Centre of Research in Animal Health (CIISA), Lisbon University, Lisboa, Portugal

[8]Faculdade de Medicina Veterinaria, Lisbon University, Lisboa, Portugal

[9]Instituto Nacional de Investigação Agrária e Veterinária (INIAV I.P.), Santarém, Portugal

[10]University of Pittsburgh, Chemical Engineering and Surgery Pittsburgh, University of Pittsburgh, Pittsburgh, PA, United States

Corresponding Author:

E-mail address: david.serrano.angelo@gmail.com (D.F. Angelo).

KEYWORDS

temporomandibular joint disorders (TMD); temporomandibular joint bioengineered disk implants; temporomandibular randomized preclinical trial protocol

ABSTRACT

Background: Preclinical trials are essential to test efficacious options to substitute the temporomandibular joint (TMJ) disk. The contemporary absence of an ideal treatment for patients with severe TMJ disorders can be related to difficulties concerning the appropriate study design to conduct preclinical trials in the TMJ field. These difficulties can be associated with the use of heterogeneous animal models, the use of the contralateral TMJ as control, the absence of rigorous randomized controlled preclinical trials with blinded outcomes assessors, and difficulties involving multidisciplinary teams.

Objective: This study aims to develop a new, reproducible, and effective study design for preclinical research in the TMJ domain, obtaining rigorous data related to (1) identify the impact of bilateral discectomy in black Merino sheep, (2) identify the impact of bilateral discopexy in black Merino sheep, and (3) identify the impact of three different bioengineering TMJ discs in black Merino sheep.

Methods: A two-phase exploratory randomized controlled preclinical trial with blinded outcomes is proposed. In the first phase, nine sheep are randomized into three different surgical bilateral procedures: bilateral discectomy, bilateral discopexy, and sham surgery. In the second phase, nine sheep are randomized to bilaterally test three different TMJ bioengineering disk implants. The primary outcome is the histological gradation of TMJ. Secondary outcomes are imaging changes, absolute masticatory time, ruminant time per cycle, ruminant kinetics, ruminant area, and sheep weight.

Results: Previous preclinical studies in this field have used the contralateral unoperated side as a control, different animal models ranging from mice to a canine model, with nonrandomized, nonblinded and uncontrolled study designs and limited outcomes measures. The main goal of this exploratory preclinical protocol is to set a new standard for future preclinical trials in oromaxillofacial surgery, particularly in the TMJ field, by proposing a rigorous design in black Merino sheep. The authors also propose to test the feasibility of pilot outcomes.

INTRODUCTION

The temporomandibular joint (TMJ) is the most frequently used joint in the human body. The TMJ opens and closes 1500 to 2000 times daily and is essential for everyday functions of the mouth, such as mastication, speech, deglutition, yawning, and snoring, involving special mandatory synergy of both articular sides [10]. The TMJ disk is an essential component in the normal TMJ and has the following functions: (1) it distributes the intra-articular load, (2) it stabilizes the joints during translation, and (3) it decreases the wear of the articular surface [70,71]. The majority of TMJ disorders (TMD) are successfully treated with reversible, conservative, and low-tech treatments such as education and counseling, therapeutic exercises, splint therapy, and pharmacotherapy [98,99]. When the TMJ disk is displaced, malformed, or damaged, it can induce serious internal pathologic processes and/or osteoarthritis [72,73]. Currently, patients suffering from severe TMD have limited validated treatment options. Most surgical approaches, such as TMJ discectomy, do not restore the structural or biological properties of the articulation and disk. This procedure may not be ideal because the TMJ is left without an important functional structure. A variety of interpositional materials have been used to replace the removed disks, including synthetic materials manufactured from silicone, teflon, polytetrafluoroethylene, and biological interpositional grafts taken from different anatomic sites [74,100–102]. These interpositional materials do not take in consideration the anatomy and biochemical and biomechanical characteristics of the TMJ native disk [103], and some of them have been associated with serious complications for the patients [102–104]. In the late 1980s, Proplast/Teflon TMJ (synthetic interpositional implant) were found to be harmful in many patients. The breakdown of the material, probably caused by TMJ high biomechanical forces, lead to fragmented particles that resulted in an immune foreign body response that caused problems ranging from severe cutaneous inflammatory reaction in the preauricular and cheek areas [105] to severe degenerative joint disease with perforation into the middle cranial fossa [106,107]. The result was a dramatic clinical spectrum of failures for these implants [74]. In December 1991, the US Food and Drug Administration's Bulletin recommended immediate removal of all previous TMJ Proplast/Teflon implants because of the mechanical failures, many resulting in progressive bone degeneration [108]. In a 1992 workshop, the American Academy of Oral and Maxillofacial Surgery instructed the discontinuation of Proplast/Teflon [108]. The absence of efficacious options to substitute the TMJ disk can be related to difficulties in the translation of animal evidence to the clinical practice in humans. These limitations are likely related to:

1. the use of heterogeneous animal models with conflicting results, possibly due to variable anatomy and intra-articular loading between species [79,109];
2. the use of the contralateral TMJ as control, which may be associated with contralateral overloading [110];
3. the biomaterials used to replace the disk do not account for the morphologic and biomechanical characteristics of the native disk;
4. absence of randomized controlled trials with blinding of outcomes' assessors; and
5. lack of multidisciplinary teams involved in the project.

Preclinical research should promote the effective translation of knowledge into practice. The previously mentioned aspects can limit the effective translation of quality scientific knowledge into clinical practice and these may present potential issues to patients, clinicians, and scientific progress. The contemporary absence of successful options to substitute the TMJ disk is still a major issue for public health. Little has changed in the past decade regarding study designs for TMJ investigation, and the treatment for patients with severe TMD remains controversial. The main objective of the Temporomandibular Joint Interposal Material Study (TEMPOJIMS) is to develop a new, reproducible, and effective study design for preclinical research in the TMJ field. The second goal is to progress in bioengineering and regenerative medicine evaluating the benefits of a TMJ bioengineering implant to substitute the damaged native TMJ disk. This preclinical exploratory study is divided into two phases. Phase 1 of this study is a blinded randomized preclinical trial, designed to investigate if the TMJ undergoes important injury in bilateral discectomy, bilateral discopexy, and sham surgery. Phase 2 intentions are to evaluate the safety and efficacy of three different TMJ bioengineering implants using the same rigorous method of phase 1.

METHODS

Study Design

The TEMPOJIMS is a two-phase exploratory randomized controlled preclinical trial planned to gather preliminary information to (1) evaluate a new study design for TMJ investigation; (2) evaluate the black Merino sheep animal model for TMJ investigation; (3) evaluate TMJ behavior under bilateral surgical intervention (discectomy and discopexy) using a histologic primary outcome (microscopic scoring of destructive changes in TMJ using a modified Mankin scoring system [111]), secondary imaging outcome (imaging scoring of TMJ); (4) testing the applicability of pilot secondary outcomes predominantly for ruminant kinetics; and (5) obtain a baseline for interpretation of TMJ disk bioengineering implants results. Phase II is aimed to test safety and efficacy of three different bilateral TMJ bioengineering disk implants (Figure 1). Outcome evaluators and analysts are blinded for surgical assessments.

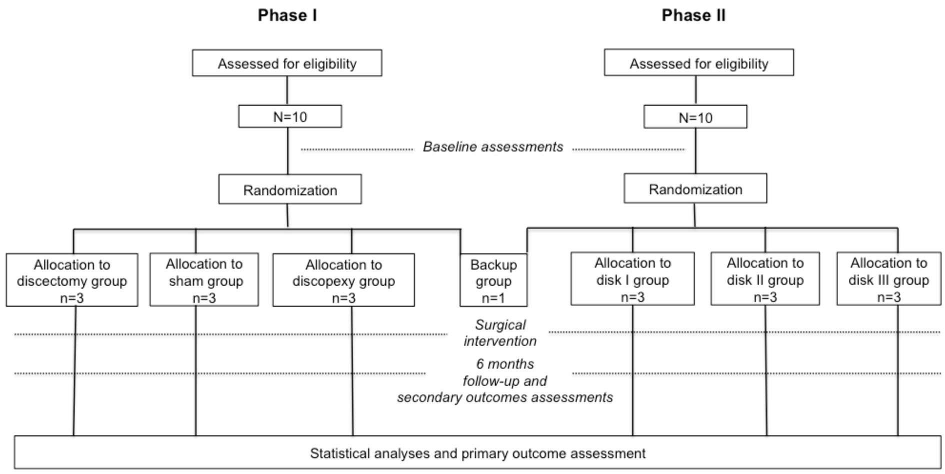


Figure 1. Study design.

Major institutions involved in this study are (1) Lisbon Faculty of Medicine for study design, coordination, and statistical analysis; (2) Interdisciplinary Centre of Research in Animal Health in Faculty of Veterinary Medicine for histological preparation and veterinary support of all animals; (3) Centre for Rapid and

Sustainable Product Development for bioengineered disk implants (disks I and II); (4) Bioengineering, Surgery, Chemical Engineering, Mechanical Engineering and Materials Science, University of Pittsburgh, for bioengineered disk implants (disk III); (5) Department of Oral and Maxillofacial-Head and Neck Surgery, University Hospital Infanta Cristina, Badajoz, Spain, for surgical support; (6) Institute of Bone and Joint Research-Northern Sydney Local Health District-Sydney Medical School Northern, University of Sydney, Australia, for histological analysis; and (7) Radiology Department of Santa Maria Hospital, Lisbon, Portugal, for imaging analysis.

Animal Model

A variety of strains/breeds of sheep have been used in TMJ investigations. To decrease biological variability, the authors recommended black Merino sheep as the animal model to conduct the study [109]. As recommended, the authors proposed to use "sheep skeletally mature" at ≥2 years of age [112]. The inclusion criteria are certified black Merino sheep, adult (age 2-5 years), female, and in good health condition (veterinary check-up is performed on all animals). Regarding the animal ethical considerations, the study design was approved by the Portuguese National Authority for Animal Health registered with number 026618. The study design and organization respect the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

Baseline and Follow-Up Evaluation

The baseline and follow-up evaluations are outlined at particular time points (Figure 2). Pilot secondary outcomes and weight are measured at days 11, 10, and 9 before surgery (details on secondary outcomes are reported in outcomes measures). Transportation to surgical facilities is performed 5 days before surgery to avoid animal stress and allow familiarization to the temporary facilities. Head computerized tomography (CT) scan is performed on the day of surgery taking advantage of pre anesthesia sedation. Ten days after surgery, animals are transported to TEMPOJIMS main facilities. Days 19, 20, and 21 after surgery, the follow-up secondary outcomes start to be recorded every 30 days for 6 months (Figure 2). At the end, animals are sacrificed and a new CT scan is performed to measure the imaging outcome and to begin the histologic preparation.

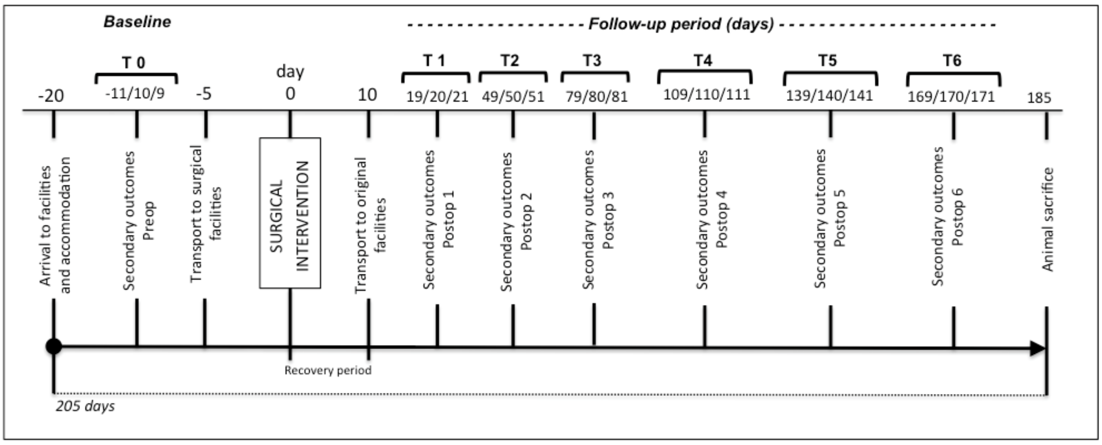


Figure 2. Study flowchart.

Randomization, Allocation, and Blinding

The randomization is performed by a statistical group not involved in the outcome assessments, managed by Lisbon Faculty of Medicine. Allocation to each randomized group is performed preoperatively by sealed envelope and separately for phase 1 and phase 2 of the study. The surgical team is not blinded to treatment allocation given the type of intervention; however, surgical team members are not involved in outcome assessments. All outcome evaluators are blinded to intervention. In phase 1, 10 sheep are allocated to the intervention group: sham surgery group (n=3), discectomy group (n=3), discopexy group (n=3), and backup group (n=1). The backup sheep is planned to be used if death occurs due to anesthesia or another complication not related to the surgical intervention. In phase 2, 10 sheep are randomly assigned to disk I group (n=3), disk II group (n=3), disk III group (n=3), and backup group (n=1) (Figure 1).

Intervention Phase

Anesthesia Protocol

Fasting and water restriction are required 24 hours before surgery. Sedation is performed with diazepam (0.5 mg/kg iv), followed by anesthesia induction with ketamine (5 mg/kg iv). Oral intubation is performed and anesthesia is maintained with isoflurane (1.5% to 2%). To assure animal analgesia, meloxicam (0.5 mg/kg iv, bid) is administered on surgery day and during 4 days postoperatively. Antibiotic prophylaxis with amoxicillin and clavulanic acid are used for 5 days.

Surgical Intervention Protocol for Phases 1 and 2

Phase 1

Bilateral discectomy (n=3): under general anesthesia, the surgical team perform a preauricular skin incision and a blunt dissection of the soft tissue covering the joint. The joint area is disclosed and the articular capsule is incised. The disk and its attachments are identified. The medial, anterior, posterior, and lateral disk attachments are detached and discectomy is performed. The wound is closed in layers.

Bilateral discopexy (n=3): under general anesthesia, the surgical team perform a preauricular skin incision and a blunt dissection of the soft tissue covering the joint. The joint area is disclosed and the articular capsule is incised. The disk and its attachments are identified. The lateral and posterior disk attachments are detached and sutured with poly- *p*-dioxanone (PDS) 3/0. The wound is closed in layers.

Sham surgery (n=3): under general anesthesia, the surgical team will perform a preauricular skin incision and a blunt dissection of the soft tissue covering the joint. The capsule is not incised. The wound is closed in layers.

Phase 2

Disk I (n=3): under general anesthesia, the surgical team perform a preauricular skin incision and a blunt dissection of the soft tissue covering the joint. The joint area is disclosed and the articular capsule is incised. The disk and its attachments are identified. The medial, anterior, posterior, and lateral disk attachments are detached and discectomy is performed. The disk I is introduced into the articular space and sutured in the lateral attachments. The wound is closed in layers. Disk I will be an alternative biomaterial and for intellectual reasons cannot be revealed in this paper.

Disk II (n=3): under general anesthesia, the surgical team perform a preauricular skin incision and a blunt dissection of the soft tissue covering the joint. The joint area is disclosed and the articular capsule is incised. The disk and its attachments are identified. The medial, anterior, posterior, and lateral disk attachments are detached and discectomy is performed. The disk II is introduced into the articular space and sutured in the lateral attachments. The wound is closed in layers. Disk II will be a porous poly(glycerol sebacate) (PGS) scaffold reinforced with polycaprolactone (PCL).

Disk III (n=3): under general anesthesia, the surgical team perform a preauricular skin incision and a blunt dissection of the soft tissue covering the joint. The joint area is disclosed and the articular capsule is incised. The disk and its attachments are identified. The medial, anterior, posterior, and lateral disk attachments are detached and discectomy is performed. The disk III is introduced into the articular space and sutured in the lateral attachment. The wound is closed in layers. Disk III will be a porous PGS scaffold prepared by a modified salt fusion method. Briefly, ground salt particles (150 mg) with a size range of 25 to 32 μm will be placed into a 3-D printed mold. The mold will be transferred to an incubator at 37°C and 90% relative humidity for 1 hour. The fused templates of salt particles will dry in a vacuum oven at 90°C and 100 millitorr (mTorr) overnight, removing salt cake carefully from the mold before further processing. Fresh-made PGS dissolved in tetrahydrofuran (THF; 20 wt%, 380 μL, salt:PGS=2:1) added to the salt cake, and the THF is allowed to evaporate completely in a fume hood for 30 minutes. The salt cake is transferred to a vacuum oven and cured at 150°C and 100 mTorr for 24 hours. The resultant PGS-impregnated salt templates are soaked in deionized water for 4 hours, and then replaced with water for 4 hours, with water exchange every 4 hours during the first 12 hours. After the 12-hour water bath, scaffolds are transferred to deionized water for another 24 hours with water exchange every 8 hours. The resultant scaffolds are frozen down at –80°C and then the lyophilization process is applied.
Ten days for recovery is contemplated for wound care and postoperative medication (see Figure 2).

Outcome Measures

The primary outcome is the microscopic scoring of destructive changes in the TMJ using a modified Mankin scoring system [111]. Secondary outcomes are imaging scoring of TMJ destructive changes, absolute masticatory time, ruminant time per cycle, ruminant kinetics, ruminant area, and sheep body weight. Primary and secondary outcome parameters are outlined in more detail in Figure 3.

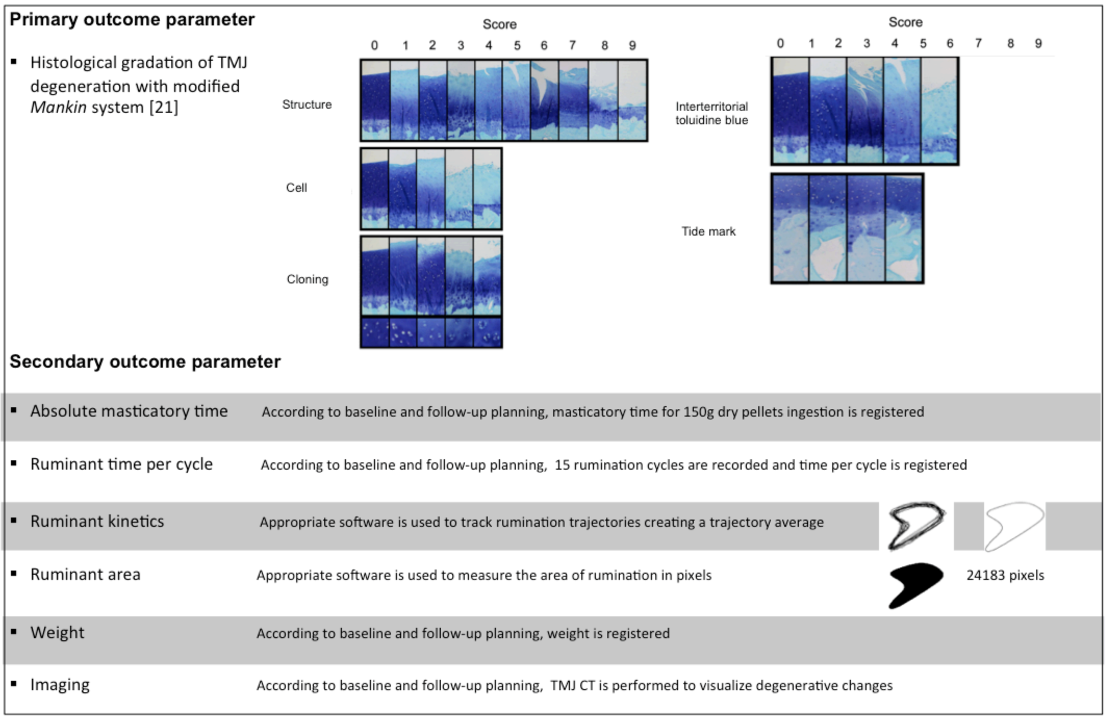


Figure 3. Primary and secondary outcome parameters.

Primary Outcome

The goal is to evaluate histologic gradation of TMJ destructive changes. The time point is 6 months following surgical intervention.

Six months after surgery, the TMJ is removed using a necropsy bone oscillatory saw according to the following anatomic references: cranial (cranial aspect of coronoid process in the union region of the zygomatic process), caudal (external to acoustic meatus), dorsal (reference is established to the squamous temporal bone), and ventral (reference is fixed 2 cm below the acoustic meatus in the zone of stylohyoid angle). The joints are fixed in 10% buffered formalin for 24 hours and stored in 70% ethanol. Decalcification is obtained by immersion in 10% formic acid in 5% formalin for up to 20 days, after which the articulations are cut sagittally through the whole condyle. After decalcifying, TMJ articulations are immersed in three graded methyl salicylate/paraffin mixtures and cut sagittally through the lateral into the central part of the TMJ. Histological sections are sent to Sydney Institute of Bone and Joint Research for histological scoring using a modified Mankin scoring system [111]. This assessment is performed and classified independent by two histologists who will be blinded to intervention. A third histologist will act as arbiter in case of disparity.

Secondary Outcomes

The features evaluated are imaging analysis, absolute masticatory time, ruminant time per cycle, ruminant kinematics, ruminant area, and sheep weight (see Multimedia Appendices 1 and 2). Time point is every month following surgical intervention for a total of 6 months. To measure secondary outcomes, a specific cage (see Figure 4) was built with a frontal window and a feeder.

Figure 4. TEMPOJIMS main facilities.

Imaging analysis: preoperative CT is performed on all sheep. After animal sacrifice, TMJ blocks are scanned by CT and imaging evaluation is performed using the criteria and score described in Table 1.

Items	Criteria	0 (no change)	1 (mild change)	2 (moderate change)	3 (severe change)
Shape	Change of joint form	May include reformed joint	Small changes; this change may include ≤2 osteophytes	Moderate changes; multiple osteophytes	Severe changes and outgrowth; marginal proliferation
Condyle erosion	Concavity in cortical	This stage includes normal joint with no signs of condyle erosion	Erosion in one-third of joint surface	Erosion in two-thirds of joint surface	Erosion over all joint surface
Temporal erosion	Concavity in cortical	This stage includes normal joint with no signs of temporal erosion	Erosion in one-third of joint surface	Erosion in two-thirds of joint surface	Erosion over all joint surface
Condyle sclerosis	Cortical thickening of condyle	This stage includes normal joint with no signs of condyle sclerosis	Sclerosis in one-third of joint surface	Sclerosis in two-thirds of joint surface	Sclerosis over all joint surface
Temporal sclerosis	Cortical thickening of temporal fossa	This stage includes normal joint with no signs of temporal sclerosis	Sclerosis in one-third of joint surface	Sclerosis in two-thirds of joint surface	Sclerosis over all joint surface
Condyle marrow	Change of underlying trabecular bone	This stage includes normal joint with no change of condyle trabecular bone	Sclerosis in less than half of trabecular bone	Sclerosis in half of trabecular bone	Sclerosis in all trabecular bone
Temporal marrow	Change of underlying trabecular bone	This stage includes normal joint with no change of temporal trabecular bone	Sclerosis in less than half of trabecular bone	Sclerosis in half of trabecular bone	Sclerosis in all trabecular bone
Calcification	Development of calcification across joint space	No calcification across joint space	Calcification in one-third of joint surface	Calcification in two-thirds of joint surface	Bony fusion across joint space
Global appreciation		Normal joint	In general, mild changes	In general, moderate changes	In general, severe changes

Table 1. TEMPOJIMS imaging evaluation criteria.

This assessment is performed and classified independently by two experienced radiologists who will be blinded to intervention. A third radiologist will act as arbiter in case of disparity.

Absolute masticatory time: respecting the flowchart (Figure 2), at 9:00 am the animals are placed in individual cages. A dose of 150 grams of dry pellets (Rico Gado A3®) are introduced in the feeder and the time until they eat all the pellets is measured with a chronometer (see Multimedia Appendix 1).

Ruminant time per cycle: respecting the timetable (Figure 2), we record 15 ruminatory cycles approximately 4 hours after 150 gram feeding. We use a Canon 7D video camera and images with 25 frames per second. Then, the number of frames per cycle are divided by 25 to obtain time in seconds per cycle (see Multimedia Appendix 2).

Ruminant kinetics: we use the software Foundry Nuke (2D tracking) to perform the ruminatory tracking and to obtain the ruminatory cycle average. With the software After Effects, we convert the 2-D tracking into a geometric form (see Multimedia Appendix 2).

Ruminant area: we determine the average of 15 cycles and create a geometric form. Using the software Image J*,* we perform a quantitative measure in pixels of the ruminant area average.

Weight: according to the timetable, after eating 150 grams of dry pellets the sheep are weighed (see Multimedia Appendix 1).

All assessments are performed by researchers who are blinded to surgical intervention.

Statistical Analyses

All statistical analyses will be performed using the SPSS version 22 (IBM Corp, Armonk, NY, USA). A cross-sectional analysis will be performed to compare the outcome variables in the three levels of the independent variable before and after the randomized treatment group assignment. In the cross-sectional analyses, one-way analysis of variance (ANOVA) will be performed, after testing all the assumptions. For longitudinal analysis, one-way ANOVA with repeated measures will be performed taking as within-subjects effects observations after surgery (months 1 to 6). Fisher least significant difference will be performed as post hoc tests to check for significant differences for the different treatments.

Reporting of Adverse Events

Adverse events related to the study will be considered, including (1) anesthesia events: idiopathic death, pneumothorax, other complications related to anesthesia; (2) surgical technique: massive bleeding, condylar fracture, other complications related to surgical technique; and (3) postoperative events: TMJ infection, suture dehiscence, decreased appetite, facial paresis, decreased rumination, decreased weight.

Discussion

This study investigates the effects and adverse effects of (1) bilateral discectomy, (2) bilateral discopexy, and (3) bioengineered disk implants. Although this preclinical study will primarily serve as a pilot study, we expect to gain a better understanding of the morphologic and histologic changes in TMJ and implications in masticatory kinetics.

So far, results on discectomy are conflicting. Previous preclinical studies in this field [48,53,64,65,68,113–117] have used the contralateral unoperated side as a control and different animal models ranging from mice to a canine model. Using the contralateral side as a control can be inappropriate considering contralateral overload influence. Theoretically, we expect to reduce this bias using a bilateral approach. Animal variability in the different studies is a warning about the importance of using the same animal model in further studies regarding TMJ implant investigations. Therefore, our group performed a previous study considering black Merino sheep as a promisor animal model for studies regarding TMJ disk implants investigation, TMJ prosthesis, and TMJ osteoarthritis model. To increase the quality of TEMPOJIMS the authors will use a sham surgery control group.

We expect to obtain valuable information related to the phase 1 discopexy group regarding if the surgical approach promotes intra-articular damage. This can improve future conclusions about attributing possible damage to the intervention itself instead of the TMJ implant. This question is important considering that a surgical approach to place TMJ implants in phase 2 will be required. Again, using a bilateral intervention could reduce a possible bias. Most preclinical studies have focused on gross morphological/histological assessments and were not designed to characterize the fundamental altered joint movement (kinetics) or functional consequences. In this study, we include pilot secondary outcomes to evaluate changes in ruminant kinetics. We expect to correlate the primary with the secondary outcomes to understand if they can be used in future TMJ studies. It may be interesting to understand several items:

1. Are there differences regarding masticatory time in the disk groups versus discectomy and discopexy?
2. Is there a correlation between histologic and imaging and kinetics results?
3. Does the ruminant area and geometry change when performing different interventions?
4. Is there a difference regarding ruminant kinetics in the disk groups versus discectomy and discopexy?

5. Do TMJ implants accelerate osteoarthritis?

Concerning phase 2, the choice of biomaterial is critical. The TMJ implant will be exposed in a mechanical, stressful environment with a limited blood supply that can limit cell migration and in situ regeneration. Testing three different bioengineering discs in vivo and correlating in vitro with in vivo behavior can seriously improve bioengineering strategies to achieve a safe and efficacious TMJ disk implant for humans. The main strength of this study is the animal model proposed; the conventional and pilot outcomes described; the study design with a randomized, blinded, and placebo control group; and the use of bilateral surgical procedures. Potential limitations of the study include the relatively small sample size. If this study confirms the feasibility of the proposed protocol and initial efficacy of the TMJ disk implants planned, a larger preclinical trial would be warranted to further determine the effectiveness of these discs and promote translation of animal evidence to clinical practice in humans.

Trial Status

At the time of submission, the surgical interventions of phase 1 were ongoing at Faculdade de Medicina Veterinária de Lisboa and TEMPOJIMS facilities in Portugal.

Acknowledgments

This preclinical trial is supported by Faculdade de Medicina Veterinária da Universidade de Lisboa, Instituto Politécnico de Leiria (Centre for Rapid and Sustainable Product Development), Centro Hospitalar de Setúbal, Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa. The authors are grateful to Joaquim Ferreira from Lisbon Faculty of Medicine for study design; to Susan Smith from Institute of Bone and Joint Research-Northern Sydney Local Health District-Sydney Medical School Northern, Australia, for histological analysis; to Pedro Nunes from Radiology Department of Centro Hospitalar Lisboa Norte; to Miguel Virgílio for kinematics video recording; and to Joaquim Ângelo and Ermelinda Ângelo for animal logistics control. This study was granted by Portuguese Grunenthal Foundation and by Secção Regional Oeste da Ordem dos Médicos. This publication was supported by the Portuguese Foundation for Science and

Technology (FCT) through the following projects: UID/Multi/04044/2013 and PTDC/EMS-SIS/7032/2014.

Authors' Contributions

The contributors, with input from the other investigators, conceived this study protocol. JF, RF, NG, AT, NG, and DA developed the protocol and study materials with input from all investigators. NG, AT, and DA participated in the randomization process. LM will conduct the statistical analyses. FM, RG, and SF will participate in the surgical interventions. CB and SC are the coordinators of the veterinary staff and responsible for the animal anesthesia and animal welfare. DC participated in organization support and was study advisor. PM, NA, and MC are dedicated to disk implants 1 and 2. WY, JE, and GJ are dedicated to disk implant 3. SR will coordinate the imaging evaluation. MP and FB are responsible for processing the histologic samples and preparing sections. LC group will coordinate histologic scoring system. All authors read and approved the final manuscript.

Conflicts of Interest

None declared.

CAPÍTULO 6. EFEITO DA DISCECTOMIA E DISCOPEXIA BILATERAL NA CINEMÁTICA MASTIGATÓRIA DA OVELHA *BLACK MERINO.* ESTUDO PRÉ-CLÍNICO RANDOMIZADO EM OCULTAÇÃO

Trabalho 4

Effects of bilateral discectomy and bilateral discopexy on Black Merino Sheep rumination kinematics: TEMPOJIMS – phase 1 - a randomized preclinical trial

David Faustino Ângelo, Florencio Monje Gil, Raúl González-García, Lisete Mónico, Carla Moura, Luís Carlos Francisco, David Sanz, Nuno Alves, Francisco Salvado, Pedro Morouço

J Craniomaxillofac Surg. 2018 Feb 46(2): 345-355.

Effects of bilateral discectomy and bilateral discopexy on Black Merino Sheep rumination kinematics: TEMPOJIMS – a randomized preclinical trial

David Faustino Ângelo [1,2], Florencio Monje Gil [3], Raúl González-García [3], Lisete Mónico [4], Carla Moura [2], Luís Carlos Francisco [2], David Sanz [4], Nuno Alves [2], Francisco Salvado [1], Pedro Morouço [2]

[1] Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal

[2] Instituto Politécnico de Leiria, Centre for Rapid and Sustainable Product Development (CDRSP), Leiria, Portugal

[3] University Hospital Infanta Cristina, Badajoz, Spain, Department of Oral and Maxillofacial Surgery, Badajoz, Spain

[4] Coimbra University, Coimbra, Portugal

Corresponding Author:

E-mail address: david.serrano.angelo@gmail.com (D.F. Angelo).

KEYWORDS

TMJ preclinical trial, TMJ discopexy, TMJ discectomy, TMJ kinematics,TMJ Black Merino Sheep, TMJ animal model

ABSTRACT

Background

The temporomandibular joint interposal study (TEMPOJIMS) is a rigorous preclinical trial divided in 2 phases. In phase 1 the authors investigated the role of the TMJ disc and in phase 2 the authors evaluated 3 different interposal materials. The present work of TEMPOJIMS - phase 1, investigated the effects of bilateral discectomy and discopexy in sheep mastication and rumination.

Methods

This randomized, blinded and controlled preclinical trial (in line with the ARRIVE guidelines) was conducted in 9 Black Merino sheep to evaluate changes in mastication and rumination after bilateral discectomy and bilateral discopexy, by comparing with a sham surgery control group. The outcomes evaluated were: (1) absolute masticatory time; (2) ruminant time per cycle; (3) ruminant kinematics, and (4) ruminant area. After baseline evaluation and surgical interventions, the outcomes were recorded over 3 successive days, every 30 days, for 6 months.

Results

The first month after intervention seemed to be the critical period for significant kinematic changes in the discectomy and discopexy groups. However, 6 months after the bilateral interventions, no significant changes were noticed when compared with the control group.

Conclusions

In this study, bilateral discectomy and discopexy had no significant effect in mastication and ruminatory movement. The introduction of kinematic evaluation presents a new challenge that may contribute to the improvement of future studies on the TMJ domain.

INTRODUCTION

The domain of temporomandibular joint (TMJ) bioengineering is growing fast and the potential for obtaining a TMJ interposal disc is immense. For that purpose, rigorous preclinical trials are needed to progress in translational medicine. However, before using valuable efforts and funds in TMJ bioengineering it is important to clarify knowledge related to the effects induced at the temporomandibular joint by surgical interventions.

TMJ discectomy is the most common intracapsular surgery performed. With overall good results, this technique remains a reasonable choice for internal derangement not responding to nonsurgical treatment [42–46]. Nevertheless, it still is a controversial technique, as it does not restore structural or biological properties of the TMJ [118]. Despite the large number of discectomy procedures performed annually, to the best of our knowledge there are no randomized controlled trials that have investigated, in human or animal, the jaw movement implication of bilateral discectomy and bilateral discopexy, using a sham surgery control group.

Small-size, mid-size and large animal model have been used to investigate the histological effects of unilateral discectomy [47,49,57,114,116], leading to diverse results. It has been stated minor degenerative changes to TMJ ankylosis reports, probably due to limitations regarding animal choice, study design and the use of a unilateral approach with contralateral side as a control, which may have induced bias on available results. As reported in the survey, commissioned by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) [58], only 59% of the 271 randomly chosen articles assessed stated the hypothesis or objective of the study, and the number and characteristics of the animals used (i.e., species/strain, sex, and age/weight). Most of the papers surveyed did not report using randomization (87%) or blinding (86%) to reduce bias in animal selection and outcome assessment. Only 70% of the publications that used statistical methods fully described them and presented the results with a measure of precision or variability [119]. These findings are a cause for concern and are consistent with reviews of many research areas, including clinical studies, published in recent years [58,120,121].

Furthermore, most of the previous studies have focused in the histologic and imaging changes, but additional inputs are mandatory to obtain a clear understanding about the functionality of the TMJ. With this paper the authors will report for the first time a high-quality preclinical study, evaluating the impact of bilateral discectomy and bilateral discopexy, comparing to a sham surgery control group, in mastication and rumination kinematics of Black Merino sheep.

The evaluation of the mastication and rumination kinematics of the sheep jaw were based in the normal processes used by ruminants to breakdown particulate dry matter: (1) initial chewing during eating, and (2) further chewing during rumination [122]. The authors discriminate the two processes and analyzed them separately. To analyze the initial chewing we proposed to examine the time spent to eat a dose of dry pellets. We named this outcome absolute masticatory time. With this outcome we expected to determine if TMJ surgical interventions could induce significant changes for the chewing time. To analyze the ruminant chewing phase a special cage was created and 15 ruminant chewing cycles were recorded with a video camera. Using Foundry Nuke (2D tracking) and Image J software ruminant movements in the frontal plane were analyzed to obtain: (1) ruminant time per cycle, (2) ruminant trajectory and (3) ruminant area. We expected to determine if TMJ surgical interventions could induce significant changes over the rumination movement. Temporomandibular Joint Interposal Material Study (TEMPOJIMS) was planned with a rigorous design according to the ARRIVE guidelines [58]. An exploratory randomized preclinical study with blinded outcomes preclinical trial was needed in this field to increase the quality of further TMJ studies, to progress in future treatment options for patients undergoing surgery for TMJ disc replacement, and to improve interpretation of future studies regarding TMJ interposal materials.

MATERIALS AND METHODS

TEMPOJIMS study is a preclinical study divided in 2 phases [123]. This manuscript focus on kinematics outcomes of phase 1, aiming to understand the role of TMJ bilateral discectomy versus TMJ bilateral discopexy, comparing to a sham surgery control group in Black Merino sheep mastication and rumination.

Study Design

The rationale and protocol for the TEMPOJIMS preclinical trial are publicly available [123].

Study Population and Sample

A variety of strains/breeds of sheep have been used in previously TMJ investigation. To decrease biological variability, the authors performed this study in Black Merino sheep strain [109]. In phase 1 the authors used 10 Black Merino sheep with the following inclusion criteria: certified Black Merino sheep, adult (aged between 2 and 5 years [112]), female and in good health condition (veterinary check-up was performed to all animals).

Randomization

The randomization process was performed by a statistical group not involved in the outcome assessments. Ten sheep were randomly allocated to intervention group: bilateral discectomy group (n=3), bilateral discopexy group (n=3), sham surgery group (n=3) and backup group (n=1). One backup sheep was planned to be used if death occurred due to anesthesia or other complication not related to surgical intervention. The allocation to each randomized group was performed preoperatively by sealed envelope (figure 1).

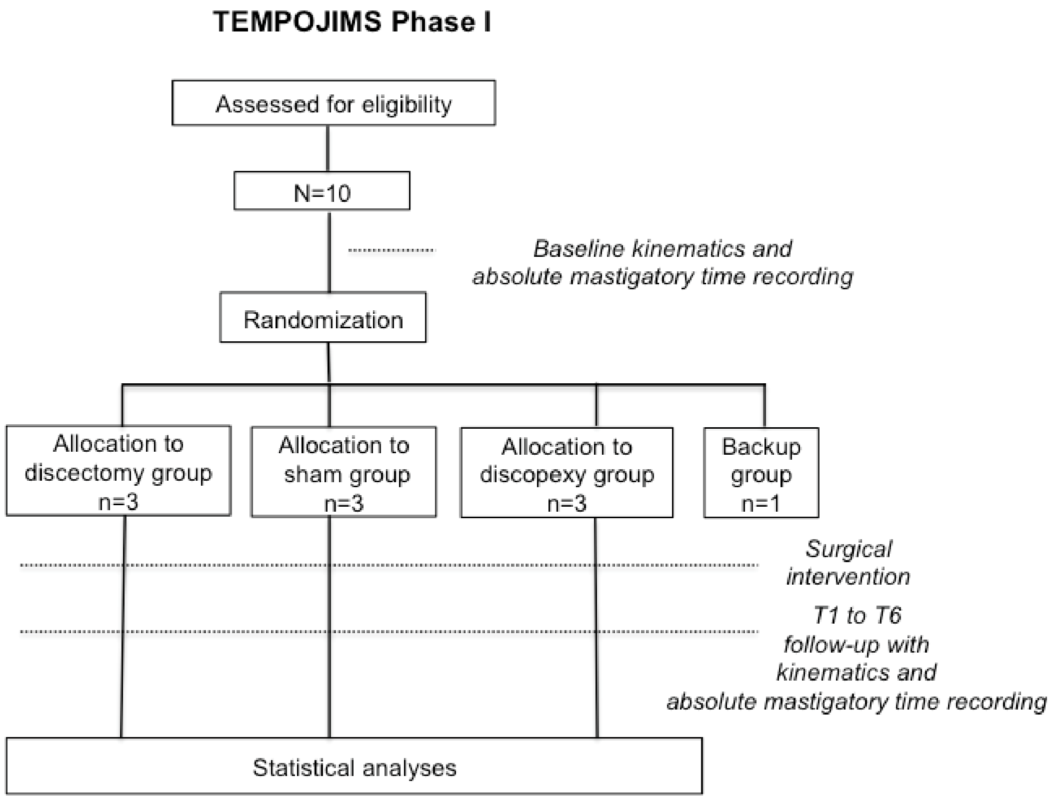


Figure 1. TEMPOJIMS phase 1 enrolment. Baseline assessments evaluated: (1) absolute masticatory time (a dose of 150 grams of dry pellets (Rico Gado A3) were introduced in the feeder and the time until they eat all the pellets were chronometered); (2) ruminant time per cycle (we recorded with a Canon 7D video camera 15 ruminant cycles approximately four hours after 150 gr feeding; (3) ruminant kinematics and (4) ruminant area (we used software Foundry Nuke (2D tracking) to make the jaw tracking and to have a With the software After Effects, we converted the 2D tracking in a geometric form).

Procedures

Ten eligible sheep were assigned to their baseline pilot secondary outcomes measured at days 11, 10, and 9 before surgery in central TEMPOJIMS facilities (figure 2). Transportation to surgical facilities was performed 5 days before surgery to avoid animal stress and allow familiarization to temporary accommodations. The surgical team was not blinded to treatment allocation given the type of intervention; however surgical team members were not involved in outcome assessment. Serious adverse events were defined as events that were fatal or life-threatening or persistent disability, which resulted in death, more than 10% weight loss per week, or clinically significant hazard or harm to the animal.

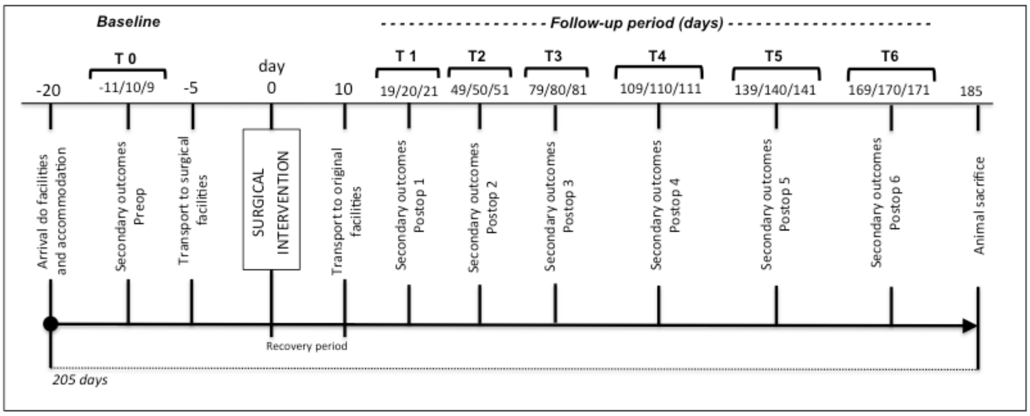


Figure 2. Flow Chart of TEMPOJIMS phase 1.

Anesthesia Protocol

Fasting and water restriction were required 24 hours before surgery. Sedation was performed with diazepam (0.5 mg / kg i.v.), followed by anesthesia induction with ketamine (5 mg / kg i.v.). Oral intubation was performed and anesthesia was maintained with isoflurane (1.5 to 2%). To assure animal analgesia, Meloxicam (0.5 mg / kg i.v, bid) was administered in surgery day and during 4 days post-operatively. Antibiotic prophylaxis with amoxicillin and clavulanic acid were administrated for 5 days.

Surgical Intervention

(A) Bilateral discectomy (n = 3): under general anesthesia, the surgical team performed a preauricular incision and a blunt dissection of the soft tissue covering the joint. The joint area was disclosed and the articular capsule was incised. The disc and its attachments were identified. The medial, anterior, posterior and lateral disc attachments were detached and discectomy was performed. The wound was closed in layers with Vicryl 3/0.

(B) Bilateral discopexy (n = 3): under general anesthesia, the surgical team performed a preauricular incision and a blunt dissection of the soft tissue covering the joint. The joint area was disclosed and the articular capsule was incised. The disc and its attachments were identified. The lateral and posterior disc attachments were detached and sutured with PDS 3/0. The wound was closed in layers with Vicryl 3/0.

(C) Sham surgery (n = 3): under general anesthesia, the surgical team performed a preauricular incision and a blunt dissection of the soft tissue covering the joint. The TMJ articular capsule was not incised. The wound was closed in layers with Vicryl 3/0.

Follow-up assessments

Baseline assessment (T0) were performed before surgery in day -11,-10,-9 (table 1).Ten days after surgery, animals were transported to TEMPOJIMS facilities. Day 19, 20 and 21 (T1) after surgery the follow-up pilot outcomes started to be recorded: every 30 days for 6 months (figure 2). T0-T6 was based on a mean of the 3 measurements. The assessments were performed by 2 specially trained assessors who were not affiliated with the intervention. All animals had bilateral scar to reduce possible bias.

Kinematic outcomes

Kinematic outcomes evaluated were: (1) absolute masticatory time; (2) ruminant time per cycle; (3) ruminant trajectory; (4) ruminant area.
To measure the referred outcomes, a specific cage was built with a frontal window and a feeder. All assessments were performed by researchers' blinded to surgical intervention, and were designed to evaluate masticatory changes related to masticatory time and to ruminant kinematics. These outcomes included:

(1) Absolute masticatory time: respecting timetable (figure 2), at 9:00 am the 10 sheep were placed in the individual cage. A dose of 150 grams of dry pellets (Rico Gado A3®) were introduced in the feeder and the time until they eat all the pellets was measured with a chronometer;

(2) Ruminant time per cycle: respecting timetable (figure 2), we recorded 15 ruminant cycles approximately 4 hours after 150 grams feeding. We used a Canon 7D Video Camera and images were recorded with 25 frames per second. Then, the number of frames per cycle was divided by 25 to obtain time in seconds per cycle;

(3) Ruminant trajectory: we used the software Foundry Nuke (2D tracking) to make the jaw tracking and calculate the ruminant cycle average. With the software After Effects, we converted the 2D tracking in a geometric form (figure 3);

(4) Ruminant area: we determined the average of 15 cycles, and created a geometric form. Using the software Image J, we performed a quantitative measure in pixels of the ruminant area average (figure 3).

Figure 3. Ruminant cycle kinematics (A) – initial position; (B) - maximum open mouth; (C) - maximum lateral movement; (D) – end of ruminant cycle.

Statistical analysis

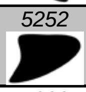
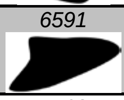
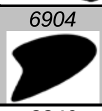
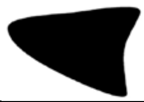
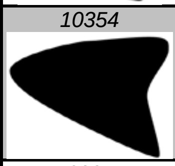
TEMPOJIMS phase 1 pilot randomized controlled preclinical trial used 9 Black Merino Sheep with a 6 months follow-up. Our primary analysis tested the effects of the independent variable (IV) of 3 experimental conditions: 1 = bilateral discectomy; 2 = bilateral discopexy; 3 = sham surgery, using series pre-test (T0) and post-test (T1 to T6). As dependent variables (outcome measures) we measured: the time to eat 150gr of pellets, the ruminant time per cycle and the ruminant area. These events were measured 3 times in the pre-test for supporting invariance concerning the outcome measures before the surgical intervention (IV). Our secondary tests (post-test) analyzed the outcomes measuring 3 times, in 6 time-points, one per month at the same place, date and hour as in pre-test (figure 2).

All statistical analyses were performed using the Statistical Package for Social Sciences (IBM SPSS, version 22.0). Shapiro-Wilk tests were performed in pre-test (T0) and post-test (T1 to T6), showing a normal distribution in all groups per time (p 241 > .0.05), except for T4 and T6 for discopexy in ruminant area (Shapiro-Wilk = .761 and .384, $p < .05$). Other outcomes showed a normal distribution ($p > .05$).

Additionally, Levene Statistics were performed for testing the homogeneity of variances and statistical significant results were found for T1, T2 and T5 in ruminant area (Levene statistics = 8.59, 6.35, and 7.82, $p < .05$), which lead to calculated non-parametric tests for these moments. For pre-test and the other times groups' variances were homogeneous ($p > .07$), which lead us to perform parametric tests. A one-way Analysis of Variance (ANOVA) (or the non-parametric equivalent Kruskal-Wallis test) was performed for cross-sectional analysis, in order to compare the outcome variables in the three levels of the IV before and after the random treatment group assignment. Fisher LSD and Games-Howell Post-hoc tests were performed for equal variances assumed and not assumed, respectively. For longitudinal analysis, Mauchly's test of sphericity was non-significant for absolute masticatory time (Mauchly's W = .004, $p = .589$), allowing to perform a parametric test one-way ANOVA with repeated measures, taking as within-subjects effects observations before (T0) and after surgery (T1 to T6) for bilateral discectomy, bilateral discopexy, and sham surgery conditions. For ruminant area a Greenhouse-Geisser corrected test was used, due to Mauchly's W = .000, $p = .011$.

RESULTS

Descriptive baseline statistics are presented in table 1. A total of 4 outcomes were analyzed: (1) absolute masticatory time; (2) ruminant time per cycle; (3) ruminant trajectory and (4) ruminant area.

Sheep ID	Age Birth-date	**Baseline** mean of 3 measures				**Allocation** randomized process
		Weight §	Absolute Masticatory Time ψ	Ruminant kinetics and Area †	Ruminant Time per cycle ‡	
8298	11.01.11	56.0 Kg	85.0	6449 	0.74 *sec*	Discopexy
9705	02.04.12	70.3 Kg	90.7	*5252* 	1.21 *sec*	Discectomy
8264	19.07.10	56.3 Kg	79.3	*7223* 	0.87 *sec*	Sham
9982	02.09.12	56.0 Kg	76.0	*6591* 	0.94 *sec*	Discectomy
3969	30.10.09	57.0 Kg	89.7	*7768* 	0.90 *sec*	Sham
8284	16.02.11	68.0 Kg	97.7	*6904* 	0.93 *sec*	Discopexy
8267	13.07.10	75.7 Kg	71.7	*8846* 	0.73 *sec*	Discectomy
9701	07.04.12	63.0 Kg	108.7	*10354* 	1.14 *sec*	Discopexy
1903	25.12.12	52.0 Kg	101.3	*6007* 	0.74 *sec*	Sham

❖ No significant differences between sheep in the reported characteristics were found at baseline, $p > .10$.

§ Weight is presented in kilograms.

ψ The absolute masticatory time is measured at 9:00 am when a dose of 150 grams of dry pellets (*Rico Gado A3®*) is introduced in the feeder and the time until they eat all the pellets is chronometered in seconds.

† The ruminant kinetics is the average tracking of 15 ruminant cycles and created a geometric form using the software *Image J*. A quantitative measure in pixels of the ruminant area average is presented.

‡ Respecting the time per cycle we recorded 15 ruminant cycles approximately four hours after 150 gr feeding. We use a Canon 7D Video Camera and images with 25 frames per second. Then, the number of frames per cycle was divided by 25 to obtain time in seconds per cycle.

Table 1. Baseline descriptive statistics

(1) ABSOLUTE MASTICATORY TIME

Cross-sectional analysis. We compared the absolute masticatory time of the 3 groups each month post-surgery (T1 to T6). A one-way ANOVA (or the non-parametric equivalent Kruskal-Wallis test) was performed, showing significant differences between the 3 groups only in T1, p = .03 (one-tailed), effect size of η2p =.736, (1 – β) = .804 (table 2), due to the higher values in the discopexy in comparison with sham surgery, as Games-Howell post-hoc test showed, p = .028. Throughout the baseline and the remaining follow-up period (T2-T6), no statistically differences were found between discectomy, discopexy, and sham surgery conditions (p > .20).

	T0	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**
	x́±dp	*x́±dp*	*x́±dp*	*x́±dp*	*x́±dp*	*x́±dp*	*x́±dp*
Discectomy	79.4±10.0	102.1±6.5	86.0±17.6	79.3±13.2	85.2±11.9	78.3±7.2	74.7±5.0
Discopexy	97.1±11.8	108.2±5.4	90.7±9.2	95.4±15.7	98.2±18.0	95.0±12.1	92.6±7.6
Sham	90.1±11.0	89.22±5.5	80.3±10.8	84.7±16.2	94.9±13.7	82.6±11.7	85.6±18.2
$F_{(2,5)}$*	1.98			0.89	0.63	2.02	1.76
K-W $X^2(2)$		5.54*	0.80				
η^2_p	.397	.74	.14	.23	.17	.40	.37
1 – β	.27	.80	.10	.14	.11	.27	.24

p = .03 one-tailed test

Table 2. Absolute masticatory time for T0 (baseline) to T1 to T6 (post-test): Descriptive, one-way ANOVA for T0 and T3 to T6 and Kruskal-Wallis for T1 and T2, effect-sizes (η^2p) and observed power (1 – β)

Longitudinal analysis. A one-way ANOVA with repeated measures was performed, taking as within-subjects effects months before (T0) and after surgery (T1 to T6) for discectomy, discopexy, and sham surgery conditions. Significant effects across time were found for discectomy, F(6, 12) = 5.67, p = .005, η2p = .739, (1 – β) = .947, but not for discopexy and sham surgery, F(6, 12) = 2.65 and 1.59, p > .07, η2p = .570 and .443, (1 – β) = .635 and .403, respectively.

	Discectomy			Discopexy			Sham Surgery		
Comparison with baseline (T0)	*F(1,2)*	η^2_p	*1-β*	*F(1,2)*	η^2_p	*1-β*	*F(1,2)*	η^2_p	*1-β*
T1 vs. T0	17.63*	.90	.60	5.03	.72	.26	0.02	.01	.05
T2 vs. T0	1.22	.38	.10	0.96	.32	.09	4.73	.70	.25
T3 vs. T0	0.00	.00	.05	0.06	.03	.05	0.40	.17	.07
T4 vs. T0	26.25*	.93	.74	0.03	.02	.05	0.56	.22	.08
T5 vs. T0	0.47	.19	.07	0.10	.05	.05	1.44	.42	.11
T6 vs. T0	1.50	.43	.12	1.56	.44	.12	0.15	.07	.06

$p \leq .05$

Table 3. Comparison of absolute masticatory between baseline and months 1 to 6: within-subjects contrasts, effect-sizes (η^2p), and observed power (1 – β)

Considering the differences in relation to the baseline (table 3), the within-subjects contrasts identified only an increase statistically significant for discectomy between T0 and T1 (effect size of 90%; observed power of .60) and between T0 and T4 (effect size of 93%; observed power of .74). For discopexy and sham surgery, despite the effect sizes, considering the low observed powers, the differences in relation to the baseline were not statistically significant. Figure 4 represents absolute masticatory time in the baseline and from T1 to T6.

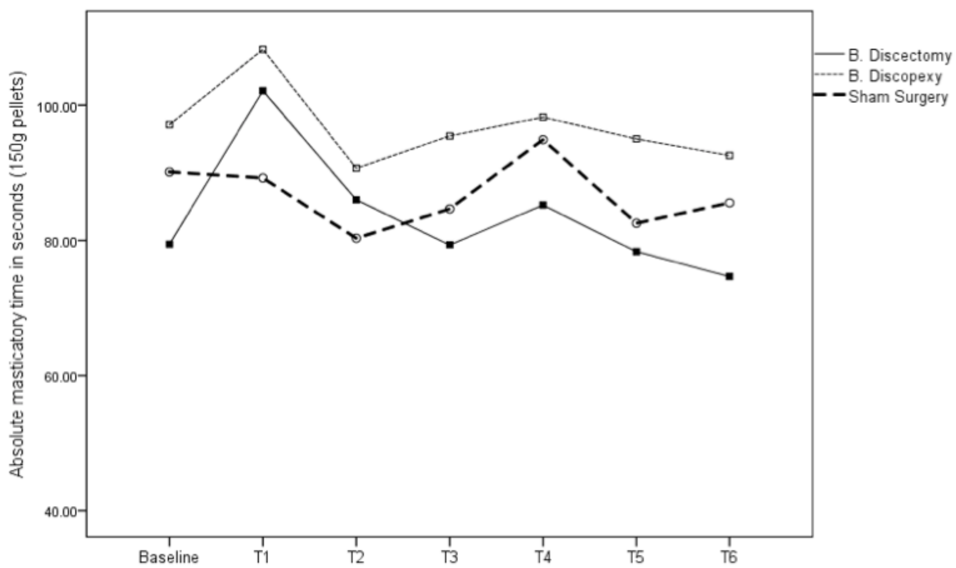


Figure 4. Absolute masticatory time from T0 (baseline) to T1 to T6 (post-test) in discectomy, discopexy, and sham surgery conditions.

(2) RUMINANT TIME PER CYCLE

Cross-sectional analysis. Ruminant time per cycle rate did not vary across groups both in the pre-test (T0) and in all times for the post-test (p > .20), as shown in table 4.

	T0	T1	T2	T3	T4	T5	T6
	x́±dp	*x́±dp*	*x́±dp*	*x́±dp*	*x́±dp*	*x́±dp*	*x́±dp*
Discectomy	.93±.03	.79±.01	.91±.06	.69±.03	.89±.09	.98±.20	1.01±.18
Discopexy	.69±.10	.76±.11	.83±.12	.62±.09	.89±.05	.91±.06	.98±.15
Sham	.80±.13	.72±.12	.85±.17	.66±.07	.76±.11	.88±.21	.84±.08
$F_{(2,5)}$*	3.02	0.29	0.20	0.67	2.02	0.10	1.92
η^2_p	.547	.103	.075	.210	.446	.037	.434
1 – β	.351	.076	.068	.112	.249	.059	.239

no significant effects, *p* > .05

Table 4. Ruminant time per cycle rate for T0 to T1-T6: descriptive, one-way ANOVA, effect-sizes (η^2p) and observed power (1 – β)

Longitudinal analysis. A one-way ANOVA with repeated measures was performed, taking as within-subjects effects the baseline and the six months after surgery for discectomy, discopexy, and sham surgery. A significant effect across time was found for discopexy and sham, respectively, F(6, 6) = 6.87 and 4.11, p < .018, η2p = .773 and .673, (1 – β) = .977 and .845, but not for discectomy, F(6, 6) = 2.70, p = .126, η2p = .730, (1 – β) = .455.

The comparison of ruminant time per cycle rate between baseline and months after the surgery identified two differences for discopexy, however only one (T5 vs. T0) with an acceptable power (effect size of 95%). For discectomy and sham surgery no significant differences were found in relation to baseline. Figure 5 illustrates the ruminant time per cycle rate in the baseline and from T1 to T6. As can be seen, lower scores were obtained in T1, T2 and T3, seeming that sheep start recovering in times T4, T5, and T6.

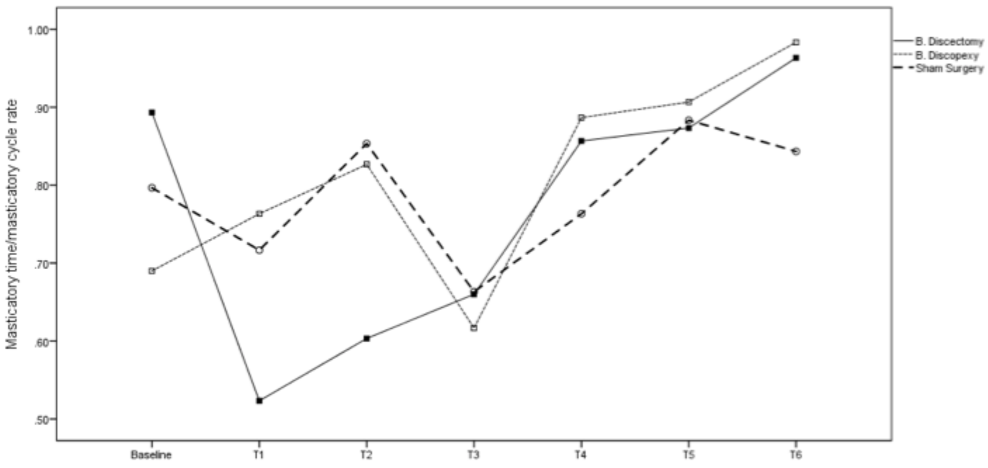


Figure 5. Ruminant time per cycle rate for T0 (pre-test) to T1 to T6 (post-test) in discectomy, discopexy, and sham surgery.

(3) RUMINANT TRAJECTORY AND AREA

Descriptive results of ruminant trajectory and average area of rumination are presented in figure 6.

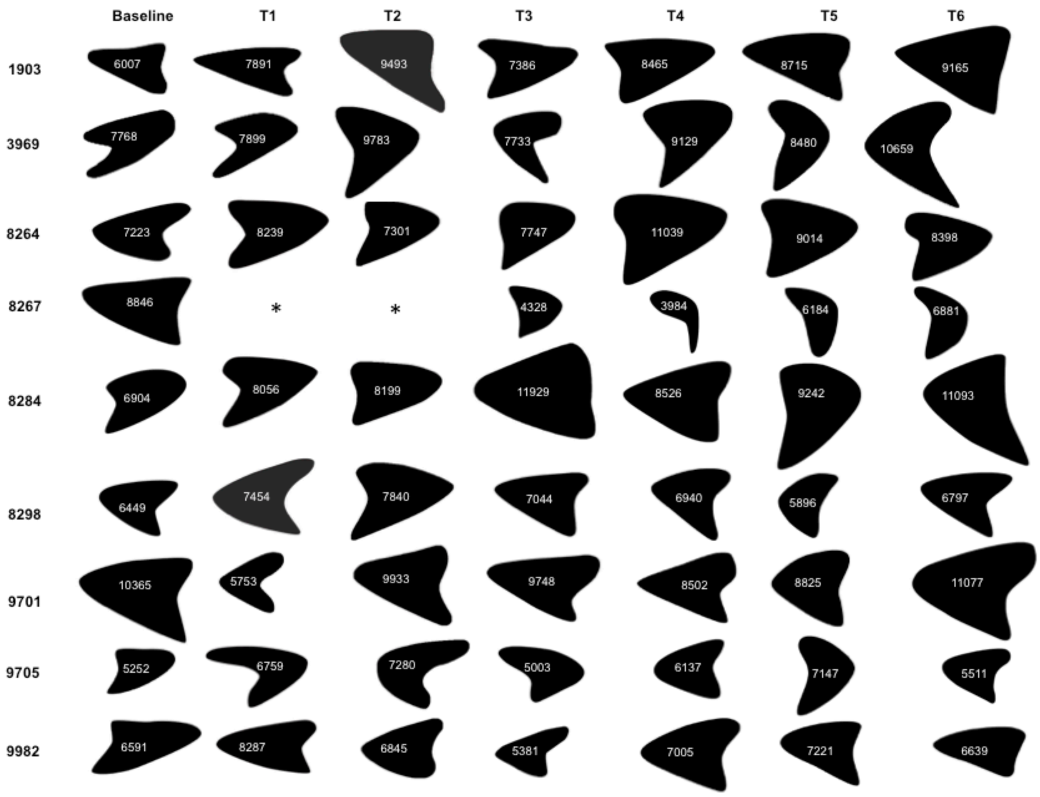


Figure 6. Ruminant kinematics and average area of rumination for the nine sheep.* no ruminant cycles were detected - ruminant area assumed value as zero.

Cross-sectional analysis. Masticatory areas only varied across groups in T3 and T4. For T3 the Fisher LSD post-hoc test identified a significant superiority in the discopexy area in comparison with the discectomy area, $p = .008$. Longitudinal analysis. A one-way ANOVA with repeated measures with Greenhouse-Geisser corrected, taking as within-subjects effects the baseline and the six months after surgery (T1 to T6) for discectomy, discopexy, and sham surgery, did not show statistically significant differences for the three conditions ($p > .10$). The differences from pre to post-test times were also no statistically significant ($p > .05$), with small power, since $(1 - \beta) < .80$, as can be seen in table 7.

	Discectomy			Discopexy			Sham Surgery		
Comparison with baseline (T0)	*F*	η^2_p	*1-β*	*F*	η^2_p	*1-β*	*F*	η^2_p	*1-β*
T1 vs. T0	0.29	.13	.06	0.19	.09	.06	3.99	.67	.22
T2 vs. T0	0.42	.17	.07	1.61	.45	.12	3.55	.64	.20
T3 vs. T0	2.37	.54	.15	0.95	.32	.09	2.52	.56	.16
T4 vs. T0	0.25	.11	.06	0.01	.00	.05	12.85[a]	.87	.49
T5 vs. T0	0.00	.00	.05	0.00	.00	.05	9.07	.82	.39
T6 vs. T0	0.61	.23	.08	2.04	.51	.14	15.02[b]	.88	.54

Table 7. Comparison of ruminant area between baseline and T1 to T6: within - subjects contrasts, effect-sizes (η^2p), and observed power (1 – β)

Figure 7 represents ruminant area for T0 (pre-test) to T1 to T6 (post-test) in discectomy, discopexy, and sham surgery. As can be seen, the baseline (pre-test) are similar for the three experimental conditions. After surgery ruminant areas are lower in discectomy condition, although the differences were not statistically significant.

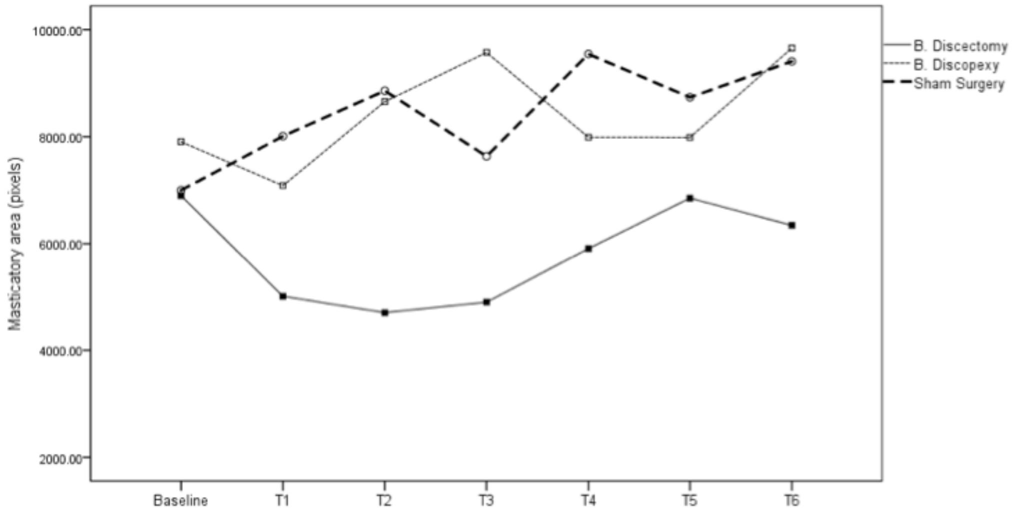


Figure 7. Ruminant area for month 0 (pre-test) to months 1 to 6 (post-test) in discectomy, discopexy, and sham surgery.

ADVERSE EVENTS

No serious adverse events were reported apart of one sheep from discectomy group that stopped rumination in T1 and T2, returning to normal function in T3 to T6.

DISCUSSION

The main goal of the present work was to examine possible effects on the sheep kinematics of mastication and rumination, induced by different types of surgery. The proposed methodology has proven to be feasible and sensitive to the interventions. Homogenous conditions were obtained in baseline, the animals behaved properly in front of the camera, guaranteeing the quality of the kinematic assessments (figure 3).

The measurement of kinematics was intended to progress in the understanding of TMJ surgery implications for the jaw movements. Theoretically, the introduction of a surgery intervention may induce relevant movement changes [20], which should be quantified to infer about the outcomes. That is why this pioneer study is relevant to establish that, independently of the procedure, sheep have the ability to adapt to survive. Regarding the absolute masticatory time, it was expected that after bilateral discectomy the animals would increase the time to eat the 150gr of pellets [124].

Accordingly, the discectomy group significantly increased time by 28% in T1. This could be related to pain in TMJ leading to a slower food intake. However, with time, these animals were able to perform a slow progression to baseline values and at the end of the study, the discectomy group was taking similar time than in baseline data (74.67 seconds) (figure 4). As above-mentioned there is a lack of studies comparing the functionality of TMJ according to interventions. Thus, it was not possible to compare this masticatory outcome measure with other investigations. Although there were no statistical differences between the time before and after surgery (ie T0 vs T1), it can be noticed a tendency to take longer time for the ingestion of the 150gr. Progressively this time started decreasing getting similar to the one performed before interventions (T0 vs T6). This outcomes suggest that sheep presented the ability to adapt to the induced constrains, highlighting the importance that function has over form [125]. The authors agree that it would be interesting in the future to analyze this outcome for a longer period. Regarding the ruminant time per cycle attractive results were achieved. In discectomy group was observed that in T1 and T2 one animal stopped rumination. This could alert for the importance of future investigations in this field, where discectomy could have a more important effect in rumination than mastication. The authors believed at first that an ankylosis process could start after bilateral discectomy, but at T3 all animals were ruminating. This output suggests that besides initial slow down related to food intake, rumination area and even one animal without the ability to ruminate, the animal were able to readapt and return to normal particulate breakdown. When analyzing figure 5 it is noticeable that all groups reduced the ruminant time per cycle in T3, without knowing the cause of any event leading to that result. However, in T4-T6 they reassumed expected values. The animals from discectomy and discopexy groups in T5 and T6 demanded more time to achieve a ruminatory cycle, suggesting a less effective rumination process. Examining the area, it is possible to notice that a faster ruminant cycle is obtained through a smaller ruminant area of each cycle. Moreover, one interesting detail is that in T3 and T4 a normalization of the results was observed for the discectomy group. This output raises the question if 3-4 months after TMJ surgical intervention is the necessary period for remodelation and adaptation?

The evaluation of trajectory and area of rumination was interesting because it was possible to identify a pattern for rumination. Each animal showed a favorite side for rumination but they switched side independently from the intervention. In every animal was noticed a triangular shape trajectory, with high resemblances with the jaw movement in the anesthetized rabbit [126]. After surgery ruminant areas became lower for the discectomy group, although the differences were not statistically significant. In conclusion, present results proved that kinematic jaw analysis is feasible in Black Merino sheep, and that the sham surgery control group was efficacious along time. Further research should be able to examine possible associations between these results with more consensual histologic, imaging and weight outcomes (e.g.[127]). We are not aware of any previous randomized, blinded, preclinical study respecting ARRIVE guidelines in TMJ domain. Using Black Merino sheep, with age and gender selection, protocol publicly available, sham control group and bilateral approach, became critical to reduce possible bias on results. In baseline, the proposed pilot outcomes were homogenous and the sham control group performed effectively in this study. Furthermore, for the present study, performed interventions were bilateral in all groups. Not only, it may clarify a better understanding of TMJ disc role and to reduce possible bias on results, but also it may avoid an adverse effect of the contralateral unoperated joint as reported when unilateral procedures are performed [47]. The first month after intervention seems to be the critical period regarding kinematic changes, with modifications related to absolute masticatory time, ruminant time per cycle and ruminant area, both in discectomy and discopexy groups. Still, after one month, TMJ bilateral discopexy does not seem to have an important impact concerning kinematic changes in Black Merino sheep. On the other hand, TMJ bilateral discectomy seems to have a significant impact, mostly in T1 and T2 but in T3 to T6 a normalization of the results can be observed. The authors agree that the rigorous study design, the animal model and bilateral intervention were the main advantages of this research. The limitations were mostly due to the small sample size, so further research should aim higher samples. The introduction of kinematics evaluation can introduce a new challenge for future studies in TMJ domain, highlighting the functionality of this critical joint.

Acknowledgements
We would like to consider the valuable work of Miguel Virgílio in the ruminant analysis.

Declarations
Funding: Portuguese Grant for Young Researcher in the pain – Grünenthal

Competing Interests: None declared

Ethical Approval: Portuguese National Authority for Animal Health registered with number 026618

Patient Consent: Not required

CAPÍTULO 7. ANÁLISE HISTOLÓGICA, IMAGIOLÓGICA E DE MASSA CORPORAL APÓS DISCECTOMIA E DISCOPEXIA BILATERAL NUM ESTUDO PRÉ-CLÍNICO RANDOMIZADO EM OCULTAÇÃO

Trabalho 5

A Pilot Preclinical Randomized Controlled Trial of Bilateral Discectomy versus Bilateral Discopexy in Black Merino Sheep Temporomandibular Joint: TEMPOJIMS Phase 1 - Histologic, Imaging and Body Weight results

David Faustino Angelo, Florencio Monje, Raúl González-García, Pedro Morouço, Rita Sousa, Lia Neto, Inês Caldeira, Margaret M. Smith, Susan M. Smith; David Sanz, Fábio Carvalho; Belmira Carrapiço, Sandra Cavaco, Mário Pinho, Carla Moura, Nuno Alves, Lisete Mónico, Francisco Salvado, Christopher B. Little

J Craniomaxillofac Surg. 2018 Apr 46(4): 688-696.

A Pilot Preclinical Randomized Controlled Trial of Bilateral Discectomy versus Bilateral Discopexy in Black Merino Sheep Temporomandibular Joint: TEMPOJIMS Phase 1 - Histologic, Imaging and Body Weight results

David Faustino Angelo[1,3], Florencio Monje[2], Raúl González-García[2], Pedro Morouço[3], Rita Sousa[4], Lia Neto[4], Inês Caldeira[4], Margaret M. Smith[5], Susan M. Smith[5]; David Sanz[6], Fábio Santos[7]; Belmira Carrapiço[7], Sandra Cavaco[8], Mário Pinho[7], Carla Moura[3], Nuno Alves[3], Lisete Mónico[6], Francisco Salvado[1], Christopher B. Little[5]

[1] Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal

[2] University Hospital Infanta Cristina, Badajoz, Spain, Department of Oral and Maxillofacial Surgery, Badajoz, Spain

[3] Instituto Politécnico de Leiria, Centre for Rapid and Sustainable Product Development (CDRSP), Leiria, Portugal

[4]Centro Hospitalar Lisboa Norte, Serviço de Neuroradilogia, Lisboa, Portugal

[5]Kolling Institute, Institute of Bone and Joint Research, University of Sydney, Sydney, Australia

[6]Coimbra University, Coimbra, Portugal

[7]Faculdade de Medicina Veterinaria, Interdisciplinary Centre of Research in Animal Health (CIISA), Lisbon University, Lisboa, Portugal

[8]Instituto Nacional de Investigação Agrária e Veterinária (INIAV I.P.), Santarém, Portugal

Corresponding Author:

E-mail address: david.serrano.angelo@gmail.com (D.F. Angelo).

KEYWORDS

TMJ cartilage; TMJ discectomy; TMJ discopexy; TMJ preclinical study; TMJ randomized study; Temporomandibular Osteoarthritis;

ABSTRACT

OBJECTIVE: Evaluate histopathologic and imaging impact of bilateral discectomy or discopexy in Black Merino sheep temporomandibular joints (TMJ), using a high-quality randomized, blinded, control trial following the ARRIVE guidelines.

DESIGN: TEMPOJIMS phase 1 is pilot randomized preclinical study, conducted in 9 Black Merino sheep designed to investigate imaging, histopathologic and body weight changes after bilateral TMJ discectomy, discopexy and sham surgery.

RESULTS: Significant changes were noticed in discectomy group, both in imaging and histopathologic analyses. Body weight changes were most pronounced in discectomy group in the first 4 months after surgery with recovery to baseline weight 6 months after surgery. Discopexy induced nonsignificant changes in both imaging and histologic scoring analyses.

CONCLUSION: This study reinforces the importance of developing an effective interposal material to substitute for the disc and the need to explore the molecular mechanisms that underlie TMJ cartilage degeneration. The study design proposed in TEMPOJIMS study represents important progress towards future rigorous TMJ investigations.

INTRODUCTION

In severe temporomandibular disorders (TMD) the standard treatment is mostly surgical [36]. However, the role of temporomandibular joint (TMJ) surgery is not well defined [128] due to a lack of quality randomized controlled clinical trials comparing TMJ surgical treatment with medical treatment and placebo [129,130]. TMJ open surgical approaches for severe disorders include mostly discectomy, discopexy and, in cases where nothing in the joint is salvable, a total joint replacement may be necessary [36]. Despite the large number of discectomy procedures performed annually, we are not aware of any rigourously-performed, randomized, controlled trials that have investigated in human or animal the effectiveness of discectomy, as compared with discopexy, bioengineered interposal material and sham surgical interventions. In 1995, Trumpy IG et al, compared three different TMJ surgical treatments: discopexy, discectomy without replacement and discectomy with replacement of the disc with an interpositional implant. They concluded the interpositional implant clearly accelerated TMJ osteoarthrosis (OA). Discectomy and implant groups developed TMJ OA in 93% and 100%, but only 62% in the discoplasty group had OA. However, TMJ discopexy was associated with frequent relapse, requiring secondary discectomy [44]. This study suggests we should improve knowledge in the role of surgery and progress for future interposal materials.

Most clinical trials use imaging to classify the TMJ degenerative process [42]. Computed tomography (CT) is a valuable tool to evaluate TMJ OA [131] and most clinical studies evaluate articular changes by CT [132–138]. Two important long-term follow-up clinical studies presented condylar flattening and sclerosis after discectomy but did not associate them with TMJ symptoms [134,137]. The *Deprez* group (1962) suggested an association of articular erosion with pain in the post-operative period [133]. However, while imaging modalities are key measures in clinical research, preclinical studies provide a unique chance to also obtain histologic pathology in order to better understand TMJ surgery-induced changes and improve knowledge in interposal materials research. Previous preclinical studies have evaluated histologic and imaging outcomes using study designs with a significant potential sources of bias (selection bias, measurement bias, non-randomization, non-blinded outcome assessment) increasing risk of errors in the results of the study and in conclusions [67,139–143].

The Temporomandibular Joint Interposal Material Study (TEMPOJIMS) was planned with a rigorous pre-published design [123] according to the ARRIVE guidelines [58]. This first high-quality randomized preclinical study, performed in Black Merino sheep, is required to increase the translational power of further studies and to progress future treatment options for patients undergoing surgery for TMJ disc replacement. TEMPOJIMS is divided into phase 1 and 2. Phase 1 is a randomized, blinded preclinical trial designed to investigate the TMJ imaging (CT), histologic, and body weight changes in sheep after bilateral discectomy (n=3), discopexy (n=3) or sham surgery (n=3). Phase 2 uses the same design to test 3 different bioengineering discs to substitute for the disc in sheep TMJ. All the assessments were performed and classified independently by two professionals from each area who were blinded to intervention, and in both phases the primary outcome is the histological grading of TMJ pathology. In this manuscript we report the phase 1 outcomes.

MATERIAL AND METHODS

Study design

This study was conducted predominantly in Portugal, but a number institutions were involved: 1) *Lisbon Faculty of Medicine* for study design, coordination and statistical analysis; 2) *Histology department of Centro Hospitalar de Setúbal* for histological preparation; 3) *Interdisciplinary Centre of Research in Animal Health in Faculty of Veterinary* for veterinary support; 4) *Department of Oral and Maxillofacial-Head and Neck Surgery, University Hospital Infanta Cristina, Badajoz, Spain* for surgical support; 5) *Radiology Department of Santa Maria Hospital, Lisbon, Portugal* for imaging analysis; 6) *Institute of Bone and Joint Research-Northern Sydney Local Health District-Sydney Medical School Northern, University of Sydney, Australia*, for histological analysis; and 7) *Coimbra University* for statistical analysis. The rationale and protocol for the TEMPOJIMS preclinical trial are publicly available [123]. An independent data and safety monitoring board unblinded preclinical results. The study was approved by the Portuguese National Authority for Animal Health registered with number 026618. The study design and organization respected the ARRIVE guidelines [58].

Study population and sample

Relevant preclinical TMJ studies have been conducted in sheep [85,88,139,144,145], and to decrease biological variability in TEMPOJIMS results, a specific purebred Black Merino sheep strain was used. In 2016, our group performed an anatomic, biomechanical and histologic study of Black Merino sheep TMJ highlighting the potential of this animal to conduct preclinical trials in the TMJ domain [109]. The following eligibility criteria were used: certified *Black Merino* sheep, adult (aged between 2 and 5 years [112]), female, and in good health condition (veterinary evaluation was performed in all animals, including ensuring normal dentition (with 32 teeth, 8 mandibular incisors 12 molars and 12 premolars).

Randomization

The randomization process was performed by a statistical group not involved in the outcome assessments. Ten sheep were randomly allocated to intervention group: bilateral sham surgery (n=3), bilateral discectomy (n=3), bilateral discopexy (n=3), and backup group (n=1). One backup sheep was planned to be used if death occurred due to anaesthesia or other complication not related to surgical intervention. The allocation to each randomized group was performed preoperatively by sealed envelope (Figure 1).

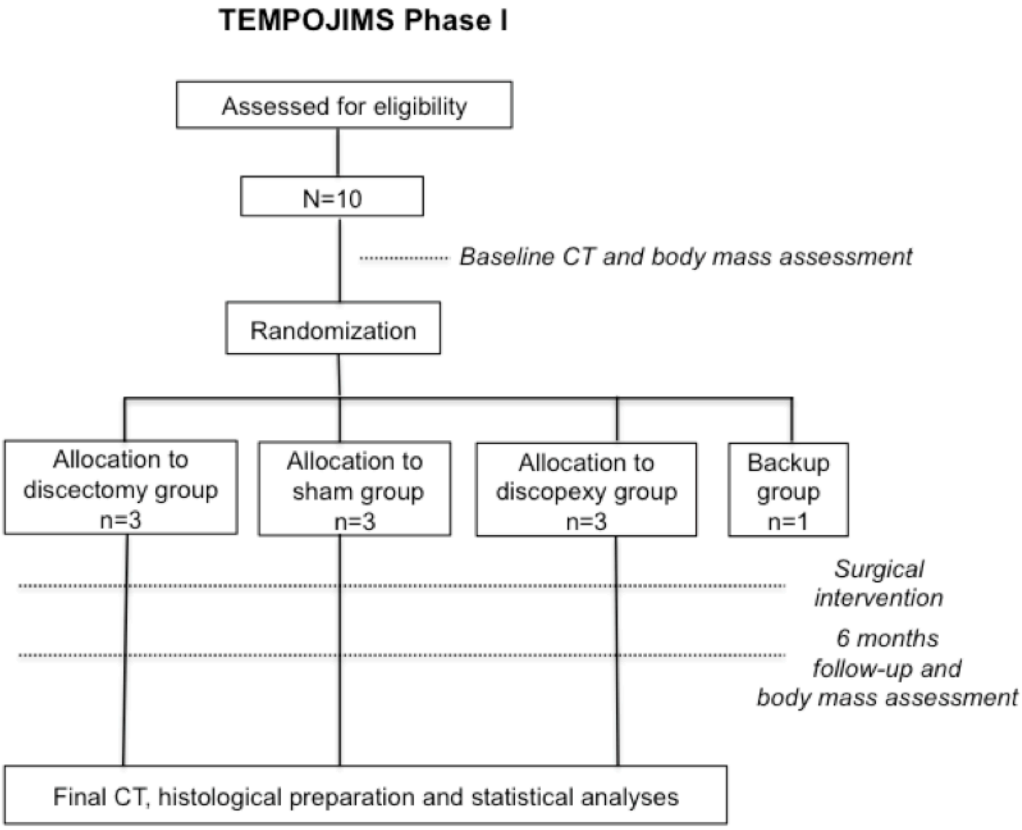


Figure 1. TEMPOJIMS phase 1 enrolment.

Procedures

Ten eligible sheep were assigned to baseline body weight measured at days 11, 10, and 9 before surgery in TEMPOJIMS facilities (Figure 3). Transportation to surgical facilities was performed 5 days before surgery to avoid animal stress and allow familiarization to the temporary accommodation. Head CT-scan was performed on the day of surgery taking advantage of pre-anaesthesia sedation (Figure 2). The surgical team was not blinded to treatment allocation given the type of intervention; however surgical team members were not involved in outcome assessment. Serious adverse events were defined as events that were fatal or life-threatening or persistent disability, that resulted in death, > 10% weight loss per week, or clinically significant hazard or harm to the animal.

Figure 2. Preoperative CT performed under pre-anesthesia sedation in Lisbon Veterinary Faculty.

Intervention phase

Anaesthesia Protocol

Fasting and water restriction were required 24 hours before surgery. Sedation was performed with diazepam (0.5 mg / kg i.v.), followed by anaesthesia induction with ketamine (5 mg / kg i.v.). Oral intubation was performed and anaesthesia was maintained with isoflurane (1.5 to 2%). To assure animal analgesia, meloxicam (0.5 mg / kg i.v, bid) was administered on the day of surgery for 4 days post-operatively. Antibiotic prophylaxis with amoxicillin and clavulanic acid (50mg / kg i.v. bid) were used for 5 days.

Surgical Intervention

In all animals the surgical site was shaved, the skin prepared with povidone iodine solution, and isolated with sterile drapes according to standard surgical procedures. With a 15-scalpel blade we performed a 6cm long pre-auricular skin incision followed by blunt dissection of the soft tissue covering the joint to expose the articular capsule and tissue retractors used to maintain exposure of the surgical field. In the *Sham Surgery GROUP (n=3)* the TMJ articular capsule was not incised, and the wound was closed in 3 layers (muscular, subcutaneous and skin) with *Vicryl* 3/0. In the remaining animals the joint capsule was incised and the disc and its attachments were identified. In *GROUP A - Discectomy* (n=3), the disc was exposed, and using iris scissors the lateral, anterior and posterior attachments were dissected allowing exposure and transection of the medial attachment and removal of the disc intact. In *GROUP B - Discopexy* (n=3) the lateral and posterior disc attachments were sharply detached using an iris scissor. A 4 mm triangular segment of the retrodiscal tissue was removed and then sutured with *PDS 3/0*. The wound, including joint capsule was closed in 4 layers (joint capsule, muscular, subcutaneous and skin) with *Vicryl* 3/0.

Follow-up assessments

Ten days after surgery, animals were transported to TEMPOJIMS facilities. Day 19, 20 and 21 (T1) after surgery the follow-up secondary outcomes were recorded, and repeated every 30 days for 6 months (T1 to T6, respectively; Figure 3). T0-T6 was based on a mean of the 3 day measurements of each month. Immediately after euthanasia all animals had a second CT scan and the TMJ block was removed to histology.

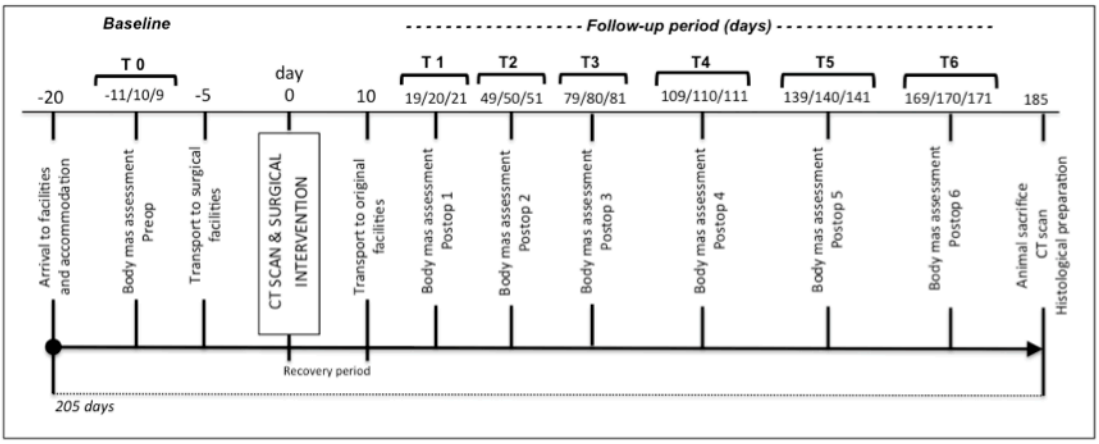


Figure 3. Flow chart of TEMPOJIMS phase 1.

Outcomes

Histologic analysis: Following euthanasia (six months after surgery) the TMJ was removed intact using a necropsy bone oscillatory saw according to the following anatomic references: *cranial* - cranial aspect of coronoid process in the union region of the zygomatic process; *caudal* - external to acoustic meatus*; dorsal* - the squamous temporal bone; and *ventral* - 2 cm below the acoustic meatus in the zone of stylohyoid angle. The joints were fixed in 10% buffered formalin for 24 hours and stored in 70% ethanol. Decalcification was obtained by immersion in 10% formic acid in 5% formalin for 20 days with solution changed every 2 days, after which the articulations were cut sagittally through the whole condyle. TMJ articulations were then immersed in three graded methyl salicylate/paraffin mixtures, embedded in paraffin and sectioned through to the central part of TMJ. Four micron sections were mounted on glass slides, heated for 1 hour at 65 degrees, de-waxed with 3 cycles of 5 minutes with xylene, and stained with toluidine blue and fast green as previously described [111]. Slides identified by a number code were randomized and shipped to the Raymond Purves Labs for scoring by 2 blinded independent assessors experienced in evaluating sheep joint histopathology (CBL, MMS).

As the normal histomorphology of the TMJ is quite distinct from cartilage in appendicular synovial joints [146] (Figure 4Aa), a modification of a published scoring system specific for the TMJ was used [56] (Table 1). Briefly, the mandibular and temporal cartilage (structure, cell number, shape and cloning, and proteoglycan content and distribution), tidemark, cement line, and subchondral bone (structure, osteocyte number, osteoblast activation, vascular invasion, and calcified cartilage islands) were separately scored from 0 (normal) to 3 (severe change, >70% abnormal). Additionally, the temporal and retrodiscal synovial hyperplasia, fibrosis and inflammatory cell infiltration were also scored from 0-3. The summed cartilage (possible maximum score 60 in each condyle), subchondral bone (possible maximum score 15 in each condyle), synovial (possible maximum score 9 in each site), and total (possible maximum score 168) histopathology scores were calculated.

Site	Structure scored
Mandibular surface	AC – lamina - structure
	AC – lamina - cell shape
	AC – lamina - cell number
	AC – lamina - cloning
	AC – lamina - PG content
	AC – lamina - PG distribution
	AC – mid layer - structure
	AC – mid layer - cell shape
	AC – mid layer - cell number
	AC – mid layer - cloning
	AC – mid layer - PG content
	AC – mid layer - PG distribution
	Tidemark (0 absent, 1 indistinct, 2 present, 3 multiple)
	AC – calcified layer - structure
	AC – calcified layer - cell shape
	AC – calcified layer - cell number
	AC – calcified layer - cloning
	AC – calcified layer - PG content
	AC – calcified layer - PG distribution
	Cement line (0 absent, 1 indistinct, 2 present, 3 multiple)
	Subchondral bone - structure
	Subchondral bone - osteocytes
	Subchondral bone - osteoblast activation
	Subchondral bone - vascular invasion
	Calcified cartilage in bone
Temporal surface	AC – lamina - structure
	AC – lamina - cell shape
	AC – lamina - cell number
	AC – lamina - cloning
	AC – lamina - PG content
	AC – lamina - PG distribution
	AC – mid layer - structure
	AC – mid layer - cell shape
	AC – mid layer - cell number
	AC – mid layer - cloning
	AC – mid layer - PG content
	AC – mid layer - PG distribution
	Tidemark (0 absent, 1 indistinct, 2 present, 3 multiple)
	AC – calcified layer - structure
	AC – calcified layer - cell shape

	AC – calcified layer - cell number
	AC – calcified layer - cloning
	AC – calcified layer - PG content
	AC – calcified layer - PG distribution
	Cement line (0 absent, 1 indistinct, 2 present, 3 multiple)
	Subchondral bone - structure
	Subchondral bone - osteocytes
	Subchondral bone - osteoblast activation
	Subchondral bone - vascular invasion
	Calcified cartilage in bone
Synovium	temporal - hyperplasia
	temporal - fibrosis
	temporal - inflammatory cells
	retrodiscal - hyperplasia
	retrodiscal - fibrosis
	retrodiscal - inflammatory cells

Table 1. Other than tidemark and cement line, all parameters are scored: 0 = normal; 1 = slightly abnormal (<30% affected); 2 = moderately abnormal (30-70% affected) ; 3 = severely abnormal (>70% affected). AC – Articular Cartilage. PGs – Proteoglycans.

***Imaging analysis**:* Imaging evaluation was performed and classified independently by 2 experienced radiologists (RS, LN) who were blinded to the intervention using the criteria outlined in Table 2.

	Criteria	**0 (no change)**	**1 (mild change)**	**2 (moderate change)**	**3 (severe change)**
Shape	Change of joint form	May include reformed joint	Small changes. This change may include <= 2 osteophytes	Moderate changes. Multiple osteophytes	Severe changes and outgrowth. Marginal proliferation
Condyle erosion	Concavity in cortical	This stage includes normal joint with no signs of condyle erosion	Erosion in one third of joint surface	Erosion in two thirds of joint surface	Erosion in all joint surface
Temporal erosion	Concavity in cortical	This stage includes normal joint with no signs of temporal erosion	Erosion in one third of joint surface	Erosion in two thirds of joint surface	Erosion in all joint surface
Condyle sclerosis	Cortical thickening of condyle	This stage includes normal joint with no signs of condyle sclerosis	Sclerosis in one third of joint surface	Sclerosis in two thirds of joint surface	Sclerosis in all joint surface
Temporal sclerosis	Cortical thickening of temporal fossa	This stage includes normal joint with no signs of temporal sclerosis	Sclerosis in one third of joint surface	Sclerosis in two thirds of joint surface	Sclerosis in all joint surface

Condyle marrow	Change of underlying trabecular bone	This stage includes normal joint with no change of condyle trabecular bone	Sclerosis in less than a half of trabecular bone	Sclerosis in one half of trabecular bone	Sclerosis in all trabecular bone
Temporal marrow	Change of underlying trabecular bone	This stage includes normal joint with no change of temporal trabecular bone	Sclerosis in less than a half of trabecular bone	Sclerosis in one half of trabecular bone	Sclerosis in all trabecular bone
Calcification	Development of calcification across joint space	No calcification across joint space	Calcification in one third of joint surface	Calcification in two thirds of joint surface	Bony fusion across joint space
Global appreciation		Normal joint	In general mild changes	In general moderate changes	In general severe changes

Table 2. Imaging assessment.

***Body mass assessment**:* Immediately after eating 150 gr of dry pellets the sheep were weighed. The body mass assessments were performed by 2 specially trained assessors who were not affiliated with the intervention. All animals had bilateral scar to reduce possible bias.

Statistical analysis

Statistical analyses were performed using either the Statistical Package for Social Sciences (IBM SPSS, version 22.0) or Statistics/data Analysis (STATA-corporation version 14.2). The histopathology scores for each parameter in each section of the 2 assessors were averaged, and following un-blinding the median scores (and score summations) for each treatment group were calculated. Differences between treatments were analysed by mixed ordinal logistic regression.

A one-way Analysis of Variance (ANOVA) was performed for cross-sectional analysis, in order to compare the outcome variables in the three levels of the IV before and after the random treatment group assignment. For longitudinal analysis a one-way ANOVA with repeated measures was performed taking as within-subjects effects observations after surgery (T1-T6) for bilateral discectomy, bilateral discopexy, and sham surgery conditions. Our primary analysis tested the effects of the independent variable (IV) surgical intervention (3 experimental conditions: 1 = bilateral discectomy; 2 = bilateral discopexy; 3 = sham surgery) using series pre-test and post-test. As dependent variables we used body mass and imaging score for degenerative process. The body mass were measured 3 times in the pre-test for supporting invariance concerning the outcome measures before the clinical intervention (IV). Our secondary analysis (post-test) analysed the outcomes measuring 3 times, in 6 time-points, one per month at the same place, date and hour as in pre-test (Figure 3).

To analyze imaging results, non-parametric tests were performed attending to the sample size and the non-normality of the distribution for the majority of variables in each group (discectomy, discopexy, and sham surgery), Shapiro-Wilk test (6) $\leq .82$, $p \leq .091$. Kruskal-Wallis tests were performed for between group comparisons. Bonferroni test was used for post-hoc multiple comparisons. Partial eta squared (η2p) and Cohen's D were used for effect size calculations. Cohen (1988) defined effect sizes as "small, d = .2," "medium, d = .5," and "large, d = .8" (p. 25).

RESULTS

Descriptive baseline statistics of the animals studied are presented in Table 3.

Sheep ID	Birth-date	Baseline mean of 3 measures	Allocation randomized process
		Body mass §	
8298	11.01.11	56,0 Kg	Discopexy
9705	02.04.12	70,3 Kg	Discectomy
8264	19.07.10	56,3 Kg	Sham
9982	02.09.12	56,0 Kg	Discectomy
3969	30.10.09	57,0 Kg	Sham
8284	16.02.11	68,0 Kg	Discopexy
8267	13.07.10	75,7 Kg	Discectomy
9701	07.04.12	63,0 Kg	Discopexy
1903	25.12.12	52,0 Kg	Sham

❖ No significant differences between sheep in the reported characteristics were found at baseline, $p > .10$.
§ Body mass is presented in kilograms.

Table 3. Baseline descriptive statistics.

1) Histologic results

The morphological appearance of the cartilage and bone in sham operated joints was consistent with that previously described as normal in the TMJ [56,146] (Figure 4Aa). The superficial half of the cartilage depth had a distinct laminar appearance, with sparse flattened cells and limited proteoglycan staining more intense with depth from the surface. Beneath this was a layer densely populated with cells that had a mesenchymal appearance, and more intense diffuse matrix proteoglycan staining. The deepest cartilage layer contained mature and/or hypertrophic chondrocytic cells often surrounded by a proteoglycan rich peri-cellular matrix but little or no inter-territorial proteoglycan. A tidemark separating the upper two layers from the deepest cartilage layers could be observed in some sections, suggesting the lower zone was calcified. An indistinct cement line demarcated the subchondral bone which contained evenly distributed osteocytes in lacunae, and completely separated the cartilage from sparse marrow spaces lined by osteoblasts in the deeper bone. The synovium in sham-operated TMJ was similar to that in the knee joint in sheep [147] with a single lining layer of synoviocytes overlying a loose connective tissue with adipocytes and sparse fibroblasts and collagen.

A variety of pathological changes of varying severity were noted in discopexy and discectomy joints (Figure 4Ab-e). The mildest changes included cartilage thickening, slightly increased matrix and peri-cellular proteoglycan staining, increased cell density, vascular activation and invasion of the sub-chondral bone and calcified cartilage layer, with both the tidemark and cement line being more distinct (Figure 4Ab). Intermediate cartilage pathology was characterized by surface roughening/fibrillation, loss of typical laminar structure, a marked increase in inter-territorial proteoglycan staining in all layers, cell cloning particularly in the upper zones, and further deep zone vascular invasion (Figure 4Ac). Further advancement of pathology was evident with erosion and loss of surface zone cartilage, decreased cell density in the mid zone but cloning in all layers, vascular invasion into the mid zone (Figure 4Ad), and ultimately complete loss of cartilage integrity and marked subchondral bone remodeling (Figure 4Ae). Accompanying the osteochondral changes, there was synovitis with hyperplasia of surface cells, sub-synovial fibrosis with loss of adipocytes and both peri-vascular and diffuse inflammatory cell (macrophages and lymphocytes) infiltration (not shown). Blinded scoring demonstrated a significant increase in total median histopathology score in discectomy compared with both sham and discopexy groups (Fig 4B). This was driven by a significant increase in pathology in cartilage, bone and synovium in discectomy compared with sham-operated joints (Figure 4C). Discopexy joints did display some evidence of cartilage and synovial pathology in particular, but this was quite variable and thus did not reach statistical significance.

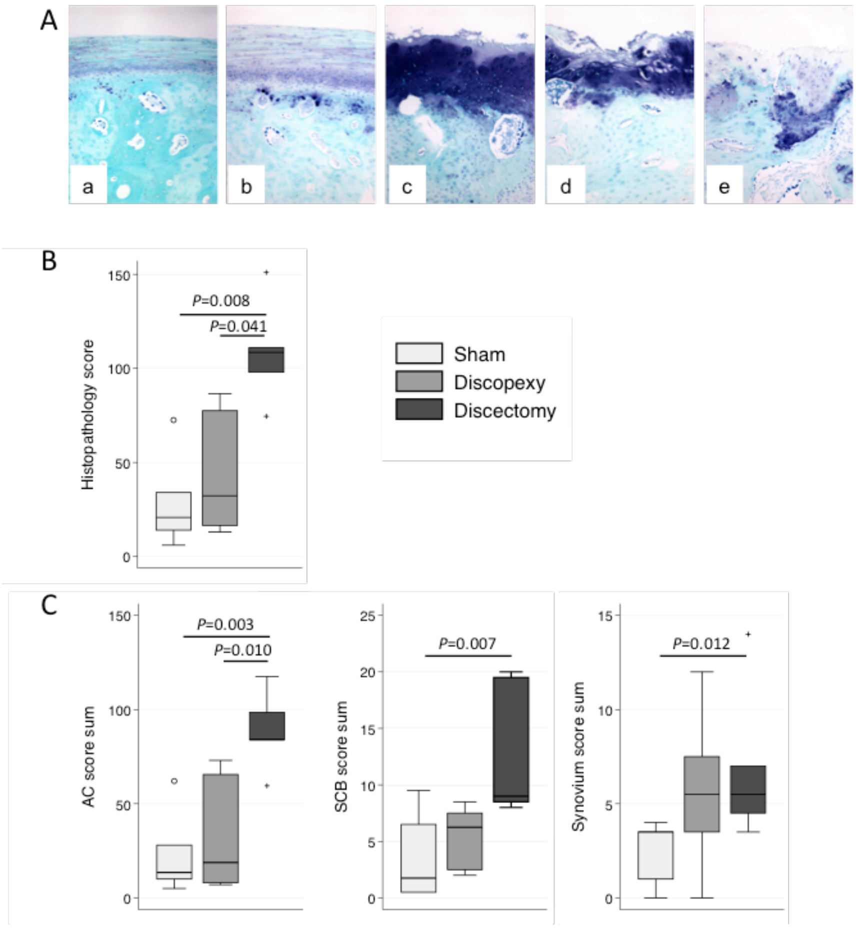


Figure 4. (A) Representative images of toluidine blue stained sections of mandibular condyles from sheep with sham-operated (a) or discopexy/discectomy (b-e) TMJs. Total histopathology (B) and individual tissue (C) scores in Sham, Discopexy and Discectomy sheep (line = median; box =25-75%, whiskers = 10-90%, dots = outside values).

2) Imaging results

The authors compared the nine outcomes between discectomy, discopexy, and sham surgery conditions (Table 2). For global appreciation differences were very high (η^2_p corresponding to 90.8%, statistical power > .999). As demonstrated in

Table 4, there was statistical differences for all outcomes, excluding calcification (M = 0 and SD = 0 for all experimental conditions). Considering each outcome, differences were higher for shape, followed by condyle sclerosis, temporal sclerosis, condyle marrow, temporal erosion, condyle erosion, and, at last, temporal marrow. The effect size of the differences ranges from 43.4% to 90.8% for global appreciation. In Figure 5 we show a representative CT imaging of sham surgery group (Figure 5A), discopexy group (Figure 5B) and discopexy (Figure 5C).

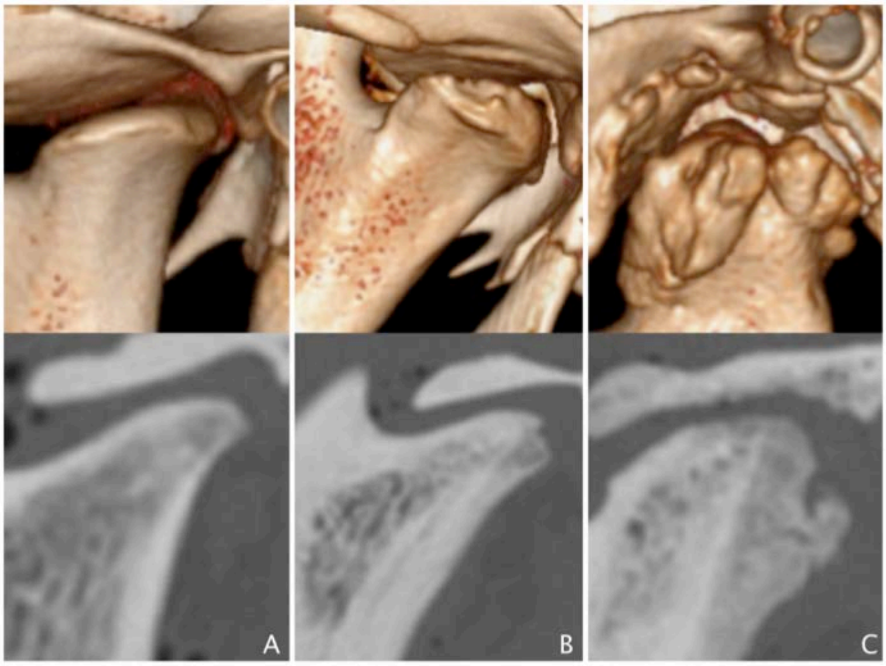


Figure 5. A- Sham group, B- Discopexy group, C- Discectomy group.

	Shape			Condyle Erosion			Temporal erosion			Condyle sclerosis			Temporal sclerosis			Condyle marrow			Temporal marrow			Calcification			Global appreciation		
Surgery:	M	SD	MR	M	SD	MR	M	SD	MR	M	SD	MR	M	SD	MR	M	SD	MR	M	SD	MR	M	SD	MR	M	SD	MR
Discectomy	2.83	0.41	15.42	1.50	0.84	13.83	2.00	1.10	14.42	2.33	0.82	14.50	2.17	0.98	14.67	2.00	.63	13.83	1.50	1.05	13.42	0.00	0.00	9.50	2.67	0.52	15.33
Discopexy	1.17	0.41	9.58	0.67	0.52	9.33	0.17	0.41	7.58	1.33	0.52	10.50	0.83	0.41	9.83	1.33	.82	10.67	0.67	0.82	9.58	0.00	0.00	9.50	1.17	0.41	9.67
Sham	0.00	0.00	3.50	0.17	0.41	5.33	0.00	0.00	6.50	0.00	0.00	3.50	0.00	0.00	4.00	0.00	0.00	4.00	0.00	0.00	5.50	0.00	0.00	9.50	0.00	0.00	3.50
$X^2(2)$		16.22***			9.30**			11.09**			14.05***			13.65***			12.05**			8.09*			0.00			15.88***	
η^2_p		.936			.490			.684			.779			.717			.700			.434			-			.908	

Table 4. Means (M), standard-deviations (SD), Means Rank (MR), Kruskal Wallis Tests, power, and effect sizes for the outcomes.

Excluding the difference between discopexy and sham surgery for temporal erosion (Cohen’s d = 0.59), all the other differences were classified as a large effect size, according to Cohen (Cohen's d > 0.80). The larger differences are between discectomy and sham surgery (R^2 corresponding to 92.9% of degeneration in global appreciation), mainly due to shape (R^2 = 96.0%), condyle marrow (R2 = 83.4%), and condyle sclerosis (R^2 = 80.1%); Condyle erosion and temporal marrow were the least affected, although with an effect size of R^2 of 50.3% and 50.8%, respectively; temporal sclerosis and temporal erosion showed effect sizes of R^2 of 71.1% and 62.3%, respectively. Discopexy also differed from sham surgery (R^2 corresponding to 80.3% of deterioration in global appreciation), although with lower effect sizes in comparison to the differences between discectomy and sham surgery, and only for shape (R^2 = 80.3%), condyle sclerosis (R^2 = 76.6%), and condyle marrow (R^2 = 56.7%) (Table 5 and Figure 6).

Outcomes	(I) VI	(J) VI	Mean Difference (I-J)	Std. Error	95% CI	Cohen's d	effect-size r	R^2
Shape	Discectomy	Discopexy	1.67***	.19	1.15 - 2.19	4.05	.897	.805
		Sham Surgery	2.83***	.19	2.31 - 3.35	9.76	.980	.960
	Discopexy	Sham Surgery	1.17***	.19	.65 -1.69	4.04	.896	.803
Condyle erosion	Discectomy	Discopexy	0.83	.35	-.12 -1.79	1.10	.482	.232
		Sham Surgery	1.33**	.35	.38 -2.29	2.01	.709	.503
	Discopexy	Sham Surgery	0.50	.35	-.46 -1.46	1.59	.623	.388
Temporal erosion	Discectomy	Discopexy	1.83**	.39	.78 -2.88	2.20	.741	.549
		Sham Surgery	2.00***	.39	.95 -3.05	2.57	.789	.623
	Discopexy	Sham Surgery	0.17	.39	-.88 -1.22	0.59	.281	.079
Condyle sclerosis	Discectomy	Discopexy	1.00*	.32	.13 -1.87	1.46	.589	.347
		Sham Surgery	2.33***	.32	1.47 -3.20	4.02	.895	.801
	Discopexy	Sham Surgery	1.33**	.32	.47 -2.20	3.62	.875	.766
Temporal sclerosis	Discectomy	Discopexy	1.33**	.35	.38 -2.29	1.78	.666	.444
		Sham Surgery	2.17***	.35	1.21 -3.12	3.13	.843	.711
	Discopexy	Sham Surgery	0.83	.35	-.12 -1.79	2.86	.820	.672
Condyle marrow	Discectomy	Discopexy	0.67	.34	-.26 -1.59	0.92	.417	.174
		Sham Surgery	2.00***	.34	1.07 -2.93	4.49	.913	.834

	Discopexy	Sham Surgery	1.33**	.34	.41 - 2.26	2.29	.753	.567
Temporal marrow	Discectomy	Discopexy	0.83	.44	-.36 -2.03	0.89	.407	.166
		Sham Surgery	1.50***	.44	.31 - 2.69	2.03	.713	.508
	Discopexy	Sham Surgery	0.67	.44	-.53 - 1.86	1.16	.500	.250
Global appreciation	Discectomy	Discopexy	1.50***	.22	.91 - 2.09	3.20	.848	.719
		Sham Surgery	2.67***	.22	2.08 - 3.26	7.26	.964	.929
	Discopexy	Sham Surgery	1.17***	.22	.58 - 1.76	4.04	.896	.803

* $p \leq .05$ ** $p \leq .01$ *** $p \leq .001$

Table 5. Mean differences between discectomy, discopexy, and sham surgery: Bonferroni test.

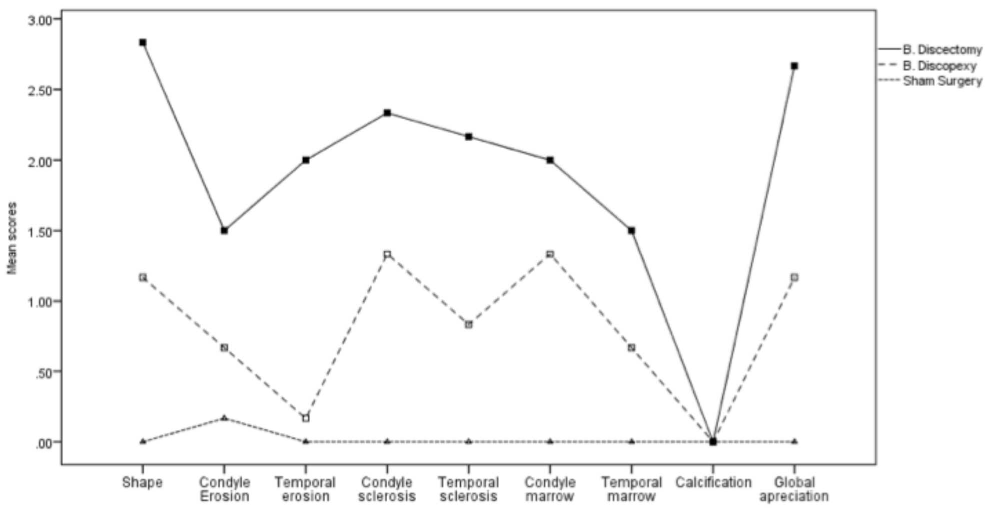


Figure 6. Mean scores for the eight outcomes and the global appreciation.

3) Body mass results

Cross-sectional analysis. Statistical differences were not found in body mass in the pre-test (T0) and in all times for the post-test ($p > .10$, Table 6).

				months												
	0			T1		T2		T3		T4		T5		T6		
	M	SD	Surgery:	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	
	67.33	10.17	Discectomy	63.56	7.40	64.33	7.31	63.78	7.24	65.00	8.19	67.33	8.97	67.56	9.25	
	62.33	6.03	Discopexy	60.67	5.33	61.44	6.17	62.00	7.55	63.33	6.51	66.22	7.00	67.33	7.51	
	55.11	2.71	Sham	54.44	9.53	54.33	8.14	53.89	7.71	55.33	8.96	58.67	8.39	59.67	8.39	
$F(2, 6)$	2.31			1.12		1.51		1.48		1.27		1.00		0.86		
η^2_p	.435			.272		.335		.331		.297		.250		.222		
$1 - \beta$	.304			.168		.212		.209		.184		.155		.138		

Table 6. Sheep body mass for T0 (pre-test) to T1-T6 (post-test): Descriptive and one-way ANOVA

In Figure 7 can be seen that in discectomy condition sheep lost weight from month 1 to month 4 and then recovery of their weight during months 5 and 6 after surgery.

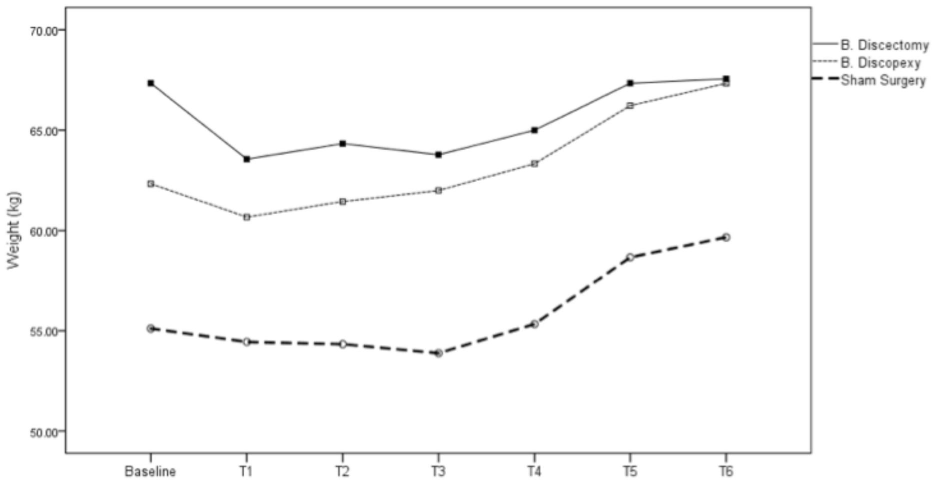


Figure 7. Sheep weight for month 0 (pre-test) to months 1 to 6 (post-test) in discectomy, discopexy, and sham surgery.

Longitudinal analysis. A one-way ANOVA with repeated measures was performed taking as within-subjects effects months after surgery (T1-T6) for discectomy, discopexy, and sham surgery. Statistically significant differences were found, $F(5, 10) = 9.69$, 27.35 and 8.07, $p < .01$, $\eta^2_p = .829$, .932, and .801, $(1 - \beta) = .992$, 1.00, and .977 for discectomy, discopexy, and sham surgery respectively, showing that sheep recovered weight from T1 to T6. The tests of within-subjects contrasts identify that the increase happened from T4 to T5 both in discectomy ($p = .04$), discopexy ($p = .01$), and sham surgery ($p = .01$). Despite this increase, only in the discopepxy and sham increased their weight over the pre-test in T5 and T6, $t(2) = -5.34$ and -5.00, $p < .04$. In discectomy and sham surgery conditions sheep didn´t exceed the weight they had in the pre-test.

DISCUSSION

This is the first temporomandibular preclinical study using a randomized, blinded design respecting ARRIVE guidelines. Using the suitable Black Merino sheep with age and gender selection, previously published protocol, sham control group and bilateral approach, the authors made an effort to reduce possible bias on results. In humans the TMJ cartilage is different from appendicular synovial joints [146], with the distinctly laminar fibrocartilage with sparse proteoglycan reminiscent of meniscus and *annulus fibrosus* of the intervertebral disc [148,149]. In sham-operated joints of Black Merino sheep the TMJ cartilage was histologically very similar to humans, supporting sheep as a good animal model. The rat [54] and goat [56] also have a typical TMJ fibrocartilage appearance with the distinct organized layers, while in the mouse [53,113] and rabbit [150] the laminar structure is less apparant.

The histopathology changes we found in the sheep TMJ after bilateral discectomy are consistent with other reports of induced osteoarthritis in various species, including mice [53], rats [54], rabbits [55] and goats [56]. Of particular interest is the increased proteoglycan and rounded cells and thickening of the cartilage commonly seen after discectomy. These changes in Black Merino sheep are similar to reports in other animals like mouse [53,66,151] and rat [54]. This is consistent with a chondroid metaplasia, potentially associated with the loss of the disc and increased direct loading in the TMJ cartilage. However, when this first protective phase fails under continued abnormal loading, the joint undergoes degeneration with cell death and cloning, surface erosion, subchondral bone changes and degeneration. This latter phase is well described and similar to that in the sheep knee joint following meniscectomy[111,152]. In the discopexy intervention group we preserved the TMJ capsule and the intra-articular environment. The result, as expected, had less severe histopathologic changes, because the disc remains interposed between the bony surfaces, dissipating the loading and protecting the TMJ cartilage. It is noteworthy that we also found more severe synovitis in discectomy compared with discopexy, indicating the inflammation it is not just a reaction to the arthrotomy but part of the OA process in the joint. The histopathological appearance of the synovitis in the TMJ was the same as that in sheep knee joints with OA [147]. However, given the underlying anatomical differences in cartilage, future studies should explore the molecular mechanisms that underlie TMJ OA pathology, to determine their similarities and differences with appendicular joints such as the knee [153]. Such studies could lead to progress in defining the pathophysiology and subsequently the management of TMJ degenerative disorders.

Radiographic morphologic changes followed discectomy was first reported by Boman in 1947, describing “flattening off the articular surface” [132]. Latter, in 1985 similar conclusions were obtained with condylar flattening and sclerosis after unilateral discectomy where no osteophytes but severe damage are described [135]. In 1988, in a 33.8 years post discectomy condyle flattening and sclerosis were the most common radiographic findings [137]. In our study we also observed severe morphological changes after bilateral discectomy. Most statistical differences were noted in shape and condyle sclerosis, corresponding to other author’s clinical findings. While the human condyle is convex and tends to flatten after discectomy, the sheep condyle is normally flat and appears to change to a more convex form after discectomy (Figure 5.C). Nevertheless, condyle sclerosis was observed in all joints after bilateral discectomy (R^2 = 80.1%), and we also detected change in underlying trabecular bone (condyle bone marrow). Cortical breakdown characterized by an initial destructive phase was reported by Agerber and Lundberg in the first 6 months post-discectomy [154]. Some authors suggest that these changes can occur if loading is not controlled during the first 6 months after discectomy [134]. Other authors raised the question of whether the lytic condylar process is precipitated by the discectomy or the overloading, since the contralateral non operated joint has similar morphologic changes [135,138,154,155]. We could not find and reports on temporal bone evaluation in patients, but our study suggests they are similar to those in the condyle. Yaillen, in 1979 described bony ankylosis between the condyle and temporal bone 1 year after unilateral discectomy in *Macaca fascicularis* [156]. Latter, Bjornland after 6 months unilateral discectomy found fibrous ankylosis [45]. In TEMPOJIMS, 6 months after bilateral discectomy we did not find any intra-articular calcification, and while with CT we cannot exclude fibrous ankylosis this was not evident histologically. We also report significant osteophyte formation, rarely described in previously studies, which may be due to imaging limitations of radiography and arthrography compared with CT.

We are not aware of any clinical or preclinical studies that have evaluated imaging after discopexy. Our results showed that TMJ open surgery is not innocuous, resulting in a mild to moderate changes in global remodeling. The condyle is more affected than the temporal bone and only for shape (R^2 = 80.3%), condyle sclerosis (R^2 = 76.6%), and condyle marrow (R^2 = 56.7%) were significant changes detected. We lack other studies to compare our results. These results are the minimum expected in TEMPOJIMS phase 2, were a surgical intervention is needed to remove the TMJ disc and insert a temporomandibular interposal implant.

In other diseases like rheumatoid arthritis [157], cancer [158], HIV [159] and surgical interventions like gastric sleeve [160] body mass has been used as a valuable outcome to evaluate progress of disease and intervention success. In TMJ this outcome has rarely been attempted. Goss (1999) reported 4% of body mass lost in 60% of the animals 3 months after unilateral discectomy with condyle and temporal surfaces removal [144]. In a study in mice, after partial discectomy no significant losses or gains in the body weight of the experimental or control mice was seen [113]. We have found 5.2% body mass lost 3 months after bilateral discectomy (all occurring in the first month) but with full recovery at 6 months follow-up. In contrast, the discopexy and sham surgery sheep increased body weight (mostly in T4-T6) finishing the study 8% and 8.2% respectively, more the baseline. The bilateral discopexy body mass change was the same as the sham surgery group, is consistent with the limited TMJ pathology. The evaluation of body mass was also a welfare control measure related to healthy and well feed, respecting the 3Rs principle (replacement, reduction, or refinement) [161]. Potential animals suffering would be reported to the veterinary team if a 10% body mass loss were detected in any animal.

Potential limitations of TEMPOJIMS study include the relatively small sample size. This pilot investigation demonstrates it is feasible using this study design to conduct preclinical trials in TMJ treatment in Black Merino Sheep. Bilateral discopexy in a healthy TMJ is not an innocuous intervention, resulting in variable cartilage and synovial pathology along with imaging changes. In contrast, bilateral discectomy induced severe TMJ changes detected with both imaging and histopathologic analysis. No fibrous or bony ankylosis was detected over the 6-month time course which would limit model utility. Beyond expected cartilage and bone changes, synovitis was shown to be part of the osteoarthritis process, providing a new outcome measure and therapeutic target. While the animals were able of recover the baseline body mass in 6 months consistent with it being an ethically appropriate model, the early weight loss may also be modifiable outcome for therapeutic trials. This study has reinforced that TMJ cartilage is different from appendicular synovial joints, and as such may require unique therapeutic approaches. We hope that the TEMPOJIMS phase 1 results reported here, will motivate other investigators to study potential biomaterials to substitute the TMJ disc, and will help in consistent study design and evaluation to improve interpretation of findings and translation to new treatments.

Acknowledgments

The authors thank Prof. Joaquim Ferreira for the contribution to study design, Dra. Matilde Gonçalves and Ana Cristina for the histologic contribution, and to Pedro Nunes for the imaging support.

Author contributions

DAF, PM, NA and DS designed the study, DAF, FS, FM and RG performed all surgical procedures, SC, BC, MP, FC contributed with the veterinary support, CL, SS and MS contributed to the histological analysis, RS, LN and IC contributed do imaging analysis, PM, MA, CM, YW, EJ and JG contributed to TEMPOJIMS phase

2, LM and DAF contributed to analysis and interpretation of data, DAF, CL and PM drafted the article and revising it. CL, MS and PM revised the article and finally approved the final version of the article to be published.

Role of the funding source

This work has been granted by Portuguese Grunenthal Foundation and by Secção Regional Oeste da Ordem dos Médicos.

Conflict of interest

There are no conflicts to declare.

CAPÍTULO 8. DISCUSSÃO GERAL E CONCLUSÕES

8.1. DISCUSSÃO GERAL

O principal objetivo da presente investigação foi contribuir para o avanço científico na compreensão do impacto morfofuncional das técnicas cirúrgicas, discectomia e discopexia. Os principais resultados do nosso estudo demonstraram que a discectomia induziu um dano degenerativo severo na ATM com alterações estatisticamente significativas nas análises histológica e radiológica. Após 6 meses, não se verificaram alterações significativas na análise da cinemática ruminatória, na análise do tempo de mastigação inicial e no peso corporal. A discopexia não induziu danos relevantes na ATM e não se verificaram alterações estatisticamente significativas em nenhuma das variáveis estudadas.

Na atualidade, é globalmente reconhecida a importância da adequada delineação dos desenhos de estudo para garantir elevada qualidade de investigação. Porém, com frequência, essa premissa é descurada [162], inibindo a formulação de conclusões coerentes e robustas. O termo desenho de estudo, ou desenho experimental, pode ter várias interpretações na literatura, sendo muitas vezes associado ao tipo de estudo utilizado para planificação de uma determinada investigação. Todavia, neste, deve estar implícito todo o protocolo e as estratégias usadas para reduzir o risco de enviesamento dos resultados [163]. Sendo os ensaios clínicos randomizados e ocultos a referência padrão para investigação clínica em humanos, existem dois protocolos de referência, com orientações para os estudos: o CONSORT [163] e o SPIRIT [61]. Estes protocolos contêm recomendações e listas de verificação a adotar, e a sua elaboração foi fundamentada pela necessidade de homogeneizar e clarificar os procedimentos na tentativa de promover ensaios clínicos de qualidade, transparentes, reduzindo o risco de enviesamento dos resultados.

Em 2010, foi possível verificar que em 271 estudos pré-clínicos, apenas 59% indicavam: os objetivos e a hipótese de investigação do estudo, o número de animais, a raça, a espécie, o género e a idade. Verificou-se ainda que apenas 13%

dos estudos foram randomizados e 14% ocultos [58]. Estes resultados são preocupantes e demonstram o panorama atual que enfrenta a investigação pré-clínica. Precisamente com o propósito de garantir qualidade superior nos estudos pré-clínicos e progredir na medicina de translação, foram elaboradas recomendações *standard* – as ARRIVE *guidelines* [58]. Assim, pretende-se que os estudos pré-clínicos modernos sejam rigorosos, transparentes e que respeitem a regra dos três R: *Replacement, Refinement and Reduction of Animals in Research* e as ARRIVE *guidelines* [58]. Quando analisados os estudos pré-clínicos mais relevantes na área da ATM (n = 32) foi possível verificar que, além das incertezas em relação ao animal mais apropriado a utilizar, nenhum estudo respeitava as ARRIVE *guidelines*. Foi objetivo do presente trabalho alterar o paradigma dos estudos pré-clínicos na área da ATM, propondo um desenho de estudo inovador, rigoroso, randomizado e oculto (capítulo 5), recorrendo para isso ao modelo animal mais adequado – a ovelha *Black Merino* (capítulo 3).

Relativamente à problemática do modelo animal mais adequado para investigação na área de regeneração intra-articular e desenvolvimento de materiais de interposição articular, a literatura não é clara nem consensual. Se por um lado o rato parece ser um modelo adequado, para estudos pré-clínicos relacionados com investigação de mecanismos inflamatórios e dor na ATM [164–169], animais de maior porte serão mais apropriados para uma investigação na área de desenvolvimento de técnicas cirúrgicas minimamente invasivas, materiais de interposição e próteses da ATM.

Entre 1989 e 2000, foram realizados 6 estudos recorrendo a primatas, com o objetivo de estudar materiais de interposição e resultados pós discectomia [48,52,57,170–172]. Cada grupo de investigadores recorreu a raças diferentes de macacos, sem referenciar sexo, peso ou idade. A escolha do macaco Cynomolgus *(macaca fascicularis)* [48,57,170–172] ou macaco Rhesus (*macaca mulatta)* [52] poderá ter originado diferenças nos resultados. Verificou-se ainda que nenhum dos grupos publicou resultados referentes à anatomia do animal ou às características biomecânicas do disco. Já em 2001, surge o primeiro estudo comparativo entre a ATM do humano e do macaco Rhesus, exibindo uma notável semelhança a nível anatómico. Estes resultados levaram os autores a concluir que o macaco Rhesus seria o modelo animal mais adequado para conduzir investigação pré-clínica na ATM [173]. No entanto, por motivos éticos e económicos, o uso dos primatas foi abandonado no início do século XX.

Durante o mesmo período temporal, uma outra linha de investigação apontava para a utilização do porco como animal experimental mais adequado para investigação na ATM [174], seguindo-se uma série de estudos descritivos *post mortem* sobre a ATM e o disco deste animal [175–191]. De facto, a elevada semelhança com a articulação humana, levou a que durante os últimos 20 anos o porco doméstico tenha sido considerado o *gold standard* da investigação pré-clínica em ATM. Contudo, quando se reveem os estudos pré-clínicos *in vivo,* é possível verificar que poucos (n = 2) foram os estudos conduzidos no porco doméstico [192,193]. Optou-se, frequentemente, por recorrer ao *mini-pig* [69,194–199]. Assim, é possível verificar que, para investigações *post mortem* no domínio do disco, foi recorrente a utilização do porco doméstico, enquanto que para estudos *in vivo* deu-se primazia ao uso do *mini-pig*; este último menos estudado, assumindo-se empiricamente que a anatomia seria semelhante à do porco doméstico. Já em 2013, foi relatado que nem todas as estruturas da ATM do porco são semelhantes às do humano, sugerindo reflexão sobre a continuidade da sua utilização no futuro [200].

Existem ainda referências a outros modelos para estudos pré-clínicos. Por exemplo, o cão foi recentemente utilizado para investigação de materiais de interposição na ATM [65,201], embora alguns estudos evidenciem diferenças anatómicas significativas entre a ATM dos caninos, do porco e do humano [202]. Adicionalmente, as sobrecargas articulares próprias dos carnívoros [82] podem ter um impacto negativo na interpretação e transferência dos resultados.

O grupo de Goss *et al* foi quem mais estudos *in viv*o realizou recorrendo à ovelha [50,86–89,145,203–216]. Seguiram-se outros estudos pré-clínicos nessa linha de investigação, com sucesso [217–220]. No entanto, ao contrário do que acontece com o porco em que existem múltiplos estudos descritivos e escassos *in vivo*, na ovelha não se encontra na literatura nenhum estudo que avalie a anatomia, a biomecânica e a histologia da ATM. Foi esse o mote para a realização do capítulo 3, obtendo-se uma descrição da anatomia da ovelha adulta *Black Merino*, da histologia e biomecânica do seu disco articular. Após esta descrição rigorosa e, cumulativamente à experiência de outros autores com este modelo, foi possível concluir que a ovelha deve ser o animal modelo para investigação experimental na ATM, sugerindo o nosso grupo a raça *Black Merino* para diminuir a variabilidade biológica inter e intra-resultados.

Considerando a correta seleção do modelo animal (capítulo 3), é fundamental proceder a uma adequada seleção das variáveis a serem estudadas. O protocolo proposto (capítulo 5) define essas variáveis, como resultado das caraterizações obtidas (capítulo 3 e 4). Em estudos anteriores, ao tentar avançar no desenvolvimento de materiais de interposição na ATM, não foi tida em consideração a morfologia do disco nativo. No presente trabalho, e ao perspetivar futuros avanços no desenvolvimento de biomateriais, foi nossa intenção caracterizar o disco da ATM da ovelha *Black Merino*, recorrendo a tecnologia de *scanning* 3D. Esta metodologia, amplamente utilizada nas mais diversas áreas da engenharia, permitiu obter uma replicação exata da morfologia do disco no seu estado natural. No entanto, além dos parâmetros morfológicos, revelou-se determinante compreender os parâmetros cinemáticos da ruminação, tempo de mastigação (capítulo 6), parâmetros imagiológicos e histológicos (capítulo 7).

Para controlar o sucesso ou insucesso das intervenções cirúrgicas em doentes com DTM, deverão ser analisadas, cumulativamente, a dor e a abertura oral. No entanto, não se tentou, no passado, extrapolar as considerações destas duas variáveis em estudos pré-clínicos na ATM. Para o presente trabalho, testou-se a hipótese de um animal com dor na ATM demorar mais tempo para ingerir uma determinada quantidade de ração. Assim, foi possível inferir que as ovelhas sujeitas a discectomia, demorando mais tempo a comer 150gr de ração, foram as únicas a alterar o seu comportamento mastigatório pela indução da dor numa fase inicial. A ausência de estudos com este tipo de análise não permite efetuar comparações, reforçando a importância de incluir a avaliação biomecânica nas investigações em torno da ATM (capítulo 3 e capítulo 4), e de garantir um apropriado procedimento experimental.

Na literatura encontramos estudos em que na remoção unilateral do disco é usado o lado contralateral como grupo de controlo [48,53,64,65,68,113-117]. Todavia, após intervenção unilateral há uma sobrecarga do lado contralateral [110], o que pode enviesar os resultados. A metodologia utilizada no presente trabalho pretendeu ultrapassar essa limitação (capítulo 5), realizando a discectomia bilateral no grupo 1, a discopexia bilateral no grupo 2, e mantendo um grupo de controlo (grupo 3). O grupo 1 teve como objetivo primário analisar o impacto da discectomia bilateral na ATM da ovelha. O grupo 2, ao qual foi seccionado o disco posteriormente e suturado na sua posição inicial, teve como objetivo perceber o efeito da intervenção na articulação. O grupo 3 (grupo controlo) funcionou como placebo, onde se realizou uma intervenção cirúrgica sob anestesia geral, sem invadir o espaço articular.

Este trabalho foi pioneiro na área da ATM, ao usar, pela primeira vez, um protocolo (capítulo 5) de randomização, ocultação, intervenção bilateral, com variáveis histológicas, radiológicas (capítulo 7), cinemáticas e funcionais (capítulo 6) no modelo animal mais adequado (capítulo 3 e capítulo 4), respeitando as ARRIVE *guidelines.*

Por último, esta investigação reforçou a importância das equipas multidisciplinares para responder a desafios na área da medicina regenerativa. Como tal, para a realização deste trabalho estiveram envolvidas equipas: (i) da biomecânica e bioengenharia, que aportaram um ganho significativo na compreensão da biomecânica da articulação e na caracterização da cinemática ruminatória; (ii) da histologia, que permitiram consolidar o impacto histológico da discectomia e da discopexia, assim como consolidar o conhecimento histológico da ATM normal; (iii) da imagiologia que permitiram uma avaliação rigorosa da ATM; (iv) da medicina veterinária que permitiram conduzir este estudo respeitando as ARRIVE *guidelines*; (v) do departamento de investigação clínica com experiência em desenhos de investigação que permitiu aperfeiçoar o desenho para estudos da ATM; (vi) e de cirurgiões experientes no tratamento de disfunções da ATM que conduziram e orientaram esta investigação para o progresso do tratamento dos doentes com DTM severa.

8.2. IMPLICAÇÕES PARA FUTURA INVESTIGAÇÃO E CLÍNICA

Os resultados obtidos após discectomia mostraram um processo degenerativo severo imagiológico e histológico, sem uma repercussão significativa na função mastigatória e ruminatória da ovelha. Estes resultados demonstraram similaridades com os apresentados na investigação pós discectomia em humanos [138,221]. No entanto, esse resultado pode estar associado a uma sobrecarga contralateral, pois, no humano a discectomia bilateral tem resultados menos satisfatórios que a discectomia unilateral [45], não sendo por isso aconselhada. A discectomia unilateral apresenta-se como uma técnica com bons resultados clínicos em estudos a 20 anos. Contudo são necessários mais estudos para avaliar se esses resultados não estão associados a uma sobrecarga contralateral [63]. Deverá dar-se continuidade à linha de investigação que tenta, já há vários anos, reproduzir um biomaterial compatível com o disco articular nativo.

A discopexia bilateral da ATM causou alterações *minor,* tanto na análise histológica como radiológica. Na análise da função ruminatória e mastigatória este grupo teve um comportamento semelhante ao grupo controlo, sugerindo ser menos invasiva e agressiva que a discectomia. No entanto, no humano esta técnica está associada a muitas recidivas. Num próximo estudo para testar na ATM possíveis biomateriais, a intervenção cirúrgica não será responsável por induzir danos significativos, devendo este grupo ser usado como controlo. O modelo animal proposto (capítulo 3) e o desenho de estudo já testado (capítulo 4) podem representar uma mais-valia, acrescentando rigor e transparência dos resultados obtidos.

8.3. CONSIDERAÇÕES FINAIS

- A ovelha *Black Merino* é um animal que apresenta as características apropriadas para ser utilizado em estudos da ATM, por evidenciar uma anatomia cirúrgica semelhante, e o disco exibir morfologia, biomecânica e histologia comparáveis. Por ser um animal ruminante poderá favorecer estudos posteriores para investigar potenciais biomateriais ou próteses da ATM que permitirão testar a fadiga do material convenientemente.

- O desenho de estudo proposto randomizado, oculto, com intervenções cirúrgicas bilaterais verificou-se exequível e reprodutível para futuras investigações na área da ATM em ovelha *Black Merino*.

- Os *outcomes* selecionados: *outcome* primário – análise histológica, *outcomes* secundários - análise radiológica, análise da mastigação, ruminação e peso corporal foram conseguidos e permitiram ampliar o conhecimento sobre o efeito de cada intervenção em cada um destes *outcomes*.

- Na análise histológica verificou-se que, 6 meses após a cirurgia, o grupo controlo apresentava características iguais às descritas como normais no capítulo 1. O grupo da discectomia apresentava diferenças estatisticamente significativas com: alterações osteocondrais, hiperplasia das células sinoviais, fibrose subsinovial e infiltração de células inflamatórias (macrófagos e linfócitos). O grupo da discopexia apresentava alterações patológicas na cartilagem e na membrana sinovial, mas sem significado estatístico.

- Na análise radiológica encontraram-se alterações estatisticamente significativas no grupo da discectomia com degeneração severa, mas sem progressão para anquilose. Na discopexia houve alterações ligeiras sem significado estatístico. O grupo controlo não apresentou alterações.

- As ovelhas do grupo da discectomia e discopexia perderam peso após a cirurgia. O grupo da discectomia conseguiu recuperar o peso inicial após 6 meses. O grupo controlo (+8.2%) e da discopexia (+8%) superaram o peso pré-operatório. Não se encontraram diferenças estatisticamente significativas.

- A avaliação do tempo de mastigação mostrou diferenças estatisticamente significativas, no 1º mês pós cirurgia, para a discectomia e discopexia. Os animais conseguiram recuperar após o 1º mês.

- A análise geral da cinemática ruminatória permitiu observar que uma ovelha do grupo da discectomia não teve movimentos de ruminação no mês 1 e 2 pós cirurgia. A análise da área de ruminação mostrou ser menor no grupo pós discectomia, sem significado estatístico.

- A bioengenharia de tecidos poderá desempenhar um papel significativo na regeneração do disco articular, melhorando as técnicas cirúrgicas atuais, nomeadamente a discectomia.

AGRADECIMENTOS

A presente tese representa um percurso clínico e científico que teve sempre um objetivo comum: contribuir para um progresso significativo no meio clínico e científico dos doentes com DTM. Por esta razão, endereço o meu primeiro agradecimento aos meus doentes, que sempre acreditaram em mim, que foram a verdadeira força motriz para este projeto se tornar real.

Nestes anos, reforcei a minha convicção de que ser médico permite-nos estabelecer uma relação ímpar com os nossos doentes. Dessa relação surge a motivação para querermos obter o melhor resultado clínico. Encontrei na investigação a melhor forma de contribuir para o meu aperfeiçoamento profissional, na esperança que daí surja um benefício significativo para conveniência destes doentes.

Esta dissertação reuniu diversos ramos da ciência, medicina, medicina dentária, medicina veterinária, bioengenharia, ciências biomédicas. Desta natureza multidisciplinar resulta a necessidade de reconhecer publicamente numerosas influências e contribuições:

- Ao Professor Doutor Francisco Salvado, Diretor do Serviço de Estomatologia do Centro Hospitalar Lisboa Norte e orientador deste projeto. Pelo cuidado que teve na minha formação enquanto interno do seu serviço e por me ter permitido explorar novos horizontes.

- Ao Professor Doutor Florencio Monje, Diretor do Serviço de Cirurgia Oromaxilofacial do Hospital Infanta Cristina em Badajoz. Nele encontrei um modelo a seguir, uma referência no que diz respeito ao rigor cirúrgico e partilha de conhecimento.

- Ao Professor Doutor Raúl González Garcia, médico assistente do Serviço de Cirurgia Oromaxilofacial do Hospital Infanta Cristina em Badajoz. O seu entusiasmo pela cirurgia maxilofacial e a humildade resultaram num fascínio especial por esta pessoa. A orientação e o contributo constantes neste projeto foram essenciais para a continuação do mesmo.

- Ao Professor Doutor Joaquim Ferreira, Diretor do Laboratório de Farmacologia Clínica e Terapêutica, pelo rigor científico transmitido. Foram várias as reuniões para planear todo o estudo e acompanhar a evolução do mesmo. Um agradecimento também a toda a sua equipa, Prof. Dr. Ricardo Fernandes, Dra. Margarida Borges, Nilza Rodrigues, Ana Teixeira e Daisy de Abreu.

- Ao Professor Doutor Nuno Alves, Diretor do CDRSP do Instituto Politécnico de Leiria (IPL). Sempre houve vontade em colaborar e no final provou-se que a Bioengenharia e a Medicina têm um caminho comum. Um agradecimento a toda a sua equipa, em especial ao Prof. Doutor Pedro Morouço que foi o meu braço direito em muitas das fases deste projeto.

- Ao Professor Doutor António Vaz Carneiro, Diretor do Centro de Medicina Baseada na Evidência, por ter acreditado em mim e neste projeto. Por diversas vezes mostrou-me o caminho a seguir e ensinou-me a acautelar, com prudência, as minhas decisões.

- Ao Professor Doutor Yadong Wang, Diretor do Departamento de Bioengenharia da Universidade Pittsburgh, por ter aceite o meu convite para participar neste projeto e por ter envolvido a sua equipa.

- Ao Professor Doutor João Eurico da Fonseca, membro do comité de tese, que reviu várias versões iniciais do projeto. Foi essencial ter o seu apoio na fase embrionária da investigação.

- Ao Professor Doutor Ivo Álvares Furtado, meu orientador de formação em Estomatologia, por me ter incutido o espírito científico. Semeou a semente deste doutoramento, obrigado!

- À Professora Doutora Cláudia Lobato, elemento do meu comité de tese que sempre se mostrou disponível para apoiar este projeto.

- Ao Fábio Santos, um companheiro nesta aventura, sempre disponível para colaborar em tudo o que foi necessário.

- Ao Serviço de Neurorradiologia do CHLN: à Dra. Rita Sousa, Prof. Dra. Lia Neto, à Dra. Inês Caldeira e técnico Pedro Nunes pela ajuda e apoio neste estudo.

- Ao Serviço de Anatomia Patológica do Centro Hospitalar de Setúbal, à Diretora de Serviço, Dra. Matilde Gonçalves e Técnica Ana Cristina pelo apoio incansável.

- Ao Dr. David Sanz, amigo e companheiro de bloco operatório, que sempre partilhou um grande entusiasmo por este projeto e, por diversas vezes, operamos doentes que seriam potenciais candidatos ao uso de um biodisco intra-articular. Este entusiasmo contribuiu significativamente para o sucesso deste estudo.

- À Prof. Dra. Belmira Carrapiço, Prof. Dra. Sandra Cavaco e Prof. Dr. Mário Pinho da Faculdade de Medicina Veterinária de Lisboa pelo incansável apoio.

- Às minhas tias, Carolina e São pelo apoio familiar que sempre me souberam dar e por confiarem no meu trabalho.

- À minha noiva, Carla, por me ter dado a energia certa nos momentos mais intensos deste estudo, e por me proporcionar uma grande estabilidade emocional, condição essencial para manter um equilíbrio saudável entre a minha vida pessoal e profissional.

- Por último aos meus pais, Ermelinda e Joaquim e ao meu irmão Hugo pelo apoio incondicional. Sem eles teria sido difícil dar ousar iniciar esta aventura. Obrigado por terem acompanhado cada passo que fui dando na minha vida, por toleraram as minhas ausências em momentos importantes e por acautelarem cada uma das minhas decisões.

- Não poderia deixar de agradecer ao Serviço de Cirurgia Experimental, ao Sr. Jorge e ao Sr. Luís. Ao Sr. António Sacaranta que me disponibilizou as suas instalações para a dissecção macroscópica das cabeças de ovelha, na fase exploratória do projeto. Certamente que haverá pessoas que colaboraram comigo, das quais no momento não me estou a recordar, para todas elas um grande obrigado.

Porque um trabalho desta natureza consome avultados recursos, quero deixar um reconhecido agradecimento a todas as instituições que contribuíram para o financiamento das diferentes etapas desta investigação:

- Banco Santander, pelo crédito cedido para dar início à investigação;
- Fundação Grünenthal Portugal;
- European Federation for Experimental Morphology;
- Secção Regional Oeste da Ordem dos Médicos;
- Faculdade de Medicina Veterinária da Universidade de Lisboa;
- CDRSP- Instituto Politécnico de Leiria;
- Serviço de Neuroradiologia do Hospital de Santa Maria;
- Schwert Iberica;
- Serviço de Anatomia Patológica do Centro Hospitalar de Setúbal;
- Associação Anatómica Portuguesa e Sociedade Anatómica Portuguesa.

PRÉMIOS E BOLSAS

- Prémio de investigação da Sociedade Anatómica Portuguesa e da Associação Anatómica Portuguesa. 14 de outubro de 2017

- Primeiro lugar no prémio de melhor comunicação oral no *24 congreso nacional de cirurgia oral y maxilofacial* com o estudo: *temporomandibular joint interposal material study* - TEMPOJIMS. SECOM. Málaga. 10 de junho de 2017

- Primeiro lugar no prémio *Investigation and Inovation Awards* com o projeto: Biodisco para Articulação Temporomandibular - Saúde Oral. 29 de setembro de 2016

- Sétimo lugar no *Born from knowledge portuguese awards* com o projecto: *Role of bioengineering and regenerative medicine in temporomandibular joint (TMJ) intra-articular reconstruction*. *Born from knowledge* – Governo de Portugal. 21 de setembro de 2016

- Primeiro prémio no concurso de investigação para internos da Ordem dos Médicos do Oeste. 25 de fevereiro de 2016

- Bolsa da *European Federation of Experimental Morphology* com o projeto *new approach for 3D virtual model of TMJ disc morphology. Preliminary results*. EFEM.10 de julho de 2015

- Segundo prémio no concurso 9º concurso de ideias de negócio de Cascais na categoria *heath medical devices ideas - DNA ideias* com o projeto TEMPOJIMS. 11 de Maio de 2015

- Bolsa da Fundação *Grünenthal* para jovem investigador na área da dor. 13 de março de 2015

REFERÊNCIAS

1. McKay GS, Yemm R, Cadden SW. The structure and function of the temporomandibular joint. Br Dent J [Internet] 1992 Sep 5;173(4):127–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1389598 PMID: 1389598
2. Guarda-Nardini L, Manfredini D, Ferronato G. Temporomandibular joint total replacement prosthesis: current knowledge and considerations for the future. Int J Oral Maxillofac Surg [Internet] 2008 Feb;37(2):103–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18022347 PMID: 18022347
3. Netter FH. Atlas of Human Anatomy. Third Edit. 2004.
4. Johns DE, Athanasiou KA. Design characteristics for temporomandibular joint disc tissue engineering: learning from tendon and articular cartilage. Proc Inst Mech Eng H 2007;221(5):509–526. PMID: 17822153
5. Design characteristics for the tissue engineering of cartilaginous tissues. Ann Biomed Eng 2004;32(1):2–17.
6. Murphy MK, Arzi B, Hu JC, Athanasiou KA. Tensile characterization of porcine temporomandibular joint disc attachments. J Dent Res [Internet] 2013 Aug;92(8):753–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23783320 PMID: 23783320
7. Clément C, Bravetti P, Plénat F, Foliguet B, Haddioui A El, Gaudy J-F, et al. Quantitative analysis of the elastic fibres in the human temporomandibular articular disc and its attachments. Int J Oral Maxillofac Surg [Internet] 2006 Dec;35(12):1120–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17011753 PMID: 17011753
8. Wang L, Lazebnik M, Detamore MS. Hyaline cartilage cells outperform mandibular condylar cartilage cells in a TMJ fibrocartilage tissue engineering application. Osteoarthr Cartil [Internet] Elsevier Ltd; 2009;17(3):346–353. Available from: http://dx.doi.org/10.1016/j.joca.2008.07.004 PMID: 18760638
9. Linss W, Möller K. Remarks on the morphology of the human temporomandibular joint in the fetal period. Ann Anat [Internet] 2007;189(4):418–22. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17696005 PMID: 17696005
10. Bae Y, Park Y. The Effect of Relaxation Exercises for the Masticator Muscles on Temporomandibular Joint Dysfunction (TMD). J Phys Ther Sci 2013;25(5):583–586.
11. Anyanechi CE. Temporomandibular joint ankylosis caused by condylar fractures: a retrospective analysis of cases at an urban teaching hospital in Nigeria. Int J Oral Maxillofac Surg [Internet] 2015 Aug;44(8):1027–33. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26008733 PMID: 26008733
12. Levorová J, Machoň V, Guha A, Foltán R. Osteoarthrosis of Temporomandibular Joint Related to the Defects of Posterior Dentition: A Retrospective Study. Prague Med Rep [Internet] 117(4):176–184. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27930895 PMID: 27930895
13. Sierwald I, John MT, Schierz O, Hirsch C, Sagheri D, Jost-Brinkmann P-G, et al. Association of temporomandibular disorder pain with awake and sleep bruxism in adults. J Orofac Orthop [Internet] 2015 Jul;76(4):305–17. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26123732 PMID: 26123732
14. Cheong RCT, Harding L. Septic Arthritis of the Temporomandibular Joint Secondary to Acute Otitis Media in an Adult: A Rare Case with Achromobacter xylosoxidans. Case Rep Otolaryngol [Internet] 2017;2017:3641642. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28458938 PMID: 28458938
15. Cordeiro PC, Guimaraes JP, de Souza VA, Dias IM, Silva JN, Devito KL, et al. Temporomandibular joint involvement in rheumatoid arthritis patients: association between clinical and tomographic data. Acta Odontol Latinoam [Internet] 2016 Dec;29(3):123–129. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28383601 PMID: 28383601
16. Betti BF, Everts V, Ket JCF, Tabeian H, Bakker AD, Langenbach GE, et al. Effect of mechanical loading on the metabolic activity of cells in the temporomandibular joint: a systematic review. Clin Oral Investig [Internet] 2017 Aug 1; Available from: http://www.ncbi.nlm.nih.gov/pubmed/28761983 PMID: 28761983
17. Nitzan DW. The process of lubrication impairment and its involvement in temporomandibular joint dics displacement. A theoretical concept. J Oral Maxillofac Surg 2001;59(1):36–45. PMID: 11152188
18. Elad S, Epstein J, Klasser G, Sroussi H. Orofacial Pain and Headache. Orofac Pain Headache 2008;321–347.
19. Rantala M a I, Ahlberg J, Suvinen TI, Nissinen M, Lindholm H, Savolainen A, et al. Temporomandibular joint related painless symptoms, orofacial pain, neck pain, headache, and psychosocial factors among non-patients. Acta Odontol Scand 2003;61(4):217–222. PMID: 14582589
20. Fuentes R, Cantin M, Ottone N, Bucchi C. Caracterización de los Componentes Óseos de la Articulación Temporomandibular. Una Revisión de la Literatura. Int J Morphol 2015;33(4):1569–1576.
21. Stelzenmueller W, Umstadt H, Weber D, Goenner-Oezkan V, Kopp S, Lisson J. Evidence ??? The intraoral palpability of the lateral pterygoid muscle ??? A prospective study. Ann Anat Elsevier GmbH.; 2016;206:89–95. PMID: 26706107
22. Pedroni CR, De Oliveira AS, Guaratini MI. Prevalence study of signs and symptoms of temporomandibular disorders in university students. J Oral Rehabil 2003;30(3):283–289. PMID: 12588501
23. Locker D, Slade G. Prevalence of symptoms associated with temporomandibular disorders in a Canadian population. Community Dent Oral Epidemiol 1988;16(5):310–3. PMID: 3263254
24. Nourallah H, Johansson a. Prevalence of signs and symptoms of temporomandibular disorders in a young male Saudi population. J Oral Rehabil 1995;22(5):343–7. PMID: 7616344

25. Gonçalves DADG, Dal Fabbro AL, Campos JADB, Bigal ME, Speciali JG. Symptoms of temporomandibular disorders in the population: an epidemiological study. J Orofac Pain 2010;24(3):270–8. PMID: 20664828
26. Pow EH, Leung KC, McMillan AS. Prevalence of symptoms associated with temporomandibular disorders in Hong Kong Chinese. J Orofac Pain 2001;15:228–234. PMID: 11575193
27. Moyaho-Bernal A, Lara-Muñoz MDC, Espinosa-De Santillana I, Etchegoyen G. Prevalence of signs and symptoms of temporomandibular disorders in children in the State of Puebla, Mexico, evaluated with the research diagnostic criteria for temporomandibular disorders (RDC/TMD). Acta Odontol Latinoam 2010;23(3):228–33. PMID: 21638964
28. Mobilio N, Casetta I, Cesnik E, Catapano S. Prevalence of self-reported symptoms related to temporomandibular disorders in an Italian population. J Oral Rehabil 2011;38(12):884–890. PMID: 21595739
29. Goulet JP, Lavigne GJ, Lund JP. Jaw pain prevalence among French-speaking Canadians in Québec and related symptoms of temporomandibular disorders. J Dent Res 1995;74(11):1738–1744. PMID: 8530734
30. Su N, Liu Y, Yang X, Shen J, Wang H. Correlation between oral health-related quality of life and clinical dysfunction index in patients with temporomandibular joint osteoarthritis. J Oral Sci 2016;58(4):483–490. PMID: 28025431
31. Yule PL, Durham J, Playford H, Moufti MA, Steele J, Steen N, et al. OHIP-TMDs: a patient-reported outcome measure for temporomandibular disorders. Community Dent Oral Epidemiol 2015 Oct;43(5):461–70. PMID: 26040190
32. Ahmed N, Mustafa HM, Catrina AI, Alstergren P. Impact of temporomandibular joint pain in rheumatoid arthritis. Mediators Inflamm 2013;2013:597419. PMID: 24363501
33. White BA, Williams LA, Leben JR. Health care utilization and cost among health maintenance organization members with temporomandibular disorders. J Orofac Pain 2001;15(2):158–69. PMID: 11443827
34. Onoriobe U, Miloro M, Sukotjo C, Mercuri LG, Lotesto A, Eke R. How Many Temporomandibular Joint Total Joint Alloplastic Implants Will Be Placed in the United States in 2030? J Oral Maxillofac Surg 2016 Aug;74(8):1531–1538.
35. Dworkin SF, LeResche L. Research diagnostic criteria for temporomandibular disorders: review, criteria, examinations and specifications, critique. J Craniomandib Disord 1992;6(4):301–355. PMID: 1298767
36. Dimitroulis G. A new surgical classification for temporomandibular joint disorders. Int J Oral Maxillofac Surg 2013 Feb;42(2):218–222.
37. Schiffman E, Ohrbach R, Truelove E, Look J, Anderson G, Goulet J-P, et al. Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) for Clinical and Research Applications: Recommendations of the International RDC/TMD Consortium Network* and Orofacial Pain Special Interest Group†. J Oral Facial Pain Headache [Internet] 2014 Jan;28(1):6–27. Available from: http://www.quintpub.com/journals/ofph/abstract.php?iss2_id=1172&article_id=13908&article=2&title=Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) for Clinical and Research Applications: Recommendations of the International RDC/TMD Consortium N
38. Wilkes CH. Internal derangements of the temporomandibular joint. Pathological variations. Arch Otolaryngol Head Neck Surg 1989 Apr;115(4):469–77. PMID: 2923691
39. Akinbami BO. Evaluation of the mechanism and principles of management of temporomandibular joint dislocation. Systematic review of literature and a proposed new classification of temporomandibular joint dislocation. Head Face Med 2011 Jun;7:10. PMID: 21676208
40. Sawhney CP. Bony ankylosis of the temporomandibular joint: follow-up of 70 patients treated with arthroplasty and acrylic spacer interposition. Plast Reconstr Surg 1986 Jan;77(1):29–40. PMID: 3941847
41. McKenna SJ. Discectomy for the treatment of internal derangements of the temporomandibular joint. J Oral Maxillofac Surg 2001;59(9):1051–1056. PMID: 11526578
42. Eriksson L, Westesson P-L. Discectomy as an effective treatment for painful temporomandibular joint internal derangement: A 5-year clinical and radiographic follow-up. J Oral Maxillofac Surg 2001 Jul;59(7):750–758. PMID: 11429734
43. Mazzonetto R, Spagnoli DB. Long-term evaluation of arthroscopic discectomy of the temporomandibular joint using the Holmium YAG laser. J Oral Maxillofac Surg 2001 Sep;59(9):1018–23; discussion 1024. PMID: 11526570
44. Trumpy IG, Lyberg T. Surgical treatment of internal derangement of the temporomandibular joint: long-term evaluation of three techniques. J Oral Maxillofac Surg 1995 Jul;53(7):740-6-7. PMID: 7595786
45. Bjørnland T, Larheim TA. Discectomy of the temporomandibular joint: 3-year follow-up as a predictor of the 10-year outcome. J Oral Maxillofac Surg 2003 Jan;61(1):55–60.
46. Nyberg J, Adell R, Svensson B. Temporomandibular joint discectomy for treatment of unilateral internal derangements—a 5 year follow-up evaluation. Int J Oral Maxillofac Surg 2004 Feb;33(1):8–12.
47. Dimitroulis G, Slavin J. The effects of unilateral discectomy and condylectomy on the contralateral intact rabbit craniomandibular joint. J Oral Maxillofac Surg 2006 Aug;64(8):1261–6. PMID: 16860220
48. Bjørnland T, Haanaes HR. Discectomy of the temporomandibular joint: an experimental study in monkeys. J Craniomaxillofac Surg 1999 Apr;27(2):113–6. PMID: 10342149
49. Tong AC, Tideman H. A comparative study on meniscectomy and autogenous graft replacement of the Rhesus monkey temporomandibular joint articular disc--Part I. Int J Oral Maxillofac Surg 2000 Apr;29(2):140–5. PMID: 10833154

50. Ogi N, Kurita K, Handa Y, Goss AN. The short-term effect of autogenous auricular cartilage graft following discectomy on the osteoarthrotic temporomandibular joint in sheep. Int J Oral Maxillofac Surg 1997 Jun;26(3):217–22. PMID: 9180235
51. Tucker MR, Watzke IM. [Autogenous auricular cartilage grafts after TMJ discectomy. A comparative study with and without silastic implants]. Dtsch Z Mund Kiefer Gesichtschir 15(6):418–20. PMID: 1667905
52. Tong AC, Tideman H. A comparative study on meniscectomy and autogenous graft replacement of the rhesus monkey temporomandibular joint articular disc--Part II. Int J Oral Maxillofac Surg 2000 Apr;29(2):146–54. PMID: 10833155
53. Cohen WA, Servais JM, Polur I, Li Y, Xu L. Articular cartilage degeneration in the contralateral non-surgical temporomandibular joint in mice with a unilateral partial discectomy. J Oral Pathol Med [Internet] 2014 Feb;43(2):162–165. Available from: http://doi.wiley.com/10.1111/jop.12113
54. Zhang M, Wang H, Zhang J, Zhang H, Yang H, Wan X, et al. Unilateral anterior crossbite induces aberrant mineral deposition in degenerative temporomandibular cartilage in rats. Osteoarthr Cartil [Internet] 2016 May;24(5):921–931. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1063458415014326
55. Embree MC, Iwaoka GM, Kong D, Martin BN, Patel RK, Lee AH, et al. Soft tissue ossification and condylar cartilage degeneration following TMJ disc perforation in a rabbit pilot study. Osteoarthr Cartil [Internet] 2015 Apr;23(4):629–639. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1063458414013880
56. Li L, Wang L, Sun Y, Yang C, He D. Establishment and histological evaluation of a goat traumatic temporomandibular joint model. J Oral Maxillofac Surg [Internet] 2015 May;73(5):943–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25883002 PMID: 25883002
57. Tucker MR, Burkes EJ. Temporary silastic implantation following discectomy in the primate temporomandibular joint. J Oral Maxillofac Surg 1989 Dec;47(12):1290–5. PMID: 2585180
58. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. PLoS Biol 2010;8(6):e1000412.
59. Krogh A. THE PROGRESS OF PHYSIOLOGY. Science [Internet] 1929 Aug 30;70(1809):200–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17732865 PMID: 17732865
60. Hirst JA, Howick J, Aronson JK, Roberts N, Perera R, Koshiaris C, et al. The need for randomization in animal trials: an overview of systematic reviews. PLoS One [Internet] 2014;9(6):e98856. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24906117 PMID: 24906117
61. Chan A-W, Tetzlaff JM, Gotzsche PC, Altman DG, Mann H, Berlin JA, et al. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. BMJ 2013 Jan;346(jan08 15):e7586–e7586.
62. Begg C, Cho M, Eastwood S, Horton R, Moher D, Olkin I, et al. Improving the quality of reporting of randomized controlled trials. The CONSORT statement. JAMA [Internet] 1996 Aug 28;276(8):637–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8773637 PMID: 8773637
63. Bekcioglu B, Bulut E, Bas B. The Effects of Unilateral Alloplastic Temporomandibular Joint Replacement on the Opposite-Side Natural Joint: A Finite-Element Analysis. J Oral Maxillofac Surg [Internet] 2017 May 24; Available from: http://www.ncbi.nlm.nih.gov/pubmed/28623683 PMID: 28623683
64. Ahtiainen K, Mauno J, Ellä V, Hagström J, Lindqvist C, Miettinen S, et al. Autologous adipose stem cells and polylactide discs in the replacement of the rabbit temporomandibular joint disc. J R Soc Interface 2013;10(85):20130287. PMID: 23720535
65. Brown BN, Chung WL, Almarza AJ, Pavlick MD, Reppas SN, Ochs MW, et al. Inductive, Scaffold-Based, Regenerative Medicine Approach to Reconstruction of the Temporomandibular Joint Disk. J Oral Maxillofac Surg 2012 Nov;70(11):2656–2668.
66. Xu L, Polur I, Lim C, Servais JM, Dobeck J, Li Y, et al. Early-onset osteoarthritis of mouse temporomandibular joint induced by partial discectomy. Osteoarthr Cartil [Internet] 2009 Jul;17(7):917–22. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19230720 PMID: 19230720
67. Choukas NC, Toto PD, Schoen JE. Gross and histologic changes in the jaws of the Macaca rhesus monkey following surgery. Oral Surg Oral Med Oral Pathol 1969 Jun;27(6):795–805. PMID: 4977743
68. Lai W-FT, Tsai Y-H, Su S-J, Su C-Y, Stockstill JW, Burch JG. Histological analysis of regeneration of temporomandibular joint discs in rabbits by using a reconstituted collagen template. Int J Oral Maxillofac Surg 2005 May;34(3):311–20. PMID: 15741041
69. Navarro R de L, Oltramari-Navarro PVP, Sant' Ana E, Henriques JFC, Taga R, Cestari TM, et al. Histologic and tomographic analyses of the temporomandibular joint after mandibular advancement surgery: study in minipigs. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009 Apr;107(4):477–84. PMID: 19121964
70. Allen KD, Athanasiou KA. Tissue Engineering of the TMJ Disc : A Review. 2006;12(5).
71. Tanaka E, Sasaki A, Tahmina K, Yamaguchi K, Mori Y, Tanne K. Mechanical properties of human articular disk and its influence on TMJ loading studied with the finite element method. J Oral Rehabil 2001;28(3):273–279. PMID: 11394374
72. Mehra P, Wolford LM. The Mitek mini anchor for TMJ disc repositioning: surgical technique and results. Int J Oral Maxillofac Surg 2001;30(6):497–503. PMID: 11829231

73. Al-Baghdadi M, Durham J, Araujo-Soares V, Robalino S, Errington L, Steele J. TMJ Disc Displacement without Reduction Management: A Systematic Review. J Dent Res [Internet] 2014 Apr 7 [cited 2014 May 3]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/24659775 PMID: 24659775
74. Henry CH, Wolford LM. Treatment outcomes for temporomandibular joint reconstruction after Proplast-Teflon implant failure. J Oral Maxillofac Surg 1993 Apr;51(4):352-8-60. PMID: 8450350
75. Westesson PL, Eriksson L, Lindström C. Destructive lesions of the mandibular condyle following diskectomy with temporary silicone implant. Oral Surg Oral Med Oral Pathol 1987;63(2):143–150.
76. Shu W, Liu L, Bao G, Kang H. Tissue engineering of the temporomandibular joint disc: current status and future trends. Int J Artif Organs [Internet] 2015;38(2):55–68. Available from: http://www.artificial-organs.com/article/tissue-engineering-of-the-temporomandibular-joint-disc--current-status-and-future-trends
77. Lavi A, Pelled G, Tawackoli W, Casap N, Gazit D, Gazit Z. Isolation and characterization of mesenchymal stromal progenitors from the temporomandibular joint disc. J Tissue Eng Regen Med [Internet] 2015; Available from: http://www.ncbi.nlm.nih.gov/pubmed/26193866 PMID: 26193866
78. Zhang S, Yap AUJ, Toh WS. Stem Cells for Temporomandibular Joint Repair and Regeneration. Stem Cell Rev [Internet] 2015; Available from: http://www.ncbi.nlm.nih.gov/pubmed/26123357 PMID: 26123357
79. Herring SW. TMJ anatomy and animal models. 2003;3(4):391–394.
80. Naples VL. Morphology, evolution and function of feeding in the giant anteater (Myrmecophaga tridactyla). J Zool [Internet] 1999 Sep;249(1):19–41. Available from: http://doi.wiley.com/10.1111/j.1469-7998.1999.tb01057.x
81. Lee YK, Moon HJ. Reciprocal influence of masticatory apparatus, craniofacial structure and whole body homeostasis. Med Hypotheses 2012;79(6):761–766. PMID: 22981594
82. Herring SW, Liu ZJ. Loading of the temporomandibular joint: Anatomical and in vivo evidence from the bones. Cells Tissues Organs 2001. p. 193–200. PMID: 11455114
83. Bosanquet A, Ishimaru J, Effect ANG, Bosanquet A, Goss AN. Effect of experimental disc perforation in sheep temporomandibular joints. 1991;2–6.
84. Bosanquet AG, Goss AN. The sheep as a model for temporomandibular joint surgery. Int J Oral Maxillofac Surg 1987;16(5):600–603. PMID: 3116116
85. Ishimaru J, Goss AN. A model for osteoarthritis of the temporomandibular joint. J Oral Maxillofac Surg 1992;50(11):1191–1195. PMID: 1403275
86. Long X, Goss AN. A Sheep Model of Intracapsular Condylar Fracture. J Oral Maxillofac Surg 2007;65(6):1102–1108. PMID: 17517292
87. Shimizu M, Kurita K, Matsuura H, Ishimaru J-I, Goss AN. The role of muscle grafts in temporomandibular joint ankylosis: short-term experimental study in sheep. Int J Oral Maxillofac Surg 2006;35(9):842–849. PMID: 16730161
88. Takaishi M, Kurita K, Matsuura H, Goss AN. Effect of Auricular Cartilage Graft in the Surgical Treatment of Temporomandibular Joint Ankylosis: An Animal Study Using Sheep. J Oral Maxillofac Surg 2007;65(2):198–204. PMID: 17236921
89. Matsuura H, Miyamoto H, Ishimaru J, Kurita K, Goss AN. Effect of partial immobilization on reconstruction of ankylosis of the temporomandibular joint with an autogenous costochondral graft:an experimental study in sheep. Br J Oral Maxillofac Surg 2001;39(3):196–203. PMID: 11384116
90. Ogi N, Kurita K, Ishimaru JI, Goss AN. Short-term effect of the use of a frozen-stored disc allograft for repair of the osteoarthritic sheep temporomandibular joint: a preliminary report. J Oral Maxillofac Surg 1999 Feb;57(2):139-44–5. PMID: 9973121
91. Tanaka E, van Eijden T, Eijden T Van, Biology CD. Biomechanical behavior of the temporomandibular joint disc. Crit Rev Oral Biol Med 2003;14(2):138–150. PMID: 12764076
92. Chin LP, Aker FD, Zarrinnia K. The viscoelastic properties of the human temporomandibular joint disc. J Oral Maxillofac Surg 1996;54(3):315-318-319. PMID: 8600239
93. Tanaka E, Eijden T Van, Biology CD. B Iomechanical B Ehavior of the. 2003;14(2):138–150.
94. Beek M, Aarnts MP, Koolstra JH, Feilzer AJ, van Eijden TM. Dynamic properties of the human temporomandibular joint disc. J DentRes 2001;80(0022–0345 (Print)):876–880. PMID: 11379888
95. Del Pozo R, Tanaka E, Tanaka M, Okazaki M, Tanne K. The regional difference of viscoelastic property of bovine temporomandibular joint disc in compressive stress-relaxation. Med Eng Phys 2002;24(3):165–171. PMID: 12062175
96. Shengyi T, Xu Y. Biomechanical properties and collagen fiber orientation of TMJ discs in dogs: Part 1. Gross anatomy and collagen fiber orientation of the discs. J Craniomandib Disord 1991;5(1):28–34. PMID: 1809767
97. Teng S, Xu Y, Cheng M, Li Y. Biomechanical properties and collagen fiber orientation of temporomandibular joint discs in dogs: 2. Tensile mechanical properties of the discs. J Craniomandib Disord 1991;5(2):107–114. PMID: 1812136
98. Martins-Júnior RL, Palma AJG, Marquardt EJ, Gondin TM de B, Kerber F de C. Temporomandibular disorders: a report of 124 patients. J Contemp Dent Pract 2010;11(5):71–8. PMID: 20978727
99. Navrátil L, Navratil V, Hajkova S, Hlinakova P, Dostalova T, Vranová J. Comprehensive treatment of temporomandibular joint disorders. Cranio 2014 Jan;32(1):24–30. PMID: 24660643
100. Mercuri LG, Giobbie-Hurder A. Long-term outcomes after total alloplastic temporomandibular joint reconstruction following exposure to failed materials. J Oral Maxillofac Surg 2004 Sep;62(9):1088–96. PMID: 15346359

101. Morouço P, Ângelo D, Francisco L, Moura C, Alves N. Tissue engineering for temporomandibular joint disc repair and regeneration: a methodological perspective. Adv Cell Mol Otolaryngol 2016 Nov;4.
102. Estabrooks LN, Fairbanks CE, Collett RJ, Miller L. A retrospective evaluation of 301 TMJ Proplast-Teflon implants. Oral Surgery, Oral Med Oral Pathol 1990 Sep;70(3):381–386.
103. Lypka M, Yamashita D-DR. Exuberant foreign body giant cell reaction to a teflon/proplast temporomandibular joint implant: report of a case. J Oral Maxillofac Surg 2007 Sep;65(9):1680–4. PMID: 17719383
104. Spagnoli D, Kent JN. Multicenter evaluation of temporomandibular joint Proplast-Teflon disk implant. Oral Surg Oral Med Oral Pathol 1992 Oct;74(4):411–21. PMID: 1408011
105. Kulber DA, Davos I, Aronowitz JA. Severe cutaneous foreign body giant cell reaction after temporomandibular joint reconstruction with Proplast-Teflon. J Oral Maxillofac Surg 1995 Jun;53(6):719-22–3. PMID: 7776062
106. Chuong R, Piper MA. Cerebrospinal fluid leak associated with proplast implant removal from the temporomandibular joint. Oral Surg Oral Med Oral Pathol 1992 Oct;74(4):422–5. PMID: 1408012
107. Berarducci JP, Thompson DA, Scheffer RB. Perforation into middle cranial fossa as a sequel to use of a Proplast-Teflon implant for temporomandibular joint reconstruction. J Oral Maxillofac Surg 1990 May;48(5):496–8. PMID: 2329399
108. Recommendations for management of patients with temporomandibular joint implants. Temporomandibular Joint Implant Surgery Workshop. J Oral Maxillofac Surg 1993 Oct;51(10):1164–72. PMID: 8410459
109. Angelo DF, Morouço P, Alves N, Viana T, Santos F, González R, et al. Choosing sheep (Ovis aries) as animal model for temporomandibular joint research: Morphological, histological and biomechanical characterization of the joint disc. Morphologie 2016 Jul;
110. Leiggener CS, Erni S, Gallo LM. Novel approach to the study of jaw kinematics in an alloplastic TMJ reconstruction. Int J Oral Maxillofac Surg 2012 Sep;41(9):1041–5. PMID: 22819692
111. Little CB, Smith MM, Cake MA, Read RA, Murphy MJ, Barry FP. The OARSI histopathology initiative - recommendations for histological assessments of osteoarthritis in sheep and goats. Osteoarthritis Cartilage 2010 Oct;18 Suppl 3:S80-92. PMID: 20864026
112. Cake MA, Appleyard RC, Read RA, Smith MM, Murrell GAC, Ghosh P. Ovariectomy alters the structural and biomechanical properties of ovine femoro-tibial articular cartilage and increases cartilage iNOS. Osteoarthritis Cartilage 2005 Dec;13(12):1066–75. PMID: 16154775
113. Xu L, Polur I, Lim C, Servais JM, Dobeck J, Li Y, et al. Early-onset osteoarthritis of mouse temporomandibular joint induced by partial discectomy. Osteoarthr Cartil 2009 Jul;17(7):917–922.
114. Sato S, Goto S, Koeda S, Motegi K. Changes of the elastic fibre network of the rabbit temporomandibular joint following discectomy. J Oral Rehabil 2002 Sep;29(9):847–52. PMID: 12366539
115. Sato S, Goto S, Motegi K. Changes of the collagen fibre arrangement of the rabbit temporomandibular joint following discectomy. J Craniomaxillofac Surg 2002 Jun;30(3):178–83. PMID: 12220997
116. Ogi N, Kurita K, Ishimaru JI, Goss AN. Short-term effect of the use of a frozen-stored disc allograft for repair of the osteoarthritic sheep temporomandibular joint: A preliminary report. J Oral Maxillofac Surg 1999;57(2):139–144. PMID: 9973121
117. Hinton RJ, Stinson JL. Effect of postoperative diet on condylar cartilage response to discectomy. J Oral Maxillofac Surg 1997 Nov;55(11):1259–64. PMID: 9371117
118. Widmark G, Dahlström L, Kahnberg KE, Lindvall AM. Diskectomy in temporomandibular joints with internal derangement: a follow-up study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1997 Mar;83(3):314–20. PMID: 9084191
119. Kilkenny C, Parsons N, Kadyszewski E, Festing MFW, Cuthill IC, Fry D, et al. Survey of the Quality of Experimental Design, Statistical Analysis and Reporting of Research Using Animals. McLeod M, editor. PLoS One [Internet] 2009 Nov 30;4(11):e7824. Available from: http://dx.plos.org/10.1371/journal.pone.0007824
120. van der Worp HB, Howells DW, Sena ES, Porritt MJ, Rewell S, O'Collins V, et al. Can Animal Models of Disease Reliably Inform Human Studies? PLoS Med [Internet] 2010 Mar 30;7(3):e1000245. Available from: http://dx.plos.org/10.1371/journal.pmed.1000245
121. Sena ES, van der Worp HB, Bath PMW, Howells DW, Macleod MR. Publication Bias in Reports of Animal Stroke Studies Leads to Major Overstatement of Efficacy. Roberts I, editor. PLoS Biol [Internet] 2010 Mar 30;8(3):e1000344. Available from: http://dx.plos.org/10.1371/journal.pbio.1000344
122. Pearce G. Changes in particle size in the reticulorumen of sheep. Aust J Agric Res [Internet] 1967;18(1):119. Available from: http://www.publish.csiro.au/?paper=AR9670119
123. Ângelo DF, Monje FG, González-García R, Little CB, Mónico L, Pinho M, et al. Bioengineered Temporomandibular Joint Disk Implants: Study Protocol for a Two-Phase Exploratory Randomized Preclinical Pilot Trial in 18 Black Merino Sheep (TEMPOJIMS). JMIR Res Protoc 2017 Mar;6(3):e37. PMID: 28254733
124. Ingawalé S, Goswami T. Temporomandibular Joint: Disorders, Treatments, and Biomechanics. Ann Biomed Eng [Internet] 2009 May 28;37(5):976–996. Available from: http://link.springer.com/10.1007/s10439-009-9659-4
125. Poveda Roda R, Bagan J V, Díaz Fernández JM, Hernández Bazán S, Jiménez Soriano Y. Review of temporomandibular joint pathology. Part I: classification, epidemiology and risk factors. Med Oral Patol Oral Cir Bucal [Internet] 2007 Aug 1;12(4):E292-8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17664915 PMID: 17664915

126. Hidaka O, Morimoto T, Masuda Y, Kato T, Matsuo R, Inoue T, et al. Regulation of masticatory force during cortically induced rhythmic jaw movements in the anesthetized rabbit. J Neurophysiol [Internet] 1997 Jun;77(6):3168–79. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9212266 PMID: 9212266

127. Zhao C, Kurita H, Kurashina K, Hosoya A, Arai Y, Nakamura H. Temporomandibular joint response to mandibular deviation in rabbits detected by 3D micro-CT imaging. Arch Oral Biol [Internet] 2010 Dec;55(12):929–937. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0003996910002074

128. Dimitroulis G. The role of surgery in the management of disorders of the temporomandibular joint: a critical review of the literature. Part 2. Int J Oral Maxillofac Surg 2005 May;34(3):231–7. PMID: 15741028

129. de Souza RF, Lovato da Silva CH, Nasser M, Fedorowicz Z, Al-Muharraqi MA. Interventions for the management of temporomandibular joint osteoarthritis. In: de Souza RF, editor. Cochrane Database Syst Rev Chichester, UK: John Wiley & Sons, Ltd; 2012.

130. Reston JT, Turkelson CM. Meta-analysis of surgical treatments for temporomandibular articular disorders. J Oral Maxillofac Surg [Internet] 2003 Jan [cited 2014 May 3];61(1):3-10–2. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12524600 PMID: 12524600

131. Cordeiro PC, Guimaraes JP, de Souza VA, Dias IM, Silva JN, Devito KL, et al. Temporomandibular joint involvement in rheumatoid arthritis patients: association between clinical and tomographic data. Acta Odontol Latinoam 2016 Dec;29(3):123–129. PMID: 28383601

132. K B. Temporomandibular joint arthrosis and its treatment by extirpation of the disc: A clinical study. Follow-up examination. Acta Chir Scand 1947;95(192).

133. KIEHN CL, DESPREZ JD. Meniscectomy for internal derangement of temporomandibular joint. Br J Plast Surg 1962 Apr;15:199–204. PMID: 14455801

134. Hall HD. Meniscectomy for damaged disks of the temporomandibular joint. South Med J 1985 May;78(5):569–72. PMID: 3992305

135. Eriksson L, Westesson PL. Long-term evaluation of meniscectomy of the temporomandibular joint. J Oral Maxillofac Surg 1985 Apr;43(4):263–9. PMID: 3856642

136. Silver CM. Long-term results of meniscectomy of the temporomandibular joint. Cranio 3(1):46–57. PMID: 6594400

137. Tolvanen M, Oikarinen VJ, Wolf J. A 30-year follow-up study of temporomandibular joint meniscectomies: a report on five patients. Br J Oral Maxillofac Surg 1988 Aug;26(4):311–6. PMID: 3166964

138. Takaku S, Toyoda T. Long-term evaluation of discectomy of the temporomandibular joint. J Oral Maxillofac Surg 1994 Jul;52(7):722-6-8. PMID: 8006736

139. Ogi N, Kurita K, Handa Y, Goss AN. The short-term effect of discectomy on the osteoarthrotic temporomandibular joint in sheep. Int J Oral Maxillofac Surg 1996 Aug;25(4):319–24. PMID: 8910121

140. Hagandora CK, Almarza AJ. TMJ disc removal: comparison between pre-clinical studies and clinical findings. J Dent Res 2012 Aug;91(8):745–52. PMID: 22744995

141. Laurell KA, Tootle R, Cunningham R, Beltran J, Simon D. Magnetic resonance imaging of the temporomandibular joint. Part II: Comparison with laminographic, autopsy, and histologic findings. J Prosthet Dent 1987 Aug;58(2):211–8. PMID: 3476729

142. Block MS, Bouvier M. Adaptive remodeling of the rabbit temporomandibular joint following discectomy and dietary variations. J Oral Maxillofac Surg 1990 May;48(5):482–6. PMID: 2329397

143. Macher DJ, Westesson PL, Brooks SL, Hicks DG, Tallents RH. Temporomandibular joint: surgically created disk displacement causes arthrosis in the rabbit. Oral Surg Oral Med Oral Pathol 1992 Jun;73(6):645–9. PMID: 1437028

144. Miyamoto H, Kurita K, Ishimaru J, Goss AN. A sheep model for temporomandibular joint ankylosis. J Oral Maxillofac Surg 1999 Jul;57(7):812–7. PMID: 10416628

145. Matsuura H, Miyamoto H, Kurita K, Goss AN. The effect of autogenous costochondral grafts on temporomandibular joint fibrous and bony ankylosis: A preliminary experimental study. J Oral Maxillofac Surg 2006 Oct;64(10):1517–25. PMID: 16982311

146. Murphy MK, MacBarb RF, Wong ME, Athanasiou KA. Temporomandibular disorders: a review of etiology, clinical management, and tissue engineering strategies. Int J Oral Maxillofac Implants [Internet] 28(6):e393-414. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24278954 PMID: 24278954

147. Smith MM, Cake MA, Ghosh P, Schiavinato A, Read RA, Little CB. Significant synovial pathology in a meniscectomy model of osteoarthritis: modification by intra-articular hyaluronan therapy. Rheumatology (Oxford) [Internet] 2008 Aug;47(8):1172–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18565987 PMID: 18565987

148. Melrose J, Fuller ES, Little CB. The biology of meniscal pathology in osteoarthritis and its contribution to joint disease: beyond simple mechanics. Connect Tissue Res [Internet] 58(3–4):282–294. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28121190 PMID: 28121190

149. Shu CC, Smith MM, Smith SM, Dart AJ, Little CB, Melrose J. A Histopathological Scheme for the Quantitative Scoring of Intervertebral Disc Degeneration and the Therapeutic Utility of Adult Mesenchymal Stem Cells for Intervertebral Disc Regeneration. Int J Mol Sci [Internet] 2017 May 12;18(5). Available from: http://www.ncbi.nlm.nih.gov/pubmed/28498326 PMID: 28498326

150. Wu G, Zhu S, Sun X, Hu J. Subchondral bone changes and chondrogenic capacity of progenitor cells from subchondral bone in the collagenase-induced temporomandibular joints osteoarthritis rabbit model. Int J Clin Exp Pathol [Internet] 2015;8(9):9782–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26617688 PMID: 26617688
151. Matías EMC, Mecham DK, Black CS, Graf JW, Steel SD, Wilhelm SK, et al. Malocclusion model of temporomandibular joint osteoarthritis in mice with and without receptor for advanced glycation end products. Arch Oral Biol [Internet] 2016 Sep;69:47–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27236646 PMID: 27236646
152. Cake MA, Read RA, Corfield G, Daniel A, Burkhardt D, Smith MM, et al. Comparison of gait and pathology outcomes of three meniscal procedures for induction of knee osteoarthritis in sheep. Osteoarthr Cartil [Internet] 2013 Jan;21(1):226–36. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23069853 PMID: 23069853
153. Young AA, Smith MM, Smith SM, Cake MA, Ghosh P, Read RA, et al. Regional assessment of articular cartilage gene expression and small proteoglycan metabolism in an animal model of osteoarthritis. Arthritis Res Ther [Internet] 2005;7(4):R852-61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15987487 PMID: 15987487
154. Agerberg G, Lundberg M. Changes in the temporomandibular joint after surgical treatment. A radiologic follow-up study. Oral Surg Oral Med Oral Pathol 1971 Dec;32(6):865–75. PMID: 5288825
155. Wilkes CH. Surgical treatment of internal derangements of the temporomandibular joint. A long-term study. Arch Otolaryngol Head Neck Surg 1991 Jan;117(1):64–72. PMID: 1986764
156. Yaillen DM, Shapiro PA, Luschei ES, Feldman GR. Temporomandibular joint meniscectomy--effects on joint structure and masticatory function in macaca fascicularis. J Maxillofac Surg 1979 Nov;7(4):255–64. PMID: 117065
157. England BR, Baker JF, Sayles H, Michaud K, Caplan L, Davis LA, et al. Body Mass Index, Weight Loss, and Cause-Specific Mortality in Rheumatoid Arthritis. Arthritis Care Res (Hoboken) 2017 Apr; PMID: 28426913
158. Lynch SM, Stricker CT, Brown JC, Berardi JM, Vaughn D, Domchek S, et al. Evaluation of a web-based weight loss intervention in overweight cancer survivors aged 50 years and younger. Obes Sci Pract 2017 Mar;3(1):83–94. PMID: 28392934
159. Malvy E, Thiébaut R, Marimoutou C, Dabis F, Groupe d'Epidemiologie Clinique du Sida en Aquitaine. Weight loss and body mass index as predictors of HIV disease progression to AIDS in adults. Aquitaine cohort, France, 1985-1997. J Am Coll Nutr 2001 Dec;20(6):609–15. PMID: 11771676
160. Casillas RA, Kim B, Fischer H, Zelada Getty JL, Um SS, Coleman KJ. Comparative effectiveness of sleeve gastrectomy versus Roux-en-Y gastric bypass for weight loss and safety outcomes in older adults. Surg Obes Relat Dis 2017 Mar; PMID: 28438494
161. Richmond J. Refinement, reduction, and replacement of animal use for regulatory testing: future improvements and implementation within the regulatory framework. ILAR J 2002;43 Suppl:S63-8. PMID: 12388854
162. D G Altman, S M Gore, M J Gardner and SJP. Statistical guidelines for contributors to medical journals. Br Med Jor 1992 Mar;286(6376):1489–1493. PMID: 1547706
163. Moher D, Schulz KF, Altman DG. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomised trials. Lancet (London, England) 2001 Apr;357(9263):1191–4. PMID: 11323066
164. Ren K. An improved method for assessing mechanical allodynia in the rat. Physiol Behav 1999 Nov;67(5):711–6. PMID: 10604842
165. Imbe H, Iwata K, Zhou QQ, Zou S, Dubner R, Ren K. Orofacial deep and cutaneous tissue inflammation and trigeminal neuronal activation. Implications for persistent temporomandibular pain. Cells Tissues Organs 2001;169(3):238–47. PMID: 11455119
166. Iwata K, Tashiro A, Tsuboi Y, Imai T, Sumino R, Morimoto T, et al. Medullary dorsal horn neuronal activity in rats with persistent temporomandibular joint and perioral inflammation. J Neurophysiol 1999 Sep;82(3):1244–53. PMID: 10482744
167. Haas DA, Nakanishi O, MacMillan RE, Jordan RC, Hu JW. Development of an orofacial model of acute inflammation in the rat. Arch Oral Biol 1992;37(5):417–22. PMID: 1610310
168. Hathaway CB, Hu JW, Bereiter DA. Distribution of Fos-like immunoreactivity in the caudal brainstem of the rat following noxious chemical stimulation of the temporomandibular joint. J Comp Neurol 1995 Jun;356(3):444–56. PMID: 7642805
169. Roveroni RC, Parada CA, Cecília M, Veiga FA, Tambeli CH. Development of a behavioral model of TMJ pain in rats: the TMJ formalin test. Pain 2001 Nov;94(2):185–91. PMID: 11690732
170. Marciani RD, Gonty AA, White DK, Traurig HH. A preliminary study of reconstruction of the monkey temporomandibular joint with autogenous tissues. J Oral Maxillofac Surg 1996 Feb;54(2):191–9. PMID: 8604069
171. Tucker MR, Watzke IM. Autogenous auricular cartilage graft for temporomandibular joint repair. A comparison of technique with and without temporary silastic implantation. J Craniomaxillofac Surg 1991 Apr;19(3):108–12. PMID: 2071692
172. Tucker MR, Kennady MC, Jacoway JR. Autogenous auricular cartilage implantation following discectomy in the primate temporomandibular joint. J Oral Maxillofac Surg 1990 Jan;48(1):38–44. PMID: 2294210
173. Tong AC-K, Tideman H. The microanatomy of the rhesus monkey temporomandibular joint. J Oral Maxillofac Surg 2001 Jan;59(1):46–52.
174. Bermejo a, González O, González JM. The pig as an animal model for experimentation on the temporomandibular articular complex. Oral Surg Oral Med Oral Pathol 1993;75(1):18–23. PMID: 8419867

175. Matuska AM, Muller S, Dolwick MF, McFetridge PS. Biomechanical and biochemical outcomes of porcine temporomandibular joint disc deformation. Arch Oral Biol 2016 Apr;64:72–9. PMID: 26774186
176. Barrientos E, Pelayo F, Tanaka E, Lamela-Rey MJ, Fernández-Canteli A. Dynamic and stress relaxation properties of the whole porcine temporomandibular joint disc under compression. J Mech Behav Biomed Mater 2016 Apr;57:109–15. PMID: 26708739
177. Cisewski SE, Zhang L, Kuo J, Wright GJ, Wu Y, Kern MJ, et al. The effects of oxygen level and glucose concentration on the metabolism of porcine TMJ disc cells. Osteoarthritis Cartilage 2015 May; PMID: 26033165
178. Wu Y, Kuo J, Wright GJ, Cisewski SE, Wei F, Kern MJ, et al. Viscoelastic shear properties of porcine temporomandibular joint disc. Orthod Craniofac Res 2015 Apr;18 Suppl 1:156–63. PMID: 25865544
179. Nickel JC, Iwasaki LR, Beatty MW, Marx DB. Tractional forces on porcine temporomandibular joint discs. J Dent Res 2009 Aug;88(8):736–40. PMID: 19734461
180. Nickel J, Spilker R, Iwasaki L, Gonzalez Y, McCall WD, Ohrbach R, et al. Static and dynamic mechanics of the temporomandibular joint: plowing forces, joint load and tissue stress. Orthod Craniofac Res 2009 Aug;12(3):159–67. PMID: 19627517
181. Cell Type and distribution in the porcine temporomandibular joint disc. J Natl Cancer Inst [Internet] 1974;53(6):1545–1551. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4474408/
182. Allen KD, Athanasiou KA. Viscoelastic characterization of the porcine temporomandibular joint disc under unconfined compression. J Biomech 2006;39(2):312–22. PMID: 16321633
183. Allen KD, Athanasiou KA. A surface-regional and freeze-thaw characterization of the porcine temporomandibular joint disc. Ann Biomed Eng 2005 Jul;33(7):951–62. PMID: 16060536
184. Almarza AJ, Bean AC, Baggett LS, Athanasiou KA. Biochemical analysis of the porcine temporomandibular joint disc. Br J Oral Maxillofac Surg 2006 Apr;44(2):124–8. PMID: 16011866
185. Detamore MS, Orfanos JG, Almarza AJ, French MM, Wong ME, Athanasiou K a. Quantitative analysis and comparative regional investigation of the extracellular matrix of the porcine temporomandibular joint disc. Matrix Biol 2005;24(1):45–57. PMID: 15749001
186. Nickel JC, Iwasaki LR, Beatty MW, Marx DB. Laboratory stresses and tractional forces on the TMJ disc surface. J Dent Res 2004 Aug;83(8):650–4. PMID: 15271976
187. Tanaka E, Kawai N, Hanaoka K, Van Eijden T, Sasaki A, Aoyama J, et al. Shear properties of the temporomandibular joint disc in relation to compressive and shear strain. J Dent Res 2004 Jun;83(6):476–9. PMID: 15153455
188. Tanaka E, Kawai N, Van Eijden T, Watanabe M, Hanaoka K, Nishi M, et al. Impulsive compression influences the viscous behavior of porcine temporomandibular joint disc. Eur J Oral Sci 2003 Aug;111(4):353–8. PMID: 12887402
189. Bao G, Kang H, Dong Y, Zhu R, Chao Y, Yi X, et al. [Effects of stress level on the biomechanical behavior of the temporomandibular joint disc in domestic pigs]. Sheng Wu Yi Xue Gong Cheng Xue Za Zhi 2000 Dec;17(4):418–20. PMID: 11211829
190. Kuboki T, Shinoda M, Orsini MG, Yamashita A. Viscoelastic properties of the pig temporomandibular joint articular soft tissues of the condyle and disc. J Dent Res 1997 Nov;76(11):1760–9. PMID: 9372793
191. Ström D, Holm S, Clemensson E, Haraldson T, Carlsson GE. Gross anatomy of the mandibular joint and masticatory muscles in the domestic pig (Sus scrofa). Arch Oral Biol 1986;31(11):763–8. PMID: 3479064
192. Proff P, Gedrange T, Franke R, Schubert H, Fanghänel J, Miehe B, et al. Histological and histomorphometric investigation of the condylar cartilage of juvenile pigs after anterior mandibular displacement. Ann Anat 2007;189(3):269–75. PMID: 17534034
193. Timmel R, Grundschober F. The interposition of Lyodura in operations for ankylosis of the temporo-mandibular joint. An experimental study using pigs. J Maxillofac Surg 1982 Nov;10(4):193–9. PMID: 6961178
194. de Lima Navarro R, Oltramari PVP, Sant'Ana E, Henriques JFC, Taga R, Cestari TM, et al. Histological and molecular temporomandibular joint analyses after mandibular advancement surgery: study in minipigs. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008 Sep;106(3):331–8. PMID: 18554945
195. Zouhary KJ, Feinberg SE. Regeneration of the mandibular condyle in minipigs. J Oral Maxillofac Surg 2006 Mar;64(3):565–6; author reply 566. PMID: 16487827
196. Thurmüller P, Troulis MJ, Rosenberg A, Chuang S-K, Kaban LB. Microscopic changes in the condyle and disc in response to distraction osteogenesis of the minipig mandible. J Oral Maxillofac Surg 2006 Feb;64(2):249–58. PMID: 16413897
197. Herford AS, Hoffman R, Demirdji S, Boyne PJ, Caruso JM, Leggitt VL, et al. A comparison of synovial fluid pressure after immediate versus gradual mandibular advancement in the miniature pig. J Oral Maxillofac Surg 2005 Jun;63(6):775–85. PMID: 15944974
198. Yagi T, Morimoto T, Hidaka O, Iwata K, Masuda Y, Kobayashi M, et al. Adjustment of the occlusal vertical dimension in the bite-raised guinea pig. J Dent Res 2003 Feb;82(2):127–30. PMID: 12562886
199. Sun Z, Liu Z-J, Herring SW. Movement of temporomandibular joint tissues during mastication and passive manipulation in miniature pigs. Arch Oral Biol 2002 Apr;47(4):293–305. PMID: 11922872
200. Štembírek J, Kyllar M, Putnová I, Stehlík L, Buchtová M. The pig as an experimental model for clinical craniofacial research. Lab Anim 2012 Oct;46(4):269–79. PMID: 22969144

201. Brown BN, Chung WL, Pavlick M, Reppas S, Ochs MW, Russell AJ, et al. Extracellular matrix as an inductive template for temporomandibular joint meniscus reconstruction: a pilot study. J Oral Maxillofac Surg 2011 Dec;69(12):e488-505. PMID: 21684655
202. Ström D, Holm S, Clemensson E, Haraldson T, Carlsson GE. Gross anatomy of the craniomandibular joint and masticatory muscles of the dog. Arch Oral Biol 1988;33(8):597–604. PMID: 3254129
203. Long X, Goss AN. Pathological changes after the surgical creation of a vertical intracapsular condylar fracture. Int J Oral Maxillofac Surg 2007 Sep;36(9):834–7. PMID: 17766087
204. Takaishi M, Kurita K, Hatano Y, Matsuura H, Borg M, Goss NA. Effects of postoperative radiotherapy for temporomandibular joint ankylosis after gap arthroplasty: an animal study using sheep. J Oral Maxillofac Surg 2010 Aug;68(8):1763–9. PMID: 20117872
205. Kurita K, Ogi N, Miyamoto K, Goss AN. Diagnostic evaluation of an ultrathin 15,000 fiberoptic arthroscope: comparison of arthroscopic and histologic findings in a sheep model. J Oral Maxillofac Surg 2005 Mar;63(3):319–22. PMID: 15742280
206. Miyamoto H, Matsuura H, Singh J, Goss AN. Regeneration of the mandibular condyle after unilateral condylectomy and myotomy of the masseter in lambs. Br J Oral Maxillofac Surg 2002 Apr;40(2):116–21. PMID: 12180201
207. Miyamoto K, Ishimaru J-I, Kurita K, Goss AN. Synovial matrix metalloproteinase-2 in different stages of sheep temporomandibular joint osteoarthrosis. J Oral Maxillofac Surg 2002 Jan;60(1):66–72. PMID: 11757011
208. Matsuura H, Miyamoto H, Ogi N, Kurita K, Goss AN. The effect of gap arthroplasty on temporomandibular joint ankylosis: an experimental study. Int J Oral Maxillofac Surg 2001 Oct;30(5):431–7. PMID: 11720046
209. Tahmasebi-Sarvestani A, Tedman R, Goss AN. The influence of experimentally induced osteoarthrosis on articular nerve fibers of the sheep temporomandibular joint. J Orofac Pain 2001;15(3):206–17. PMID: 11575191
210. Ishimaru JI, Ogi N, Mizuno S, Goss AN. Quantitation of chondroitin-sulfates, disaccharides and hyaluronan in normal, early and advanced osteoarthritic sheep temporomandibular joints. Osteoarthr Cartil 2001 May;9(4):365–70. PMID: 11399101
211. Matsuura H, Miyamoto H, Ishimaru JI, Kurita K, Goss AN. Costochondral grafts in reconstruction of the temporomandibular joint after condylectomy: an experimental study in sheep. Br J Oral Maxillofac Surg 2001;39(3):189–195. PMID: 11384115
212. Miyamoto H, Kurita K, Ogi N, Ishimaru J, Goss AN. The effect of an intra-articular bone fragment in the genesis of temporomandibular joint ankylosis. Int J Oral Maxillofac Surg 2000 Aug;29(4):290–5. PMID: 11030402
213. Miyamoto H, Kurita K, Ogi N, Ishimaru JI, Goss AN. Effect of limited jaw motion on ankylosis of the temporomandibular joint in sheep. Br J Oral Maxillofac Surg 2000 Apr;38(2):148–53. PMID: 10864712
214. Miyamoto K, Vickers R, Ishimaru JI, Ogi N, Kurita K, Goss AN. Effect of unilateral condylectomy on the sheep temporomandibular joint. Br J Oral Maxillofac Surg 1999 Oct;37(5):401–4. PMID: 10577756
215. Miyamoto H, Kurita K, Ogi N, Ishimaru JI, Goss AN. The role of the disk in sheep temporomandibular joint ankylosis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999 Aug;88(2):151–8. PMID: 10468457
216. Neo H, Ishimaru JI, Kurita K, Goss AN. The effect of hyaluronic acid on experimental temporomandibular joint osteoarthrosis in the sheep. J Oral Maxillofac Surg 1997 Oct;55(10):1114–9. PMID: 9331236
217. Ciocca L, Donati D, Fantini M, Landi E, Piattelli A, Iezzi G, et al. CAD-CAM-generated hydroxyapatite scaffold to replace the mandibular condyle in sheep: preliminary results. J Biomater Appl 2013 Aug;28(2):207–18. PMID: 22492196
218. Yan Y-B, Zhang Y, Gan Y-H, An J-G, Li J-M, Xiao E. Surgical induction of TMJ bony ankylosis in growing sheep and the role of injury severity of the glenoid fossa on the development of bony ankylosis. J Craniomaxillofac Surg 2013 Sep;41(6):476–86. PMID: 22507296
219. Wang X, Zhang Y, Li J. [Experimental establishment of animal model of temporomandibular joint ankylosis secondary to condylar sagittal fracture]. Beijing Da Xue Xue Bao 2011 Dec;43(6):903–7. PMID: 22178844
220. Yan Y-B, Li J-M, Xiao E, An J-G, Gan Y-H, Zhang Y. A pilot trial on the molecular pathophysiology of traumatic temporomandibular joint bony ankylosis in a sheep model. Part I: Expression of Wnt signaling. J Craniomaxillofac Surg 2014 Mar;42(2):e15-22. PMID: 23707780
221. Takaku S, Sano T, Yoshida M. Long-term magnetic resonance imaging after temporomandibular joint discectomy without replacement. J Oral Maxillofac Surg [Internet] 2000 Jul;58(7):739–45. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10883688 PMID: 10883688

FACSIMILE

Morphologie (2016) **100**, 223—233

Disponible en ligne sur
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com

ORIGINAL ARTICLE

Histology;
Biomechanical
characterization

analysis) and biomechanical characterization (tension and compression tests) of sheep TMJ comparing the obtained results with human data. Results showed that sheep processus condylaris and fossa mandibularis are anatomically similar to the same human structures. TMJ disc has an elliptical perimeter, thinner in the center than in periphery. Peripheral area acts as a ring structure supporting the central zone. The disc cells display both fibroblast and chondrocyte-like morphology. Marginal area is formed by loose connective tissue, with some chondrocyte-like cells and collagen fibers in diverse orientations. Discs obtained a tensile modulus of 3.97 ± 0.73 MPa and 9.39 ± 1.67 MPa, for anteroposterior and mediolateral assessment. The TMJ discs presented a compressive modulus (E) of 446.41 ± 5.16 MPa and their maximum stress value (σmax) was 18.87 ± 1.33 MPa. Obtained results suggest that these animals should be considered as a prime model for TMJ research and procedural training. Further investigations in the field of oromaxillofacial surgery involving TMJ should consider sheep as a good animal model due to its resemblance of the same joint in humans.

MOTS CLÉS
Mouton ;
Articulation temporo-mandibulaire ;
Anatomie ;
Histologie ;
Biomécanique

Résumé Les essais précliniques sont essentiels pour le développement des technologies scientifiques. Des recherches moléculaires et cellulaires remarquables ont été réalisées sur de petits modèles animaux. Toutefois, des différences significatives existent en ce qui concerne le comportement articulaire entre ces modèles et l'Homme. Ainsi, les modèles de gros animaux peuvent être plus appropriés pour effectuer des essais concernant l'articulation temporomandibulaire (ATM). Le but de ce travail était de faire une description morphologique (dissection anatomique et imagerie photonique 3D), histologique (ATM en bloc a été prélevée) et biomécanique (tests de traction et de compression) sur des ATM de mouton en comparant les résultats obtenus avec les données connues chez l'Homme. Les résultats ont montré que le processus condylaris et la fossa mandibularis sont anatomiquement semblables aux structures humaines. Le disque de l'ATM présente un périmètre elliptique, plus mince au centre que dans la périphérie. La zone périphérique agit comme une structure annulaire de support de la zone centrale. Les cellules du disque ont un aspect à la fois fibroblastique et chondrocytaire. La zone marginale est formée par un tissu conjonctif lâche, avec quelques cellules chondroïdes et des fibres de collagène dans diverses orientations. Les disques avaient un module de traction de 3,97 ± 0,73 MPa et de 9,39 ± 1,67 MPa pour les mesures en antéropostérieur et en médiolatéral. Les disques ATM avaient un module en compression (E) de 446,41 ± 5,16 MPa et leur valeur en contrainte maximale (σmax) était de 18,87 ± 1,33 MPa. Les résultats obtenus suggèrent que ces animaux peuvent être considérés comme un modèle de choix pour les recherches sur l'ATM. D'autres études dans le domaine de la chirurgie oro-maxillofaciale impliquant l'ATM pourraient envisager le mouton comme un bon modèle animal en raison de sa ressemblance avec l'ATM humaine.

Introduction

To improve human health, scientific discoveries and technologies must be translated into practical applications. Such advances classically begin with basic research and then progress to the clinical level. Inherent to the development of new technologies is the role of preclinical trials using animal models. Although no animal model can fully replicate human conditions, animal models are key for the evaluation of mechanisms of disease, testing new technologies and applying new procedures. Temporomandibular joint (TMJ) is the most frequently used joint in the human body. TMJ opens and closes 1500—2000 times daily and is essential for everyday functions of the mouth such as mastication, speech, deglutition, yawning and snoring involving special mandatory synergy of both articular sides [1]. Joint surfaces are convex and, therefore, smooth joint movements are only possible due to an intra-articular disc between them. TMJ disc is an essential component in the normal TMJ and has the following functions: it distributes the intra-articular load, stabilizes the joints during translation and decreases the wear of the articular surface [2,3]. TMJ disc displaced, malformed or damaged, can induce pathologic processes of internal derangement and/or osteoarthritis [4,5]. Currently patients suffering from severe temporomandibular dysfunction (TMD) have few treatment options. Without safe, effective TMJ disc implants, many patients undergo discectomy: a surgical procedure that removes the injured TMJ disc aiming to reduce severe TMD symptoms. This procedure may not be the ideal as the TMJ is left without an important functional structure. Since the previous problems associated with alloplastic materials used to substitute TMJ disc such as silicone and Proplast-Teflon (PTIPI, Vitek, Inc, Houston, Texas, USA) [6,7], many groups discarded investigation in this field. However, the potential impact of a synthetic temporomandibular interposal implant (TII) is immense. Failures of the synthetic TII have generally been attributed to the lack of knowledge concerning the TMJ biomechanical and biochemical aspects. The development of new technologies for scaffolds engineering regarding TMJ disc is growing [8—11] and the ideal animal model for TMJ research should be well characterized. The choice of an animal for experimental design is not straightforward. Due to physiological and anatomical differences between the human TMJ and that of experimental animals, there is no animal model that is valid per se. TMJ is a cardinal feature that defines the class *Mammalia* and separates mammals from other vertebrates [12]. TMJ shows remarkable morphological and functional variation between different species, reflecting not only the great mammalian adaptation to feeding mechanisms but also different biomechanical behavior [13]. The morphological variations are either correlates of loading (e.g. size of articular surfaces) or movement (e.g. orientation of the joint), or both. Loading of the TMJ is a reaction force arising from the contraction of masticatory muscles; its magnitude depends strongly on the position of the bite point relative to the muscle action line [14]. Many commonly used laboratory animals, especially rodents, fall in the category of minimal TMJ loading, especially during chewing. In contrast, carnivores such as dogs sustain TMJ loads that are higher than those of primates [15]. Opening of the jaw usually involves a combination of rotation and forward sliding (translation), but some carnivores have lost the ability to slide and some specialized anteaters instead use a rotation around the long axis of the curved mandible [13]. The most extreme evolutionary variants include:

- loss of the synovial cavity in some baleen whales;
- loss (or possibly primitive absence) of the disc in monotremes, some marsupials, and some edentates (anteaters and sloths);
- variations in the orientation of the joint cavity from sagittal (many rodents) to transverse (many carnivores);
- reversal of the usual convex/concave relationship so that the processus condylaris becomes the female element (many artiodactyl ungulates such as sheep and cattle).

In addition, the relative size of the joint is exceedingly variable. Sheep, rabbit and monkey have been used as TMJ disc defect models in many studies [16—23]. Monkey model is barely used in recent years, considering the high cost, difficult surgical operation and ethical approval. Rabbit is an excellent option for TMJ disc anterior dislocation studies but the small size of TMJ increases the difficulty for surgical approach and disc manipulation. The authors agree with others studies considering sheep is a valid option for TMJ studies due to TMJ size, processus condylaris and fossa mandibularis shape, disc size, morphology and attachments [12]. However, a deep biochemical and biomechanical characterization of the sheep TMJ is lacking in the available literature. Hence, the aim of the present study was to examine the morphological, histological and biomechanical properties of TMJ discs extracted from sheep (*Ovis aries*). It was hypothesized that these discs would present high similarity with available data on human TMJ.

Materials and methods

The material used for this study was obtained from sheep slaughtered for meat consumption. A total of 15 heads from *Black Merino* female sheep, 40 to 50 kg, were used: 6 for morphological characterization, 4 for histological characterization and 5 for biomechanical testing. One of the major requirements for this study was to use fresh TMJ discs; for that reason a team of certified surgeons was available 5 days weekly to collect fresh TMJ up to a maximum of 5 hours after death.

Regarding the animal ethical considerations, the present study design was approved by the Portuguese National Authority for Animal Health.

Morphological characterization

For morphological characterization 12 fresh TMJ discs were collected from six sheep heads. A surgical discectomy was performed exposing and identifying TMJ anatomical structures. All muscular attachments were removed to obtain clean TMJ discs. Discs were submersed for 5 minutes in a ColorBond solution, an extremely fast-curing infiltrant, designed to rapidly strengthen 3D-printed parts. This submersion was essential to maintain the correct morphology for the 3D scanning. A white light 3D scanning system (Steinbichler — COMET 5®) and the appropriate software were used to replicate the discs in a 3D virtual model. Once the discs removed, two of the skulls were boiled in water (120 °C) for 2 h to allow the procurement of complete clean crania.

Histological characterization

Four sheep heads were used to conduct the histological investigation. The TMJ were removed using a necropsy bone oscillatory saw according to the following anatomic references: cranial — cranial aspect of processus coronoideus in the section of the arcus zygomaticus; caudal — external to the meatus acusticus. The dorsal reference was established to the squamous temporal bone. The ventral reference was 2 cm ventral to the meatus acusticus in the zone of angulus stylohyoideus.

The joints were fixed in 10% buffered formalin for ten days. Decalcification was obtained by immersion in 10% formic acid for three weeks, after which the articulations were cut sagittally and transversally through the whole processus condylaris. After intensive washing the fragments were submitted to routine tissue processing with paraffin embedding. Four-micron sections were stained with hematoxylin and eosin (H&E) and with Orcein to show elastic fibers in the disc. Digital images were obtained with an Olympus DP21 camera.

Biomechanical testing

Five sheep heads were used for biomechanical studies. TMJ discs were removed and immersed in a saline solution for transport up to the bioengineering facilities (1 hour maximum). All muscular attachments and ligaments were removed to obtain a clean fibrocartilaginous disc. Ten clean discs were obtained but one was excluded due to surgical damaging. Consequently, 9 discs were randomized in 3 groups and tested in different mechanical tests: Tensile modulus (E), tensile strength and elongation were tested in: anteroposterior tests (APT) and mediolateral tests (MDT).

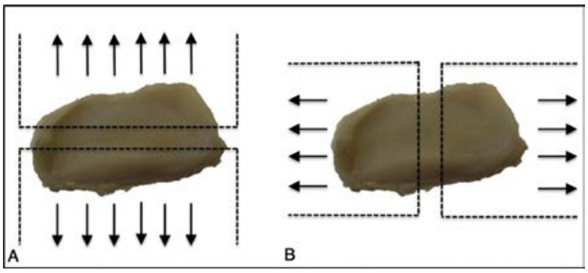


Figure 1 Direction of loading on: (A) anteroposterior and (B) mediolateral tensile tests. The dotted line represents the limit used to fix temporomandibular joint (TMJ) discs in grips. Tension tests used a strain rate of 0.5 mm/min with an initial distance between grips (L0) of 2 mm. All tests were conducted on a Zwick Z100 strength-testing machine (Zwick GmbH & Co. kg, Germany) equipped with a 10 kN load cell. For the compression tests the same rate was applied.
Direction de la charge sur les tests en : (A) antéropostérieur et (B) en traction médiolatéral. La ligne pointillée représente la limite utilisée pour fixer les disques de l'articulation temporomandibulaire (ATM) dans les mors. Les essais de traction ont utilisé une vitesse de déformation de 0,5 mm/min avec une distance initiale entre les mors (L0) de 2 mm. Tous les tests ont été effectués sur un appareil Zwick Z100 (Zwick GmbH & Co. KG, Allemagne) équipé d'une cellule 10 kN de charge. Pour les tests de compression, les mêmes paramètres ont été appliqués.

Compression tests (CT) were performed using a stress-strain tests. In case of anteroposterior tensile test, during loading, the TMJ discs were stretched in the direction represented on Fig. 1A, while in mediolateral tensile test the direction of stretching was as shown on Fig. 1B.

Results

Morphological characterization

In the sheep heads studied, the TMJ was located, as expected, in the posterior segment of the side of the face, cranioventral to the external meatus acusticus, being a diarthrodial, bicondylar joint that allows normal opening and closing of the mandible. It comprised the superior articulating face, the fossa mandibularis of temporal bone, and the processus condylaris, as the inferior articulating surface (Figs. 2 and 6). A protruding processus coronoideus was noted (Fig. 2).

The superior articulating surface (fossa mandibularis) was located in the inferior zone of temporal bone, lateral of foramen ovale and anterior to the external meatus acusticus. The fossa mandibularis was anteroposterior larger than mediolateral with a convexity downwards. The inferior articulating surface (Fig. 3) is represented by the processus condylaris, with ellipsoidal shape with the longer axis in the mediolateral position, the mean measures being 23.47 mm long ($\sigma = 0.87$) and 8.32 mm wide ($\sigma = 1.54$). The processus

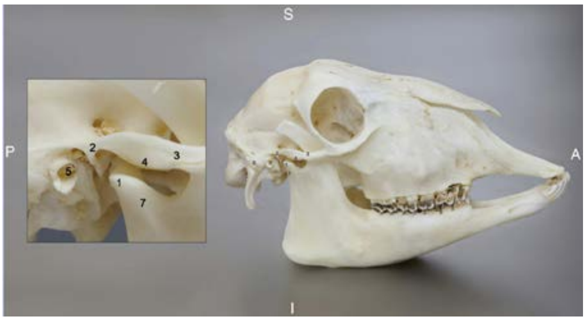


Figure 2 Right view of a sheep skull used in the present study. (1) Processus condylaris, (2) fossa mandibularis, (3) arcus zygomaticus, (4) eminentia articularis, (5) external meatus acusticus, (6) processus mastoideus, (7) collum mandibulae. P: posterior; A: anterior; S: superior; I: inferior.
Vue droite du crâne de mouton utilisé dans la présente étude. (1) Processus condylaris, (2) fossa mandibularis, (3) arcus zygomaticus, (4) eminentia articularis, (5) méat externe acoustique, (6) processus mastoïdien, (7) collum mandibulae. P : postérieur ; A : antérieur ; S : supérieure ; I : inférieure.

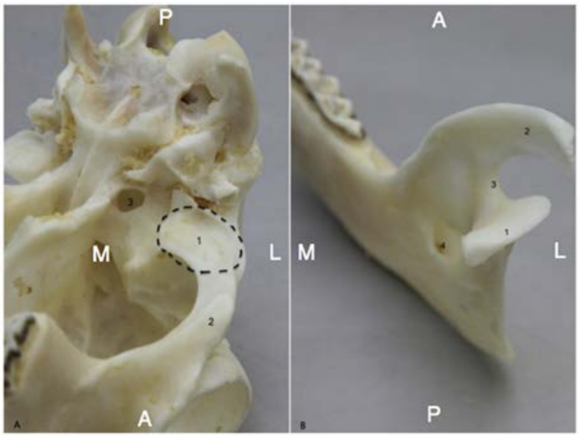


Figure 3 Articular surfaces of the temporomandibular joint (TMJ). A. Superior articular surface: (1) fossa mandibularis, (2) arcus zygomaticus, (3) foramen ovale. B. Inferior articular surface: (1) processus condylaris, (2) processus coronoideus, (3) incisura mandibulae, (4) foramen mandibulae. P: posterior; A: anterior; M: medial; L: lateral.
Surfaces articulaires de l'articulation temporomandibulaire. A. Surface articulaire supérieure : (1) fossa mandibularis, (2) arcus zygomaticus, (3) foramen ovale. B. Surface articulaire inférieure : (1) processus condylaris, (2) processus coronoideus, (3) incisura mandibulae, (4) foramen mandibulaire. P : postérieur ; A : antérieur ; M : médial ; L : latéral.

condylaris was mediolateral concave. The fossa mandibularis receives the processus condylaris.

With an easy surgical approach the authors located the fibrocartilaginous joint disc interposed between the fossa mandibularis and the processus condylaris (Fig. 4). This disc separates an upper joint cavity from a lower one. The first was consistently larger than the second. The bony structures were coated with cartilage more evident in the processus condylaris.

In the ewes studied, the joint disc had an elliptical shape, being substantially thinner in the center than at the periphery. TMJ disc regions are commonly classified as anterior band, posterior band, and intermediate zone (Fig. 5). The intermediate zone exhibits differences from its lateral to medial aspects, being often subdivided into lateral, medial and central region. The bands discs are thicker than the intermediate zone.

The mean length and width of the 12 analyzed fresh TMJ discs were 21.23 mm ($\sigma = 1.53$) and 11.49 mm ($\sigma = 0.62$), respectively. Anterior and posterior band thicknesses were 1.05 mm ($\sigma = 0.07$) and 1.27 mm ($\sigma = 0.04$), respectively. Mean central thickness was 0.76 mm ($\sigma = 0.09$).

The same measures obtained from the 3D virtual models were totally similar to the ones registered in the fresh discs. An important report and consistent with all TMJ was the presence of viscous fluid in upper and lower compartment. This fluid was not analyzed.

Histological characterization

The histological study of the sheep TMJ revealed that the articular disc was attached anteriorly and posteriorly to

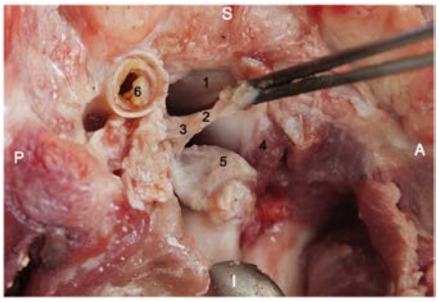


Figure 4 View of the right temporomandibular joint (TMJ). To improve visualization the authors pulled down the processus condylaris. (1) Cartilage surface of fossa mandibularis in the upper joint compartment, (2) temporomandibular joint disc, (3) retrodiscal tissue, (4) muscle pterygoideus lateralis (5) cartilage surface of the processus condylaris (6) external meatus acusticus. P: posterior; A: anterior; M: medial; L: lateral.
Vue de l'articulation temporomandibulaire (ATM) droite. Pour améliorer la visualisation, les auteurs ont tiré sur le processus condylaris. (1) Surface du cartilage de la fossa mandibularis dans le compartiment supérieur de l'articulation (2), disque de l'articulation temporomandibulaire, (3) tissu rétrodiscal, (4) muscle pterygoideus lateralis (5) surface de cartilage du processus condylaris, (6) orifice du conduit auditif externe. P : postérieur ; A : antérieur ; M : médial ; L : latéral.

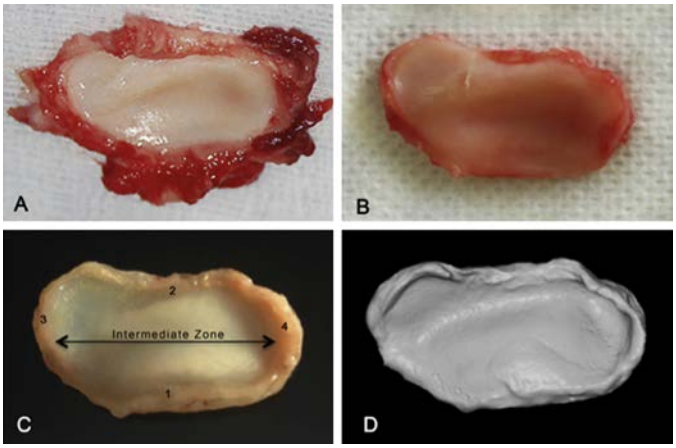


Figure 5 Temporomandibular joint (TMJ) disc. A. Fresh disc with attachments. B. Fresh disc without attachments. C. TMJ disc submitted to ColorBond treatment: (1) anterior band, (2) posterior band, (3) medial band, (4) lateral band. D. TMJ disc 3D virtual model.
Disque de l'articulation temporomandibulaire (ATM). A. Disque frais avec ses ligaments. B. Disque frais sans ligament. C. ATM soumise au protocole ColorBond : (1) bande antérieure, (2) bande postérieure, (3) bande médiane, (4) bande latérale. D. Disque ATM : modèle virtuel 3D.

Table 1 Length, width and thickness of the 9 discs tested.
Longueur, largeur et épaisseur des 9 disques testés.

Sample	Dimensions (mm)					Test
	Length	Width	Thickness			
			Posterior band	Central	Anterior band	
APT1	22.71	11.06	1.23	0.55	1.14	Anteroposterior
APT2	23.89	10.69	1.12	0.77	0.98	
APT3	20.43	11.29	1.23	0.79	1.12	
MDT1	19.60	12.63	1.24	0.82	0.94	Mediolateral
MDT2	20.57	10.56	1.36	0.72	1.02	
MDT3	20.05	11.39	1.26	0.85	1.10	
CT1	20.75	10.07	1.12	0.81	1.02	Compression
CT2	20.49	11.93	1.25	0.79	0.97	
CT3	19.94	10.44	1.29	0.80	1.03	

APT: anteroposterior tests; MDT: mediolateral tests; CT: compression tests.

the articular capsule composed by fibrous tissue. Both the fossa mandibularis and the processus condylaris surfaces were covered by a fibrocartilaginous layer. However, the fibrocartilaginous layer covering the processus condylaris was considerably thicker than the layer covering the fossa mandibularis (Fig. 6).

The central thin part of the disc consisted of scattered fibroblasts and densely packed, thick collagen fiber bundles arranged mainly in an anteroposterior direction. The collagen fibers were not straight but showed evidence of a wavy outline. The anterior and posterior disc portions were in turn occupied by collagen fiber bundles with diverse orientations (Fig. 7). In some areas, these two portions showed chondrocyte-like cells residing in lacunae distributed among less compact collagen fibers (Fig. 7). Each lacuna was surrounded by minimal amount of amorphous matrix. The posterior band blended, in the retrodiscal space, with loose connective tissue with profuse blood and nerve supply. A few small caliber blood vessels, surrounded by loose connective tissue, were observed in all parts of the disc

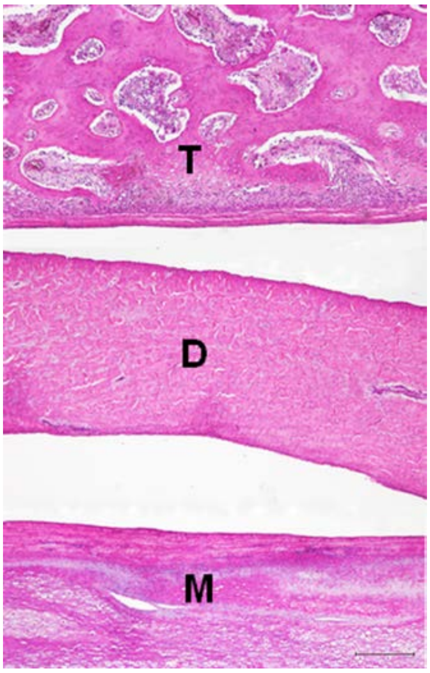


Figure 6 Microscopic overview of a sagittal section of the temporomandibular joint (TMJ) stained with haematoxylin-eosin. T: temporal bone; D: central region of the intermediate area of the joint disc; M: processus condylaris (bar = 10 μm).
Aspect microscopique d'une coupe sagittale d'articulation temporomandibulaire (ATM) colorée par l'hématoxyline-éosine. T : os temporal ; D : région centrale de la zone intermédiaire du disque commun ; M : processus condylaris (la barre = 10 μm).

(Fig. 7). Also occasional unilocular adipocytes were present at both the anterior and posterior attachments of the disc.

Orcein-positive elastic fibers were found throughout the disc, being apparently more abundant in the thinnest central portion. In this disc area, elastic fibers were arranged mostly in parallel to the collagen bundles (Fig. 8). Instead, in the anterior and posterior disc portions, elastic fibers showed a reticular distribution among collagen fibers and chondrocyte-like cells (Fig. 8).

Biomechanical characterization

In Table 1, the measures of the discs used in the mechanical tests are presented.

Tensile tests performed revealed that TMJ discs presented different behaviors for anteroposterior and mediolateral directions (Fig. 9).

The obtained results demonstrated that the tensile modulus of mediolateral tensile tests is higher than anteroposterior tensile tests, as well as the tensile strength and elongation at break (Figs. 10 and 11).

In Table 2 the results obtained for the tested discs for tensile modulus, tensile strength and elongation at break are summarized.

Mechanical testing under compression was performed to evaluate the macro-mechanical performances of the TMJ discs. Fig. 12 demonstrates the compressive stress-strain curves of the tested discs.

The TMJ discs presented a compressive modulus (E) of 446.41 ± 5.16 MPa and their maximum stress value (σmax) was 18.87 ± 1.33 MPa.

Discussion

TMJ disc is a specialized fibrocartilaginous tissue, located between the processus condylaris and the fossa mandibularis [2,8,24] as shown in our sheep morphologic characterization. In humans TMJ disc has an elliptical perimeter, thinner in the center than on periphery. Disc periphery acts like a ring structure supporting the central zone. The same was observed in sheep disc morphology. The functions of the TMJ disc are:

- to improve the fit between bony surfaces;
- to provide stability during mandibular movements;
- to distribute masticatory forces [25].

This capacity is due to the high concentration of collagen fibers. This ring structure around the disc is an important structural aspect to support disc connections. The connection area is rich in elastic fibers, which is essential to disc mobility in the joint. As it was shown in the morphological characterization of the sheep TMJ, this anatomical structure revealed several similar characteristics with the TMJ in humans, including the mediolateral diameter being longer than the anteroposterior, the long axis of the processus condylaris directing backwards, and larger anterior condylar slope. One of the main differences is the concave form of the mediolateral processus condylaris that is convex in humans. The processus condylaris forms a small anteroposterior and mediolateral depression to fit exactly in the fossa mandibularis, unlike the human processus condylaris, which is rounded anteroposterior and mediolateral. The fossa mandibularis is anteroposterior larger than mediolateral with a convexity downwards contrarily to the fossa mandibularis in humans that is concave upwards. The fossa mandibularis allows for the free mediolateral movement of the processus condylaris for rumination. The articular tubercle, a special feature in humans, is rudimentary in the sheep, since the path of the processus condylaris movement is mediolateral, contrarily to the one in humans, which is mostly anteroposterior. Comparatively, the fossa and processus condylaris of the sheep are much like edentulous human TMJ, much flatter. Architecturally, the processus condylaris in both species also has a thin external cortex that surrounds the medullary bone that is made up of trabecular bone. There is also a thin layer of fibrocartilage covering the condylar surface and entire fossa mandibularis,

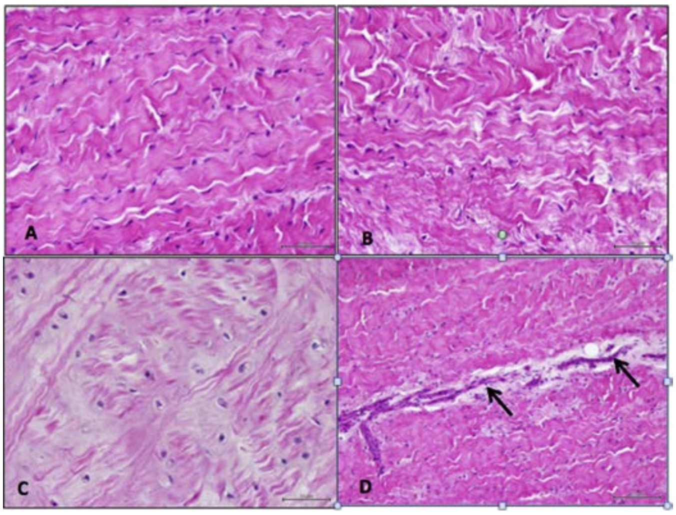


Figure 7 Photomicrographs of various regions of the sheep temporomandibular joint (TMJ) disc stained with haematoxylin-eosin. A. Tightly packed collagen fibers with parallel arrangement interspersed by fibroblasts in the central portion of the TMJ disc (bar = 50 μm). B. Haphazardly arranged collagen fiber bundles in the posterior band of the TMJ disc (bar = 50 μm). C. Chondrocyte-like cells in the anterior band of the TMJ disc (×200, bar = 50 μm). D. Small caliber blood vessels (arrows) in the TMJ disc (×100, bar = 100 μm).

Microphotographies de différentes régions du disque articulation temporomandibulaire (ATM) de mouton ; coloration à l'hématoxyline-éosine. A. Fibres de collagène denses avec un agencement parallèle intercalées par des fibroblastes dans la partie centrale du disque de l'ATM (×200, la barre = 50 μm). B. Agencement au hasard des faisceaux de fibres de collagène dans la bande postérieure du disque de l'ATM (la barre = 50 μm). C. Cellules chondrocytes-like dans la bande antérieure du disque de ATM (×200, la barre = 50 μm). D. Les petits vaisseaux sanguins de calibre (flèches) dans le disque ATM (la barre = 100 μm).

indicating parts of the temporomandibular joint that are subject to highest loading. TMJ relation with the external acusticus meatus, foramen ovale and the joint disc position interposing processus condylaris and fossa mandibularis are similar to human TMJ anatomy. TMJ disc morphology is very similar to human TMJ disc. The choice of sheep as an animal model for TMJ studies has been used for several years [16—23]. TMJ disc implants can be an efficacious

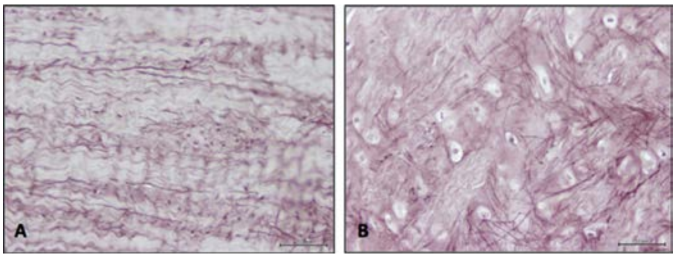


Figure 8 Photomicrographs of the central zone (A) and (B) anterior band of sheep temporomandibular joint (TMJ) disc stained with orcein for detection of elastic fibers (bar = 50 μm). A. Longitudinal elastic fibers follow the wavy structure of collagen bundles. B. Loose mesh elastic fibers distributed between chondrocyte-like cells.

Microphotographies de la zone centrale (A) et (B) antérieure du disque d'une articulation temporomandibulaire (ATM) de mouton colorées par l'orcéine pour la détection des fibres élastiques (la barre = 50 μm). A. Les fibres élastiques longitudinales suivent la disposition ondulée des faisceaux de collagène. B. Réseau lâche de fibres élastiques en vrac réparties entre les cellules chondroïdes.

Table 2 Mechanical tensile properties of TMJ discs.
Propriétés mécaniques en traction des disques d'ATM.

Tensile test	Tensile modulus E (MPa)	Tensile strength (MPa)	Elongation at break (%)
Anteroposterior	3.97 ± 0.73	4.34 ± 1.22	170.92 ± 47.87
Mediolateral	9.39 ± 1.67	13.21 ± 0.85	195.23 ± 20.44

TMJ: temporomandibular joint. Tensile modulus (E), tensile strength and elongation at break are reported as mean value ± standard deviation.

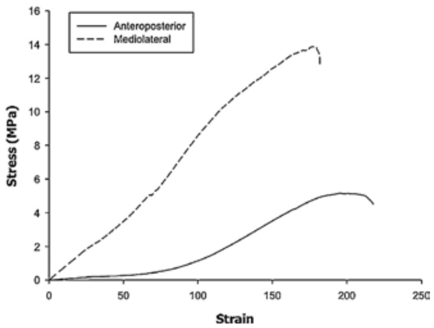


Figure 9 Tensile mechanical performance of temporomandibular joint (TMJ) discs in anteroposterior and mediolateral directions.
Résistances mécaniques à la traction des disques de l'articulation temporomandibulaire (ATM) dans les directions antéropostérieures et médiolatérales.

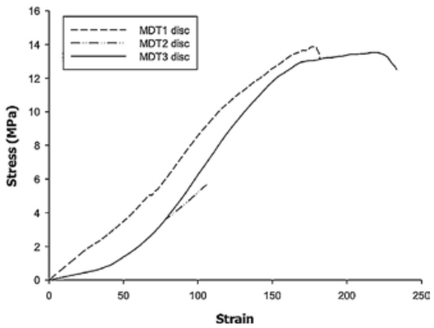


Figure 10 Mediolateral tensile mechanical performance of temporomandibular joint (TMJ) discs. Tension tests used a strain rate of 0.5 mm/min with an initial distance between grips (L0) of 2 mm. All tests were conducted on a Zwick Z100 strength-testing machine equipped with a 10 kN load cell.
Résistance mécanique médiolatérale des disques de l'articulation temporomandibulaire (ATM). Les essais en traction ont utilisé une vitesse de déformation de 0,5 mm/min avec une distance initiale entre les mors (L0) de 2 mm. Tous les tests ont été effectués sur un Zwick Z100 équipé d'une cellule de charge 10 kN.

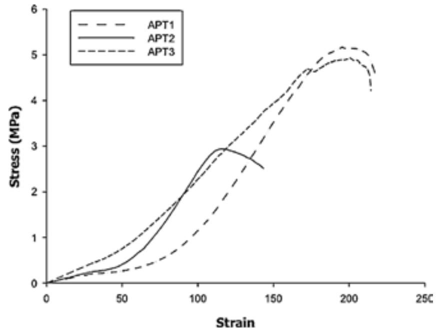


Figure 11 Anteroposterior tensile mechanical performance of temporomandibular joint (TMJ) discs. Tension tests used a strain rate of 0.5 mm/min with an initial distance between grips (L0) of 2 mm. All tests were conducted on a Zwick Z100 strength-testing machine equipped with a 10 kN load cell.
Résistance mécanique antéropostérieure en traction des disques articulation temporomandibulaire (ATM). Les essais en traction ont utilisé une vitesse de déformation de 0,5 mm/min avec une distance initiale entre les mors (L0) de 2 mm. Tous les tests ont été effectués sur un Zwick Z100 résistance dans les essais (Zwick GmbH & Co. KG, Allemagne) équipé d'une cellule de charge 10 kN.

complement in bioengineered joint reconstruction and animal models may offer the possibility to conduct informative preclinical studies. One of the most important problems to create an effective TII is to replicate the biomechanics characteristics of the native disc. Therefore, information on the biomechanical properties of the substitute material is indispensable for further investigation in TMJ disc tissue engineering. During mandibular movements the TMJ disc is subject to a multitude of different loading regimens. TMJ disc behaves as a viscoelastic structure acting as a stress absorber and a stress distributor [24,27]. Elastic fibers play an important role providing the disc with the necessary viscoelastic structure. During every type of loading, the disc undergoes a deformation, while internal forces are produced within the tissue [24]. The internal forces are quantified by the amount of stress, which is defined as force per unit area in Pa (1 Pa = 1 N/m2). There are only two studies available on bovine TMJ disc in which tensile and compressive modulus have been compared using the same experimental protocol and material [28,29]. In these studies tensile modulus ranged between 22 and 26 MPa, and compressive

joint ankylosis: an animal study using sheep. J Oral Maxillofac Surg 2007;65:198—204.
[22] Matsuura H, Miyamoto H, Ishimaru J, Kurita K, Goss AN. Effect of partial immobilization on reconstruction of ankylosis of the temporomandibular joint with an autogenous costochondral graft: an experimental study in sheep. Br J Oral Maxillofac Surg 2001;39:196—203.
[23] Ogi N, Kurita K, Ishimaru JI, Goss AN. Short-term effect of the use of a frozen-stored disc allograft for repair of the osteoarthritic sheep temporomandibular joint: a preliminary report. J Oral Maxillofac Surg 1999;57:139—44.
[24] Tanaka E, van Eijden T. Biomechanical behavior of the temporomandibular joint disc. Crit Rev Oral Biol Med 2003;14:138—50.
[25] Chin LP, Aker FD, Zarrinnia K. The viscoelastic properties of the human temporomandibular joint disc. J Oral Maxillofac Surg 1996;54:315—8.
[27] Beek M, Aarnts MP, Koolstra JH, Feilzer AJ, van Eijden TM. Dynamic properties of the human temporomandibular joint disc. J Dent Res 2001;80:876—80.
[28] Tanaka E, van Eijden TM, Van Eijden T, Biology CD. Biomechanical behavior of the temporomandibular joint disc. Crit Rev Oral Biol Med 2003;14:138—50.
[29] Del Pozo R, Tanaka E, Tanaka M, Okazaki M, Tanne K. The regional difference of viscoelastic property of bovine temporomandibular joint disc in compressive stress-relaxation. Med Eng Phys 2002;24:165—71.
[30] Shengyi T, Xu Y. Biomechanical properties and collagen fiber orientation of TMJ discs in dogs: part 1. Gross anatomy and collagen fiber orientation of the discs. J Craniomandib Disord 1991;5:28—34.
[31] Teng S, Xu Y, Cheng M, Li Y. Biomechanical properties and collagen fiber orientation of temporomandibular joint discs in dogs: 2. Tensile mechanical properties of the discs. J Craniomandib Disord 1991;5:107—14.
[32] Beatty MW, Bruno MJ, Iwasaki LR, Nickel JC. Strain rate dependent orthotropic properties of pristine and impulsively loaded porcine temporomandibular joint disk. J Biomed Mater Res 2001;57:25—34.

Novel approach for 3D virtual model of TMJ disc morphology. Preliminary results

D. F. Angelo, P. Morouco, N. Alves, T. Carvalho, D. Bonaparte, B. Carrapicio, F. Monje, I. A. Furtado, F. Salvado

Centro Hospitalar Lisboa Norte, Centre for Rapid and Sustainable Product Development, Faculdade de Medicina Veterinária, IMM, Portugal

david.serrano.angelo.phd@gmail.com

Introduction: Regenerative medicine is an immense field with extreme and challenging obstacles. The first challenge is to understand the morphological, histological, biochemical and biomechanical characteristics of the structure to reproduced. This multi task and multidisciplinary approach is essential to determine the success of regenerative medicine. The authors present a new method to reproduce 3D morphology of anatomical structures with sheep TMJ disc as an example.
Objective: The main objective of the authors was to reproduce a 3D virtual model of six sheep TMJ disc.
Methods: A medical surgeon performed surgical discectomy of TMJ disc in fresh sheep cadaver with microscope. The second step was related to remove all disc muscular attachments to obtain a clean cartilage disc. The disc was submersed in a solution to maintain the correct morphology. With a white light 3D scanning system and appropriate software we reproduce the morphology of six shep TMJ disc.
Conclusion: 3D virtual model of TMJ disc were successfully reproduced using a white light 3D scanning system. This technique have economic and time related advantages. For precision and detailed results we need to conduct more studies.

The use of platysma for orbital reanimation

Iona Underwood, Matthew Carder, Paul Rea

Laboratory of Human Anatomy; School of Life Sciences, College of Medical, Veterinary and Life Sciences; University of Glasgow, UK

1102202u@student.gla.ac.uk

Lagophthalmos is the result of the paralysis of orbicularis oculi in facial palsy, which can lead to ulceration of the eye and blindness. Unlike static procedures, muscle transfer surgery allows the restoration of involuntary blink reflexes of the orbicularis oris. As the platysma is like the orbicularis oculi in thickness, it is hypothesised the platysma would be an ideal candidate for muscle transfer surgery. The aim was to determine the neurovasculature of the platysma for reanimation of the orbit. Results were consistent between one platysma from cadaver A (A) and one platysma from cadaver B (B). The numbers of arterial branches identified in each platysma were as follows: facial artery A-7, B-2; submental artery A-3, B-1; and occipital artery A-1, B-1. The venous drainage branches identified were: anterior jugular vein A-1, B-2; external jugular vein A-2, B-1; and facial vein A-2, B-1. All nerves were identified as branches of the facial nerve: A-7, B-7. From the dissection it was found the posterosuperior lateral portion of platysma was more vascular rich; containing branches from the facial artery and facial vein, as well as the facial nerve. This portion of the muscle therefore has potential for use as a muscle transfer flap in surgery, as the rich neurovascular allows adequate rewiring for dynamic restoration. In particular, the muscle transfer flaps would allow the application of the trouser graft procedure, for reanimation of the whole orbit.

Anatomic variation in the pterygopalatine angle of the maxillary sinus—a cone beam CT study

Mugurel Constantin Rusu, Cătălina Craiu, Sorin Hostiuc, Mihai Săndulescu, Andreea Derjac-Aramă

"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

Ioana-anatomon@gmail.com

A retrospective cone beam CT study was performed in 50 patients to evaluate the possibilities of variation in the anatomical situs of the orbital process of the palatine bone (OPPB). In that situs were found posterior ethmoid air cells, such as Sieur's and Onodi's cells, the maxillary recess of the sphenoidal sinus (MRSS) and the sphenoidal recess of the maxillary sinus (SRMS). The middle and, respectively, superior nasal meatus were also projecting lateral recesses in the postero-supero-medial angle of the maxillary sinus, these being previously unknown variants. In a single case the atrophic left maxillary bone was bicameral, with an anterior hypoplastic maxillary sinus and the posterior chamber being a huge downshifted posterior ethmoid air cell. We determined which of these variant pneumatizations produced a maxillary bulla (MB) within the maxillary sinus. The most frequently occurring pneumatizations were Sieur's cells (58 % on the right side, 64 % on the left) and MRSSs (20 % on the right side, 22 % on the left), and these were those producing, but not

Protocol

Bioengineered Temporomandibular Joint Disk Implants: Study Protocol for a Two-Phase Exploratory Randomized Preclinical Pilot Trial in 18 Black Merino Sheep (TEMPOJIMS)

David Faustino Ângelo[1,2,3], MD; Florencio Gil Monje[4], MD, PhD; Raúl González-García[4], MD, PhD; Christopher B Little[5], MVM, PhD; Lisete Mónico[6], PhD; Mário Pinho[7], MVM, PhD; Fábio Abade Santos[7]; Belmira Carrapiço[8], MVM; Sandra Cavaco Gonçalves[9], MVM; Pedro Morouço[2], PhD; Nuno Alves[2], MEng, PhD; Carla Moura[2], MEng (Biomed Eng), PhD; Yadong Wang[10], MEng (Biomed Eng), PhD; Eric Jeffries[10], MEng (Biomed Eng), PhD; Jin Gao[10], MEng (Biomed Eng), PhD; Rita Sousa[3], MD; Lia Lucas Neto[3], MD, PhD; Daniel Caldeira[3], MD; Francisco Salvado[3], MD, PhD

[1]Centro Hospitalar de Setúbal, EPE, Stomatology Department, Setúbal, Portugal
[2]Instituto Politécnico de Leiria, Centre for Rapid and Sustainable Product Development (CDRSP), Leiria, Portugal
[3]Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal
[4]University Hospital Infanta Cristina, Badajoz, Spain, Department of Oral and Maxillofacial Surgery, Badajoz, Spain
[5]Kolling Institute, Institute of Bone and Joint Research, University of Sydney, Sydney, Australia
[6]Coimbra University, Coimbra, Portugal
[7]Faculdade de Medicina Veterinaria, Interdisciplinary Centre of Research in Animal Health (CIISA), Lisbon University, Lisboa, Portugal
[8]Faculdade de Medicina Veterinaria, Lisbon University, Lisboa, Portugal
[9]Instituto Nacional de Investigação Agrária e Veterinária (INIAV I.P.), Santarém, Portugal
[10]University of Pittsburgh, Chemical Engineering and Surgery Pittsburgh, University of Pittsburgh, Pittsburgh, PA, United States

Corresponding Author:
David Faustino Ângelo, MD
Centro Hospitalar de Setúbal, EPE
Stomatology Department
Rua Camilo Castelo Branco
Setúbal, 2919-446
Portugal
Phone: 351 265 549 00
Fax: 351 265 238 066
Email: david.serrano.angelo@gmail.com

Abstract

Background: Preclinical trials are essential to test efficacious options to substitute the temporomandibular joint (TMJ) disk. The contemporary absence of an ideal treatment for patients with severe TMJ disorders can be related to difficulties concerning the appropriate study design to conduct preclinical trials in the TMJ field. These difficulties can be associated with the use of heterogeneous animal models, the use of the contralateral TMJ as control, the absence of rigorous randomized controlled preclinical trials with blinded outcomes assessors, and difficulties involving multidisciplinary teams.

Objective: This study aims to develop a new, reproducible, and effective study design for preclinical research in the TMJ domain, obtaining rigorous data related to (1) identify the impact of bilateral discectomy in black Merino sheep, (2) identify the impact of bilateral discopexy in black Merino sheep, and (3) identify the impact of three different bioengineering TMJ discs in black Merino sheep.

Methods: A two-phase exploratory randomized controlled preclinical trial with blinded outcomes is proposed. In the first phase, nine sheep are randomized into three different surgical bilateral procedures: bilateral discectomy, bilateral discopexy, and sham surgery. In the second phase, nine sheep are randomized to bilaterally test three different TMJ bioengineering disk implants. The primary outcome is the histological gradation of TMJ. Secondary outcomes are imaging changes, absolute masticatory time, ruminant time per cycle, ruminant kinetics, ruminant area, and sheep weight.

Results: Previous preclinical studies in this field have used the contralateral unoperated side as a control, different animal models ranging from mice to a canine model, with nonrandomized, nonblinded and uncontrolled study designs and limited outcomes measures. The main goal of this exploratory preclinical protocol is to set a new standard for future preclinical trials in oromaxillofacial surgery, particularly in the TMJ field, by proposing a rigorous design in black Merino sheep. The authors also intend to test the feasibility of pilot outcomes. The authors expect to increase the quality of further studies in this field and to progress in future treatment options for patients undergoing surgery for TMJ disk replacement.

Conclusions: The study has commenced, but it is too early to provide results or conclusions.

(JMIR Res Protoc 2017;6(3):e37) doi:10.2196/resprot.6779

KEYWORDS

temporomandibular joint disorders (TMD); temporomandibular joint bioengineered disk implants; temporomandibular randomized preclinical trial protocol

Introduction

The temporomandibular joint (TMJ) is the most frequently used joint in the human body. The TMJ opens and closes 1500 to 2000 times daily and is essential for everyday functions of the mouth, such as mastication, speech, deglutition, yawning, and snoring, involving special mandatory synergy of both articular sides [1]. The TMJ disk is an essential component in the normal TMJ and has the following functions: (1) it distributes the intra-articular load, (2) it stabilizes the joints during translation, and (3) it decreases the wear of the articular surface [2,3]. The majority of TMJ disorders (TMD) are successfully treated with reversible, conservative, and low-tech treatments such as education and counseling, therapeutic exercises, splint therapy, and pharmacotherapy [4,5].

When the TMJ disk is displaced, malformed, or damaged, it can induce serious internal pathologic processes and/or osteoarthritis [6,7]. Currently, patients suffering from severe TMD have limited validated treatment options. Most surgical approaches, such as TMJ discectomy, do not restore the structural or biological properties of the articulation and disk. This procedure may not be ideal because the TMJ is left without an important functional structure. A variety of interpositional materials have been used to replace the removed disks, including synthetic materials manufactured from silicone, Teflon, polytetrafluoroethylene, and biological interpositional grafts taken from different anatomic sites [8-11]. These interpositional materials do not take in consideration the anatomy and biochemical and biomechanical characteristics of the TMJ native disk [12], and some of them have been associated with serious complications for the patients [8,13,14]. In the late 1980s, Proplast/Teflon TMJ (synthetic interpositional implant) were found to be harmful in many patients. The breakdown of the material, probably caused by TMJ high biomechanical forces, lead to fragmented particles that resulted in an immune foreign body response that caused problems ranging from severe cutaneous inflammatory reaction in the preauricular and cheek areas [15] to severe degenerative joint disease with perforation into the middle cranial fossa [16,17]. The result was a dramatic clinical spectrum of failures for these implants [10]. In December 1991, the US Food and Drug Administration's Bulletin recommended immediate removal of all previous TMJ Proplast/Teflon implants because of the mechanical failures, many resulting in progressive bone degeneration [18]. In a 1992 workshop, the American Academy of Oral and Maxillofacial Surgery instructed the discontinuation of Proplast/Teflon [18].

The absence of efficacious options to substitute the TMJ disk can be related to difficulties in the translation of animal evidence to the clinical practice in humans. These limitations are likely related to:

1. the use of heterogeneous animal models with conflicting results, possibly due to variable anatomy and intra-articular loading between species [19,20];

2. the use of the contralateral TMJ as control, which may be associated with contralateral overloading [21];

3. the biomaterials used to replace the disk do not account for the morphologic and biomechanical characteristics of the native disk;

4. absence of randomized controlled trials with blinding of outcomes' assessors; and

5. lack of multidisciplinary teams involved in the project.

Preclinical research should promote the effective translation of knowledge into practice. The previously mentioned aspects can limit the effective translation of quality scientific knowledge into clinical practice and these may present potential issues to patients, clinicians, and scientific progress.

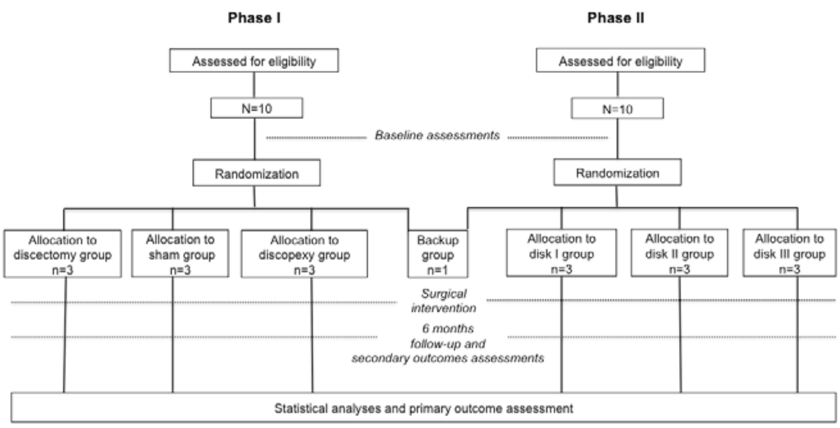
The contemporary absence of successful options to substitute the TMJ disk is still a major issue for public health. Little has changed in the past decade regarding study designs for TMJ investigation, and the treatment for patients with severe TMD remains controversial. The main objective of the Temporomandibular Joint Interposal Material Study (TEMPOJIMS) is to develop a new, reproducible, and effective study design for preclinical research in the TMJ field. The second goal is to progress in bioengineering and regenerative medicine evaluating the benefits of a TMJ bioengineering implant to substitute the damaged native TMJ disk. This preclinical exploratory study is divided into two phases. Phase 1 of this study is a blinded randomized preclinical trial, designed to investigate if the TMJ undergoes important injury in bilateral discectomy, bilateral discopexy, and sham surgery. Phase 2 intentions are to evaluate the safety and efficacy of three different TMJ bioengineering implants using the same rigorous method of phase 1.

Methods

Study Design

The TEMPOJIMS is a two-phase exploratory randomized controlled preclinical trial planned to gather preliminary information to (1) evaluate a new study design for TMJ investigation; (2) evaluate the black Merino sheep animal model for TMJ investigation; (3) evaluate TMJ behavior under bilateral surgical intervention (discectomy and discopexy) using a histologic primary outcome (microscopic scoring of destructive changes in TMJ using a modified Mankin scoring system [22]), secondary imaging outcome (imaging scoring of TMJ); (4) testing the applicability of pilot secondary outcomes predominantly for ruminant kinetics; and (5) obtain a baseline for interpretation of TMJ disk bioengineering implants results. Phase II is aimed to test safety and efficacy of three different bilateral TMJ bioengineering disk implants (Figure 1). Outcome evaluators and analysts are blinded for surgical assessments.

Figure 1. Study design.



Major institutions involved in this study are (1) Lisbon Faculty of Medicine for study design, coordination, and statistical analysis; (2) Interdisciplinary Centre of Research in Animal Health in Faculty of Veterinary Medicine for histological preparation and veterinary support of all animals; (3) Centre for Rapid and Sustainable Product Development for bioengineered disk implants (disks I and II); (4) Bioengineering, Surgery, Chemical Engineering, Mechanical Engineering and Materials Science, University of Pittsburgh, for bioengineered disk implants (disk III); (5) Department of Oral and Maxillofacial-Head and Neck Surgery, University Hospital Infanta Cristina, Badajoz, Spain, for surgical support; (6) Institute of Bone and Joint Research-Northern Sydney Local Health District-Sydney Medical School Northern, University of Sydney, Australia, for histological analysis; and (7) Radiology Department of Santa Maria Hospital, Lisbon, Portugal, for imaging analysis.

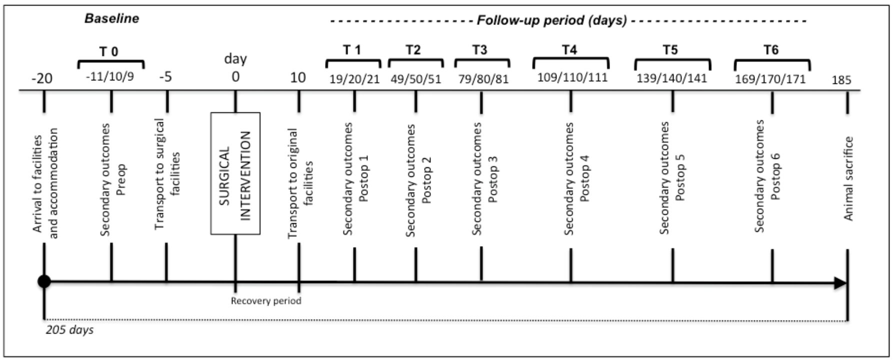
Animal Model

A variety of strains/breeds of sheep have been used in TMJ investigations. To decrease biological variability, the authors recommended black Merino sheep as the animal model to conduct the study [20]. As recommended, the authors proposed to use “sheep skeletally mature” at ≥2 years of age [23]. The inclusion criteria are certified black Merino sheep, adult (age 2-5 years), female, and in good health condition (veterinary check-up is performed on all animals). Regarding the animal ethical considerations, the study design was approved by the Portuguese National Authority for Animal Health registered with number 026618. The study design and organization respect the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

Baseline and Follow-Up Evaluation

The baseline and follow-up evaluations are outlined at particular time points (Figure 2). Pilot secondary outcomes and weight are measured at days 11, 10, and 9 before surgery (details on secondary outcomes are reported in outcomes measures). Transportation to surgical facilities is performed 5 days before surgery to avoid animal stress and allow familiarization to the temporary facilities. Head computerized tomography (CT) scan is performed on the day of surgery taking advantage of preanesthesia sedation. Ten days after surgery, animals are transported to TEMPOJIMS main facilities. Days 19, 20, and 21 after surgery, the follow-up secondary outcomes start to be recorded every 30 days for 6 months (Figure 2). At the end, animals are sacrificed and a new CT scan is performed to measure the imaging outcome and to begin the histologic preparation.

Figure 2. Study flowchart.



Randomization, Allocation, and Blinding

The randomization is performed by a statistical group not involved in the outcome assessments, managed by Lisbon Faculty of Medicine. Allocation to each randomized group is performed preoperatively by sealed envelope and separately for phase 1 and phase 2 of the study. The surgical team is not blinded to treatment allocation given the type of intervention; however, surgical team members are not involved in outcome assessments. All outcome evaluators are blinded to intervention. In phase 1, 10 sheep are allocated to the intervention group: sham surgery group (n=3), discectomy group (n=3), discopexy group (n=3), and backup group (n=1). The backup sheep is planned to be used if death occurs due to anesthesia or another complication not related to the surgical intervention. In phase 2, 10 sheep are randomly assigned to disk I group (n=3), disk II group (n=3), disk III group (n=3), and backup group (n=1) (Figure 1).

Intervention Phase

Anesthesia Protocol

Fasting and water restriction are required 24 hours before surgery. Sedation is performed with diazepam (0.5 mg/kg iv), followed by anesthesia induction with ketamine (5 mg/kg iv). Oral intubation is performed and anesthesia is maintained with isoflurane (1.5% to 2%). To assure animal analgesia, meloxicam (0.5 mg/kg iv, bid) is administered on surgery day and during 4 days postoperatively. Antibiotic prophylaxis with amoxicillin and clavulanic acid are used for 5 days.

Surgical Intervention Protocol for Phases 1 and 2

Phase 1

Bilateral discectomy (n=3): under general anesthesia, the surgical team perform a preauricular skin incision and a blunt dissection of the soft tissue covering the joint. The joint area is disclosed and the articular capsule is incised. The disk and its attachments are identified. The medial, anterior, posterior, and lateral disk attachments are detached and discectomy is performed. The wound is closed in layers.

Bilateral discopexy (n=3): under general anesthesia, the surgical team perform a preauricular skin incision and a blunt dissection of the soft tissue covering the joint. The joint area is disclosed and the articular capsule is incised. The disk and its attachments are identified. The lateral and posterior disk attachments are detached and sutured with poly- *p*-dioxanone (PDS) 3/0. The wound is closed in layers.

Sham surgery (n=3): under general anesthesia, the surgical team will perform a preauricular skin incision and a blunt dissection of the soft tissue covering the joint. The capsule is not incised. The wound is closed in layers.

Phase 2

Disk I (n=3): under general anesthesia, the surgical team perform a preauricular skin incision and a blunt dissection of the soft tissue covering the joint. The joint area is disclosed and the articular capsule is incised. The disk and its attachments are identified. The medial, anterior, posterior, and lateral disk attachments are detached and discectomy is performed. The disk I is introduced into the articular space and sutured in the lateral attachments. The wound is closed in layers. Disk I will be an alternative biomaterial and for intellectual reasons cannot be revealed in this paper.

Disk II (n=3): under general anesthesia, the surgical team perform a preauricular skin incision and a blunt dissection of the soft tissue covering the joint. The joint area is disclosed and the articular capsule is incised. The disk and its attachments are identified. The medial, anterior, posterior, and lateral disk attachments are detached and discectomy is performed. The disk II is introduced into the articular space and sutured in the lateral attachments. The wound is closed in layers. Disk II will be a porous poly(glycerol sebacate) (PGS) scaffold reinforced with polycaprolactone (PCL).

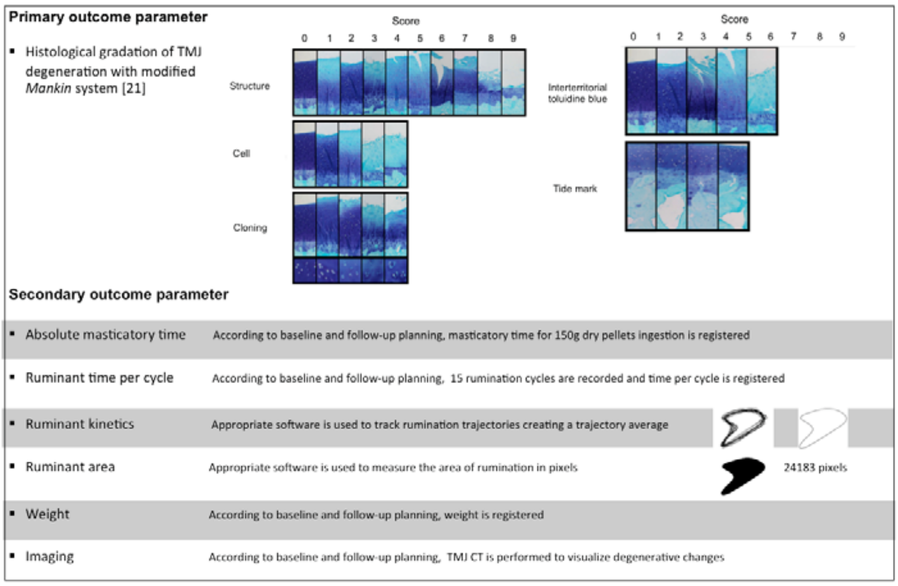
Disk III (n=3): under general anesthesia, the surgical team perform a preauricular skin incision and a blunt dissection of the soft tissue covering the joint. The joint area is disclosed and the articular capsule is incised. The disk and its attachments are identified. The medial, anterior, posterior, and lateral disk attachments are detached and discectomy is performed. The disk III is introduced into the articular space and sutured in the lateral attachment. The wound is closed in layers. Disk III will be a porous PGS scaffold prepared by a modified salt fusion method. Briefly, ground salt particles (150 mg) with a size range of 25 to 32 µm will be placed into a 3-D printed mold. The mold will be transferred to an incubator at 37°C and 90% relative humidity for 1 hour. The fused templates of salt particles will dry in a vacuum oven at 90°C and 100 millitorr (mTorr) overnight, removing salt cake carefully from the mold before further processing. Fresh-made PGS dissolved in tetrahydrofuran (THF; 20 wt%, 380 µL, salt:PGS=2:1) added to the salt cake, and the THF is allowed to evaporate completely in a fume hood for 30 minutes. The salt cake is transferred to a vacuum oven and cured at 150°C and 100 mTorr for 24 hours. The resultant PGS-impregnated salt templates are soaked in deionized water for 4 hours, and then replaced with water for 4 hours, with water exchange every 4 hours during the first 12 hours. After the 12-hour water bath, scaffolds are transferred to deionized water for another 24 hours with water exchange every 8 hours. The resultant scaffolds are frozen down at –80°C and then the lyophilization process is applied.

Ten days for recovery is contemplated for wound care and postoperative medication (see Figure 2).

Outcome Measures

The primary outcome is the microscopic scoring of destructive changes in the TMJ using a modified Mankin scoring system [22]. Secondary outcomes are imaging scoring of TMJ destructive changes, absolute masticatory time, ruminant time per cycle, ruminant kinetics, ruminant area, and sheep body weight. Primary and secondary outcome parameters are outlined in more detail in Figure 3.

Figure 3. Primary and secondary outcome parameters.



Primary Outcome

The goal is to evaluate histologic gradation of TMJ destructive changes. The time point is 6 months following surgical intervention.

Six months after surgery, the TMJ is removed using a necropsy bone oscillatory saw according to the following anatomic references: cranial (cranial aspect of coronoid process in the union region of the zygomatic process), caudal (external to acoustic meatus), dorsal (reference is established to the squamous temporal bone), and ventral (reference is fixed 2 cm below the acoustic meatus in the zone of stylohyoid angle). The joints are fixed in 10% buffered formalin for 24 hours and stored in 70% ethanol. Decalcification is obtained by immersion in 10% formic acid in 5% formalin for up to 20 days, after which the articulations are cut sagittally through the whole condyle. After decalcifying, TMJ articulations are immersed in three graded methyl salicylate/paraffin mixtures and cut sagittally through the lateral into the central part of the TMJ. Histological sections are sent to Sydney Institute of Bone and Joint Research for histological scoring using a modified Mankin scoring system [22]. This assessment is performed and classified independent by two histologists who will be blinded to intervention. A third histologist will act as arbiter in case of disparity.

Secondary Outcomes

The features evaluated are imaging analysis, absolute masticatory time, ruminant time per cycle, ruminant kinematics, ruminant area, and sheep weight (see Multimedia Appendices 1 and 2). Time point is every month following surgical intervention for a total of 6 months.

To measure secondary outcomes, a specific cage (see Figure 4) was built with a frontal window and a feeder.

Imaging analysis: preoperative CT is performed on all sheep. After animal sacrifice, TMJ blocks are scanned by CT and imaging evaluation is performed using the criteria and score described in Table 1.

Table 1. TEMPOJIMS imaging evaluation criteria.

Items	Criteria	0 (no change)	1 (mild change)	2 (moderate change)	3 (severe change)
Shape	Change of joint form	May include reformed joint	Small changes; this change may include ≤2 osteophytes	Moderate changes; multiple osteophytes	Severe changes and outgrowth; marginal proliferation
Condyle erosion	Concavity in cortical	This stage includes normal joint with no signs of condyle erosion	Erosion in one-third of joint surface	Erosion in two-thirds of joint surface	Erosion over all joint surface
Temporal erosion	Concavity in cortical	This stage includes normal joint with no signs of temporal erosion	Erosion in one-third of joint surface	Erosion in two-thirds of joint surface	Erosion over all joint surface
Condyle sclerosis	Cortical thickening of condyle	This stage includes normal joint with no signs of condyle sclerosis	Sclerosis in one-third of joint surface	Sclerosis in two-thirds of joint surface	Sclerosis over all joint surface
Temporal sclerosis	Cortical thickening of temporal fossa	This stage includes normal joint with no signs of temporal sclerosis	Sclerosis in one-third of joint surface	Sclerosis in two-thirds of joint surface	Sclerosis over all joint surface
Condyle marrow	Change of underlying trabecular bone	This stage includes normal joint with no change of condyle trabecular bone	Sclerosis in less than half of trabecular bone	Sclerosis in half of trabecular bone	Sclerosis in all trabecular bone
Temporal marrow	Change of underlying trabecular bone	This stage includes normal joint with no change of temporal trabecular bone	Sclerosis in less than half of trabecular bone	Sclerosis in half of trabecular bone	Sclerosis in all trabecular bone
Calcification	Development of calcification across joint space	No calcification across joint space	Calcification in one-third of joint surface	Calcification in two-thirds of joint surface	Bony fusion across joint space
Global appreciation		Normal joint	In general, mild changes	In general, moderate changes	In general, severe changes

This assessment is performed and classified independently by two experienced radiologists who will be blinded to intervention. A third radiologist will act as arbiter in case of disparity.

Absolute masticatory time: respecting the flowchart (Figure 2), at 9:00 am the animals are placed in individual cages. A dose of 150 grams of dry pellets (Rico Gado A3) are introduced in the feeder and the time until they eat all the pellets is measured with a chronometer (see Multimedia Appendix 1).

Ruminant time per cycle: respecting the timetable (Figure 2), we record 15 ruminatory cycles approximately 4 hours after 150 gram feeding. We use a Canon 7D video camera and images with 25 frames per second. Then, the number of frames per cycle are divided by 25 to obtain time in seconds per cycle (see Multimedia Appendix 2).

Ruminant kinetics: we use the software Foundry Nuke (2D tracking) to perform the ruminatory tracking and to obtain the ruminatory cycle average. With the software After Effects , we convert the 2-D tracking into a geometric form (see Multimedia Appendix 2).

Ruminant area: we determine the average of 15 cycles and create a geometric form. Using the software Image J , we perform a quantitative measure in pixels of the ruminant area average.

Weight: according to the timetable, after eating 150 grams of dry pellets the sheep are weighed (see Multimedia Appendix 1).

All assessments are performed by researchers who are blinded to surgical intervention.

Figure 4. TEMPOJIMS main facilities.

Statistical Analyses

All statistical analyses will be performed using the SPSS version 22 (IBM Corp, Armonk, NY, USA). A cross-sectional analysis will be performed to compare the outcome variables in the three levels of the independent variable before and after the randomized treatment group assignment. In the cross-sectional analyses, one-way analysis of variance (ANOVA) will be performed, after testing all the assumptions. For longitudinal analysis, one-way ANOVA with repeated measures will be performed taking as within-subjects effects observations after surgery (months 1 to 6). Fisher least significant difference will be performed as post hoc tests to check for significant differences for the different treatments.

Reporting of Adverse Events

Adverse events related to the study will be considered, including (1) anesthesia events: idiopathic death, pneumothorax, other complications related to anesthesia; (2) surgical technique: massive bleeding, condylar fracture, other complications related to surgical technique; and (3) postoperative events: TMJ infection, suture dehiscence, decreased appetite, facial paresis, decreased rumination, decreased weight.

Discussion

This study investigates the effects and adverse effects of (1) bilateral discectomy, (2) bilateral discopexy, and (3) bioengineered disk implants. Although this preclinical study will primarily serve as a pilot study, we expect to gain a better understanding of the morphologic and histologic changes in TMJ and implications in masticatory kinetics.

So far, results on discectomy are conflicting. Previous preclinical studies in this field [24-33] have used the contralateral unoperated side as a control and different animal models ranging from mice to a canine model. Using the contralateral side as a control can be inappropriate considering contralateral overload influence. Theoretically, we expect to reduce this bias using a bilateral approach. Animal variability in the different studies is a warning about the importance of using the same animal model in further studies regarding TMJ implant investigations. Therefore, our group performed a previous study considering black Merino sheep as a promisor animal model for studies regarding TMJ disk implants investigation, TMJ prosthesis, and TMJ osteoarthritis model. To increase the quality of TEMPOJIMS the authors will use a sham surgery control group.

We expect to obtain valuable information related to the phase 1 discopexy group regarding if the surgical approach promotes intra-articular damage. This can improve future conclusions about attributing possible damage to the intervention itself instead of the TMJ implant. This question is important considering that a surgical approach to place TMJ implants in phase 2 will be required. Again, using a bilateral intervention could reduce a possible bias.

Most preclinical studies have focused on gross morphological/histological assessments and were not designed to characterize the fundamental altered joint movement (kinetics) or functional consequences. In this study, we include pilot secondary outcomes to evaluate changes in ruminant kinetics. We expect to correlate the primary with the secondary outcomes to understand if they can be used in future TMJ studies. It may be interesting to understand several items:

1. Are there differences regarding masticatory time in the disk groups versus discectomy and discopexy?

2. Is there a correlation between histologic and imaging and kinetics results?

3. Does the ruminant area and geometry change when performing different interventions?

4. Is there a difference regarding ruminant kinetics in the disk groups versus discectomy and discopexy?

5. Do TMJ implants accelerate osteoarthritis?

Concerning phase 2, the choice of biomaterial is critical. The TMJ implant will be exposed in a mechanical, stressful environment with a limited blood supply that can limit cell migration and in situ regeneration. Testing three different bioengineering discs in vivo and correlating in vitro with in vivo behavior can seriously improve bioengineering strategies to achieve a safe and efficacious TMJ disk implant for humans.

The main strength of this study is the animal model proposed; the conventional and pilot outcomes described; the study design with a randomized, blinded, and placebo control group; and the use of bilateral surgical procedures. Potential limitations of the study include the relatively small sample size. If this study confirms the feasibility of the proposed protocol and initial efficacy of the TMJ disk implants planned, a larger preclinical trial would be warranted to further determine the effectiveness of these discs and promote translation of animal evidence to clinical practice in humans.

Trial Status

At the time of submission, the surgical interventions of phase 1 were ongoing at Faculdade de Medicina Veterinária de Lisboa and TEMPOJIMS facilities in Portugal.

Acknowledgments

This preclinical trial is supported by Faculdade de Medicina Veterinária da Universidade de Lisboa, Instituto Politécnico de Leiria (Centre for Rapid and Sustainable Product Development), Centro Hospitalar de Setúbal, Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa. The authors are grateful to Joaquim Ferreira from Lisbon Faculty of Medicine for study design; to Susan Smith from Institute of Bone and Joint Research-Northern Sydney Local Health District-Sydney Medical School Northern, Australia, for histological analysis; to Pedro Nunes from Radiology Department of Centro Hospitalar Lisboa Norte; to Miguel Virgílio for kinematics video recording; and to Joaquim Ângelo and Ermelinda Ângelo for animal logistics control. This study was granted by Portuguese Grunenthal Foundation and by Secção Regional Oeste da Ordem dos Médicos. This publication was supported by the Portuguese Foundation for Science and Technology (FCT) through the following projects: UID/Multi/04044/2013 and PTDC/EMS-SIS/7032/2014.

Authors' Contributions

The contributors, with input from the other investigators, conceived this study protocol. JF, RF, NG, AT, NG, and DA developed the protocol and study materials with input from all investigators. NG, AT, and DA participated in the randomization process. LM will conduct the statistical analyses. FM, RG, and SF will participate in the surgical interventions. CB and SC are the coordinators of the veterinary staff and responsible for the animal anesthesia and animal welfare. DC participated in organization support and was study advisor. PM, NA, and MC are dedicated to disk implants 1 and 2. WY, JE, and GJ are dedicated to disk implant 3. SR will coordinate the imaging evaluation. MP and FB are responsible for processing the histologic samples and preparing sections. LC group will coordinate histologic scoring system. All authors read and approved the final manuscript.

Conflicts of Interest

None declared.

Multimedia Appendix 1

Outcomes assessments in TEMPOJIMS main facilities, absolute masticatory time and weight.

[MP4 File (MP4 Video), 230MB - resprot_v6i3e37_app1.mp4]

Multimedia Appendix 2

Outcomes assessments in TEMPOJIMS main facilities. After recording 15 ruminant cycles with a Canon 7D Video Camera we used the software Foundry Nuke (2D tracking) to make the ruminant tracking to obtain the ruminant cycle average in each time period.

[MP4 File (MP4 Video), 4MB - resprot_v6i3e37_app2.mp4]

References

1. Bae Y, Park Y. The effect of relaxation exercises for the masticator muscles on temporomandibular joint dysfunction (TMD). J Phys Ther Sci 2013;25(5):583-586. [doi: 10.1589/jpts.25.583]
2. Allen K, Athanasiou K. Tissue engineering of the TMJ disc: a review. Tissue Eng 2006;12(5):1183-1196. [doi: 10.1089/ten.2006.12.1183]
3. Tanaka E, Sasaki A, Tahmina K, Yamaguchi K, Mori Y, Tanne K. Mechanical properties of human articular disk and its influence on TMJ loading studied with the finite element method. J Oral Rehabil 2001 Mar;28(3):273-279. [Medline: 11394374]
4. Martins-Júnior RL, Palma Antônio JC, Marquardt EJ, Gondin TM, Kerber FD. Temporomandibular disorders: a report of 124 patients. J Contemp Dent Pract 2010 Oct 14;11(5):71-78. [Medline: 20978727]
5. Navrátil L, Navratil V, Hajkova S, Hlinakova P, Dostalova T, Vranová J. Comprehensive treatment of temporomandibular joint disorders. Cranio 2014 Jan;32(1):24-30. [doi: 10.1179/0886963413Z.0000000002] [Medline: 24660643]
6. Mehra P, Wolford LM. The Mitek mini anchor for TMJ disc repositioning: surgical technique and results. Int J Oral Maxillofac Surg 2001 Dec;30(6):497-503. [doi: 10.1054/ijom.2001.0163] [Medline: 11829231]
7. Al-Baghdadi M, Durham J, Araujo-Soares V, Robalino S, Errington L, Steele J. TMJ disc displacement without reduction management: a systematic review. J Dent Res 2014 Jul;93(7 Suppl):37S-51S [FREE Full text] [doi: 10.1177/0022034514528333] [Medline: 24659775]
8. Estabrooks L, Fairbanks C, Collett R, Miller L. A retrospective evaluation of 301 TMJ Proplast-Teflon implants. Oral Surg Oral Med Oral Pathol 1990 Sep;70(3):381-386. [Medline: 2216373]
9. Henry CH, Wolford LM. Treatment outcomes for temporomandibular joint reconstruction after Proplast-Teflon implant failure. J Oral Maxillofac Surg 1993 Apr;51(4):352-358; discussion 359. [Medline: 8450350]
10. Mercuri LG, Giobbie-Hurder A. Long-term outcomes after total alloplastic temporomandibular joint reconstruction following exposure to failed materials. J Oral Maxillofac Surg 2004 Sep;62(9):1088-1096. [Medline: 15346359]
11. Morouço P, Ângelo D, Francisco L, Moura C, Alves N. Tissue engineering for temporomandibular joint disc repair and regeneration: a methodological perspective. Adv Cell Mol Otolaryngol 2017 Jan 17;4(1):33709. [doi: 10.3402/acmo.v4.33709]
12. Lypka M, Yamashita DR. Exuberant foreign body giant cell reaction to a teflon/proplast temporomandibular joint implant: report of a case. J Oral Maxillofac Surg 2007 Sep;65(9):1680-1684. [doi: 10.1016/j.joms.2006.09.030] [Medline: 17719383]
13. Spagnoli D, Kent JN. Multicenter evaluation of temporomandibular joint Proplast-Teflon disk implant. Oral Surg Oral Med Oral Pathol 1992 Oct;74(4):411-421. [Medline: 1408011]
14. Fricton JR, Look JO, Schiffman E, Swift J. Long-term study of temporomandibular joint surgery with alloplastic implants compared with nonimplant surgery and nonsurgical rehabilitation for painful temporomandibular joint disc displacement. J Oral Maxillofac Surg 2002 Dec;60(12):1400-1411; discussion 1411. [doi: 10.1053/joms.2002.36091] [Medline: 12465000]
15. Kulber DA, Davos I, Aronowitz JA. Severe cutaneous foreign body giant cell reaction after temporomandibular joint reconstruction with Proplast-Teflon. J Oral Maxillofac Surg 1995 Jun;53(6):719-722; discussion 722. [Medline: 7776062]
16. Chuong R, Piper MA. Cerebrospinal fluid leak associated with proplast implant removal from the temporomandibular joint. Oral Surg Oral Med Oral Pathol 1992 Oct;74(4):422-425. [Medline: 1408012]
17. Berarducci JP, Thompson DA, Scheffer RB. Perforation into middle cranial fossa as a sequel to use of a Proplast-Teflon implant for temporomandibular joint reconstruction. J Oral Maxillofac Surg 1990 May;48(5):496-498. [Medline: 2329399]
18. [No authors listed]. Recommendations for management of patients with temporomandibular joint implants. Temporomandibular Joint Implant Surgery Workshop. J Oral Maxillofac Surg 1993 Oct;51(10):1164-1172. [Medline: 8410459]
19. Herring S. TMJ anatomy and animal models. J Musculoskelet Neuronal Interact 2003 Dec;3(4):391-394; discussion 406 [FREE Full text] [Medline: 15758330]
20. Angelo D, Morouço P, Alves N, Viana T, Santos F, González R. Choosing sheep (Ovis aries) as animal model for temporomandibular joint research: Morphological, histological and biomechanical characterization of the joint disc. Morphologie 2016 Jul:223-233 [FREE Full text] [doi: 10.1016/j.morpho.2016.06.002] [Medline: 27450042]
21. Leiggener CS, Erni S, Gallo LM. Novel approach to the study of jaw kinematics in an alloplastic TMJ reconstruction. Int J Oral Maxillofac Surg 2012 Sep;41(9):1041-1045. [doi: 10.1016/j.ijom.2012.06.014] [Medline: 22819692]
22. Little CB, Smith MM, Cake MA, Read RA, Murphy MJ, Barry FP. The OARSI histopathology initiative - recommendations for histological assessments of osteoarthritis in sheep and goats. Osteoarthritis Cartilage 2010 Oct;18 Suppl 3:S80-S92 [FREE Full text] [doi: 10.1016/j.joca.2010.04.016] [Medline: 20864026]
23. Cake MA, Appleyard RC, Read RA, Smith MM, Murrell GA, Ghosh P. Ovariectomy alters the structural and biomechanical properties of ovine femoro-tibial articular cartilage and increases cartilage iNOS. Osteoarthritis Cartilage 2005 Dec;13(12):1066-1075 [FREE Full text] [doi: 10.1016/j.joca.2005.07.001] [Medline: 16154775]
24. Cohen W, Servais J, Polur I, Li Y, Xu L. Articular cartilage degeneration in the contralateral non-surgical temporomandibular joint in mice with a unilateral partial discectomy. J Oral Pathol Med 2014 Feb;43(2):162-165. [doi: 10.1111/jop.12113] [Medline: 24044578]

25. Ahtiainen K, Mauno J, Ellä V, Hagström J, Lindqvist C, Miettinen S, et al. Autologous adipose stem cells and polylactide discs in the replacement of the rabbit temporomandibular joint disc. J R Soc Interface 2013 Aug 06;10(85):20130287 [FREE Full text] [doi: 10.1098/rsif.2013.0287] [Medline: 23720535]
26. Brown B, Chung W, Almarza A, Pavlick M, Reppas S, Ochs M. Inductive, scaffold-based, regenerative medicine approach to reconstruction of the temporomandibular joint disk. J Oral Maxillofac Surg 2012 Nov;70(11):2656-2668. [doi: 10.1016/j.joms.2011.12.030]
27. Xu L, Polur I, Lim C, Servais J, Dobeck J, Li Y, et al. Early-onset osteoarthritis of mouse temporomandibular joint induced by partial discectomy. Osteoarthritis Cartilage 2009 Jul;17(7):917-922. [doi: 10.1016/j.joca.2009.01.002] [Medline: 19230720]
28. Lai WT, Tsai Y, Su S, Su C, Stockstill JW, Burch JG. Histological analysis of regeneration of temporomandibular joint discs in rabbits by using a reconstituted collagen template. Int J Oral Maxillofac Surg 2005 May;34(3):311-320. [doi: 10.1016/j.ijom.2004.05.003] [Medline: 15741041]
29. Sato S, Goto S, Koeda S, Motegi K. Changes of the elastic fibre network of the rabbit temporomandibular joint following discectomy. J Oral Rehabil 2002 Sep;29(9):847-852. [Medline: 12366539]
30. Sato S, Goto S, Motegi K. Changes of the collagen fibre arrangement of the rabbit temporomandibular joint following discectomy. J Craniomaxillofac Surg 2002 Jun;30(3):178-183. [Medline: 12220997]
31. Bjørnland T, Haanaes HR. Discectomy of the temporomandibular joint: an experimental study in monkeys. J Craniomaxillofac Surg 1999 Apr;27(2):113-116. [Medline: 10342149]
32. Ogi N, Kurita K, Ishimaru JI, Goss AN. Short-term effect of the use of a frozen-stored disc allograft for repair of the osteoarthritic sheep temporomandibular joint: a preliminary report. J Oral Maxillofac Surg 1999 Feb;57(2):139-144; discussion 144. [Medline: 9973121]
33. Hinton R, Stinson J. Effect of postoperative diet on condylar cartilage response to discectomy. J Oral Maxillofac Surg 1997 Nov;55(11):1259-1264. [Medline: 9371117]

Abbreviations

CT: computerized tomography
PCL: polycaprolactone
PDS: poly-p-dioxanone
PGS: poly(glycerol sebacate)
TEMPOJIMS: Temporomandibular Joint Interposal Material Study
THF: tetrahydrofuran
TMD: temporomandibular joint disorders
TMJ: temporomandibular joint

Edited by G Eysenbach; submitted 10.10.16; peer-reviewed by W Cohen, A Almarza, P Boffano; comments to author 08.12.16; accepted 16.12.16; published 02.03.17

Please cite as:
Ângelo DF, Monje FG, González-García R, Little CB, Mónico L, Pinho M, Santos FA, Carrapiço B, Gonçalves SC, Morouço P, Alves N, Moura C, Wang Y, Jeffries E, Gao J, Sousa R, Neto LL, Caldeira D, Salvado F
Bioengineered Temporomandibular Joint Disk Implants: Study Protocol for a Two-Phase Exploratory Randomized Preclinical Pilot Trial in 18 Black Merino Sheep (TEMPOJIMS)
JMIR Res Protoc 2017;6(3):e37
URL: http://www.researchprotocols.org/2017/3/e37/
doi:10.2196/resprot.6779
PMID:28254733

ARTICLE IN PRESS

Journal of Cranio-Maxillo-Facial Surgery xxx (2017) 1–10

Contents lists available at ScienceDirect

Journal of Cranio-Maxillo-Facial Surgery

journal homepage: www.jcmfs.com

Effects of bilateral discectomy and bilateral discopexy on black Merino sheep rumination kinematics: TEMPOJIMS – phase 1 – pilot blinded, randomized preclinical study

David Faustino Ângelo [a, d, *], Florencio Monje Gil [b], Raúl González-García [b], Lisete Mónico [c], Rita Sousa [a, b, c, d, e], Lia Neto [a, b, c, d, e], Inês Caldeira [a, b, c, d, e], Carla Moura [d], Luís Carlos Francisco [d], David Sanz [c], Nuno Alves [d], Francisco Salvado [a], Pedro Morouço [d]

[a] Faculty of Medicina, Lisbon University - Av. Prof. Egas Moniz, 1649-035 Lisboa, Portugal
[b] Infanta Cristina Hospital, Faculty of Medicina, University of Extremadura - Av. Elvas, S/N, 06006 Badajoz, Spain
[c] Coimbra Univesity. Av. Bissaya Barreto, Bloco de Celas 3000-075 Coimbra, Portugal
[d] Centre for Rapid and Sustainable Product Development, Polytechnic Institute of Leiria, Portugal - Rua de Portugal, Zona Industrial Marinha Grande, 2430-028 Marinha Grande, Portugal
[e] Neuroradiology Department of Santa Maria Hospital - Av. Prof Egas Moniz, 1649-035, Lisboa, Portugal

ARTICLE INFO

Article history:
Paper received 13 June 2017
Accepted 20 November 2017
Available online xxx

Keywords:
TMJ
Preclinical trial
Discopexy
Discectomy
Kinematics
Black merino sheep

ABSTRACT

Background: The temporomandibular joint interposal study (TEMPOJIMS) is a rigorous preclinical trial divided in 2 phases. In phase 1 the authors investigated the role of the TMJ disc and in phase 2 the authors evaluated 3 different interposal materials. The present work of TEMPOJIMS - phase 1, investigated the effects of bilateral discectomy and discopexy in sheep mastication and rumination.
Methods: This randomized, blinded and controlled preclinical trial (in line with the ARRIVE guidelines) was conducted in 9 Black Merino sheep to evaluate changes in mastication and rumination after bilateral discectomy and bilateral discopexy, by comparing with a sham surgery control group. The outcomes evaluated were: (1) absolute masticatory time; (2) ruminant time per cycle; (3) ruminant kinematics, and (4) ruminant area. After baseline evaluation and surgical interventions, the outcomes were recorded over 3 successive days, every 30 days, for 6 months.
Results: The first month after intervention seemed to be the critical period for significant kinematic changes in the discectomy and discopexy groups. However, 6 months after the bilateral interventions, no significant changes were noticed when compared with the control group.
Conclusions: In this study, bilateral discectomy and discopexy had no significant effect in mastication and ruminatory movement. The introduction of kinematic evaluation presents a new challenge that may contribute to the improvement of future studies on the TMJ domain.

1. Introduction

The area of temporomandibular joint (TMJ) bioengineering is growing fast, and the potential for developing a TMJ interposal disc is immense. Rigorous preclinical trials are therefore needed for the normal progress of translational medicine. However, before using valuable resources and funds for TMJ bioengineering, it is important to improve our understanding of the effects induced at the temporomandibular joint by common surgical interventions.

TMJ discectomy is the most performed intracapsular surgery. With good overall results, this technique remains a reasonable choice for internal derangement not responding to nonsurgical treatment (Nyberg et al., 2004; Eriksson and Westesson, 2001; Mazzonetto and Spagnoli, 2001; Bjørnland and Larheim, 2003; Trumpy and Lyberg, 1995). Nevertheless, it still is a controversial technique because it does not restore structural or biological properties of the TMJ (Takaku et al., 2000). TMJ discopexy is a less invasive surgery technique used to restore ideal TMJ disc position, but with variable results (Sharma et al., 2010).

* Corresponding author. Lisbon Faculty of Medicine, 1649-028 Lisbon, Portugal. Fax: +351 21 798 5110.
E-mail address: david.serrano.angelo@gmail.com (D.F. Ângelo).

https://doi.org/10.1016/j.jcms.2017.11.022
1010-5182/© 2017 European Association for Cranio-Maxillo-Facial Surgery. Published by Elsevier Ltd. All rights reserved.

Despite the large number of discectomy and discopexy procedures performed annually, to the best of our knowledge there have been no randomized, blinded, controlled trials that have investigated, in human or animal, the jaw movement implications of bilateral discectomy and bilateral discopexy.

Small-size, mid-size, and large animal models have been used to investigate the histological effects of unilateral discectomy (Bjørnland and Haanaes, 1999; Dimitroulis and Slavin, 2006; Ogi et al., 1999; Sato et al., 2002; Tong and Tideman, 2000), leading to diverse results, from minor degenerative changes to TMJ ankylosis. This heterogeneous results are probably due to limitations regarding animal choice, study design, and the use of a unilateral approach with the contralateral side as a control, which may have induced bias in available results (Cohen et al., 2014).

As reported in a survey, commissioned by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) (Kilkenny et al., 2009), only 59% of the 271 randomly chosen articles assessed stated the hypothesis or objective of the study, and the number and characteristics of the animals used (i.e., species/strain, sex, and age/weight). Most of the papers surveyed did not report using randomization (87%) or blinding (86%) to reduce bias in animal selection and outcome assessment. Only 70% of the publications that used statistical methods fully described them and presented the results with a measure of precision or variability (Kilkenny et al., 2009). These findings are a cause for concern, and are consistent with reviews of many research areas, including clinical studies, published in recent years (Kilkenny et al., 2009; Sharma et al., 2010; Van der Worp et al., 2010). Furthermore, most of the previous studies have focused on histological and imaging differences, but additional inputs are essential to obtain a clear understanding of TMJ functionality.

In this paper the authors report, for the first time, on a high-quality, preclinical study that evaluates the impacts of bilateral discectomy and bilateral discopexy on mastication and rumination kinematics in black Merino sheep, in comparison with a sham surgery control group.

The evaluation of mastication and rumination kinematics of the sheep jaw was based on the normal processes used by ruminants to break down particulate dry matter: (1) initial chewing during eating and (2) further chewing during rumination (Pearce, 1967). The authors discriminated between the two processes and analyzed them separately. To analyze the initial chewing the authors examined the time taken to eat a dose of dry pellets, naming this outcome absolute masticatory time. With this outcome the authors expected to determine if TMJ surgical interventions could induce significant changes in the initial chewing time. To analyze the ruminant chewing phase, a special cage was created and 15 ruminant chewing cycles were recorded with a video camera. Using Foundry Nuke (2D tracking) and Image J software, ruminant movements in the frontal plane were analyzed to obtain: (1) rumination time per cycle, (2) rumination kinematics, and (3) rumination area.

The temporomandibular joint interposal material study (TEMPOJIMS) was planned with a rigorous design, in line with the ARRIVE guidelines (Kilkenny et al., 2009). A Randomized, preclinical study with blinded outcomes, was needed in this field in order to increase the quality of further TMJ studies, improve future treatment options for patients undergoing surgery for TMJ disc replacement, and facilitate interpretation of future studies regarding TMJ interposal materials using TEMPOJIMS design.

2. Materials and methods

TEMPOJIMS study was a preclinical study divided into two phases (Ângelo et al., 2017). This paper focuses on the kinematic outcomes of phase 1, aiming to understand the impact of TMJ bilateral discectomy versus TMJ bilateral discopexy, in comparison with a sham surgery control group, on black Merino sheep.

2.1. Study design

The rationale and protocol for the TEMPOJIMS preclinical trial are publicly available (Ângelo et al., 2017).

2.2. Study population and sample

A variety of strains/breeds of sheep have been used in previous TMJ investigations. To reduce biological variability, the authors performed this study in a black Merino sheep strain (Angelo et al., 2016). In phase 1 the authors used 10 black Merino sheep with the following inclusion criteria: certified black Merino sheep, adult (aged between 2 and 5 years), female, good health condition (veterinary checks were performed on all animals), and normal dentition (with 32 teeth—8 mandibular incisors, 12 premolars, and 12 molars).

2.3. Randomization

The randomization process was performed by a statistical team not involved in the outcome assessments. Ten sheep were randomly allocated to intervention groups as follows: bilateral discectomy group ($n = 3$), bilateral discopexy group ($n = 3$), sham surgery group ($n = 3$), and back-up group ($n = 1$). The one back-up sheep was in case of death occurring as a result of anesthesia or other complications not related to surgical intervention. The allocation to each randomized group was performed preoperatively using sealed envelopes (Fig. 1).

2.4. Procedures

Ten eligible sheep were assigned to their baseline pilot secondary outcomes, measured at days 11, 10, and 9 before surgery in TEMPOJIMS facilities (Fig. 2). Transportation to surgical facilities occurred 5 days before surgery to avoid animal stress and allow familiarization with temporary accommodation. The surgical team was not blinded to treatment allocation, given the type of intervention; however, the surgical team members were not involved in outcome assessment. Serious adverse events were defined as events that were fatal or that resulted in life-threatening or persistent disability, more than 10% weight loss per week, or clinically significant hazards/harm to the animal.

2.5. Anesthesia protocol

Fasting and water restriction were required 24 h before surgery. Sedation was performed with diazepam (0.5 mg/kg IV), followed by anesthesia induction with ketamine (5 mg/kg IV). Oral intubation was performed and anesthesia was maintained with isoflurane (1.5–2%). To assure animal analgesia, Meloxicam (0.5 mg/kg IV/bid) was administered on the surgery day and over the following 4 days. Antibiotic prophylaxis with amoxicillin and clavulanic acid was administrated for 5 days.

2.6. Surgical intervention

(A) Bilateral discectomy group ($n = 3$): during general anesthesia, the surgical team performed a preauricular incision and a blunt dissection of the soft tissue covering the joint. The joint area was disclosed and the articular capsule was incised. The disc and its attachments were identified. The medial, anterior, posterior, and lateral disc attachments were

Please cite this article in press as: Ângelo DF, et al., Effects of bilateral discectomy and bilateral discopexy on black Merino sheep rumination kinematics: TEMPOJIMS – phase 1 – pilot blinded, randomized preclinical study, Journal of Cranio-Maxillo-Facial Surgery (2017), https://doi.org/10.1016/j.jcms.2017.11.022

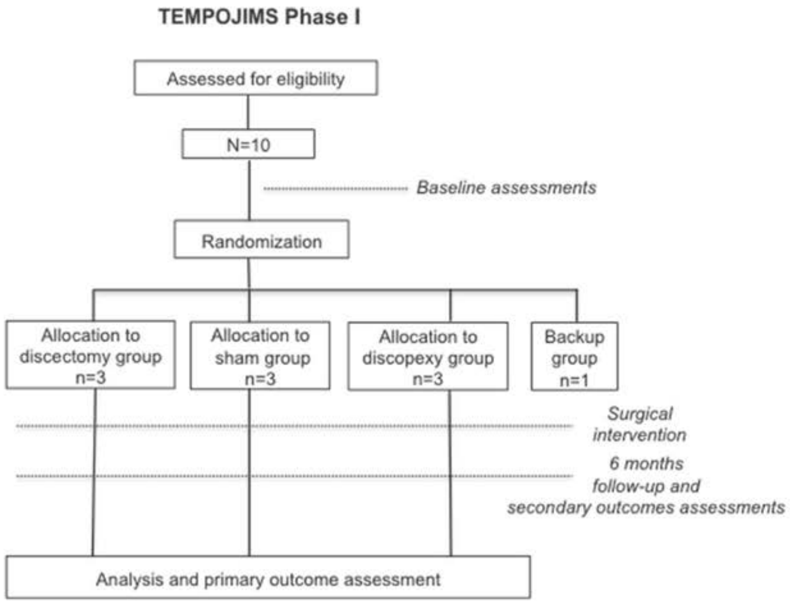


Fig. 1. TEMPOJIMS phase 1 enrolment (Ângelo et al., 2017). Baseline assessments: (1) *absolute masticatory time* a dose of 150 g of dry pellets (Rico Gado A3) was introduced in the feeder and the time taken to eat all the pellets was measured; (2) *rumination time per cycle* (the authors used a Canon 7D video camera to record 15 rumination cycles approximately 4 h after the 150 g feed; (3) *rumination kinematics* and (4) *rumination area* (the authors used Foundry Nuke software (2D tracking) to track the jaw and used After Effects software to convert the 2D tracking into a geometric form).

detached and discectomy was performed. The wound was closed in layers with Vicryl 3/0.

(B) Bilateral discopexy group ($n = 3$): during general anesthesia, the surgical team performed a preauricular incision and a blunt dissection of the soft tissue covering the joint. The joint area was disclosed and the articular capsule was incised. The disc and its attachments were identified. The lateral and posterior disc attachments were detached and sutured with PDS 3/0. The wound was closed in layers with Vicryl 3/0.

(C) Sham surgery group ($n = 3$): during general anesthesia, the surgical team performed a preauricular incision and a blunt dissection of the soft tissue covering the joint. The TMJ articular capsule was not incised. The wound was closed in layers with Vicryl 3/0.

2.7. Follow-up assessments

Baseline assessment (T0) was performed before surgery on days −11, −10, and −9 (Table 1). Ten days after surgery, animals were transported to TEMPOJIMS facilities. Follow-up recording of outcomes began on days 19, 20, and 21 after surgery (T1) and was repeated every 30 days for 6 months (Fig. 2). T0–T6 were based on the means of the three measurements. The assessments were

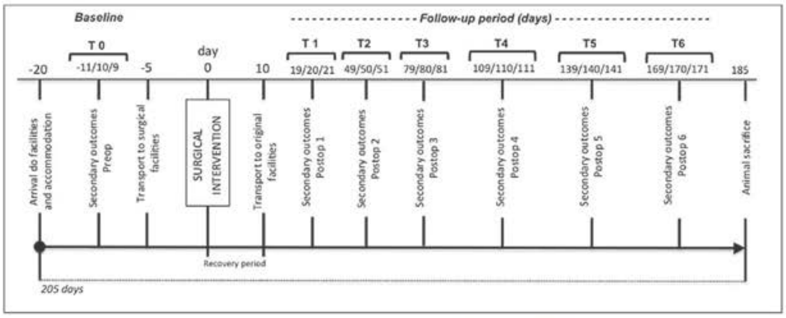


Fig. 2. Flow chart of TEMPOJIMS – phase 1 (Ângelo et al., 2017).

Please cite this article in press as: Ângelo DF, et al., Effects of bilateral discectomy and bilateral discopexy on black Merino sheep rumination kinematics: TEMPOJIMS – phase 1 – pilot blinded, randomized preclinical study, Journal of Cranio-Maxillo-Facial Surgery (2017), https://doi.org/10.1016/j.jcms.2017.11.022

Fig. 3. Ruminant cycle kinematics (A) – initial position (B) – maximum open mouth (C) – maximum lateral movement (D) – end of ruminant cycle.

performed by two specially trained assessors who were not affiliated with the interventions. All animals had bilateral scars to reduce possible bias.

2.8. Kinematic outcomes

Kinematic outcomes evaluated were: (1) absolute masticatory time; (2) ruminatory time per cycle; (3) ruminatory kinematics; and (4) ruminatory area.

To measure the referred outcomes, a specific cage was built with a frontal window and a feeder. All assessments were performed by researchers blinded to surgical intervention, and were designed to evaluate masticatory time changes and to ruminatory kinematics. These outcomes were as follows:

(1) Absolute masticatory time: based on the assessment timetable (Fig. 2), at 9:00 am the 10 sheep were placed in their individual cages. A dose of 150 g of dry pellets (Rico Gado A3) was introduced in the feeder and the time taken to eat all the pellets was measured with a chronometer.
(2) Ruminatory time per cycle: based on the assessment timetable (Fig. 2), we recorded 15 ruminant cycles approximately 4 h after the 150g feed. We used a Canon 7D video camera to record images at 25 frames per second. The number of frames per cycle was then divided by 25 to obtain time in seconds per cycle.
(3) Ruminatory kinematics: we used Foundry Nuke (2D tracking) software to track jaw movements and calculate the average ruminant cycle. Using After Effects software, we converted the 2D tracking into a geometric form.
(4) Ruminatory area: we determined an average for 15 cycles, and created a geometric representation. Using the software Image J, we performed a quantitative measurement, in pixels, of the average ruminatory area.

2.9. Statistical analysis

The TEMPOJIMS phase 1 randomized, controlled, preclinical trial used ten black Merino sheep, with a 6-month follow-up. The primary analysis tested the effects of the independent variable (IV) for three experimental conditions: 1 = bilateral discectomy; 2 = bilateral discopexy; 3 = sham surgery, using a series of pre-tests (T0) and post-tests (T1 to T6). The dependent variables (outcome measures) were: the time to eat 150 g of pellets; the ruminant time per cycle; and the ruminant area. These events were measured three times in the pre-tests to promote invariance in the outcome measures before surgical intervention (IV). The secondary tests (post-tests) analyzed the outcomes using three measurements, at six time intervals (T1 to T6), at the same place, and hour as the pre-tests (Fig. 2).

All statistical analyses were performed using the Statistical Package for Social Sciences (IBM SPSS, version 22.0). Shapiro–Wilk tests were performed for pre-tests (T0) and post-tests (T1 to T6), showing a normal distribution in all groups for all tests ($p > 0.05$), except for T4 and T6 rumination areas for discopexy (Shapiro–Wilk = 0.761 and 0.384; $p < 0.05$).

Please cite this article in press as: Ângelo DF, et al., Effects of bilateral discectomy and bilateral discopexy on black Merino sheep rumination kinematics: TEMPOJIMS – phase 1 – pilot blinded, randomized preclinical study, Journal of Cranio-Maxillo-Facial Surgery (2017), https://doi.org/10.1016/j.jcms.2017.11.022

Table 1
Baseline descriptive statistics.

Sheep ID	Birth date	Baseline Mean of three measures				Allocation randomized process
		Weight/kg	Absolute masticatory time/s[a]	Rumination kinematics and average area/pixels[b]	Rumination time per cycle/s[c]	
8298	11.01.11	56.0	85.0	6449 	0.74	Discopexy
9705	02.04.12	70.3	90.7	5252 	1.21	Discectomy
8264	19.07.10	56.3	79.3	7223 	0.87	Sham
9982	02.09.12	56.0	76.0	6591 	0.94	Discectomy
3969	30.10.09	57.0	89.7	7768 	0.90	Sham
8284	16.02.11	68.0	97.7	6904 	0.93	Discopexy
8267	13.07.10	75.7	71.7	8846 	0.73	Discectomy
9701	07.04.12	63.0	108.7	10354 	1.14	Discopexy
1903	25.12.12	52.0	101.3	6007 	0.74	Sham

❖ No significant differences between sheep for the reported characteristics were found at baseline; $p > 0.10$.
[a] The absolute masticatory time was measured from 9:00 am, when a dose of 150 g of dry pellets (*Rico Gado A3*) was introduced in the feeder, until all pellets were eaten.
[b] Ruminant kinematics refers to the average tracking of 15 ruminant cycles and the creation of a geometric form using the software *Image J*.
[c] A Canon 7D video camera set at 25 frames per second was used to record 15 ruminatory cycles approximately 4 h after the 150 g feed. The number of frames per cycle was divided by 25 to obtain the time in seconds per cycle.

Additionally, Levene statistics were performed to test the homogeneity of variances. Statistically significant results were found at T1, T2, and T5 for rumination area (Levene statistics = 8.59, 6.35, and 7.82; $p < 0.05$), which led to non-parametric tests being calculated for these times. For the pre-tests and other time groups, variances were homogeneous ($p > 0.07$), leading to parametric tests.

A one-way analysis of variance (ANOVA) (or the non-parametric equivalent Kruskal–Wallis test) was performed for cross-sectional analysis to compare the outcome variables at the three levels of the IV before and after the random treatment group assignment. Fisher LSD and Games-Howell Post-hoc tests were performed for equal variances assumed and not assumed, respectively. For longitudinal analysis, Mauchly's test of sphericity was non-significant for absolute masticatory time (Mauchly's $W = 0.004$; $p = 0.589$), allowing a parametric one-way ANOVA test with repeated measures, taking as within-subject effects observations before (T0) and after (T1 to T6) surgery for bilateral discectomy, bilateral discopexy, and sham surgery conditions. For rumination area, a Greenhouse-Geisser corrected test was used, due to a Mauchly's W value of 0.000; $p = 0.011$.

3. Results

Descriptive baseline statistics are presented in Table 1. Four outcomes were analyzed: (1) absolute masticatory time; (2) ruminatory time per cycle; (3) ruminatory kinematics; and (4) ruminatory area.

Please cite this article in press as: Ângelo DF, et al., Effects of bilateral discectomy and bilateral discopexy on black Merino sheep rumination kinematics: TEMPOJIMS – phase 1 – pilot blinded, randomized preclinical study, Journal of Cranio-Maxillo-Facial Surgery (2017), https://doi.org/10.1016/j.jcms.2017.11.022

Table 2
Absolute masticatory time for T0 (baseline) to T1–T6 (post-test): descriptive one-way ANOVA for T0 and T3–T6, and Kruskal–Wallis test for T1 and T2; effect-sizes (η^2_p) and observed power ($1-\beta$).

	T0	T1	T2	T3	T4	T5	T6
	$\bar{x}$±dp	$\bar{x}$±dp	$\bar{x}$±dp	$\bar{x}$±dp	$\bar{x}$±dp	$\bar{x}$±dp	$\bar{x}$±dp
Discectomy	79.4 ± 10.0	102.1 ± 6.5	86.0 ± 17.6	79.3 ± 13.2	85.2 ± 11.9	78.3 ± 7.2	74.7 ± 5.0
Discopexy	97.1 ± 11.8	108.2 ± 5.4	90.7 ± 9.2	95.4 ± 15.7	98.2 ± 18.0	95.0 ± 12.1	92.6 ± 7.6
Sham	90.1 ± 11.0	89.22 ± 5.5	80.3 ± 10.8	84.7 ± 16.2	94.9 ± 13.7	82.6 ± 11.7	85.6 ± 18.2
$F_{(2,5)}$*	1.98			0.89	0.63	2.02	1.76
K–W $X^2(2)$		5.54*	0.80				
η^2_p	0.397	0.74	0.14	0.23	0.17	0.40	0.37
$1-\beta$	0.27	0.80	0.10	0.14	0.11	0.27	0.24

*$p = 0.03$; one-tailed test.

(1) Absolute masticatory time

Cross-sectional analysis: The authors compared the absolute masticatory times for the three groups each month, post-surgery (T1 to T6). A one-way ANOVA (or the non-parametric equivalent Kruskal–Wallis test) was performed, showing significant differences between the three groups only in T1—$p = 0.03$ (one-tailed), effect size of $\eta 2p = 0.736$ $(1-\beta) = 0.804$ (Table 2)—due to the higher values for discopexy in comparison with sham surgery, as shown using a Games-Howell post-hoc test ($p = 0.028$). Throughout the baseline and the remaining follow-up period (T2–T6), no statistically significant differences were found between discectomy, discopexy, and sham surgery conditions ($p > 0.20$).

Longitudinal analysis: A one-way ANOVA with repeated measures was performed, taking as within-subject effects months before (T0) and after surgery (T1 to T6) for discectomy, discopexy, and sham surgery conditions. Significant effects across time were found for discectomy—$F(6, 12) = 5.67$, $p = 0.005$, $\eta 2p = 0.739$ $(1-\beta) = 0.947$—but not for discopexy and sham surgery—$F(6, 12) = 2.65$ and 1.59, $p > 0.07$, $\eta 2p = 0.570$ and 0.443 $(1-\beta) = 0.635$ and 0.403, respectively. Considering the differences in relation to the baseline (Table 3), the within-subject contrasts identified a statistically significant increase only for discectomy between T0 and T1 (effect size of 90%; observed power of 0.60) and between T0 and T4 (effect size of 93%; observed power of 0.74). For discopexy and sham surgery, despite the effect sizes and considering the low observed powers, the differences in relation to the baseline were not statistically significant. Fig. 4 represents absolute masticatory time for the baseline and from T1 to T6.

(2) Rumination time per cycle

Cross-sectional analysis: Rumination time per cycle rate did not vary across groups both in the pre-test (T0) and in all times for the post-test ($p > 0.20$), as shown in Table 4.

Longitudinal analysis: A one-way ANOVA with repeated measures was performed, taking as within-subject effects the baseline and the 6 months after surgery for discectomy, discopexy, and sham surgery (see Table 5). A significant effect across time was found for discopexy and sham surgery—$F(6, 6) = 6.87$ and 4.11, $p < 0.018$, $\eta 2p = 0.773$ and 0.673 $(1-\beta) = 0.977$ and 0.845, respectively—but not for discectomy—$F(6, 6) = 2.70$, $p = 0.126$, $\eta 2p = 0.730$ $(1-\beta) = 0.455$.

The comparison of rumination time per cycle rate between the baseline and months after the surgery identified two differences for discopexy, (T5 vs. T0) with an acceptable power (effect size of 95%). For discectomy and sham surgery no significant differences were found in relation to baseline. Fig. 5 illustrates the rumination time per cycle rate in the baseline, and from T1 to T6. As can be seen, lower scores were obtained for times T1, T2, and T3, suggesting that sheep TMJ recovery started at T4.

(3) Ruminatory kinematics and area

Descriptive results for rumination kinematics and average area of rumination are presented in Fig. 6.

Cross-sectional analysis: Rumination areas only varied across groups in T3 and T4. For T3, the Fisher LSD post-hoc test identified a significant superiority for the discopexy area compared with the discectomy area ($p = 0.008$) (see Table 6).

Longitudinal analysis: A one-way ANOVA with repeated measures, with Greenhouse-Geisser correction, taking as within-subject effects the baseline and the 6 months after surgery (T1 to T6) for discectomy, discopexy, and sham surgery, did not show statistically significant differences for the three conditions ($p > 0.10$). The differences between pre-test and post-test times were also not statistically significant ($p > 0.05$), with low power, since $(1-\beta) < 0.80$, as can be seen in Table 7. Fig. 7 represents rumination area for T0 (pre-test) and T1 to T6 (post-test), for discectomy, discopexy, and sham surgery. The baseline results are similar for the three experimental conditions. After surgery, rumination areas were lower in the discectomy condition, although the differences were not statistically significant.

3.1. Adverse events

No serious adverse events were reported, apart from one sheep in the discectomy group that stopped rumination in T1 and T2, but returned to normal function in T3 to T6.

Table 3
Comparison of absolute masticatory times between baseline and T1–T6: within-subjects contrasts, effect-sizes (η^2_p), and observed power ($1-\beta$).

Comparison with baseline (T0)	Discectomy			Discopexy			Sham surgery		
	$F(1,2)$	η^2_p	$1-\beta$	$F(1,2)$	η^2_p	$1-\beta$	$F(1,2)$	η^2_p	$1-\beta$
T1 vs T0	17.63*	0.90	0.60	5.03	0.72	0.26	0.02	0.01	0.05
T2 vs T0	1.22	0.38	0.10	0.96	0.32	0.09	4.73	0.70	0.25
T3 vs T0	0.00	0.00	0.05	0.06	0.03	0.05	0.40	0.17	0.07
T4 vs T0	26.25*	0.93	0.74	0.03	0.02	0.05	0.56	0.22	0.08
T5 vs T0	0.47	0.19	0.07	0.10	0.05	0.05	1.44	0.42	0.11
T6 vs T0	1.50	0.43	0.12	1.56	0.44	0.12	0.15	0.07	0.06

*$p < 0.05$.

Please cite this article in press as: Ângelo DF, et al., Effects of bilateral discectomy and bilateral discopexy on black Merino sheep rumination kinematics: TEMPOJIMS – phase 1 – pilot blinded, randomized preclinical study, Journal of Cranio-Maxillo-Facial Surgery (2017), https://doi.org/10.1016/j.jcms.2017.11.022

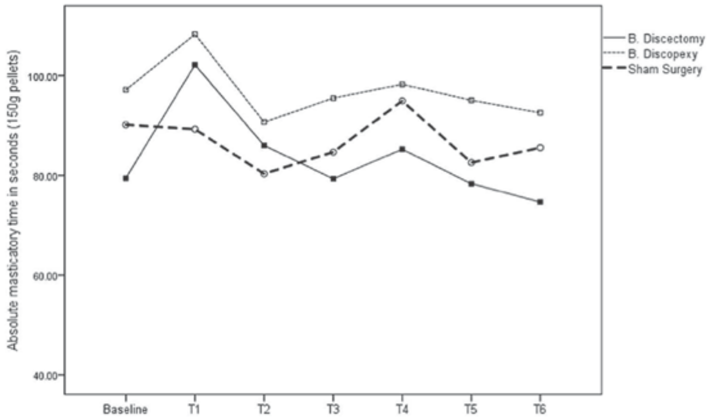


Fig. 4. Absolute masticatory time from T0 (baseline) to T1–T6 (post-test) in discectomy, discopexy, and sham surgery conditions.

Table 4
Rumination time per cycle for T0 to T1–T6: descriptive, one-way ANOVA; effect-sizes (η^2_p), and observed power ($1-\beta$).

	T0	T1	T2	T3	T4	T5	T6
	$\bar{x}$±dp	$\bar{x}$±dp	$\bar{x}$±dp	$\bar{x}$±dp	$\bar{x}$±dp	$\bar{x}$±dp	$\bar{x}$±dp
Discectomy	0.93 ± 0.03	0.79 ± 0.01	0.91 ± 0.06	0.69 ± 0.03	0.89 ± 0.09	0.98 ± 0.20	1.01 ± 0.18
Discopexy	0.69 ± 0.10	0.76 ± 0.11	0.83 ± 0.12	0.62 ± 0.09	0.89 ± 0.05	0.91 ± 0.06	0.98 ± 0.15
Sham	0.80 ± 0.13	0.72 ± 0.12	0.85 ± 0.17	0.66 ± 0.07	0.76 ± 0.11	0.88 ± 0.21	0.84 ± 0.08
$F_{(2,5)}$*	3.02	0.29	0.20	0.67	2.02	0.10	1.92
η^2_p	0.547	0.103	0.075	0.210	0.446	0.037	0.434
$1-\beta$	0.351	0.076	0.068	0.112	0.249	0.059	0.239

*No significant effects; $p > 0.05$.

4. Discussion

The main goal of this study was to analyze the effects of different types of surgery on sheep mastication and rumination. The proposed methodology has proven to be feasible and sensitive to the interventions. Homogenous conditions were obtained in baseline and the animals behaved naturally in front of the camera, guaranteeing the quality of the kinematic assessments (Fig. 3).

The measurement of kinematics was designed to advance understanding of the implications of TMJ surgery on jaw movements. Theoretically, bilateral TMJ surgery may cause jaw movement changes, but these outcomes need to be quantified.

Regarding absolute masticatory time, it was expected that, after bilateral discectomy, the animals would require more time to eat the 150 g of pellets (Ingawale and Goswami, 2009). Accordingly, the discectomy group increased the masticatory time by 28% in T1. This could be related to TMJ pain, leading to a slower food intake. By the end of the study these animals were able to recover to baseline values (74.67 s) (Fig. 4). As mentioned previously, there is a lack of studies evaluating the effects of interventions on functionality of the TMJ. Thus, it was not possible to compare this masticatory outcome measured with other results. Although there were no statistical differences between masticatory time before and after surgery (i.e. T0 vs T1), the difference was noticeable. After T1, the subsequent recover to baseline values suggests that sheep presented the ability to adapt to the induced constraints, highlighting the importance that function has over form (Poveda et al., 2007). Sheep, as with other animals, have the ability to adapt in order to survive, even in the case of TMJ major interventions, where severe dysfunction could lead to disastrous consequences for the animal.

Table 5
Comparison of rumination time per cycle rate between baseline and T1–T6: within-subjects contrasts, effect-sizes (η^2_p), and observed power ($1-\beta$).

Comparison with baseline (T0)	Discectomy			Discopexy			Sham surgery		
	F	η^2_p	$1-\beta$	F	η^2_p	$1-\beta$	F	η^2_p	$1-\beta$
T1 vs T0	2.69	0.57	0.17	17.29*	0.90	0.59	2.63	0.57	0.16
T2 vs T0	1.19	0.37	0.10	4.20	0.68	0.23	1.68	0.46	0.12
T3 vs T0	1225.00***	0.99	1.00	11.26	0.85	0.45	7.69	0.79	0.35
T4 vs T0	0.54	0.21	0.07	7.62	0.79	0.34	0.33	0.14	0.07
T5 vs T0	0.04	0.02	0.05	38.76*	0.95	0.86	2.61	0.57	0.16
T6 vs T0	0.35	0.15	0.07	6.75	0.77	0.32	0.42	0.17	0.07

*$p \leq 0.05$;
***$p < 0.001$.

Please cite this article in press as: Ângelo DF, et al., Effects of bilateral discectomy and bilateral discopexy on black Merino sheep rumination kinematics: TEMPOJIMS – phase 1 – pilot blinded, randomized preclinical study, Journal of Cranio-Maxillo-Facial Surgery (2017), https://doi.org/10.1016/j.jcms.2017.11.022

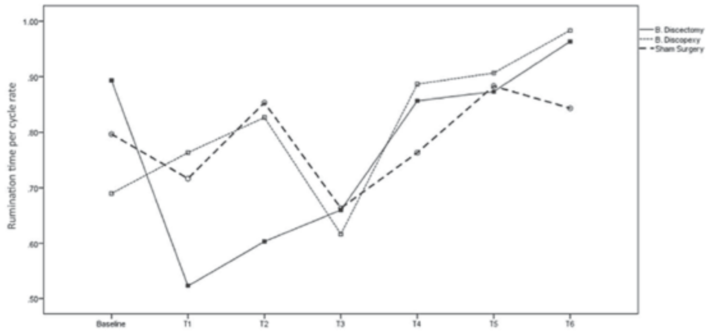


Fig. 5. Rumination time per cycle for T0 (baseline) to T1–T6 (post-test) in bilateral discectomy, discopexy, and sham surgery conditions.

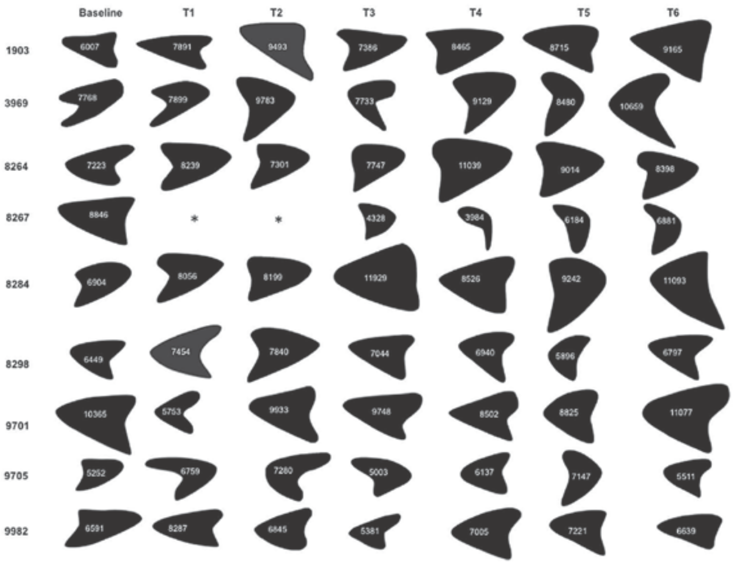


Fig. 6. Rumination geometry and average area of rumination for T0 (baseline) to T1–T6 (post-test) in bilateral discectomy, discopexy, and sham surgery conditions. *No rumination cycles were detected – rumination area value assumed to be zero.

Table 6
Rumination area for T0 (baseline) to T1–T6 (post-test): descriptive one-way ANOVA for T0 and T3–T6, and Kruskal–Wallis test for T1 and T2; effect-sizes (η^2_p) and observed power ($1-\beta$).

	Time						
	T0	T1	T2	T3	T4	T5	T6
Surgery	x±dp	x±dp	x±dp	x±dp	x±dp	x±dp	x±dp
Discectomy	6896.33 ± 1816.3	5015.33 ± 4410.09	4708.33 ± 4083.33	4904.00 ± 533.44	5908.67 ± 1672.19	6850.67 ± 578.53	6343.67 ± 731.19
Discopexy	7906.00 ± 2141.67	7087.67 ± 1194.40	8657.33 ± 1119.25	9573.67 ± 2447.16	7989.33 ± 908.83	7987.67 ± 1823.40	9655.67 ± 2475.69
Sham	6999.33 ± 901.55	8009.67 ± 198.65	8859.00 ± 1357.04	7635.33 ± 216.32	9544.33 ± 1336.32	8736.33 ± 267.64	9407.33 ± 1149.81
$F(2, 6)$	0.32	-	-	7.84*	-	-	-
K–W $X^2(2)$	-	1.07	5.42[a]	-	5.07[a]	2.49	4.36
η^2_p	0.096	0.252	0.454	0.723	0.649	0.420	0.561
$1-\beta$	0.081	0.156	0.324	0.777	0.625	0.288	0.469

[a] $p < 0.08$.

Please cite this article in press as: Ângelo DF, et al., Effects of bilateral discectomy and bilateral discopexy on black Merino sheep rumination kinematics: TEMPOJIMS – phase 1 – pilot blinded, randomized preclinical study, Journal of Cranio-Maxillo-Facial Surgery (2017), https://doi.org/10.1016/j.jcms.2017.11.022

Table 7
Comparison of rumination areas between baseline and T1–T6: within-subjects contrasts, effect-sizes (η^2_p), and observed power ($1-\beta$).

Comparison with baseline (T0)	Discectomy			Discopexy			Sham surgery		
	F	η^2_p	$1-\beta$	F	η^2_p	$1-\beta$	F	η^2_p	$1-\beta$
T1 vs T0	0.29	0.13	0.06	0.19	0.09	0.06	3.99	0.67	0.22
T2 vs T0	0.42	0.17	0.07	1.61	0.45	0.12	3.55	0.64	0.20
T3 vs T0	2.37	0.54	0.15	0.95	0.32	0.09	2.52	0.56	0.16
T4 vs T0	0.25	0.11	0.06	0.01	0.00	0.05	12.85[a]	0.87	0.49
T5 vs T0	0.00	0.00	0.05	0.00	0.00	0.05	9.07	0.82	0.39
T6 vs T0	0.61	0.23	0.08	2.04	0.51	0.14	15.02[b]	0.88	0.54

[a] $p < .05$.
[b] $p < .001$.

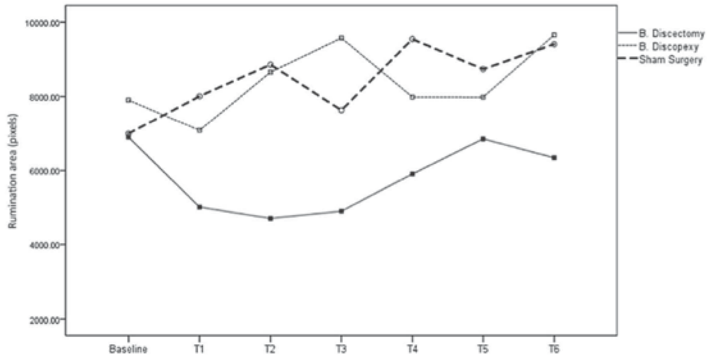


Fig. 7. Rumination area for T0 (baseline) to T1–T6 (post-test) in discectomy, discopexy, and sham surgery conditions.

The authors agree that it would be interesting in the future to analyze this outcome for a longer period.

Regarding the rumination time per cycle, notable results were achieved. In the discectomy group one animal stopped rumination through T1 and T2. This suggests a need for future investigations in this field, to understand for example if TMJ could have a more important impact on rumination than on mastication. The authors believed at first that ankylosis might develop after bilateral discectomy, but at T3 all animals were ruminating. This outcome suggests that in spite of an initial slow down related to food intake, rumination area, and even one animal being unable to ruminate, the sheep were able to readapt and return to normal particulate breakdown. When analyzing Fig. 5, it is noticeable that all groups reduced the rumination time per cycle in T3, without knowing the cause of any event leading to that result. However, in T4–T6 the sheep reassumed expected values. The animals from both discectomy and discopexy groups in T5 and T6 needed more time to achieve a ruminatory cycle, suggesting a less effective rumination process.

Regarding rumination area, it is noticeable that a faster ruminant cycle is obtained through a smaller rumination area. Another interesting detail is that in T3 and T4 a normalization of the rumination kinematics was observed for the discectomy group. This outcome suggests that remodelling and adaptation occurs 3–4 months after TMJ surgical intervention. Although ruminant areas were reduced in the discectomy group after surgery, the differences were not statistically significant.

The evaluation of trajectory and area of rumination was interesting because it was possible to identify a pattern. Each animal showed a favorite side for rumination but switched sides independently of the intervention. Every animal displayed a triangular trajectory, similar to the jaw movements demonstrated in anesthetized rabbits (Hidaka et al., 1997).

Further research should be able to examine possible associations between these results and histological, imaging, and weight outcomes (Zhao et al., 2010).

5. Conclusions

The authors are not aware of any previous randomized, blinded, preclinical studies of the TMJ domain that follow the ARRIVE guidelines. Using black Merino sheep, with age and gender selection, a publicly available protocol, sham control group, and a bilateral approach, we intended to minimising possible bias. The bilateral approach also avoided any adverse effects of the unoperated contralateral joint, as have been reported with unilateral procedures (Dimitroulis and Slavin, 2006). The proposed baseline outcomes were homogeneous and the sham control group performed effectively.

The first month after intervention seems to be the critical period regarding kinematic changes, with modifications related to absolute masticatory time, rumination time per cycle, and rumination area, both in discectomy and discopexy groups. After 1 month, TMJ bilateral discopexy does not seem to have an important kinematic impact in black Merino sheep. TMJ bilateral discectomy does seem to have a significant impact, mostly in T1 and T2, but from T3 to T6 normalization of results is observed.

The authors agree that the rigorous study design, the animal model, and bilateral intervention were the main advantages of this research. The limitations were mostly due to the small sample size, so further research should aim for larger samples. The introduction of kinematic evaluation highlights the importance of kinematics

Please cite this article in press as: Ângelo DF, et al., Effects of bilateral discectomy and bilateral discopexy on black Merino sheep rumination kinematics: TEMPOJIMS – phase 1 – pilot blinded, randomized preclinical study, Journal of Cranio-Maxillo-Facial Surgery (2017), https://doi.org/10.1016/j.jcms.2017.11.022

study in TMJ domain, and represents a new approach for future studies.

Ethical Approval

Portuguese National Authority for Animal Health; registration number 026618.

Funding

Portuguese Grünenthal Grant for Pain Research.

Competing Interests

None declared.

Patient Consent

Not required.

Acknowledgements

We would like to acknowledge Miguel Virgilio for his valuable work on rumination analysis.

References

Ângelo DF, Monje FG, Gonzalez-Garcia R, Little CB, Monico L, Pinho M, et al: Bioengineered temporomandibular joint disk implants: study protocol for a two-phase exploratory randomized preclinical pilot trial in 18 black Merino sheep (TEMPOJIMS). JMIR Res Protoc 6: e37, **2017**

Angelo DF, Morouco P, Alves N, Viana T, Santos F, Gonzalez R, et al: Choosing sheep (*Ovis aries*) as animal model for temporomandibular joint research: morphological, histological and biomechanical characterization of the joint disc. Morphologie 100: 223–233, **2016**

Bjørnland T, Haanaes HR: Discectomy of the temporomandibular joint: an experimental study in monkeys. J Craniomaxillofac Surg 27: 113–116, **1999**

Bjørnland T, Larheim TA: Discectomy of the temporomandibular joint: 3-year follow-up as a predictor of the 10-year outcome. J Oral Maxillofac Surg 61: 55–60, **2003**

Cohen WA, Servais JM, Polur I, Li Y, Xu L: Articular cartilage degeneration in the contralateral non-surgical temporomandibular joint in mice with a unilateral partial discectomy. J Oral Pathol Med 43: 162–165, **2014**

Dimitroulis G, Slavin J: The effects of unilateral discectomy and condylectomy on the contralateral intact rabbit craniomandibular joint. J Oral Maxillofac Surg 64: 1261–1266, **2006**

Eriksson L, Westesson P-L: Discectomy as an effective treatment for painful temporomandibular joint internal derangement: a 5-year clinical and radiographic follow-up. J Oral Maxillofac Surg 59: 750–758, **2001**

Hidaka O, Morimoto T, Masuda Y, Kato T, Matsuo R, Inoue T, et al: Regulation of masticatory force during cortically induced rhythmic jaw movements in the anesthetized rabbit. J Neurophysiol 77: 3168–3179, **1997**

Ingawale S, Goswami T: Temporomandibular joint: disorders, treatments, and biomechanics. Ann Biomed Eng 37: 976–996, **2009**

Kilkenny C, Parsons N, Kadyszewski E, Festing MFW, Cuthill IC, Fry D, et al: Survey of the quality of experimental design, statistical analysis and reporting of research using animals. PLoS One 4: e7824, **2009**

Mazzonetto R, Spagnoli DB: Long-term evaluation of arthroscopic discectomy of the temporomandibular joint using the holmium yag laser. J Oral Maxillofac Surg 59: 1018–1023, **2001**

Nyberg J, Adell R, Svensson B: Temporomandibular joint discectomy for treatment of unilateral internal derangements – a 5 year follow-up evaluation. Int J Oral Maxillofac Surg 33: 8–12, **2004**

Ogi N, Kurita K, Ishimaru J, Goss AN: Short-term effect of deep-frozen stored auricular cartilage allograft repair on the osteoarthritic sheep temporomandibular joint. Int J Oral Maxillofac Surg 28: 393–397, **1999**

Pearce G: Changes in particle size in the reticulorumen of sheep. Aust J Agric Res 18: 119, **1967**

Poveda RR, Bagan JV, Diaz Fernandez JM, Hernandez Bazan S, Jimenez SY: Review of temporomandibular joint pathology. Part I: classification, epidemiology and risk factors. Med Oral Patol Oral Cir Bucal 12: e292–e298, **2007**

Sato S, Goto S, Koeda S, Motegi K: Changes of the elastic fibre network of the rabbit temporomandibular joint following discectomy. J Oral Rehabil 29: 847–852, **2002**

Sharma R, Sinha R, Menon PS: Meniscopexy for internal derangement of temporomandibular joint. J Maxillofac Oral Surg 13: 261–265, **2010**

Takaku S, Sano T, Yoshida M: Long-term magnetic resonance imaging after temporomandibular joint discectomy without replacement. J Oral Maxillofac Surg 58: 739–745, **2000**

Tong AC, Tideman H: A comparative study on meniscectomy and autogenous graft replacement of the rhesus monkey temporomandibular joint articular disc: Part I. Int J Oral Maxillofac Surg 29: 140–145, **2000**

Trumpy IG, Lyberg T: Surgical treatment of internal derangement of the temporomandibular joint: long-term evaluation of three techniques. J Oral Maxillofac Surg 53: 740–747, **1995**

Van der Worp HB, Howells DW, Sena ES, Porritt MJ, Rewell S, O'Collins V, et al: Can animal models of disease reliably inform human studies? PLoS Med 7: e1000245, **2010**

Zhao C, Kurita H, Kurashina K, Hosoya A, Arai Y, Nakamura H: Temporomandibular joint response to mandibular deviation in rabbits detected by 3D micro-CT imaging. Arch Oral Biol 55: 929–937, **2010**

Please cite this article in press as: Ângelo DF, et al., Effects of bilateral discectomy and bilateral discopexy on black Merino sheep rumination kinematics: TEMPOJIMS – phase 1 – pilot blinded, randomized preclinical study, Journal of Cranio-Maxillo-Facial Surgery (2017), https://doi.org/10.1016/j.jcms.2017.11.022

Preclinical randomized controlled trial of bilateral discectomy versus bilateral discopexy in Black Merino sheep temporomandibular joint: TEMPOJIMS — Phase 1- histologic, imaging and body weight results

David Faustino Ângelo [a,b,*], Pedro Morouço [b], Florencio Monje Gil [c], Lisete Mónico [d], Raúl González-Gárcia [c], Rita Sousa [a], Lia Neto [a], Inês Caldeira [a], Margaret Smith [e], Susan Smith [e], David Sanz [d], Fábio Abade dos Santos [f], Mário Pinho [f], Belmira Carrapiço [f], Sandra Cavaco [g], Carla Moura [b], Nuno Alves [b], Francisco Salvado [a], Christopher Little [e]

[a] *Faculty of Medicine of Lisbon University, Av. Prof. Egas Moniz, 1649-035 Lisboa, Portugal*
[b] *Centre for Rapid and Sustainable Product Development, Polytechnic Institute of Leiria, Portugal, Rua de Portugal, Zona Industrial, Marinha Grande, 2430-028, Marinha Grande, Portugal*
[c] *Infanta Cristina Hospital, Faculty of Medicina, University of Extremadura, Av. Elvas, S/N, 06006 Badajoz, Spain*
[d] *Faculty of Medicine of Coimbra University, Portugal*
[e] *Kolling Institute, Institute of Bone and Joint Research, University of Sydney, Australia*
[f] *Faculty of Veterinary Medicine of Lisbon University, Portugal*
[g] *Instituto Nacional de Investigação Agrária e Veterinária (INIAVIP), Portugal*

ARTICLE INFO

Article history:
Paper received 26 November 2017
Accepted 22 January 2018
Available online xxx

Keywords:
Preclinical study
TMJ discectomy
TMJ discopexy
TMJ imaging
TMJ histology
TMJ disc

ABSTRACT

Introduction: The role of temporomandibular joint (TMJ) surgery is not well defined due to a lack of quality randomized controlled clinical trials, comparing different TMJ surgical treatments with medical and placebo interventions. The temporomandibular joint interposal study (TEMPOJIMS) is a rigorous preclinical trial divided in 2 phases. In phase 1 the authors investigated the role of the TMJ disc and in phase 2 the authors evaluated 3 different interposal materials. The present work of TEMPOJIMS — phase 1, aims to evaluate histopathologic and imaging changes of bilateral discectomy and discopexy in Black Merino sheep TMJ, using a high-quality trial following the ARRIVE guidelines.
Material and methods: This randomized, blinded and controlled preclinical trial was conducted in 9 Black Merino sheep to investigate histopathologic (primary outcome), imaging and body weight (secondary outcomes) changes after bilateral discectomy, discopexy and sham surgery.
Results: Significant changes were noticed in discectomy group, both in imaging and histopathologic analyses. Body weight changes were most pronounced in the discectomy group in the first 4 months after surgery with recovery to baseline weight 6 months after surgery. Discopexy induced nonsignificant changes in histopathologic, imaging and body weight analyses.
Conclusions: This study reinforces the importance of developing an effective interposal material to substitute the TMJ disc and the need to explore the molecular mechanisms that underlie TMJ cartilage degeneration. The study design proposed in TEMPOJIMS represents an important progress towards future rigorous TMJ investigations.

* Corresponding author. Fax: +351 21 798 5110.
E-mail addresses: david.serrano.angelo@gmail.com (D.F. Ângelo), pedro.morouco@ipleiria.pt (P. Morouço), fmonje@oralmaxilofacial.com (F. Monje Gil), lisetemonico@gmail.com (L. Mónico), Raulmaxilo@gmail.com (R. González-Gárcia), ritafsousa1@gmail.com (R. Sousa), lia_neto@yahoo.com (L. Neto), maria_caldeira@hotmail.com (I. Caldeira), mobsmith@sydney.edu.au (M. Smith), susan.smith@sydney.edu.au (S. Smith), d.sanz@hotmail.com (D. Sanz), faas@outlook.pt (F. Abade dos Santos), mpinho@fmv.ulisboa.pt (M. Pinho), belmira@fmv.ulisboa.pt (B. Carrapiço), sandracavaco1@gmail.com (S. Cavaco), carla.moura@ipleiria.pt (C. Moura), nuno.alves@ipleiria.pt (N. Alves), fjsalvado2002@yahoo.com (F. Salvado), christopher.little@sydney.edu.au (C. Little).

1. Introduction

In severe temporomandibular disorders (TMD) the standard treatment is mostly surgical (Dimitroulis, 2013). However, the role of temporomandibular joint (TMJ) surgery is not well defined (Dimitroulis, 2005) due to a lack of quality randomized controlled clinical trials, comparing TMJ surgical treatment with medical treatment and placebo (Reston and Turkelson, 2003; Souza et al., 2012). TMJ open surgical approaches for severe disorders include mostly discectomy or discopexy. In cases where nothing in the joint is salvageable, a total joint replacement may be necessary (Dimitroulis, 2013). Despite the large number of discectomy procedures performed annually, we are not aware of any rigorously performed, randomized, controlled trials that have investigated, in human or animal, the effectiveness of discectomy, compared with discopexy, bioengineered interposal material and sham surgical interventions. Previous studies stated a significant increase in TMJ osteoarthrosis (OA), following discectomy with and without replacement of the disc with an interpositional implant (93% and 100%, respectively). These authors presented a reduced incidence of OA (62%) when using discopexy. Still, this technique was associated with frequent relapse, requiring a secondary discectomy (Trumpy and Lyberg, 1995). These outcomes clearly demonstrate the importance of further studies to deeper understand the effects of surgery and progress for future development of interposal materials.

Most clinical trials use imaging to classify the TMJ degenerative process (Eriksson and Westesson, 2001). Computed tomography (CT) is a valuable tool to evaluate TMJ OA (Cordeiro et al., 2016) and it is used by most clinical studies to evaluate articular changes (Boman, 1947; Eriksson and Westesson, 1985; Hall, 1985; Kiehn and Desprez, 1962; Silver, 1984; Takaku et al., 1994; Tolvanen et al., 1988). Two important long-term follow-up clinical studies presented condylar flattening and sclerosis after discectomy, but these were not associated with TMJ symptoms (Eriksson and Westesson, 1985; Hall, 1985; Silver, 1984; Tolvanen et al., 1988). Opposingly, The *Desprez* group (1962) suggested an association of articular erosion with pain in the post-operative period (Kiehn and Desprez, 1962). While imaging modalities are key measures in clinical research, preclinical studies provide a unique chance to also obtain histologic pathology to better understand TMJ surgery-induced changes and improve knowledge for interposal materials research. Previous preclinical studies have evaluated histologic and imaging outcomes using study designs with a potential sources of bias (selection bias, measurement bias, non-randomization, non-blinded outcome assessment) increasing risk of errors in the results of the study, and in further conclusions (Block and Bouvier, 1990; Choukas et al., 1969; Hagandora and Almorza, 2012; Laurell et al., 1987; Macher et al., 1992; Ogi et al., 1996).

The Temporomandibular Joint Interposal Material Study (TEMPOJIMS) was planned with a rigorous pre-published design (Ângelo et al., 2017) according to the ARRIVE guidelines (Kilkenny et al., 2010). This first high-quality randomized preclinical study, performed in Black Merino sheep, was required to increase the translational power of further studies and to progress in future treatment options for patients undergoing surgery for TMJ disc replacement. TEMPOJIMS is divided into phase 1 and 2. Phase 1 was a randomized, blinded preclinical trial designed to investigate the TMJ imaging (CT), histopathologic, and body weight changes in sheep after bilateral discectomy, discopexy or sham surgery. Phase 2 uses the same design to test different bioengineering scaffolds to replace the TMJ disc in sheep. It is critical that all assessments are performed and classified independently by two professionals, from each area, who are blinded to intervention. In both phases the primary outcome was the histological grading of TMJ pathology. The main goal of the present investigation was to examine the effects of bilateral surgery over the phase 1 outcomes.

2. Material and methods

2.1. Study design

The rationale and protocol for the TEMPOJIMS preclinical trial are publicly available (Ângelo et al., 2017). An independent data and safety monitoring board unblinded preclinical results. The study was approved by the Portuguese National Authority for Animal Health registered with number 026618. The study design and organization respected the ARRIVE guidelines (Kilkenny et al., 2010).

2.2. Study population and sample

Relevant preclinical TMJ studies have been conducted in sheep (Ishimaru and Goss, 1992; Matsuura et al., 2006; Miyamoto et al., 1999; Ogi et al., 1996; Takaishi et al., 2007), and to decrease biological variability in TEMPOJIMS results, a specific purebred Black Merino sheep strain was used (Ângelo DF et., 2017). In 2016, our group performed an anatomic, biomechanical and histologic study of Black Merino sheep TMJ highlighting the potential of this animal to conduct preclinical trials in the TMJ domain (Angelo et al., 2016). The following eligibility criteria were used: certified *Black Merino* sheep, adult (aged between 2 and 5 years), female, and in good health condition (evaluation was performed by veterinaries, also confirming normal dentition).

2.3. Randomization

The randomization process was performed by a statistical group, not enrolled in the outcome assessments. Ten sheep were randomly allocated to the intervention group: bilateral sham surgery ($n = 3$), bilateral discectomy ($n = 3$), bilateral discopexy ($n = 3$), and backup group ($n = 1$). One backup sheep was planned to be used if death occurred due to anaesthesia, or other complication not related to surgical intervention. The allocation to each randomized group was performed preoperatively by sealed envelope.

2.4. Procedures

Ten eligible sheep were selected and baseline body weight was measured at days 11, 10, and 9 before surgery. Transportation to surgical facilities was performed 5 days before surgery to avoid animal stress and allow familiarization to the temporary accommodation. Head CT-scan was performed on the day of surgery taking advantage of pre-anaesthesia sedation (supplementary material doc 1). The surgical team was not blinded to treatment allocation given the type of intervention; however surgical team members were not involved in outcome assessment. Serious adverse events were defined as events that were fatal or life-threatening or persistent disability, or that resulted in death, over 10% weight loss per week, or clinically significant hazard or harm to the animal.

2.5. Intervention phase

2.5.1. Anaesthesia protocol

Fasting and water restriction were required 24 h before surgery. Sedation was performed with diazepam (0.5 mg/kg i.v.), followed by anaesthesia induction with ketamine (5 mg/kg i.v.). Oral intubation was performed and anaesthesia was maintained with isoflurane (1.5–2%). To guarantee animal analgesia, meloxicam

Please cite this article in press as: Ângelo DF, et al., Preclinical randomized controlled trial of bilateral discectomy versus bilateral discopexy in Black Merino sheep temporomandibular joint: TEMPOJIMS – Phase 1- histologic, imaging and body weight results, Journal of Cranio-Maxillo-Facial Surgery (2018), https://doi.org/10.1016/j.jcms.2018.01.006

(0.5 mg/kg i.v, bid) was administered on the day of surgery up until 4 days post-operatively. Antibiotic prophylaxis with amoxicillin and clavulanic acid (50 mg/kg i.v. bid) was used for 5 days, after surgeries.

2.5.2. Surgical intervention

In all animals, the surgical site was shaved, the skin prepared with povidone iodine solution, and isolated with sterile drapes according to standard surgical procedures. With a 15-scalpel blade a 6 cm long pre-auricular skin incision was performed followed by blunt dissection of the soft tissue covering the joint, to expose the articular capsule. Tissue retractors were used to maintain exposure of the surgical field. In the sham group (n = 3) TMJ articular capsule was not incised, and the wound was closed in 3 layers (muscular, subcutaneous and skin) with Vicryl 3/0. In the remaining animals, the joint capsule was incised and the disc and its attachments were identified. In the discectomy group (n = 3), the disc was exposed and using iris scissors the lateral, anterior and posterior attachments were dissected, allowing exposure and transection of the medial attachment and removal of the intact disc. In the discopexy group (n = 3), the lateral and posterior disc attachments were sharply detached using an iris scissor. A 4-mm triangular segment of the retrodiscal tissue was removed and then sutured with *PDS 3/0*. The wound, including joint capsule, was closed in 4 layers (joint capsule, muscular, subcutaneous and skin) with Vicryl 3/0.

2.5.3. Follow-up assessments

Ten days after surgery, animals were transported to TEMPOJIMS facilities (Ângelo et al., 2017). From the 19th to 21st day after surgery, follow-up secondary outcomes were recorded, which were repeated every 30 days for 6 months (T1 to T6, respectively). Data from T0-T6 were calculated on a mean of the 3-day measurements of each month. Six months after the intervention, immediately after euthanasia, all animals had a second CT scan and the TMJ block was removed to histology.

2.5.4. Outcomes

Histologic analysis: Intact TMJ was removed using a necropsy bone oscillatory saw according to the following anatomic references: *cranial* – cranial aspect of coronoid process in the union region of the zygomatic process; *caudal* – external to acoustic meatus; *dorsal* – the squamous temporal bone; and *ventral* - 2 cm below the acoustic meatus in the zone of stylo-hyoid angle. The joints were fixed in 10% buffered formalin for 24 h and stored in 70% ethanol. Decalcification was obtained by immersion in 10% formic acid in 5% formalin for 20 days with solution changed every 2 days, after which the articulations were cut sagittally through the whole condyle. TMJ articulations were then immersed in three graded methyl salicylate/paraffin mixtures, embedded in paraffin and sectioned through to the central part of TMJ. Four micron sections were mounted on glass slides, heated for 1 h at 65 °C, de-waxed with 3 cycles of 5 min with xylene, and stained with toluidine blue and fast green as previously described (Little et al., 2010). Slides identified by a number code were randomized and shipped to the Raymond Purves Labs for scoring by 2 blinded independent assessors experienced in evaluating sheep joint histopathology (CBL, MMS).

As the normal histomorphology of the TMJ is quite distinct from cartilage in appendicular synovial joints (Murphy et al., 2013) (Fig. 1Aa), a modification of a published scoring system specific for the TMJ was used (Li et al., 2014) (supplementary material doc2). Briefly, the mandibular and temporal cartilage (structure, cell number, shape and cloning, and proteoglycan content and distribution), tidemark, cement line, and subchondral bone (structure, osteocyte number, osteoblast activation, vascular invasion, and calcified cartilage islands) were separately scored from 0 (normal) to 3 (>70% abnormal). Additionally, the temporal and retrodiscal synovial hyperplasia, fibrosis and inflammatory cell infiltration were also scored from 0 to 3. The summed cartilage (possible maximum score 60 in each condyle), subchondral bone (possible maximum score 15 in each condyle), synovial (possible maximum score 9 in each site), and total (possible maximum score 168) histopathology scores were calculated.

Imaging analysis: Imaging evaluation was performed and classified independently by 2 experienced radiologists (RS, LN) who were blinded to the intervention using the outlined criteria (supplementary material doc 3).

Body mass assessment: Sheep were weighed immediately after eating 150-gr of dry pellets. Body mass assessments were performed by 2 trained evaluators who were not affiliated with the intervention.

2.5.5. Statistical analysis

Statistical analyses were performed using either the Statistical Package for Social Sciences (IBM SPSS, version 22.0) or Statistics/data Analysis (STATA-corporation version 14.2). The histopathology scores for each parameter in each section of the 2 evaluators were averaged, and following un-blinding the median scores (and score summations) for each treatment group were calculated. Differences between treatments were analysed by mixed ordinal logistic regression.

A one-way Analysis of Variance (ANOVA) was performed for cross-sectional analysis, to compare the outcome variables in the three levels of the independent variable before and after the random treatment group assignment. For longitudinal analysis, a one-way ANOVA with repeated measures was performed taking as within-subjects effects observations after surgery (T1-T6) for all conditions. Primary analysis tested the effects of the surgical intervention using series pre-test and post-test. Body mass and imaging score were used as dependent variables for degenerative process. Body mass was measured 3 times in the pre-test for supporting invariance concerning the outcome measures before the clinical intervention. The secondary analysis (post-test) assessed the outcomes measuring 3 times, in 6 time-points, one per month at the same place, date and hour as in pre-test (Ângelo et al., 2017).

To analyse imaging results, non-parametric tests were performed attending to the sample size and the non-normality of the distribution for most variables in each group, Shapiro–Wilk test $\leq .82$, $p \leq .091$. Kruskal–Wallis tests were performed for group comparisons, with Bonferroni test for post-hoc multiple comparisons. Partial eta squared (η^2_p) and Cohen's *d* were used for effect size calculations. Cohen's categories were used to evaluate the magnitude of these effect sizes (small if $0 \leq |d| \leq 0.5$, medium if $0.5 < |d| \leq 0.8$, and large if $|d| > 0.8$).

3. Results

At baseline, no differences between groups were observed in body mass (sham group: 55.1 ± 2.7 kg, discopexy group: 62.3 ± 6.0 kg, discectomy group: 67.3 ± 10.2, p > .10).

3.1. Histologic results

The morphological appearance of the cartilage and bone in sham operated joints was consistent with that previously described as normal TMJ (Murphy et al., 2013; Li et al., 2015) (Fig. 1Aa). The superficial half of the cartilage depth had a distinct laminar appearance, with sparse flattened cells and limited proteoglycan staining more intense with depth. Beneath this, there was a layer densely populated with cells that had a

Please cite this article in press as: Ângelo DF, et al., Preclinical randomized controlled trial of bilateral discectomy versus bilateral discopexy in Black Merino sheep temporomandibular joint: TEMPOJIMS – Phase 1- histologic, imaging and body weight results, Journal of Cranio-Maxillo-Facial Surgery (2018), https://doi.org/10.1016/j.jcms.2018.01.006

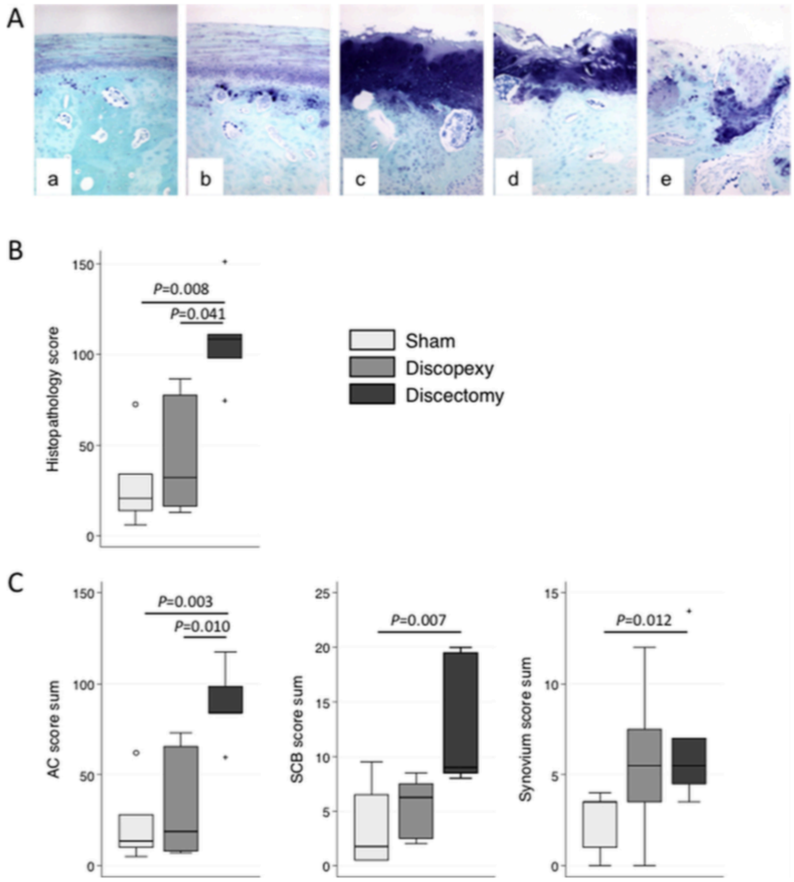


Fig. 1. (A) Representative images of toluidine blue stained sections of mandibular condyles from sheep with sham-operated (a) or discopexy/discectomy (b–e) TMJs. Total histopathology (B) and individual tissue (C) scores in sham, discopexy and discectomy sheep (line = median; box = 25–75%, whiskers = 10–90%, dots = outside values).

mesenchymal appearance, and more intense diffuse matrix proteoglycan staining. The deepest cartilage layer contained mature and/or hypertrophic chondrocytes often surrounded by a proteoglycan rich peri-cellular matrix but little or no inter-territorial proteoglycan. A tidemark separating the upper two layers from the deepest cartilage layers could be observed in some sections, suggesting the lower zone was calcified. An indistinct cement line demarcated the subchondral bone which contained evenly distributed osteocytes in lacunae, and completely separated the cartilage from sparse marrow spaces lined by osteoblasts in the deeper bone. The synovium in sham-operated TMJ was similar to that in the knee joint in sheep (Smith et al., 2008) with a single lining layer of synoviocytes overlying a loose connective tissue with adipocytes and sparse fibroblasts and collagen.

A variety of pathological changes of varying severity were noted in discopexy and discectomy joints (Fig. 1Ab–e). The mildest changes included cartilage thickening, slightly increased matrix and peri-cellular proteoglycan staining, increased cell density, vascular activation and invasion of the sub-chondral bone and calcified cartilage layer, with both the tidemark and cement line being more distinct (Fig. 1Ab). Intermediate cartilage pathology was characterized by surface roughening/fibrillation, loss of typical laminar structure, a marked increase in inter-territorial proteoglycan staining in all layers, cell cloning particularly in the upper zones, and further deep zone vascular invasion (Fig. 1Ac). Further advancement of pathology was evident with erosion and loss of surface zone cartilage, decreased cell density in the mid zone but cloning in all layers, vascular invasion into the mid zone (Fig. 1Ad), and ultimately complete loss of cartilage integrity and marked subchondral bone remodelling (Fig. 1Ae). Accompanying the osteochondral changes, there was synovitis with hyperplasia of surface cells, sub-synovial fibrosis with loss of adipocytes and both peri-vascular and diffuse inflammatory cell infiltration (not shown). Blinded scoring demonstrated a significant increase in total median histopathology score in discectomy compared with the other groups (Fig. 1B). This was driven by a significant increase

Please cite this article in press as: Ângelo DF, et al., Preclinical randomized controlled trial of bilateral discectomy versus bilateral discopexy in Black Merino sheep temporomandibular joint: TEMPOJIMS – Phase 1- histologic, imaging and body weight results, Journal of Cranio-Maxillo-Facial Surgery (2018), https://doi.org/10.1016/j.jcms.2018.01.006

in pathology in cartilage, bone and synovium in discectomy compared with sham-operated joints (Fig. 1C). Discopexy joints displayed some evidence of cartilage and synovial pathology, but this was quite variable and did not reach statistical significance.

3.2. Imaging results

The authors compared the outcomes of all surgery conditions (Table 1). In general, differences were very high (η^2_p corresponding to 90.8%, statistical power > .999) for all outcomes, excluding calcification. Considering each outcome, differences were higher for shape, followed by condyle sclerosis, temporal sclerosis, condyle marrow, temporal erosion, condyle erosion, and temporal marrow. The effect size of the differences ranged from 43.4% to 90.8%. Fig. 2 is a representative CT imaging of sham surgery group (Fig. 2A), discopexy group (Fig. 2B) and discectomy (Fig. 2C).

Excluding the difference between discopexy and sham surgery for temporal erosion ($d = 0.59$), all the other differences were classified as large ($d > 0.80$). The larger differences were between discectomy and sham surgery (R^2 corresponding to 92.9% of degeneration in global appreciation), mainly due to shape ($R^2 = 96.0\%$), condyle marrow ($R^2 = 83.4\%$), and condyle sclerosis ($R^2 = 80.1\%$). Condyle erosion and temporal marrow were the least affected, despite an effect size of R^2 of 50.3% and 50.8%, respectively. Temporal sclerosis and temporal erosion showed R^2 effect sizes of 71.1% and 62.3%, respectively. Discopexy also differed from sham surgery (R^2 corresponding to 80.3% of deterioration in global appreciation), although with lower effect sizes in comparison to the differences between discectomy and sham surgery, and only for shape ($R^2 = 80.3\%$), condyle sclerosis ($R^2 = 76.6\%$), and condyle marrow ($R^2 = 56.7\%$) (Table 2 and Fig. 3).

3.3. Body mass results

Cross-sectional analysis. Statistical differences were not found in body mass in the pre-test (T0) and in all times for the post-test ($p > .10$, Table 3).

In Fig. 4 can be seen that in the discectomy condition sheep lost weight from month 1 to month 4 and recovered their weight during months 5 and 6 after surgery.

Longitudinal analysis. A one-way ANOVA with repeated measures was performed taking as within-subjects effects months after surgery (T1-T6) for discectomy, discopexy, and sham surgery. Statistically significant differences were found, $F(5, 10) = 9.69$, 27.35 and 8.07, $p < .01$, $\eta^2_p = .829$, .932, and .801, $(1 - \beta) = .992$, 1.00, and .977 for discectomy, discopexy, and sham surgery respectively, showing that sheep recovered weight from T1 to T6. The tests of within-subjects contrasts identified that the increase happened from T4 to T5 both in discectomy ($p = .04$), discopexy ($p = .01$), and sham surgery ($p = .01$). Despite this increase, only those in the discopexy and sham groups increased their weight over the pre-test in T5 and T6, $t(2) = -5.34$ and -5.00, $p < .04$. In discectomy and sham surgery conditions sheep did not exceed their weight at baseline.

4. Discussion

This is the first temporomandibular preclinical study using a randomized, blinded design respecting ARRIVE guidelines. Using the suitable Black Merino sheep with age and gender selection, sham control group and bilateral approach, the authors aimed to reduce possible bias on results. In humans, TMJ cartilage is different from appendicular synovial joints (Murphy et al., 2013), with the distinctly laminar fibrocartilage with sparse proteoglycan

Table 1
Means (M), standard-deviations (SD), Means Rank (MR), Kruskal Wallis Tests, power, and effect sizes for the imaging results.

	Surgery:	Shape			Condyle Erosion			Temporal erosion			Condyle sclerosis			Temporal sclerosis			Condyle marrow			Temporal marrow			Calcification			Global appreciation		
		M	SD	MR	M	SD	MR	M	SD	MR	M	SD	MR	M	SD	MR	M	SD	MR	M	SD	MR	M	SD	MR	M	SD	MR
	Discectomy	2.83	0.41	15.42	1.50	0.84	13.83	2.00	1.10	14.42	2.33	0.82	14.50	2.17	0.98	14.67	2.00	.63	13.83	1.50	1.05	13.42	0	0	9.50	2.67	0.52	15.33
	Discopexy	1.17	0.41	9.58	0.67	0.52	9.33	0.17	0.41	7.58	1.33	0.52	10.50	0.83	0.41	9.83	1.33	.82	10.67	0.67	0.82	9.58	0	0	9.50	1.17	0.41	9.67
	Sham	0	0	3.50	0.17	0.41	5.33	0	0	6.50	0	0	3.50	0	0	4.00	0	0	4.00	0	0	5.50	0	0	9.50	0	0	3.50
$X^2(2)$		16.22***			9.30**			11.09**			14.05***			13.65***			12.05**			8.09*			0			15.88***		
η^2_p		.936			.490			.684			.779			.717			.700			.434			-			.908		

*p<.05, ** p < .01, *** p < .001.

Please cite this article in press as: Ângelo DF, et al., Preclinical randomized controlled trial of bilateral discectomy versus bilateral discopexy in Black Merino sheep temporomandibular joint: TEMPOJIMS – Phase 1- histologic, imaging and body weight results, Journal of Cranio-Maxillo-Facial Surgery (2018), https://doi.org/10.1016/j.jcms.2018.01.006

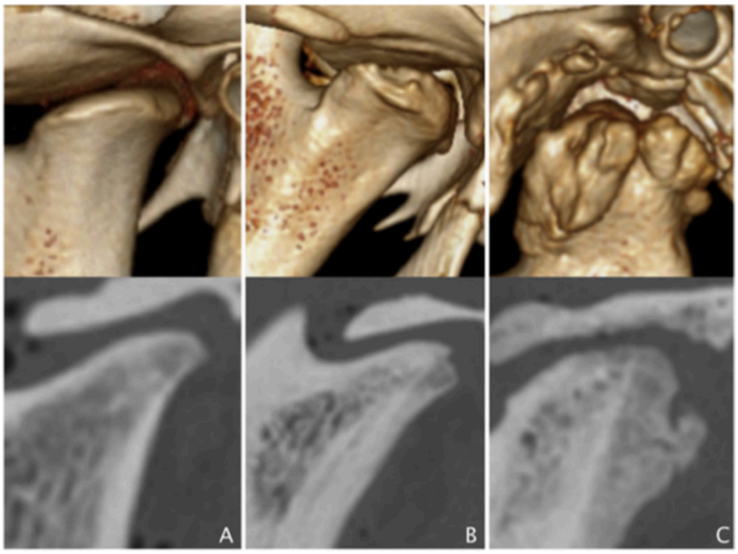


Fig. 2. Representative CT sagittal image of TMJ: A-sham group, B- discopexy group, C- discectomy group.

Table 2
Mean differences between discectomy, discopexy, and sham surgery: Bonferroni test.

Outcomes	(I) VI	(J) VI	Mean Difference (I-J)	Std. Error	95% CI	Cohen's d	Effect-size r	R^2
Shape	Discectomy	Discopexy	1.67***	.19	1.15–2.19	4.05	.897	.805
		Sham Surgery	2.83***	.19	2.31–3.35	9.76	.980	.960
	Discopexy	Sham Surgery	1.17***	.19	.65–1.69	4.04	.896	.803
Condyle erosion	Discectomy	Discopexy	0.83	.35	minus;.12–1.79	1.10	.482	.232
		Sham Surgery	1.33**	.35	.38–2.29	2.01	.709	.503
	Discopexy	Sham Surgery	0.50	.35	minus;.46–1.46	1.59	.623	.388
Temporal erosion	Discectomy	Discopexy	1.83**	.39	.78–2.88	2.20	.741	.549
		Sham Surgery	2.00***	.39	.95–3.05	2.57	.789	.623
	Discopexy	Sham Surgery	0.17	.39	minus;.88–1.22	0.59	.281	.079
Condyle sclerosis	Discectomy	Discopexy	1.00*	.32	.13–1.87	1.46	.589	.347
		Sham Surgery	2.33***	.32	1.47–3.20	4.02	.895	.801
	Discopexy	Sham Surgery	1.33**	.32	.47–2.20	3.62	.875	.766
Temporal sclerosis	Discectomy	Discopexy	1.33**	.35	.38–2.29	1.78	.666	.444
		Sham Surgery	2.17***	.35	1.21–3.12	3.13	.843	.711
	Discopexy	Sham Surgery	0.83	.35	–.12–1.79	2.86	.820	.672
Condyle marrow	Discectomy	Discopexy	0.67	.34	–.26–1.59	0.92	.417	.174
		Sham Surgery	2.00***	.34	1.07–2.93	4.49	.913	.834
	Discopexy	Sham Surgery	1.33**	.34	.41–2.26	2.29	.753	.567
Temporal marrow	Discectomy	Discopexy	0.83	.44	.36–2.03	0.89	.407	.166
		Sham Surgery	1.50***	.44	.31–2.69	2.03	.713	.508
	Discopexy	Sham Surgery	0.67	.44	minus;.53–1.86	1.16	.500	.250
Global appreciation	Discectomy	Discopexy	1.50***	.22	.91–2.09	3.20	.848	.719
		Sham Surgery	2.67***	.22	2.08–3.26	7.26	.964	.929
	Discopexy	Sham Surgery	1.17***	.22	.58–1.76	4.04	.896	.803

$^{*}p \leq .05$, $^{**}p \leq .01$, $^{***}p \leq .001$.

reminiscent of meniscus and *annulus fibrosus* of the intervertebral disc (Melrose et al., 2017; Shu et al., 2017). In sham-operated joints of Black Merino sheep the TMJ cartilage was histologically very like humans, supporting sheep as a good animal model. Rat (Zhang et al., 2016) and goat (Li et al, 2015) also have a typical TMJ fibrocartilage appearance with the distinct organized layers, while in the mouse (Cohen et al., 2014; Xu et al., 2009) and rabbit (Wu et al., 2015) the laminar structure is less apparent.

The histopathology changes obtained in the sheep TMJ after bilateral discectomy were consistent with other investigations using various species, including mice (Cohen et al., 2014), rats (Zhang et al., 2016), rabbits (Embree et al., 2015) and goats (Li et al., 2015). The authors noticed an increase in proteoglycan and rounded cells and thickening of the cartilage after discectomy. These changes in Black Merino sheep are similar to reports in other animals (e.g. mouse (Cohen et al., 2014; Matías et al., 2016; Xu et al., 2009) and

Please cite this article in press as: Ângelo DF, et al., Preclinical randomized controlled trial of bilateral discectomy versus bilateral discopexy in Black Merino sheep temporomandibular joint: TEMPOJIMS – Phase 1- histologic, imaging and body weight results, Journal of Cranio-Maxillo-Facial Surgery (2018), https://doi.org/10.1016/j.jcms.2018.01.006

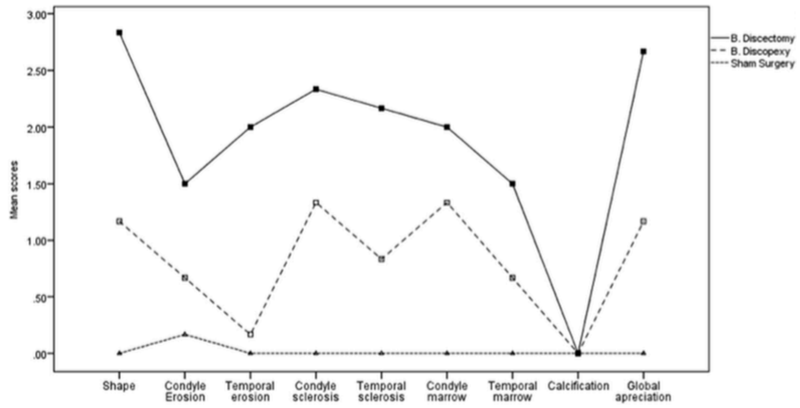


Fig. 3. Mean scores for TMJ imaging score.

Table 3
Sheep body mass for T0 (pre-test) to T1-T6 (post-test): Descriptive and one-way ANOVA.

	Months														
	T0			T1		T2		T3		T4		T5		T6	
	M	SD	Surgery:	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
	67.33	10.17	Discectomy	63.56	7.40	64.33	7.31	63.78	7.24	65.00	8.19	67.33	8.97	67.56	9.25
	62.33	6.03	Discopexy	60.67	5.33	61.44	6.17	62.00	7.55	63.33	6.51	66.22	7.00	67.33	7.51
	55.11	2.71	Sham	54.44	9.53	54.33	8.14	53.89	7.71	55.33	8.96	58.67	8.39	59.67	8.39
$F(2, 6)$	2.31			1.12		1.51		1.48		1.27		1.00		0.86	
η^2_p	.435			.272		.335		.331		.297		.250		.222	
$1 - \beta$	.304			.168		.212		.209		.184		.155		.138	

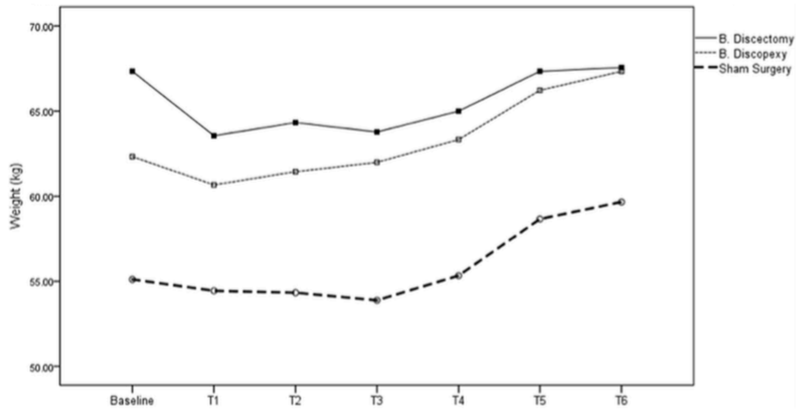


Fig. 4. Sheep weight for T0 (pre-test) to T1 to T6 (post-test) in sham surgery, discopexy and discectomy.

rat (Zhang et al., 2016)). They are consistent with a chondroid metaplasia, which is potentially associated with the loss of the disc and increased direct loading in the TMJ cartilage. However, when this first protective phase fails under continued abnormal loading, the joint undergoes degeneration with cell death and cloning, surface erosion, subchondral bone changes and degeneration. This latter phase is well described and similar to that in the sheep knee joint following meniscectomy (Cake et al., 2013; Little et al., 2010). In the discopexy intervention group, the TMJ capsule and the intra-articular environment was preserved. The result, as expected, had less severe histopathologic changes, because the disc remained interposed between the bony surfaces, dissipating loading and

Please cite this article in press as: Ângelo DF, et al., Preclinical randomized controlled trial of bilateral discectomy versus bilateral discopexy in Black Merino sheep temporomandibular joint: TEMPOJIMS – Phase 1- histologic, imaging and body weight results, Journal of Cranio-Maxillo-Facial Surgery (2018), https://doi.org/10.1016/j.jcms.2018.01.006

protecting the TMJ cartilage. It is noteworthy that we also found more severe synovitis in discectomy compared with discopexy, indicating that the inflammation is not just a reaction to the arthrotomy, but part of the OA process in the joint. The histopathological appearance of the synovitis in the TMJ was the same as that in sheep knee joints with OA (Smith et al., 2008). Still, given the underlying anatomical differences in cartilage, future studies should explore the molecular mechanisms that underlie TMJ OA pathology, to determine their similarities and differences with appendicular joints such as the knee (Young et al., 2005). Such studies could lead to progress in defining the pathophysiology and the management of TMJ degenerative disorders.

Radiographic morphologic changes caused by discectomy were first reported by Boman in 1947, describing "flattening off the articular surface" (Boman, 1947). In 1985, similar conclusions were obtained with condylar flattening and sclerosis after unilateral discectomy, where no osteophytes but severe damage were described (Eriksson and Westesson, 1985). Concomitantly, condyle flattening and sclerosis were the most common radiographic findings in a 33.8 years post discectomy investigation (Tolvanen et al., 1988). In the present study, these outcomes are reinforced with severe morphological changes observed after bilateral discectomy. Most statistical differences were noted in shape and condyle sclerosis, corresponding to other authors' clinical findings (Eriksson and Westesson, 1985; Takaku et al., 2000). While the human condyle is convex and tends to flatten after discectomy, the sheep condyle is normally flat and tend to a more convex form after discectomy (Fig. 2C). Nevertheless, condyle sclerosis in all joints after bilateral discectomy ($R^2 = 80.1\%$) and change in underlying trabecular bone (condyle bone marrow) were also detected. Cortical breakdown characterized by an initial destructive phase was reported by Agerber and Lundberg in the first 6 months post-discectomy (Agerberg and Lundberg, 1971). Some authors suggest that these changes can occur if loading is not controlled during those 6 months (Hall, 1985). Other authors raised the question of whether the lytic condylar process is precipitated by discectomy or overloading, since the contralateral nonoperated joint has similar morphologic changes (Agerberg and Lundberg, 1971; Eriksson and Westesson, 1985; Takaku and Toyoda, 1994; Wilkes, 1991). Yaillen, in 1979 described bony ankylosis between the condyle and temporal bone 1 year after unilateral discectomy in *Macaca fascicularis* (Yaillen et al., 1979). Later, Bjornland found fibrous ankylosis 6 months after unilateral discectomy (Bjornland and Larheim, 2003). In TEMPOJIMS, 6 months after bilateral discectomy no intra-articular calcification was found, and while fibrous ankylosis cannot be excluded with CT, this was not evident histologically. Significant osteophyte formation is also reported, rarely described in previous studies, which may be due to imaging limitations of radiography and arthrography compared with CT.

To the best of our knowledge, there are no clinical or preclinical studies assessing imaging after discopexy. Results showed that TMJ open surgery is not innocuous, resulting in mild to moderate changes in global remodelling. The condyle is more affected than the temporal bone and only for shape ($R^2 = 80.3\%$), condyle sclerosis ($R^2 = 76.6\%$), and condyle marrow ($R^2 = 56.7\%$).

In other diseases like rheumatoid arthritis (England et al., 2017), cancer (Lynch et al., 2017), HIV (Malvy et al., 2001) and surgical interventions like gastric sleeve (Casillas et al., 2017), body mass has been used as a valuable outcome to evaluate progress of disease and intervention success. However, for TMJ disorders this outcome has rarely been used. A 4% decrease of body mass in 60% of the animals, 3 months after unilateral discectomy with condyle and temporal surfaces removal, has been reported (Miyamoto et al., 1999). In a study in mice, after partial discectomy no significant losses or gains in the body weight of the experimental or control mice were seen (Xu et al., 2009). In this study, after bilateral discectomy there was 5.2% body mass loss (all occurring in the first month) but with full recovery at 6 months follow-up. In contrast, the discopexy and sham surgery sheep increased body weight (mostly in T4-T6), finishing the study 8% and 8.2% above the baseline, respectively. It is consistent with the limited TMJ pathology. The evaluation of body mass was also a welfare control measure related to healthy and well fed, respecting the 3 Rs principle (replacement, reduction, or refinement) (Richmond, 2002).

5. Conclusion

This pilot study design demonstrates it is feasible to conduct surgical TMJ preclinical trials in Black Merino sheep. In this study, the authors observed: (1) bilateral discopexy in a healthy TMJ is not an innocuous intervention, resulting in variable cartilage and synovial pathology along with imaging changes; (2) bilateral discectomy induced severe TMJ changes detected with both imaging and histopathologic analysis; (3) no fibrous or bony ankylosis was detected over the 6-month period after bilateral discectomy and discopexy. And (4) beyond expected cartilage and bone changes, synovitis was shown to be part of the osteoarthritis process, providing a new outcome measure and therapeutic target.

This study has reinforced that: (1) TMJ cartilage is different from appendicular synovial joints, and as such may require unique therapeutic approaches; (2) future investigations are needed to study an effective interposal material to substitute the TMJ disc and (3) future investigations are needed to explore the molecular mechanisms that underlie TMJ cartilage degeneration.

Grants

This study was granted by Portuguese Grunenthal Foundation, Secção Regional Oeste da Ordem dos Médicos, Sociedade Anatómica Portuguesa e Associação Anatómica Portuguesa.

Author's contributions

All the listed authors were involved in drafting the article or revising it critically for important intellectual content, and all the authors approved the final version to be published. Ângelo DF, Ferreira J, Monje F, Salvado F and González R designed the study and carried out the surgical protocol. Ângelo DF, Morouço P, Moura C, Alves N, Virgílio M contributed to data acquisition. Neto L, Sousa R and Caldeira I contributed to imaging blinded scoring. Fábio S, Cavaco S contributed to the animal veterinary support during the investigation. Smith M, Smith S and Little C coordinated the histologic blinded scoring. Monico L contributed to data analysis. Ângelo DF, Little C and Morouço P contributed to manuscript preparation.

Conflicts of interest

There are no conflicts of interest between this research and any of the authors herein listed.

Acknowledgment

This preclinical trial is supported by Faculdade de Medicina Veterinária da Universidade de Lisboa, Instituto Politécnico de Leiria(Centre for Rapid and Sustainable Product Development), Instituto Português da Face Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa. The authors are grateful to Joaquim Ferreira from Lisbon Faculty of Medicine for valuable contribution in study design; to Pedro Nunes from Radiology Department of Centro Hospitalar Lisboa Norte for his valuable contribution; and to Joaquim Ângelo and Ermelinda Ângelo for contribution to animal logistics control.

Please cite this article in press as: Ângelo DF, et al., Preclinical randomized controlled trial of bilateral discectomy versus bilateral discopexy in Black Merino sheep temporomandibular joint: TEMPOJIMS – Phase 1- histologic, imaging and body weight results, Journal of Cranio-Maxillo-Facial Surgery (2018), https://doi.org/10.1016/j.jcms.2018.01.006

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jcms.2018.01.006.

References

Agerberg G, Lundberg M: Changes in the temporomandibular joint after surgical treatment. A radiologic follow-up study. Oral Surg Oral Med Oral Pathol 32(6): 865–875, 1971

Angelo DF, Morouço P, Alves N, Viana T, Santos F, González R, et al: Choosing sheep (Ovis aries) as animal model for temporomandibular joint research: morphological, histological and biomechanical characterization of the joint disc. Morphologie 100(331): 223–233, 2016

Ângelo DF, Gil FM, González-Garcia R, Mónico L, Sousa R, Neto L, et al: Effects of bilateral discectomy and bilateral discopexy on black Merino sheep rumination kinematics: TEMPOJIMS - phase 1-pilot blinded, randomized preclinical study. J Craniomaxillofac Surg, 2017a S1010–5182(17)30415-8

Ângelo DF, Monje FG, Gonzalez-Garcia R, Little CB, Monico L, Pinho M, et al: Bioengineered temporomandibular joint disk implants: study protocol for a two-phase exploratory randomized preclinical pilot trial in 18 black Merino sheep (TEMPOJIMS). JMIR Res Protoc 6(3): e37, 2017b

Bjornland T, Larheim TA: Discectomy of the temporomandibular joint: 3-year follow-up as a predictor of the 10-year outcome. J Oral Maxillofac Surg 61(1): 55–60, 2003

Block MS, Bouvier M: Adaptive remodeling of the rabbit temporomandibular joint following discectomy and dietary variations. J Oral Maxillofac Surg 48(5): 482–486, 1990

Boman K: Temporomandibular joint arthrosis and its treatment by extirpation of the disc: a clinical study. Follow-up examination. Acta Chir Scand 95(192), 1947

Cake MA, Read RA, Corfield G, et al: Comparison of gait and pathology outcomes of three meniscal procedures for induction of knee osteoarthritis in sheep. Osteoarthr Cartil 21(1): 226–236, 2013

Casillas RA, Kim B, Fisher H, Zelada JL, Um SS, Coleman KJ: Comparative effectiveness of sleeve gastrectomy versus Roux-en-Y gastric bypass for weight loss and safety outcomes in older adults. Surg Obes Relat Dis 13(9): 1476–1483, 2017

Choukas NC, Toto PD, Schoen JE: Gross and histologic changes in the jaws of the Macaca rhesus monkey following surgery. Oral Surg Oral Med Oral Pathol 27(6): 795–805, 1969

Cohen WA, Servais JM, Polur I, Li Y, Xu L: Articular cartilage degeneration in the contralateral non-surgical temporomandibular joint in mice with a unilateral partial discectomy. J Oral Pathol Med 43(2): 162–165, 2014

Cordeiro PC, Guimaraes JP, de Souza VA, Dias IM, Silva JN, Devito KL, et al: Temporomandibular joint involvement in rheumatoid arthritis patients: association between clinical and tomographic data. Acta Odontol Latinoam 29(3): 123–129, 2016

Dimitroulis G: The role of surgery in the management of disorders of the temporomandibular joint: a critical review of the literature. Part 2. Int J Oral Maxillofac Surg 34(3): 231–237, 2005

Dimitroulis G: A new surgical classification for temporomandibular joint disorders. Int J Oral Maxillofac Surg 42: 218–222, 2013

Embree MC, Iwaoka GM, Kong D, Martin BN, Patel RK, Lee AH, et al: Soft tissue ossification and condylar cartilage degeneration following TMJ disc perforation in a rabbit pilot study. Osteoarthr Cartil 23(4): 629–639, 2015

England BR, Baker JF, Sayles H, Michaud K, Caplan L, Davis LA: Body mass index, weight loss, and cause-specific mortality in rheumatoid arthritis. Arthritis Care Res, Apr 20. 2017

Eriksson L, Westesson PL: Long-term evaluation of meniscectomy of the temporomandibular joint. J Oral Maxillofac Surg 43(4): 263–269, 1985

Eriksson L, Westesson P-L: Discectomy as an effective treatment for painful temporomandibular joint internal derangement: a 5-year clinical and radiographic follow-up. J Oral Maxillofac Surg 59(7): 750–758, 2001

Hagandora CK, Almarza AJ: TMJ disc removal: comparison between pre-clinical studies and clinical findings. J Dent Res 91(8): 745–752, 2012

Hall HD: Meniscectomy for damaged disks of the temporomandibular joint. South Med J 78(5): 569–572, 1985

Ishimaru J, Goss AN: A model for osteoarthritis of the temporomandibular joint. J Oral Maxillofac Surg 50(11): 1191–1195, 1992

Kiehn CL, Desprez JD: Meniscectomy for internal derangement of temporomandibular joint. Br J Plast Surg 15: 199–204, 1962

Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG: Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS Biol 8(6): e1000412, 2010

Laurell KA, Tootle R, Cunningham R, Beltran J, Simon D: Magnetic resonance imaging of the temporomandibular joint. Part II: comparison with laminographic, autopsy, and histologic findings. J Prosthet Dent 58(2): 211–218, 1987

Li L, Wang L, Sun Y, Yang C, He D: Establishment and histological evaluation of a goat traumatic temporomandibular joint model. J Oral Maxillofac Surg 73(5): 943–950, 2015

Little CB, Smith MM, Cake MA, Read RA, Murphy MJ, Barry FP: The OARSI histopathology initiative – recommendations for histological assessments of osteoarthritis in sheep and goats. Osteoarthr Cartil 18(Suppl 3): S80–S92, 2010

Lynch SM, Stricker CT, Brown JC, Berardi JM, Vaughn D, Domchek S, et al: Evaluation of a web-based weight loss intervention in overweight cancer survivors aged 50 years and younger. Obes Sci Pract 3(1): 83–94, 2017

Macher DJ, Westesson PL, Brooks SL, Hicks DG, Tallents RH: Temporomandibular joint: surgically created disk displacement causes arthrosis in the rabbit. Oral Surg Oral Med Oral Pathol 73(6): 645–649, 1992

Malvy E, Thiébaut R, Marimoutou C, Dabis F, Groupe d'Epidemiologie Clinique du Sida en Aquitaine: Weight loss and body mass index as predictors of HIV disease progression to AIDS in adults. Aquitaine cohort, France, 1985-1997. J Am Coll Nutr 20(6): 609–615, 2001

Matías EM, Mecham DK, Black CS, Graf JW, Steel SD, Wilhelm SK, et al: Malocclusion model of temporomandibular joint osteoarthritis in mice with and without receptor for advanced glycation end products. Arch Oral Biol 69: 47–62, 2016

Matsuura H, Miyamoto H, Kurita K, Goss AN: The effect of autogenous costochondral grafts on temporomandibular joint fibrous and bony ankylosis: a preliminary experimental study. J Oral Maxillofac Surg 64(10): 1517–1525, 2006

Melrose J, Fuller ES, Little CB: The biology of meniscal pathology in osteoarthritis and its contribution to joint disease: beyond simple mechanics. Connect Tissue Res 58(3–4): 282–294, 2017

Miyamoto H, Kurita K, Ishimaru J, Goss AN: A sheep model for temporomandibular joint ankylosis. J Oral Maxillofac Surg 57(7): 812–817, 1999

Murphy MK, MacBarb RF, Wong ME, Athanasiou KA: Temporomandibular disorders: a review of etiology, clinical management, and tissue engineering strategies. Int J Oral Maxillofac Implant 28(6): e393–414, 2013

Ogi N, Kurita K, Handa Y, Goss AN: The short-term effect of discectomy on the osteoarthrotic temporomandibular joint in sheep. Int J Oral Maxillofac Surg 25(4): 319–324, 1996

Reston JT, Turkelson CM: Meta-analysis of surgical treatments for temporomandibular articular disorders. J Oral Maxillofac Surg 61(1), 2003 3–10-2

Richmond J: Refinement, reduction, and replacement of animal use for regulatory testing: future improvements and implementation within the regulatory framework. ILAR J 43(Suppl): S63–S68, 2002

Shu CC, Smith MM, Smith SM, Dart AJ, Little CB, Melrose J: A histopathological scheme for the quantitative scoring of intervertebral disc degeneration and the therapeutic utility of adult mesenchymal stem cells for intervertebral disc regeneration. Int J Mol Sci 18(5), 2017

Silver CM: Long-term results of meniscectomy of the temporomandibular joint. Cranio 3(1): 46–57, 1984

Smith MM, Cake MA, Ghosh P, Schiavinato A, Read RA, Little CB: Significant synovial pathology in a meniscectomy model of osteoarthritis: modification by intra-articular hyaluronan therapy. Rheumatology (Oxford) 47(8): 1172–1178, 2008

Souza RF, Lovato da Silva CH, Nasser M, Fedorowicz Z, Al-Muharraqi MA: Interventions for the management of temporomandibular joint osteoarthritis. In: de Souza RF (ed.), Cochrane database of systematic reviews; 2012, 2012

Takaishi M, Kurita K, Matsuura H, Goss AN: Effect of auricular cartilage graft in the surgical treatment of temporomandibular joint ankylosis: an animal study using sheep. J Oral Maxillofac Surg 65(2): 198–204, 2007

Takaku S, Toyoda T: Long-term evaluation of discectomy of the temporomandibular joint. J Oral Maxillofac Surg 52(7), 1994 722–726-8

Takaku S, Sano T, Yoshida M: Long-term magnetic resonance imaging after temporomandibular joint discectomy without replacement. J Oral Maxillofac Surg 58(7): 739–745, 2000

Tolvanen M, Oikarinen VJ, Wolf JA: 30-year follow-up study of temporomandibular joint meniscectomies: a report on five patients. Br J Oral Maxillofac Surg 26(4): 311–316, 1988

Trumpy IG, Lyberg T: Surgical treatment of internal derangement of the temporomandibular joint: long-term evaluation of three techniques. J Oral Maxillofac Surg 53(7), 1995 740–746-7

Wilkes CH: Surgical treatment of internal derangements of the temporomandibular joint. A long-term study. Arch Otolaryngol Head Neck Surg 117(1): 64–72, 1991

Wu G, Zhu S, Sun X, Hu J: Subchondral bone changes and chondrogenic capacity of progenitor cells from subchondral bone in the collagenase-induced temporomandibular joints osteoarthritis rabbit model. Int J Clin Exp Pathol 8(9): 9782–9789, 2015

Xu L, Polur I, Lim C, Servais JM, Dobeck J, Li Y, et al: Early-onset osteoarthritis of mouse temporomandibular joint induced by partial discectomy. Osteoarthr Cartil 17(7): 917–922, 2009

Yaillen DM, Shapiro PA, Luschei ES, Feldman GR: Temporomandibular joint meniscectomy–effects on joint structure and masticatory function in macaca fascicularis. J Maxillofac Surg 7(4): 255–264, 1979

Young AA, Smith MM, Smith SM, Cake MA, Ghosh P, Read RA, et al: Regional assessment of articular cartilage gene expression and small proteoglycan metabolism in an animal model of osteoarthritis. Arthritis Res Ther 7(4): 852–861, 2005

Zhang M, Wang H, Zhang J, Zhang H, Yang H, Wan X, et al: Unilateral anterior crossbite induces aberrant mineral deposition in degenerative temporomandibular cartilage in rats. Osteoarthr Cartil 24(5): 921–931, 2016

Please cite this article in press as: Ângelo DF, et al., Preclinical randomized controlled trial of bilateral discectomy versus bilateral discopexy in Black Merino sheep temporomandibular joint: TEMPOJIMS – Phase 1- histologic, imaging and body weight results, Journal of Cranio-Maxillo-Facial Surgery (2018), https://doi.org/10.1016/j.jcms.2018.01.006