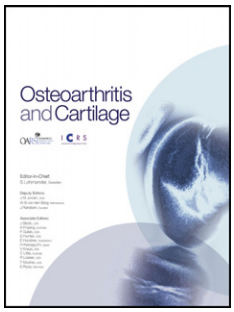
Accepted Manuscript



One-stage focal cartilage defect treatment with bone marrow mononuclear cells and chondrocytes leads to better macroscopic cartilage regeneration compared to microfracture in goats

J.E.J. Bekkers, L.B. Creemers, A.I. Tsuchida, M.H.P. van Rijen, R.J.H. Custers, W.J.A. Dhert, D.B.F. Saris

PII: S1063-4584(13)00756-5

DOI: 10.1016/j.joca.2013.03.015

Reference: YJOCA 2863

To appear in: *Osteoarthritis and Cartilage*

Received Date: 7 January 2013

Revised Date: 24 March 2013

Accepted Date: 31 March 2013

Please cite this article as: Bekkers J, Creemers L, Tsuchida A, van Rijen M, Custers R, Dhert W, Saris D, One-stage focal cartilage defect treatment with bone marrow mononuclear cells and chondrocytes leads to better macroscopic cartilage regeneration compared to microfracture in goats, *Osteoarthritis and Cartilage* (2013), doi: 10.1016/j.joca.2013.03.015.

One-stage focal cartilage defect treatment with bone marrow mononuclear cells and chondrocytes leads to better macroscopic cartilage regeneration compared to microfracture in goats

Running head: one-stage cell-based defect treatment

[1]JEJ Bekkers (j.bekkers@umcutrecht.nl)

[1]LB Creemers (l.b.creemers@umcutrecht.nl)

[1]AI Tsuchida (a.tsuchida@umcutrecht.nl)

[1]MHP van Rijen (m.rijen@umcutrecht.nl)

[1]RJH Custers (r.j.h.custers-3@umcutrecht.nl)

[1,2]WJA Dhert (w.dhert@umcutrecht.nl)

[1,3]DBF Saris (d.saris@umcutrecht.nl)

[1]Department of Orthopaedics, UniversityMedicalCenterUtrecht, Utrecht, the Netherlands

[2]Faculty of Veterinary Medicine, UniversityMedicalCenterUtrecht, Utrecht, the Netherlands

[3]MIRA institute, Department of Tissue Regeneration, University of Twente, Enschede, The Netherlands

Corresponding author:

Prof. dr. D.B.F. Saris, Orthopaedic surgeon

Department of Orthopaedics, University Medical Center

POB 85500, 3508 GA, Utrecht, the Netherlands

Telephone: 0031-88-7551133, Fax: 0031-30-2510638

E-mail: d.saris@umcutrecht.nl

Abstract

Objective

The combination of chondrocytes and mononuclear fraction (MNF) cells might solve the expansion induced dedifferentiation problem of reimplanted cells in autologous chondrocytes implantation as sufficient cells would be available for direct, one-stage, implantation. Earlier in vitro work already showed a positive stimulation of cartilage specific matrix production when chondrocytes and MNF cells were combined. Therefore, this study aimed to evaluate cartilage regeneration using a one-stage procedure combining MNF cells and primary chondrocytes for the treatment of focal cartilage lesions in goats compared to microfracture treatment.

Design

Freshly created focal cartilage defects were treated with either a combination of chondrocytes and MNF cells embedded in fibrin glue or microfracture treatment. After 6 months follow-up local regeneration as well as the general joint cartilage health were evaluated using validated scores and biochemical assays.

Results

Macroscopic ($p=0.015$) scores for the cartilage surface at the treated defect were, after 6 months, significantly higher for the chondrocyteMNF treatment compared to microfracture-treated defects, but microscopic scores were not ($p=0.067$). The articulating cartilage showed more ($p=0.005$) degeneration following microfracture treatment compared to chondrocyte-MNF treatment. Biochemical GAG evaluation did not reveal differences between the treatments. Both treatments had resulted in a slight to moderate cartilage degeneration at other locations in the joint.

Conclusion

In conclusion, treatment of focal articular cartilage lesions in goats using a combination of mononuclear fraction cells from bone marrow and unexpanded chondrocytes leads to better

macroscopic regeneration compared to microfracture, however needs further fine-tuning to decrease the negative influence on other joint compartments.

Keywords:

Mononuclear fraction

Articular cartilage

ACI

Chondrocyte

Goat

Regeneration

Introduction

Autologous chondrocyte implantation (ACI) is frequently used to treat focal cartilage lesions in the knee. Due to the limited number of cells that can be harvested from a biopsy, in vitro cell expansion followed by reimplantation of this precultured population is inevitable and considered to be the main drawback of this relatively successful procedure. A combination of cells could be an alternative for the expansion of harvested chondrocytes in ACI. When isolated chondrocytes are directly combined with another cell type, which also would improve the chondrogenic potential of reimplanted cells, the whole procedure could be performed within one surgery and the quality of regenerated cartilage improved.

The combination of cells derived from native tissue with other cell types has recently gained attention to address currently defined challenges in regenerative medicine.[1] For example, vascular endothelial cells and urothelial cells were both able to differentiate adipose-derived stem cells into respectively the osteogenic and urothelial lineage in order to enhance bone and urothelial tissue engineering.[2,3] Articular cartilage matrix production of expanded articular chondrocytes has been shown to be positively influenced by primary chondrocytes and human adipose derived and embryonic stem cells in vitro.[4-6] In addition, the chondrogenic phenotype of dedifferentiated chondrocytes was improved when cocultured with mesenchymal stromal cells (MSC).[7-9] As these effects were also achieved when conditioned medium and non-contact culture systems were used, the underlying mechanism could be explained by the communication between the cells through trophic factors.[5,8,10] The mononuclear fraction (MNF) in bone marrow is a major source of trophic factors and can easily be obtained in the timeframe of one surgery.[11] Delivery of this bone marrow fraction to full-thickness cartilage defects in horses showed good cartilage healing.[12] For this reason, combination of primary isolated chondrocytes and MNF cells could be the basis for a one-stage cell-based regenerative treatment for focal articular cartilage defects. Therefore, this study aimed to evaluate cartilage regeneration using a one-stage procedure combining MNF cells and primary chondrocytes for the treatment of focal cartilage lesions in goats compared to microfracture treatment.

Materials and Methods

Experimental design:

This experiment was approved by and conducted following the guidelines of the animal care committee of the University of Utrecht under number DEC 2011.III.03.026. A full-thickness chondral lesion was created in the medial condyle of both knees in female Dutch milk goats. Per goat one defect was treated with a combination of chondrocytes and mononuclear fraction cells in fibrin glue (Beriplast®, Nycomed, CSL Behring, the Netherlands) and the other by microfracture. The animals were euthanized at 6 months after surgery. Defect healing and the quality of articular cartilage in all other compartments of the joint were determined using macroscopic, microscopic and biochemical analysis.

Animals:

A total of 9adult Dutch milk goats (age 3-5 years old, weight 75 ± 10 kg) were used in this study. The necessary number of animal (n=9) was determined by a power analysis (power of 0.90 and an α of 0.05) based on the estimated amount of glycosaminoglycans (GAG) (32.2 ± 2.3) at 6 months after cartilage surgery.[13]Once arrived at the animal facility the goats were allowed to acclimatize for at least one week before surgery. During follow-up food was provided ad libitum and general health was assessed by the veterinarian of the institutional animal care facility. Up to two weeks after surgery an additional health scoring system, focusing on wound infection, limping and general activity, was scored.

Surgery:

One day prior to surgery, the goats were weighed and prophylactic pain medication was provided by a fentanyl skin patch. Intravenous premedication (0.4 mg/kg detomidine hydrochloride (Pfizer, the Netherlands) and a single dose of Augmentin(GlaxoSmithKline, the Netherlands) was followed by induction anaesthesia using thiopental (6 mg/kg, Rhône Mérieux, France). During surgery, anaesthesia was maintained by a combination of midazolam 0.8 mg/kg(Abbott Laboratories, the

Netherlands) and sufenta forte 0.007 mg/kg (ASTPharma, the Netherlands) and, if necessary, isoflurane or propofol (Abbott Laboratories).

Surgery was performed under aseptic conditions. Bone marrow was obtained by needle aspiration from the iliac crest. For this a Jamshidi® needle was tapped into the iliac crest. Bone marrow was aspirated using 20ml sterile syringes and stored in heparin coated tubes. After this the whole bone marrow was spun down (300g, 10min) and the cell pellet diluted 50 times in red blood cell lysis buffer (Sigma, the Netherlands) during 45 minutes. Following this the remaining cells were spun down by centrifugation and washed twice in PBS, thus producing the MNF fraction.

A medial parapatellar approach was performed to expose the medial femur condyle. After macroscopic scoring of the medial cartilage surface, using the Mastbergen score[14], a 5mm cylindrical chondral lesion was created in the central weight bearing region using a hand-operated drill. A bone curette was used to debride the remnants of articular cartilage and to create a stable defect rim. The debrided defect cartilage was digested using a rapid-digestion which resulted in chondrocytes with similar chondrogenic potential, in terms of cartilage matrix formation, compared to chondrocytes obtained after standard overnight isolation (data not shown).. For this, cartilage was cut into small pieces and digested during 45 minutes in 2% collagenase type II solution (Worthington, Lakewood, USA) in Dulbecco's modified Eagle's medium (DMEM, GIBCO, USA) with penicillin/streptomycin (100 U/ml/100 µg/ml, Invitrogen, The Netherlands) under continuous shaking at 37ºC. Following digestion the collagen was washed out by 3 washing steps. This digestion protocol resulted in a digestion efficiency of $1.37 \pm 0.50*10^6$ viable chondrocytes per gram debrided tissue. Meanwhile, the debrided defect was treated using the microfracture technique by creating 4 holes through the subchondral bone using a 1.5 mm K-wire. Microfracture treatment was confirmed by the presence of blood entering the defect from the drilled holes. Following this the knee was closed in layers.

The other knee was opened, the medial cartilage surface scored and a chondral defect created using similar procedures as described above. A mixture of 10% rapidly isolated chondrocytes and 90% MNF cells were suspended in the fibrinogen component of Beriplast® (Nycomed) at a final concentration of $1*10^6$ cells per milliliter. The fibrinogen component and thrombin component of the Beriplast® were prepared for application following the manufacturers' protocol and injected into the cartilage defect. After five minutes of gelation time the knee was flexed 10 times to check the stability of the graft into the defect and the knee was closed in layers.

Direct full-weight bearing was allowed following surgery. The animals were housed individually for a period of 24 hours. Additional pain medication and antibiotics were provided, based on the judgment of the veterinarian, with approval of JEJB. The animals were euthanized after 6 months using an overdose of pentobarbital (Euthesate®) and both hind legs explanted for further analysis.

Macroscopic cartilage evaluation:

Soft tissues were removed from the explanted hind legs and high resolution pictures obtained from the medial (MFC) and lateral femoral condyle(LFC) and tibial cartilage surfaces (medial (MTP, lateral LTP) and from the cartilage defect. Pictures were coded for blinded scoring by two observers. The articular cartilage of the medial and lateral tibia and femur as well as the medial tibia cartilage that directly articulated with the treated defect were scored using the macroscopic Mastbergen score.[14] The Mastbergen score is a 4-point scale ranging from a macroscopically healthy and smooth cartilage surface (0 points) to cartilage degeneration characterized by deep grooves and surrounding cartilage damage (4 points). The close-up pictures from the cartilage defect were scored using the ICRS macroscopic evaluation of cartilage repair score.[15]This score (0-12 points scale) evaluates the macroscopic cartilage repair on degree of defect repair and fill, integration into border zone and macroscopic appearance. The higher the score the better the macroscopic cartilage repair. For both macroscopic scoring systems the scores of the two observers were averaged. Where individual items differed more than 2 points between observers consensus was reached.

Microscopic cartilage evaluation:

Microscopic evaluation was performed on osteochondral samples derived from the central weight bearing lateral femur and tibia, the medial tibia cartilage that articulated with the defect and from the posterior half of the defect. Samples were fixed in 10% buffered formalin for 48 h followed by a decalcification process using Luthra's solution (3.2% 11 M HCl, 10% formic acid in distilled water). Following this samples were dehydrated by graded alcohol series, immersed in xylene and embedded in paraffin. Sections (5 µm) were stained with safranin-O (Merck, Germany) for GAG. Counterstaining for nuclei and cytoplasm were performed with respectively Weigert's haematoxylin (Klinipath, the Netherlands) and 0.4% fast green (Merck, Germany). The microscopic quality of cartilage regeneration was assessed using the O'Driscoll score.[16,17] This score (range 0-24points) evaluates the regenerated

cartilage on the amount of Safranin-O staining in the matrix, cellular morphology and clustering, the structural characteristics of the tissue and degenerative changes in adjacent tissue. The higher the score the better the microscopic cartilage regeneration. All other osteochondral cartilage samples were evaluated using the Mankin score which ranges from normal appearing articular cartilage (0 points) to tissue with complete disorganization, no matrix staining and hypocellularity or cloning (14 points). Similar score processing from the two observers was performed as with the macroscopic scoring.

DNA and GAG content:

Full-thickness articular cartilage was, if present, obtained from the anterior part of the treated defect, the cartilage that articulated with the defect and from the lateral weight bearing femur and tibia cartilage. Samples were weighed and digested overnight in papain (250µg/ml papain (Sigma-Aldrich) in 50 mM EDTA and 5 mM L-cysteine) at 56ºC.GAG content was determined using a dimethylmethylene blue (DMMB) assay[18] where the complexation of GAGs with DMMB was measured spectophotometrically at 540 nm, using 595 nm as a reference. Chondroitin sulphate (shark; Sigma-Aldrich) was used as a standard. Per sample, the DNA content was also determined from the papain digest using a Picogreen DNA assay (Invitrogen) in accordance with the manufacturer's instructions.

Statistical analysis:

All analyses were performed using SPSS version 15.0 (Chicago, Illinois, USA). Outcome variables showed a normal distribution tested by the Kolmogorov-Smirnov test ($p>0.05$) and equality of variance as determined by the Levene's test ($p>0.05$). Differences between the two treatments in macroscopic score, microscopic score, GAG/gram tissue and GAG/DNA were tested using a paired samples t-test. A $p<0.05$ was considered statistically significant. All outcomes in graphs are presented as mean and 95% CI. Biochemical GAG analysis are described using mean ± standard deviation.

Results

Animal health and follow-up:

No signs of surgery-related complications like wound or joint infection were observed after surgery and all animals were able to move their joints and load their limbs without limitations. After two weeks no additional pain medication was provided. However, 2 animals died. One animal died directly after surgery due to an already existing, unrecognized, pulmonary infection. The other goat was euthanized at 2 months follow-up due to progressive weight loss and increased infection parameters which were, after obduction, related to a large intra-abdominal cyst. The maximal weight loss of the goats that reached 6 months follow-up was 4.5 ± 3.0% compared to their preoperative weight.

Macroscopic evaluation:

Peroperative macroscopic evaluation of the articular surfaces showed superficial fibrillations that were related to the central weight bearing cartilage of the medial femoral condylein 3 cases. Peroperative macroscopic scores of femur cartilage surfaces did not differ between the two groups receiving the treatments ($p=0.62$). Post-treatment macroscopic scores of the articulating tibia cartilage were statistically significantly ($p=0.005$) higher in the microfracture-treated defects compared to the chondrocyteMNF-treated defects. This indicates increased cartilage degeneration of the articulating tibia cartilage following microfracture treatment of a defect in the femur compared to chondrocyteMNF treatment. Other joint locations (lateral tibia and femur cartilage and medial femoral condyle) did not show statistically significant differences, however, the medial compartment showed relatively high Mastbergen scores indicative for moderate degeneration(Figure 1). Overall, microfracture treatment showed less defect fill at 6 months compared to chondrocyteMNF treatment(Figure 2). Also when the quality of macroscopic cartilage regeneration was scored, the chondrocyteMNF treatment scored statistically significantly ($p=0.015$) higher compared to the microfracture-treated defects.

Microscopic evaluation:

Mankin microscopic score for cartilage degeneration showed no statistically significant differences between the two treatments for cartilage surfaces adjacent to the treated defects (Figure 3). The

microscopic appearance of the regenerated tissue in the cartilage defects treated with chondrocyteMNF appeared better compared to the microfracture treated defects although still incomplete defect fill was present after chondrocyteMNF treatment (Figure 4). The O'Driscoll score for the chondrocyteMNF-treated defects appeared to be higher compared to the microfracture treated defects, although this did not fully reach statistical significance (p=0.067).The cartilage derived from the lateral compartment and cartilage surrounding the defect showed slight cartilage degeneration while those from the articulating medial tibia cartilage showed moderate cartilage degeneration for both treatments (Figure 3).

Biochemical evaluation:

GAG production per gram of regenerated tissue did not show statistically significant differences between the two treatments (25.61 ± 14.95 mg GAG per gram tissue versus 23.51 ± 6.82 mg GAG per gram tissue for the microfracture and chondrocyteMNF respectively). Also for the other joint locations, the microfracture treatment (lateral femur 27.26 ± 7.09 mg GAG per gram tissue, lateral tibia 30.90 ± 9.89 mg GAG per gram tissue, articulating cartilage 28.34 ± 10.11 mg GAG per gram tissue) did not result in statistically significant differences in GAGs per gram tissue compared to chondrocyteMNF treatment (lateral femur 33.87 ± 7.04 mg GAG per gram tissue, lateral tibia 25.04 ± 11.76 mg GAG per gram tissue, articulating cartilage 25.73 ± 10.51 mg GAG per gram tissue). Also for GAG content normalized to DNA content, no difference between the treatments was observed (microfracture: lateral femur 284 ± 113 mg GAG/mg DNA, lateral tibia 390 ± 332 mg GAG / mg DNA, articulating cartilage 462 ± 278 mg GAG / mg DNA vschondrocyteMNF: lateral femur 313 ± 144 mg GAG / mg DNA, lateral tibia 215 ± 116 mg GAG / mg DNA, articulating cartilage 416 ± 208 mg GAG / mg DNA).

Discussion

This study shows that treatment of a freshly created cartilage defect using a combination of mononuclear fraction cells and primary chondrocytes, mixed in fibrin glue, leads to better macroscopic and a trend towards better microscopic cartilage regeneration compared to microfracture after 6 months follow-up in a goat model. Microfracture treatment resulted in more macroscopic cartilage degeneration of the directly opposing articulating tibia cartilage compared to chondrocyteMNF treatment. After 6 months follow-up both treatments had resulted in slight to moderate cartilage degeneration at more remote locations in the joint. GAG and DNA content did not differ between the two treatments at the regenerated defect cartilage nor for cartilage samples from other locations in the joint.

Recent studies have shown that an incongruent cartilage surface introduces high peak forces to the directly articulating cartilage resulting in cartilage degeneration.[19] These effects were not only observed after elevated implantation of osteochondral implants but also after microfracture or due to untreated defects.[19-22] Inability to restore the articular cartilage surface will make the partly regenerated cartilage prone to damage as a non-continuous cartilage surface has a higher tendency to deform which initiates accelerated matrix damage and tissue loss.[23-25]Also, an incongruent cartilage surface and incomplete defect fill, which was mainly observed after microfracture but also present in chondrocyteMNF treated defects, could have contributed to increased shear forces at the surface of the articulating cartilage, thereby introducing cartilage degeneration. This will initiate a cascade of matrix damage in the treated joint compartment and eventually the contralateral compartment and whole knee. For this reason, the incomplete defect fill in this study could have contributed to the slight to -moderate degeneration seen after 6 months follow-up in both treatments. In addition to this, the bilateral approach could also have added to the slight degeneration observed in both treatments at distant locations in the joint. This is important as most treatment failures or insufficient clinical improvement after cartilage therapy can eventually be brought down to inadequate defect fill and tissue regeneration.[26] Short term follow-up in this study at least shows better defect fills as resembled by the higher macroscopic scores obtained after chondrocyteMNF treatment. However, definite and more objective conclusions could be observed after longer follow-up (12-24 months) which should be considered in future animal studies of this approach.

The factor of success in the combination of chondrocytes, MNF cells and fibrin glue is difficult to determine. Concentrated bone marrow, derived from spinning down whole bone marrow aspirates, showed better macroscopic and microscopic cartilage formation compared to microfracture in an equine model.[12]Also, bone marrow nucleated cells combined with a collagen hydrogel contributed to the repair of full-thickness focal lesions in minipigs.[27] These studies show that the use of crude bone marrow cells in cartilage repair has potential. However, results so far did not lead to a clinical translation. If concentrated bone marrow would be that potent, in terms of cartilage regeneration, the added value of adding chondrocytes could be questioned as well. Others already showed the added value of adding cells from mesenchymal origin to chondrocytes in culture.[8-10,28] These studies mainly evaluated the combinations of chondrocytes and MSCs and also show that MSCs disappear during culture. This supports the idea of chondrocytes being responsible for tissue regeneration whereas added cells rather steer this chondrogenic response by trophic factors. MNF is speculated to be a potent source of trophic factors as well.[11] How these trophic factors influence the fast digested chondrocytes in this study is still subject to speculation, however growth factors and stimulating cytokines could play a major role. The presented study design is insufficient to answer such mechanistic questions. Ideally, without taking animal ethics into account, another two experimental groups should have been added to the study. One that only consists of rapidly digested chondrocytes and another that consists of only MNF cells in fibrin glue. Additionally, the chondrocyteMNF group could use labeled cells in order to provide data on cell faith and contribution in the newly formed cartilage.

The role of the carrier for the cell combinations should be considered as well. A carrier of cells should provide stability during the first periods of cartilage regeneration and degrade inversely related to the cartilage matrix that is being formed. For this study we selected fibrin glue as a carrier of cells because it is already clinically applied, easy to handle and, most important, fibrin sealants facilitate cartilage regeneration and show a high (>90 %) chondrocyte viability.[29] New techniques also show that fibrin glue can be used as a vehicle for chondrocyte migration from minced cartilage pieces,[30] while fibrin glue could also be used as an augmentation of microfracturing to provide stability to the bone marrow entering the knee.

In conclusion, this study shows that the treatment of a focal articular cartilage lesion using a combination of mononuclear fraction cells from bone marrow and unexpanded chondrocytes leads to

statistical significantly higher macroscopic regeneration scores compared to microfracture, however needs further fine-tuning to improve defect fill and decrease the negative influence on other joint compartments before clinical translation if safe and useful.

Acknowledgements:

The authors greatly acknowledge the support of the TeRM Smart Mix Program of the Netherlands Ministry of Economic Affairs and the Netherlands Ministry of Education, Culture and Science.
L.B.C is funded by the Dutch Arthritis Association

Contributions:

JEJ Bekkers, LB Creemers, AI Tsuchida, MHP van Rijen have all contributed substantially to the conception and design of the study and the acquisition or interpretation of data.
All authors have substantially contributed to drafting and critically revising the article and have approved the final version of the article.

Role of funding sources:

None of the funding sources had any influence on the design of the study, acquisition and interpretation of the data, drafting the manuscript or decisions on where to submit the manuscript.

Conflict of interest statement:

None of the authors have any conflict of interest related to the work presented.

Reference List

(1) Hendriks J, Riesle J, van Blitterswijk CA. Co-culture in cartilage tissue engineering. J Tissue Eng Regen Med 2007;1(3):170-8.

(2) Shi JG, Fu WJ, Wang XX, Xu YD, Li G, Hong BF, et al. Tissue engineering of ureteral grafts by seeding urothelial differentiated hADSCs onto biodegradable ureteral scaffolds. J Biomed Mater Res A 2012;May 21.

(3) Zhao X, Liu L, Wang FK, Zhao DP, Dai XM, Han XS. Coculture of Vascular Endothelial Cells and Adipose-Derived Stem Cells as a Source for Bone Engineering. Ann Plast Surg 2012;May 23.

(4) Bigdeli N, Karlsson C, Strehl R, Concaro S, Hyllner J, Lindahl A. Coculture of human embryonic stem cells and human articular chondrocytes results in significantly altered phenotype and improved chondrogenic differentiation. Stem Cells 2009 Aug;27(8):1812-21.

(5) Gan L, Kandel RA. In vitro cartilage tissue formation by Co-culture of primary and passaged chondrocytes. Tissue Eng 2007 Apr;13(4):831-42.

(6) Hildner F, Concaro S, Peterbauer A, Wolbank S, Danzer M, Lindahl A, et al. Human adipose-derived stem cells contribute to chondrogenesis in coculture with human articular chondrocytes. Tissue Eng Part A 2009 Dec;15(12):3961-9.

(7) Chen WH, Lai MT, Wu AT, Wu CC, Gelovani JG, Lin CT, et al. In vitro stage-specific chondrogenesis of mesenchymal stem cells committed to chondrocytes. Arthritis Rheum 2009 Feb;60(2):450-9.

(8) Mo XT, Guo SC, Xie HQ, Deng L, Zhi W, Xiang Z, et al. Variations in the ratios of co-cultured mesenchymal stem cells and chondrocytes regulate the expression of cartilaginous and osseous phenotype in alginate constructs. Bone 2009 Jul;45(1):42-51.

(9) Tsuchiya K, Chen G, Ushida T, Matsuno T, Tateishi T. The effect of coculture of chondrocytes with mesenchymal stem cells on their cartilaginous phenotype in vitro. Mater Sci Eng 2004;C24:391-6.

(10) Wu L, Leijten JC, Georgi N, Post JN, van Blitterswijk CA, Karperien M. Trophic Effects of Mesenchymal Stem Cells Increase Chondrocyte Proliferation and Matrix Formation. Tissue Eng Part A 2011 Feb 28.

(11) Balakumaran A, Robey PG, Fedarko N, Landgren O. Bone marrow microenvironment in myelomagenesis: its potential role in early diagnosis. Expert Rev Mol Diagn 2010;10(4):465-80.

(12) Fortier LA, Potter HG, Rickey EJ, Schnabel LV, Foo LF, Chong LR, et al. Concentrated bone marrow aspirate improves full-thickness cartilage repair compared with microfracture in the equine model. J Bone Joint Surg Am 2010;92(10):1927-37.

(13) Saris DB, Dhert WJ, Verbout AJ. Joint homeostasis. The discrepancy between old and fresh defects in cartilage repair. J Bone Joint Surg Br 2003 Sep;85(7):1067-76.

(14) Mastbergen SC, Marijnissen AC, Vianen ME, Zoer B, van Roermund PM, Bijlsma JW, et al. Inhibition of COX-2 by celecoxib in the canine groove model of osteoarthritis. Rheumatology (Oxford) 2006 Apr;45(4):405-13.

(15) van den Borne MP, Raijmakers NJ, Vanlauwe J, Victor J, de Jong SN, Bellemans J, et al. International Cartilage Repair Society (ICRS) and Oswestry macroscopic cartilage evaluation

scores validated for use in Autologous Chondrocyte Implantation (ACI) and microfracture. Osteoarthritis Cartilage 2007 Dec;15(12):1397-402.

(16) O'Driscoll SW, Keeley FW, Salter RB. The chondrogenic potential of free autogenous periosteal grafts for biological resurfacing of major full-thickness defects in joint surfaces under the influence of continuous passive motion. An experimental investigation in the rabbit. J Bone Joint Surg Am 1986 Sep;68(7):1017-35.

(17) O'Driscoll SW, Keeley FW, Salter RB. Durability of regenerated articular cartilage produced by free autogenous periosteal grafts in major full-thickness defects in joint surfaces under the influence of continuous passive motion. A follow-up report at one year. J Bone Joint Surg Am 1988 Apr;70(4):595-606.

(18) Farndale RW, Buttle DJ, Barrett AJ. Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethylmethylene blue. Biochim Biophys Acta 1986 Sep 4;883(2):173-7.

(19) Becher C, Huber R, Thermann H, Paessler HH, Skrbensky G. Effects of a contoured articular prosthetic device on tibiofemoral peak contact pressure: a biomechanical study. Knee Surg Sports Traumatol Arthrosc 2008 Jan;16(1):56-63.

(20) Custers RJ, Dhert WJ, van Rijen MH, Verbout AJ, Creemers LB, Saris DB. Articular damage caused by metal plugs in a rabbit model for treatment of localized cartilage defects. Osteoarthritis Cartilage 2007 Aug;15(8):937-45.

(21) Custers RJ, Saris DB, Dhert WJ, Verbout AJ, van Rijen MH, Mastbergen SC, et al. Articular cartilage degeneration following the treatment of focal cartilage defects with ceramic metal implants and compared with microfracture. J Bone Joint Surg Am 2009;91(4):900-10.

(22) Custers RJ, Dhert WJ, Saris DB, Verbout AJ, van Rijen MH, Mastbergen SC, et al. Cartilage degeneration in the goat knee caused by treating localized cartilage defects with metal implants. Osteoarthritis Cartilage 2010;18(3):377-88.

(23) Braman JP, Bruckner JD, Clark JM, Norman AG, Chansky HA. Articular cartilage adjacent to experimental defects is subject to atypical strains. Clin Orthop Relat Res 2005 Jan;(430):202-7.

(24) Gratz KR, Wong BL, Bae WC, Sah RL. The effects of focal articular defects on intra-tissue strains in the surrounding and opposing cartilage. Biorheology 2008;45(3-4):193-207.

(25) Gratz KR, Wong BL, Bae WC, Sah RL. The effects of focal articular defects on cartilage contact mechanics. J Orthop Res 2009 May;27(5):584-92.

(26) Nehrer S, Spector M, Minas T. Histologic analysis of tissue after failed cartilage repair procedures. Clin Orthop Relat Res 1999 Aug;(365):149-62.

(27) Zhang Y, Wang F, Chen J, Ning Z, Yang L. Bone marrow-derived mesenchymal stem cells versus bone marrow nucleated cells in the treatment of chondral defects. Int Orthop 2012;36(5):1079-86.

(28) Wu L, Prins HJ, Helder MN, van Blitterswijk CA, Karperien M. Trophic effects of Mesenchymal Stem Cells in Chondrocyte Co-Cultures are independent of Culture Conditions and Cell Sources. Tissue Eng Part A 2012.

(29) Dare EV, Griffith M, Poitras P, Wang T, Dervin GF, Giulivi A, et al. Fibrin sealants from freshl/frozen plasma as scaffolds for in vivo articular cartilage regeneration. Tissue Eng Part A 2012;15(8):2285-97.

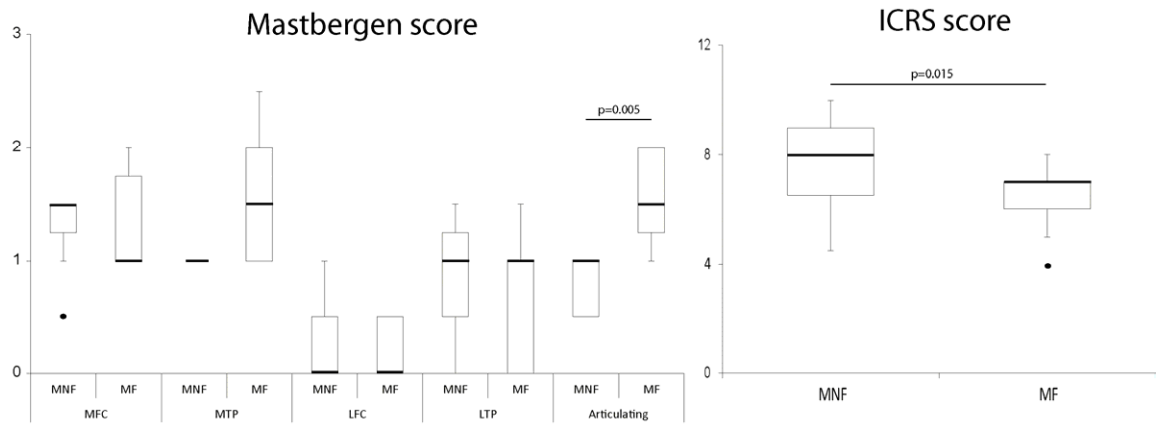
(30) Ahmed TA, Hincke MT. Strategies for articular cartilage lesion repair and functional restoration. Tissue Eng part B Rev 2010;16(3):305-29.

Figure 1:
Title: Macroscopic scores 6 months after treatment
Legend: Macroscopic scores show no differences between the treatments at 6 months follow-up for the medial femur (MFC) and tibia (MTP) surfaces, lateral femur (LFC) and tibia (LTP) surfaces and the articulating defect surface. At the treated defect site the chondrocyteMNF-treatment showed a statistically significant (p=0.015) higher macroscopic ICRS score compared to the microfracture treated defects. Scores are presented as boxplots.

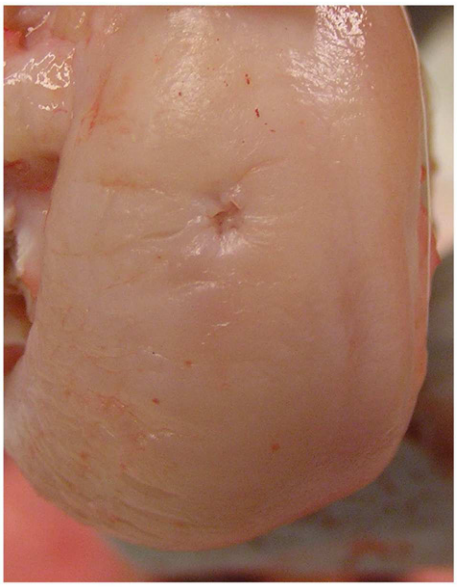
Figure 2:
Title: Representative examples of macroscopic repair after 6 months follow-up
Legend: Macroscopic examples of defect fill at 6 months follow-up for both treatments. Microfracture treatment showed incomplete macroscopic defect fill while the chondrocyteMNF treatment showed complete macroscopic fill.

Figure 3:
Title: Microscopic scores 6 months after treatment.
Legend: Microscopic scores for the articular cartilage surrounding and directly articulating with the treated defect and the lateral femur (LFC) and tibia (LTP) surfaces for both the chondrocyteMNF (MNF) and microfracture (MF) treatment. No statistically significant differences were observed in microscopic score between the two treatments for the mentioned scores. However, a trend towards statistical significance (p=0.067) was observed in microscopic score between chondrocyteMNF and microfracture treatment at the treated defect. Scores are presented as boxplots.

Figure 4:
Title: Best, mean and worst result of microscopic repair after 6 months follow-up.
Legend: Cross-sectional slices perpendicular to the center of the treated defect at 6 months follow-up for the best, mean and worst result of both treatments showing incomplete defect fill for the microfracture treatment while nearly complete defect fill following chondrocyteMNF was observed in some cases.



Microfracture



ChondrocyteMNF