Dissertation

Mesenchymal Stem Cell Constructs for Repair of Focal Cartilage Defects in an Ovine Model

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Abstract

Focal cartilage defects (FCD) of the knee joint remain a difficult area of treatment for orthopaedic surgeons, as they often progress to generalized osteoarthritis (OA). Osteochondral autograft transfer (OAT) to the damaged cartilage area has shown promise, but this has been associated with pain and bleeding at the site of graft harvest. The use of mesenchymal stem cells (MSCs) in a matrix to regenerate articular cartilage has been proposed. This work describes a prospective case-control series comparing OAT with a novel, MSC-seeded scaffold graft in the stifle joints of healthy merino sheep. The triphasic grafts were composed of a beta-tricalcium phosphate osseous phase, an intermediate activated plasma phase and a collagen I hydrogel cartilage phase. The osseous and cartilage phases were seeded with autologous MSCs. All sheep underwent creation of a full-thickness, 4.0 mm diameter FCD (n=20) followed by six weeks of unrestricted activity, allowing the defects to degenerate naturally. At six weeks, half of the lesions were treated with OAT and half with the triphasic engineered grafts.

At 6-month and 12-month follow-up, no significant differences were noted between groups with regard to overall histological scores. Macroscopic and biomechanical analysis at 12 months showed no significant differences between groups. In summary, autologous MSC-seeded implants showed comparable repair quality to OAT without the associated donor site morbidity.

I. Table of Contents

II. List of abbreviations

ACI	Autologous Chondrocyte Implantation
AMIC	Autologous Matrix-Induced Chondrogenesis
CT	Computed Tomography
DGOU	German Society of Orthopaedic Surgery and Traumatology
FCD	Focal Cartilage Defect
ICRS	International Cartilage Repair Society
MACI	Matrix-associated Autologous Chondrocyte Implantation
MSC	Mesenchymal Stem Cell
OA	Osteoarthritis
OAT	Osteochondral Autograft Transfer
PEG	Polyethylene Glycol
PLGA	Poly(Lactic-co-Glycolic Acid)
TCP	Beta-Tricalcium Phosphate
VHS1	Visual Histological Scale-1

III. Foreword

This dissertation uses the cumulative form of promotion, as the related publication appeared in the Journal of Orthopaedic Research in December 2010. This publication, which includes the background, methods, results and discussion of the experimental data, is included in this work.

Publication details:

Marquass B[1], Somerson JS[1], Hepp P, Aigner T, Schwan S, Bader A, Josten C., Zscharnack M, Schulz RM. A novel MSC-seeded triphasic construct for the repair of osteochondral defects. Journal of Orthopaedic Research. 2010 Dec; 28(12): 1586-99.
[1]These authors contributed equally to the work.

1. Introduction

Injuries to the articular cartilage represent an area of medicine in which current therapies cannot fully restore a joint to a native, pre-injury state. For this reason, new treatment modalities are necessary to prevent these injuries from progressing to osteoarthritis (OA), a disease state that substantially restricts health-related quality of life and employment capacity.

Current options for treatment include techniques that stimulate the underlying bone below the cartilage layer to produce new tissue, transplantation of existing tissue into the defect, joint replacement using conventional prostheses and implantation of engineered tissue into the defect. The limitations of each of these approaches will be discussed in brief, followed by a review of the osteochondral tissue-engineering approach used in the present work and opportunities for future development.

1.1 Epidemiology and clinical background

Focal cartilage defects (FCDs) are a frequent cause of disability and functional limitations. A review of over 30,000 knee arthroscopies identified FCDs in 63% of patients, with an average of 2.7 lesions per patient (Curl et al., 1997). In the acute phase, these injuries cause substantial limitations and reductions in quality of life, with objective clinical scores similar to those of patients with end-stage arthritis (Heir et al., 2010). A high economic burden from absenteeism and work-related disability has also been demonstrated (Lindahl et al., 2014). Of additional concern is the potential for focal lesions to develop into generalized OA. FCDs have a limited potential for spontaneous healing given the poor vascular supply and hypocellularity of joint cartilage (O'Driscoll, 1998). As a result, these injuries have the potential to initiate a degenerative cascade with progressive development of OA.

A longitudinal study demonstrated increased cartilage loss in healthy adults with existing FCDs, indicating that the presence of these defects is a risk factor for degenerative joint disease (Cicuttini et al., 2005). The burden of disease and poor prognosis without treatment have led to development of numerous surgical management strategies.

1.2 Marrow-stimulating techniques

In response to lack of vascularity in articular cartilage, surgeons have developed various techniques that seek to bring blood supply to the damaged cartilage by penetrating subchondral bone. These techniques are grouped under the term “marrow-stimulating techniques”, as they share the goal of recruiting marrow-based stem cells to the repair site (see Figure 1). The mechanism of this technique has recently been shown to involve chondroinduction with collagen II deposition in the deep and middle cartilage zones (Hoemann et al., 2015). Early animal studies in large and small animal models demonstrated the need to penetrate the subchondral bone to obtain defect coverage with fibrocartilage tissue (Kim et al., 1991; Vachon et al., 1986). The technique in most common use today is microfracture, in which the defect area is treated with penetration of subchondral bone using small hand-held picks in an arthroscopic procedure (Steadman et al., 1997). A recent systematic review of Level I and II studies demonstrated good short-term results of microfracture for small lesions, but worsening outcomes after two to five years (Goyal et al., 2013). Despite this, the widespread use of microfracture in clinical practice as well as the low cost and simplicity of the procedure make it a commonly used gold standard by which other cartilage repair techniques are measured. Modifications to this method include the addition of a matrix material to the site of microfracture to better contain the released marrow elements, a practice known as autologous matrix-induced chondrogenesis (AMIC) (Behrens, 2005).

Clinical trials have shown superior cartilage repair quality at one and five year follow-up with AMIC compared to standard microfracture, but no difference in clinical scores has been demonstrated (Shive et al., 2015; Stanish et al., 2013).

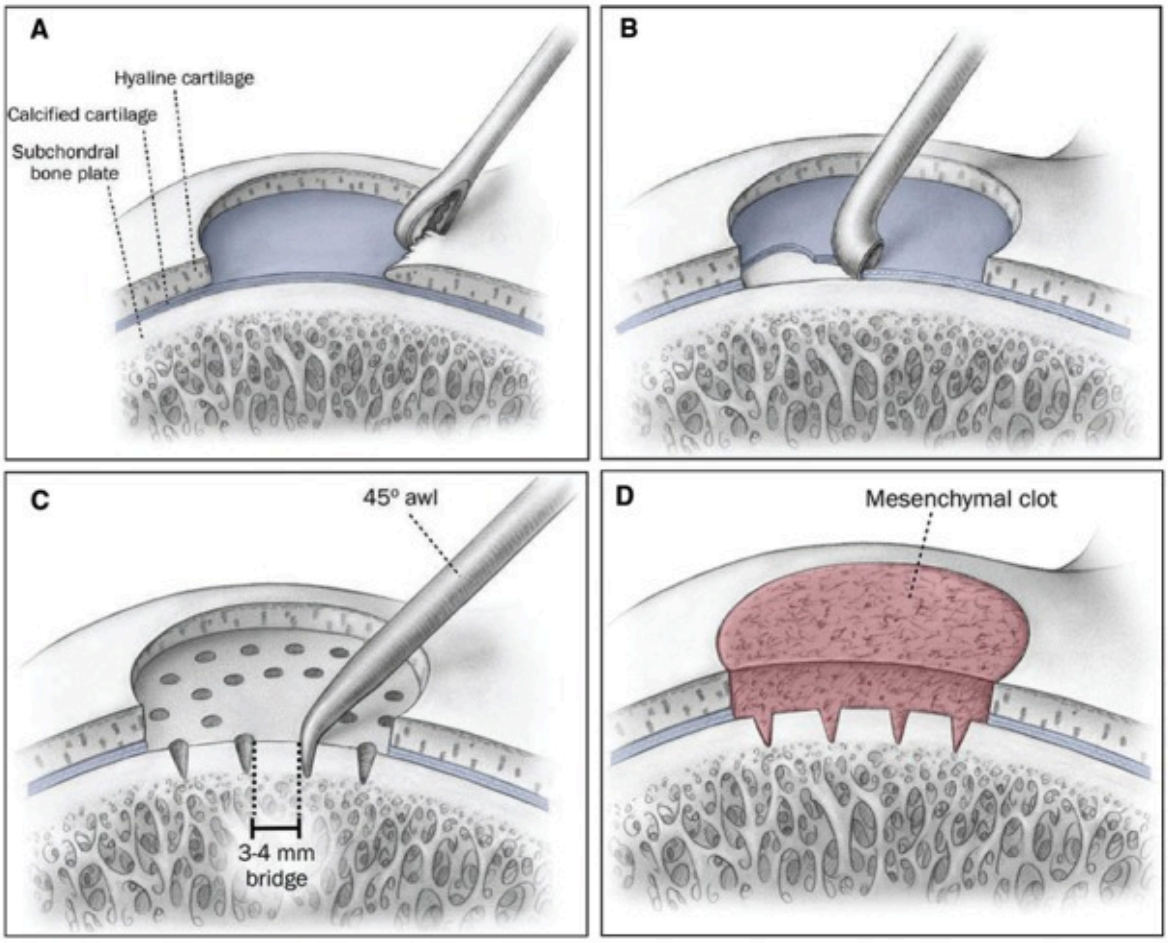

Figure 1: Microfracture technique. (A) Debridement of margins, (B) Removal of calcified cartilage layer, (C) Perforation of subchondral bone, (D) Resulting mesenchymal clot (Figure taken from Mithoefer et al., 2006).

1.3 Autologous chondrocyte or stem cell implantation

Autologous chondrocyte implantation (ACI) was initially described in the 1990s as a method for harvesting cartilage tissue, expanding the harvested chondrocytes, and injecting the cultured cells into cartilage defects under a periosteal membrane as shown in Figure 2 (Brittberg et al., 1994). Long-term case series using this method have demonstrated significant improvements in functional outcome scores at five to twenty years after implantation (Beris et al., 2012; Minas et al., 2013; Peterson et al., 2010). Comparison of ACI to OAT in a prospective randomized trial demonstrated a lower failure rate at long-term follow-up in the ACI group (17%) compared to OAT (55%) (Bentley et al., 2012).

More recently, researchers have implanted the harvested cells after seeding them onto a collagen matrix, giving rise to the name “matrix-associated autologous chondrocyte implantation”, or MACI (Bartlett et al., 2005). The superiority of the matrix technique over the standard periosteal membrane technique has not been demonstrated in randomized controlled trials (Gooding et al., 2006, p. 20; Zeifang et al., 2010). Although ACI/MACI has the recommendation of the German Society of Orthopaedic Surgery and Traumatology (DGOU) for focal cartilage injuries larger than 3-4 cm^2 (Niemeyer et al., 2013), significant negative aspects of the technique include a two-stage surgery in order to harvest graft tissue, the expense of chondrocyte culture and a high rate of reoperation due to hypertrophy of the graft in periosteal techniques (Richter et al., 2015).

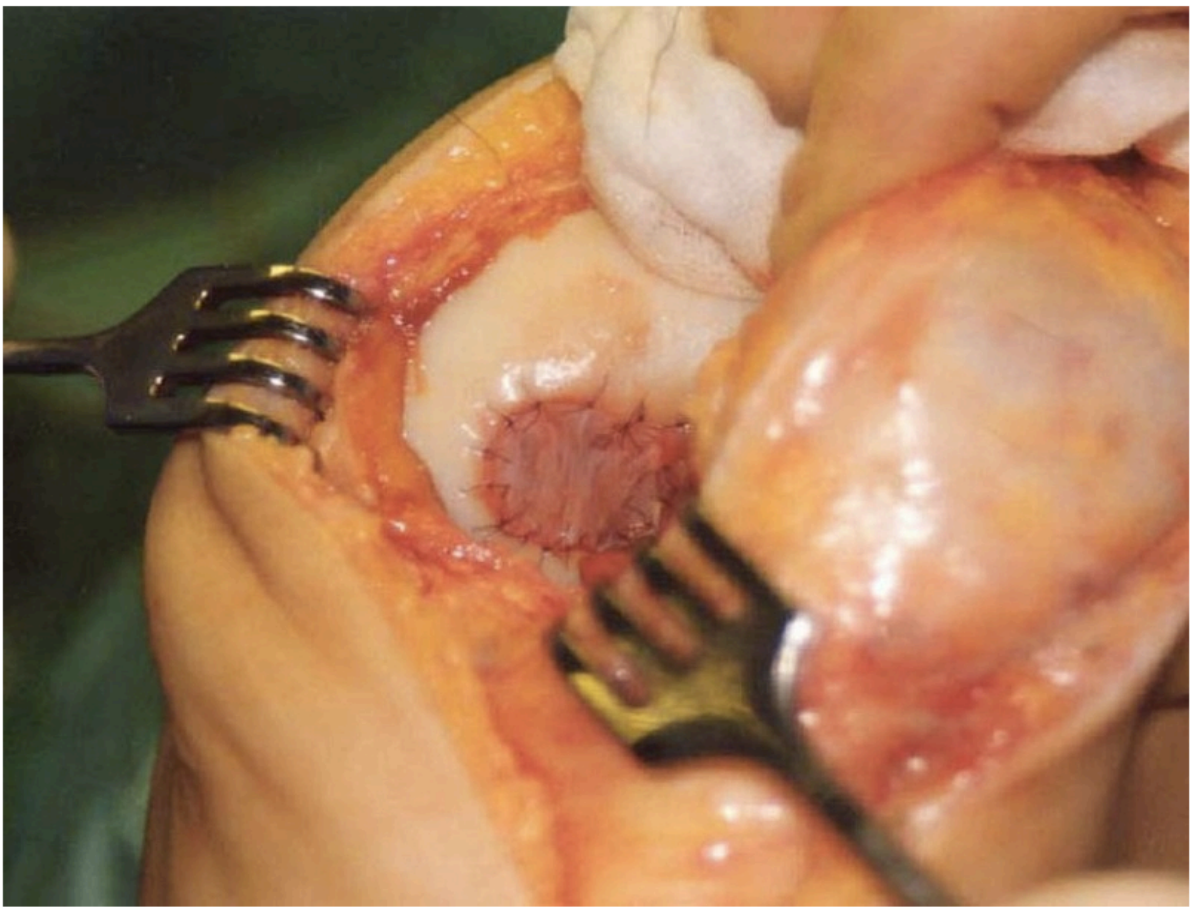

Figure 2: Intraoperative image of a focal cartilage defect treated with first-generation autologous chondrocyte implantation (Figure taken from Mithoefer et al., 2012).

1.4 Osteochondral autograft transfer

The principle of OAT involves harvest of one or more osteochondral grafts from non-weight-bearing portions of the knee and transfer into weight-bearing defect regions as shown in Figure 3. Hangody et al. coined the term “mosaicplasty” to describe the practice of shaping multiple autografts to fill a single defect (Hangody et al., 1997). A report of 831 patients undergoing mosaicplasty reported good-to-excellent results in 92% of patients (Hangody and Füles, 2003). However, a systematic review comparing OAT to microfracture demonstrated no significant differences in activity levels, functional outcome scores, or cost-efficacy (Miller et al., 2015). The OAT technique may cause morbidity at the donor site where the plugs are harvested, which also limits the size of defects that can be repaired (LaPrade and Botker, 2004). In order to avoid this, researchers have sought other approaches including osteochondral tissue-engineered scaffolds.

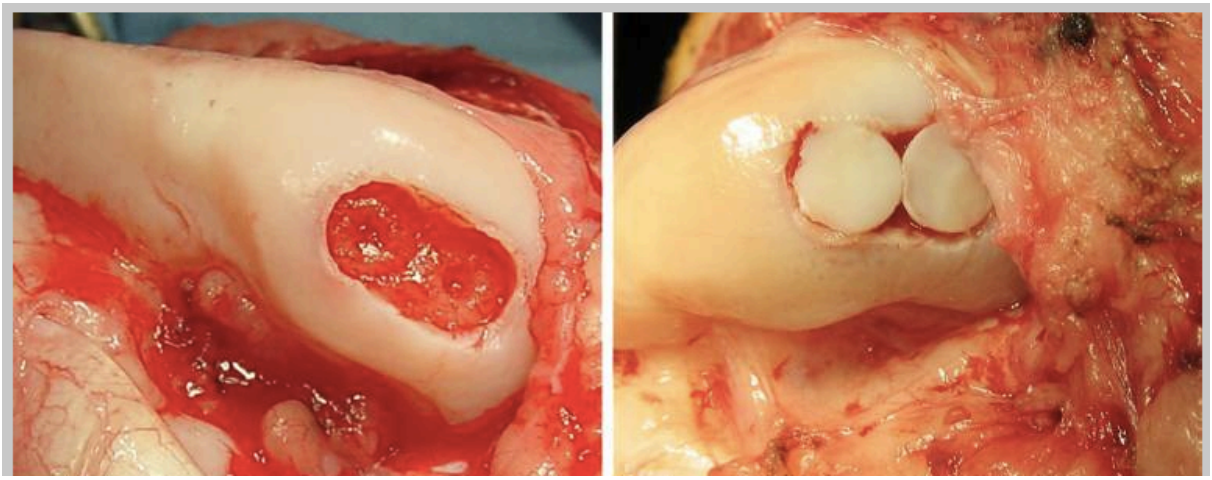

Figure 3: Clinical example of a large cartilage defect treated with osteochondral allograft transfer (Figure taken from Kock et al., 2011).

1.5 Osteochondral tissue-engineered scaffolds

To mimic the native layers of cartilage and subchondral bone found in the knee, multilayer synthetic scaffolds have been developed and tested in preclinical animal models. These scaffolds can be seeded with chondrocytes or mesenchymal stem cells (MSCs) to promote regeneration of healthy cartilage tissue (Caplan and Goldberg, 1999). The layers of these scaffolds are composed of materials designed to replicate the mechanical and biological qualities of cartilage and bone. For the cartilage layer, materials have included polyethylene glycol (PEG) (Alhadlaq and

Mao, 2005), calcium polyphosphate (Kandel et al., 2006), hyaluronic acid (Ahn et al., 2009; Kon et al., 2014; Miot et al., 2012), chitosan-gelatin (Chen et al., 2011), chondroitin sulfate/hyaluronic acid/gelatin (Deng et al., 2014) as well as collagen (Kon et al., 2010; Schleicher et al., 2013). Of these, type 1 collagen (col1) was chosen for the present study given its mechanical properties and ability to allow MSC adhesion (Zhang et al., 2013).

With regard to the subchondral osseous phase, a material must be chosen with adequate initial mechanical strength, good cell ingrowth properties and integration with native surrounding bone (Shimomura et al., 2014). Researchers have reported on cell-seeded osseous materials including hyaluronic acid/chitosan/gelatin (Chen et al., 2011), ceramic bovine bone/gelatin (Deng et al., 2014) as well as beta-tricalcium phosphate (TCP) (Mellor et al., 2015; Meng et al., 2014; Seo et al., 2015). The observed quality of TCP to resorb and be replaced by native bone makes it an excellent choice for tissue-engineering approaches seeking to restore native anatomy (Artzi et al., 2004).

1.6 Cartilage restoration approaches with mesenchymal stem cells

MSCs have shown great promise for cartilage regeneration techniques for a variety of reasons. For one, MSCs can be pre-differentiated prior to implantation to direct them to a chondrocyte lineage, as has been reported by several pre-clinical studies (Lam et al., 2014; Lee et al., 2015; Marquass et al., 2011; Spaas et al., 2015; Tang et al., 2015; Zscharnack et al., 2010). In contrast to chondrocytes, which dedifferentiate with culture expansion, MSCs treated with predifferentiation accumulate cartilage-specific matrix properties after implantation (Rackwitz et al., 2014). Contaminating hematopoietic cells and heterogeneity of MSCs isolated from bone marrow has been a concern, but advances in isolation, selection and culture methods have resulted in efficient and homogeneous cell populations (Baustian et al., 2015). Finally, the immune-privileged status of MSCs creates the possibility of off-the-shelf allogeneic transplantation of cells, uncoupling the cell source from the patients who are treated (Smith et al., 2015). This approach has been supported clinically by a study demonstrating regeneration of meniscus tissue and no adverse immune effects with injection of allogeneic MSCs into the knee joint (Vangsness et

al., 2014).

1.7 Clinical applications of cell-seeded osteochondral scaffolds to date

Studies using cell-seeded bilayer scaffolds in small animal model have showed good formation of regenerated tissue and integration between the graft and surrounding native cartilage (Gao et al., 2002; Lam et al., 2014). However, based on the International Cartilage Repair Society (ICRS) guidelines for preclinical testing of cartilage repair techniques, larger animal models such as goat, sheep or horse are recommended for pivotal studies (Hurtig et al., 2011). These results have been replicated in large animal models with mid-term follow-up (Kandel et al., 2006; Kon et al., 2010). A recent study using scaffolds made of poly(lactic-co-glycolic acid) (PLGA) seeded with MSCs showed good quality of cartilage regeneration in a sheep model at 12 months, with better results seen in MSC-seeded scaffolds compared to those seeded with mature cartilage cells (Caminal et al., 2015).

1.8. Aim of the dissertation

Despite many advances in the field of cartilage repair, there remains a need for a technique that is single-stage, cost-effective and provides reliable long-term outcomes (Richter et al., 2015). To this end, further large animal studies are needed prior to clinical experimentation in humans. Use of a tissue-engineered osteochondral implant seeded with MSCs obtained from bone marrow aspiration was chosen as a potential answer to the problems of donor site morbidity and surface area limitations of OAT techniques.

A cooperative project between the Department of Orthopedics, Plastic Surgery and Traumatology at the University Clinic of Leipzig and the Translational Center for Regenerative Medicine at the University of Leipzig was initiated to compare two methods of repair of chronic, critical-size focal cartilage lesions in a sheep model: (1) repair using an OAT and (2) repair using a tissue-engineered triphasic construct consisting of a TCP osseous phase, an intermediate activated plasma phase and a collagen I hydrogel phase. Outcomes were determined based on validated histological scoring systems at six and twelve months. The study was performed after obtaining authorization from the local legal representative for animal studies.

2. Publication

A Novel MSC-Seeded Triphasic Construct for the Repair of Osteochondral Defects

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ABSTRACT: Mesenchymal stem cells (MSC) are increasingly replacing chondrocytes in tissue engineering based research for treatment of osteochondral defects. The aim of this work was to determine whether repair of critical-size chronic osteochondral defects in an ovine model using MSC-seeded triphasic constructs would show results comparable to osteochondral autografting (OATS). Triphasic implants were engineered using a beta-tricalcium phosphate osseous phase, an intermediate activated plasma phase, and a collagen I hydrogel chondral phase. Autologous MSCs were used to seed the implants, with chondrogenic predifferentiation of the cells used in the cartilage phase. Osteochondral defects of 4.0 mm diameter were created bilaterally in ovine knees ($n = 10$). Six weeks later, half of the lesions were treated with OATS and half with triphasic constructs. The knees were dissected at 6 or 12 months. With the chosen study design we were not able to demonstrate significant differences between the histological scores of both groups. Subcategory analysis of O'Driscoll scores showed superior cartilage bonding in the 6-month triphasic group compared to the autograft group. The 12-month autograft group showed superior cartilage matrix morphology compared to the 12-month triphasic group. Macroscopic and biomechanical analysis showed no significant differences at 12 months. Autologous MSC-seeded triphasic implants showed comparable repair quality to osteochondral autografts in terms of histology and biomechanical testing. J. Orthop. Res. 28: 1586–1599, 2010

Keywords: cartilage; osteoarthritis; osteochondral autografting; animal model; mesenchymal stem cells

Localized articular cartilage defects are frequently encountered by practicing orthopedic surgeons, but their proper treatment remains a controversial subject. It is widely held that cartilage lesions larger than a critical size result in an increased risk of progression to osteoarthritis (OA).[1] Osteochondral autograft techniques such as the Osteochondral Autograft Transfer System (OATS) or mosaicplasty are used in clinical practice for repair of localized femoral condyle cartilage lesions and have shown good long-term results.[2,3] However, this method presents a number of limitations. For one, morbidity at the donor site limits the size of defects that can be repaired to an optimal maximum size of 1–4 cm^2.[4] Post-operative symptoms related to the donor site such as persistent pain have been reported by several authors.[5–7] Donor site bleeding has been implicated as a cause of early hemarthrosis.[4,8] In addition, the surgical difficulty of shaping host tissue to fit the defect area is a limiting factor.[9] Inadequate bonding of the graft cartilage to surrounding tissue is a common finding noted in second-look arthroscopy in clinical subjects[10] as well as histological analysis in animal models.[11,12]

Synthetic tissue-engineered multiphasic implants consisting of distinct cartilage and bone layers are an area of increasing research interest. This treatment method has the potential to restore the anatomical osteochondral junction without iatrogenic injury at donor sites and with the flexibility to treat larger lesions.[13] Biphasic structures cultivated with autologous osteogenic and/or chondrogenic cells have shown promising results in vitro.[14–16] The majority of in vivo studies investigating multiphasic scaffolds have been performed on small animal models.[17–20] Kandel et al.[21] investigated biphasic constructs in a sheep model showing in vivo results after 9 months. This study suggests that biphasic constructs may be suitable to repair joint defects. However, this study used autologous chondrocytes for the cartilage layer instead of bone marrow-derived MSCs. In addition, nearly all of this research is done on a model of acute cartilage injury in which generation of a defect is immediately followed by repair in a single surgery. To more accurately model the chronic cartilage damage seen in human subjects undergoing cartilage repair, use of a chronic injury model may be more appropriate.[22] Based on superior in vivo results for MSC-seeded constructs in comparison to chondrocyte-seeded implants[23] and due to the demonstrated osteogenic and chondrogenic potential of MSCs,[15,18,24–28] we developed a new MSC-based triphasic construct. The objective of this study was to compare the clinically established OATS technique with triphasic MSC-seeded implants for treatment of chronic osteochondral defects. We hypothesized that repair of critical-size chronic osteochondral defects using

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Table 1. O'Driscoll Histological Score

	Cell Morphology (0–4)	Toluidine Blue Staining (0–3)	Surface Regularity (0–3)	Structural Integrity (0–2)	Thickness (0–2)	Bonding to Adjacent Cartilage (0–2)	Hypocellularity (0–3)	Chondrocyte Clustering (0–2)	Freedom From Degenerative Changes (0–3)	Total
OATS 6 months (mean ± SD)	2.8[a] ± 0.9	1.8 ± 0.4	1.0 ± 0.8	0.7[d] ± 0.4	0.9 ± 0.5	0.5[a] ± 0.4	2.4 ± 0.3	1.7 ± 0.3	1.4[d] ± 0.6	13.1 ± 2.6
Triphasic 6 months (mean ± SD)	1.9 ± 0.3	1.5[c] ± 0.3	1.5 ± 0.8	0.8 ± 0.6	0.7 ± 0.4	1.5 ± 0.5	2.0 ± 0.4	2.0 ± 0.0	1.5 ± 0.3	13.5 ± 2.4
OATS 12 months (mean ± SD)	2.8[b] ± 0.7	2.2 ± 0.2	1.7 ± 0.6	1.4 ± 0.4	1.1 ± 0.5	1.3 ± 0.9	2.7 ± 0.6	1.3[b] ± 0.0	2.1 ± 0.4	16.5 ± 2.6
Triphasic 12 months (mean ± SD)	1.5 ± 0.7	2.3 ± 0.6	1.0 ± 1.0	1.1 ± 0.6	0.5 ± 0.5	1.5 ± 0.5	2.2 ± 0.9	1.8 ± 0.3	1.6 ± 0.4	13.5 ± 4.5

[a] $p < 0.05$ compared with triphasic 6 months.
[b] $p < 0.05$ compared with triphasic 12 months.
[c] $p < 0.05$ compared 6 versus 12 months inside the triphasic group.
[d] $p < 0.05$ compared 6 versus 12 months inside the OATS group.

MSC-seeded triphasic constructs would show cartilage properties comparable to osteochondral autografting as well as good integration of the osseous phase. To the best of the authors' knowledge, no prior studies showing 12-month results of MSC-based multiphasic cartilage repair in a chronic large animal defect model have been published.

MATERIALS AND METHODS

A complete description of the methodology used in this study can be found in Appendix A.

Animals

Ten skeletally mature and healthy female merino sheep with an age of 2–2.5 years and an average weight of 65 kg (57–75 kg) were used for this study. The sheep were randomly divided into two groups with follow-ups of 6 and 12 months. The right hind knee of each sheep was treated with a triphasic scaffold and the left knee was treated with an osteochondral autograft. The animals were treated in accordance with applicable animal protection laws (Paragraph 8, Section 1) and authorization by the local legal representative was granted (TVV/07).

Bone Marrow Aspiration and Triphasic Implant Production

Prior to the first surgery, bone marrow aspirates were obtained from each sheep and MSCs were isolated and expanded for 4 weeks to passage one. Venous blood was also collected from each sheep and autologous EDTA plasma and serum were isolated. For the chondral phase, 4.0×10^5 MSCs/mL were mixed with a clinically approved collagen type I hydrogel (CaReS®, Arthro Kinetics Biotechnology, Krems, Austria) and cultured for 14 days in a chondrogenic medium (Chondrogenic Bullet Kit®, Lonza, Cambridge) with 10 ng/mL TGF-β3. Simultaneously, 1×10^6 MSCs were mixed with autologous EDTA plasma and 0.1 M $CaCl_2$ and seeded onto resorbable β-TCP implants (CERASORB®, Curasan, Kleinostheim, Germany) with a diameter of 6 mm and length of 10 mm. The cylinders contained four parallel-oriented, vertical macropores with a diameter of 1 mm, resulting in a true porosity of 65%. The osseous constructs were then cultured for 14 days in autologous expansion medium (Fig. 1).

In Vitro Analysis of Constructs

The viability of the MSCs in both composites was determined following culture using fluorometric live/dead staining. Gene expression profiles of the chondral constructs were determined using RT-PCR with primers as shown in Table 1 of Appendix A. Scanning electron microscopy (SEM) was performed to confirm cell morphology and distribution on the constructs. Immunohistochemistry of cryosections of the chondral phase was performed for aggrecan and collagen type II (Fig. 1).

Surgical Techniques

In the primary surgery, bilateral full-thickness defects with a diameter of 4 mm were created to 2 mm below the calcified layer in the medial femoral condyles of the sheep using a medial arthrotomy (Fig. 2A). After 6 weeks, the animals were returned for a second surgery with implantation of the constructs. The original arthrotomy was reopened and the initial defects (Fig. 2B) were cored out using a 6.4 mm harvesting drill to a depth of 12 mm. In the right knee, an MSC-seeded

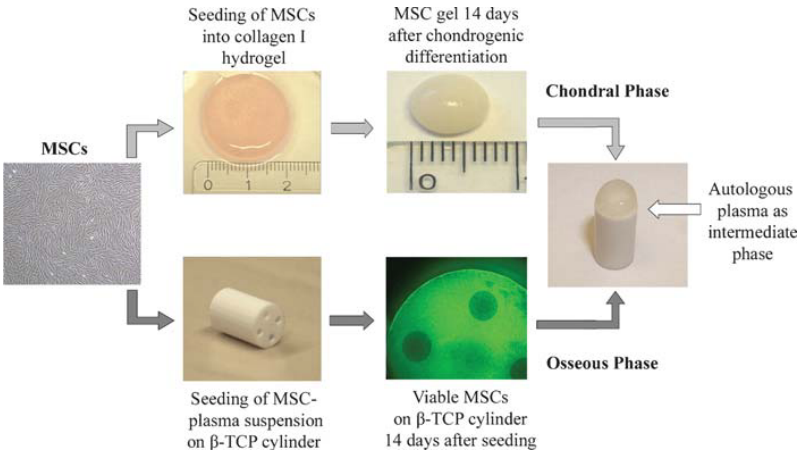

Figure 1. Overview of triphasic implant production.

β-TCP cylinder was inserted (Fig. 2C). Autologous cancellous bone taken from the harvested cylinder was used to fill the 200 μm gap between the β-TCP cylinder and the surrounding native bone. The intermediate activated autologous plasma phase was applied and a seeded collagen I gel phase was affixed and fitted to the condyle surface (Fig. 2D). In the left knee, the patella was released medially and laterally subluxated to allow for harvest of an osteochondral cylinder of 6.6 mm in diameter and 12 mm depth. The cylinder was implanted using a press-fit technique. After 6 or 12 months, the hind knees were explanted for analysis of regeneration. Each sample was first visually graded by a specialist orthopedist using the Brittberg ICRS Visual Scale.[29,30] This scale assesses the degree of defect repair, integration to the border zone and macroscopic surface appearance with a rating of 0–4, where 4 represents the best appearance. After biomechanical analysis of the samples using a ball indentation test, the samples underwent plastination for preparation of histological slides. Toluidine blue[31] and Levai–Laczko[32,33] stains were used. The slides were graded independently by three reviewers using the ICRS Visual Histological Scale,[34] the O'Driscoll Scale[35] and the semiquantitative Siebert score.[36]

High-resolution micro-computed tomography (μCT) scans were made of all triphasic constructs (La Theta™ LCT-100, Aloka, Inc., Tokyo, Japan). The slice thickness was set to 0.1 mm. Measurements of the sinking distance of the β-TCP cylinders were then made using computer software (ImageJ, NIH, Bethesda, MD) by drawing a tangent to the subchondral bone surface and measuring perpedicular from this to the highest point on the β-TCP cylinder.

Statistics

For assessment of statistical significance, non-parametric Mann–Whitney *U* tests were done using SPSS (SPSS, Inc., Chicago, IL). Statistical significance was set at $p < 0.05$.

RESULTS

See Appendix B for cell viability and extracellular marker accumulation/expression results.

Assessment of Tissue Regeneration

Macroscopy

Visual evaluation according to the ICRS Brittberg score revealed significantly higher scoring in terms of "macroscopic appearance" (OATS 6 months: 2.6 ± 0.6, 12 months: 2.8 ± 1.1; triphasic 6 months: 1.4 ± 0.6, 12 months: 2.0 ± 0.7) and "degree of defect repair" (OATS 6 months: 4.0 ± 0.0, 12 months: 3.8 ± 0.5; triphasic 6 months: 3.2 ± 0.8, 12 months: 3.2 ± 1.3) for the OATS group after 6 months (Fig. 3A and B). At 12 months the OATS group showed higher values for both parameters without being statistically significant. Nearly no difference in terms of "bonding to adjacent cartilage" was observed macroscopically at 6 and 12 months.

Histological Analysis

ICRS Visual Histological Scale

We found no statistical differences in overall scores between the OATS and triphasic groups after 6 and 12

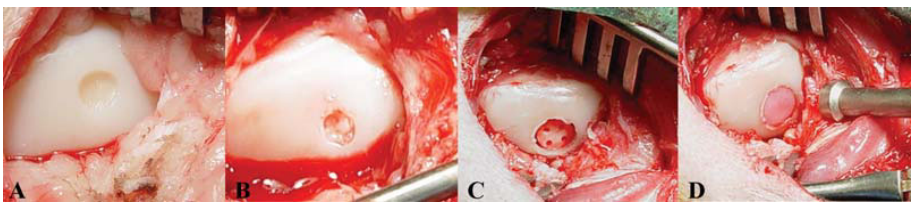

Figure 2. Implantation of triphasic construct. (A) Fresh defect immediately after creation. (B) Chronic defect at re-arthrotomy after 6 weeks. (C) Osseous phase with visible β-TCP cylinder. (D) Chondral phase after implantation.

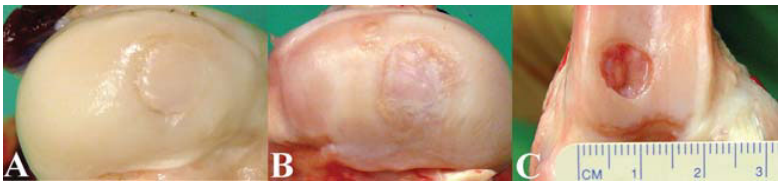

Figure 3. Macroscopic views after 6 months of OATS (A), triphasic (B), and repair tissue at donor site (C).

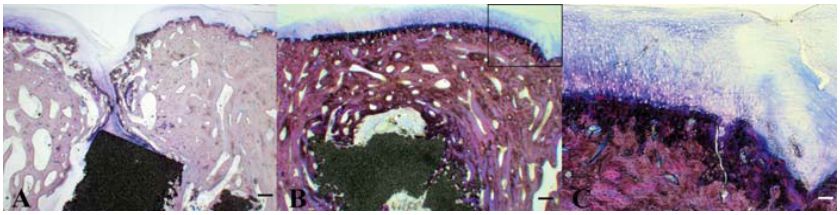

Figure 4. Histology of triphasic construct repair tissue (Levai–Laczko stain). (A) Sinking of the osseous construct with incomplete subchondral bone coverage (6-month specimen, 500 μm bar). (B) Complete subchondral bone coverage (12-month specimen, 500 μm bar). (C) Five times magnification of rectangle shown in (B) (100 μm bar).

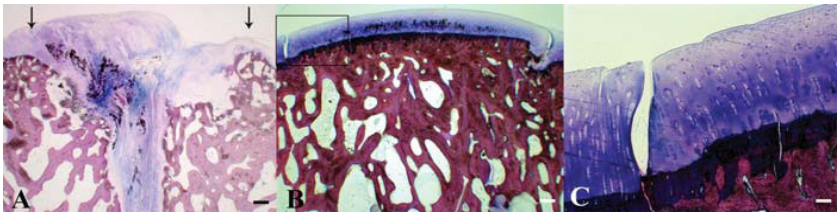

Figure 5. Histology of OATS specimens (Levai–Laczko stain). (A) Severe cyst formation was noted in this 6-month specimen (500 μm bar). Arrows indicate original osteochondral implant borders. (B) 12-month specimen with incomplete cartilage bonding (500 μm bar) and (C): 5× magnification of rectangle shown in (B) (100 μm bar).

months using the ICRS scale (Figs. 4–6). Analyzing the subcategories individually, we observed significantly higher scores in the OATS group for "matrix composition," "cell distribution," and "cell population" after 12 months (Fig. 7). *O'Driscoll score*: No significant differences between the total scores of the two groups could be observed (Fig. 8 and Table 1). As with ICRS scoring, we found significant differences here in "cell morphology" with higher values for the OATS group after 6 and 12 months. "Bonding to adjacent cartilage" was significantly better for triphasic implants than autografts after 6 months but not after 12 months. Within the triphasic group, the repair tissue showed significantly higher "toluidine blue staining" after 12 months than after 6 months. Within the OATS group, better "structural integrity" for the cylinder was observed after 1 year compared to the results after 6 months. In addition, less degenerative changes were found after 12 months. *Siebert Semiquantitative Score*: Total scores and subcategory scores showed no significant differences between the two groups at either endpoint. Although "bonding to adjacent cartilage" was greater in the triphasic implants at 6 months (OATS: 0.7 ± 0.6; triphasic: 1.5 ± 0.5), this difference was not statistically significant ($p = 0.071$). For the triphasic group we observed higher scores ($p < 0.01$) for "subchondral reconstruction" after 1 year (3.2 ± 0.7) compared to 6 months (0.8 ± 1.0) (Figs. 4–8, Table 1).

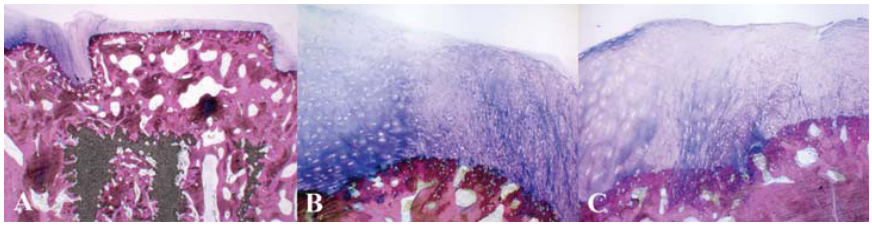

Figure 6. Histology of triphasic specimen (A). Magnification of the same triphasic (B) and OATS (C) histology (Levai–Laczko stain) at 5× showing the native/regenerated cartilage border. Native cartilage is seen on the left in (B) and (C).

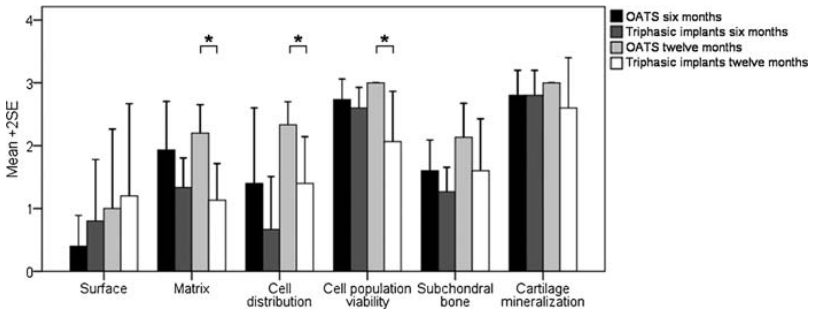

Figure 7. ICRS visual histological score by subcategories showing 2× standard error (2SE). Differences of statistical significance are marked with the * symbol.

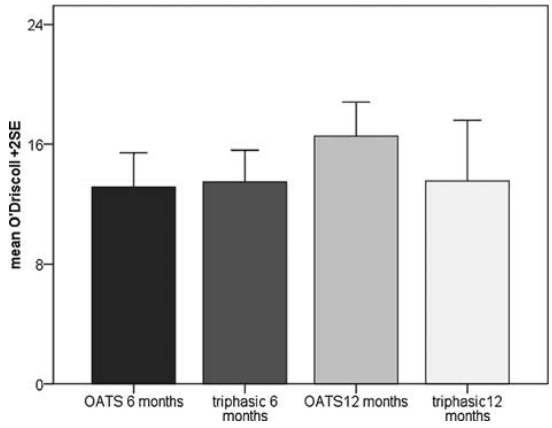

Figure 8. O'Driscoll score, group totals showing 2× standard error (2SE).

Biomechanical Testing

Indentation forces showed no significant differences between the 6-month triphasic (0.09 ± 0.04 N) and OATS (0.15 ± 0.10 N) groups or between the 12-month triphasic (0.15 ± 0.02 N) and OATS (0.11 ± 0.05 N) groups (Fig. 9). One measurement from the 12-month triphasic group was not included in the analysis due to a technical error. Significantly ($p < 0.047$) softer repair tissue in comparison to native cartilage (0.16 ± 0.04 N) was measured for the 6-month triphasic group (0.09 ± 0.04 N). At 12 months, no statistically significant difference between triphasic regenerated cartilage and native cartilage (0.13 ± 0.03 N) was observed.

Micro-CT

Measurements revealed that in total four triphasic implants were sunken more than 3 mm below the subchondral bone plate. The average distance between the tip of the β-TCP implant and the subchondral bone was 2.6 mm after 6 months and 2.4 mm after 12 months.

DISCUSSION

The concept of a triphasic scaffold arose from experimentation with MSC-seeded matrix-associated autologous chondrocyte implantation (MACI) methods for repair of osteochondral defects that showed frequent dislocation

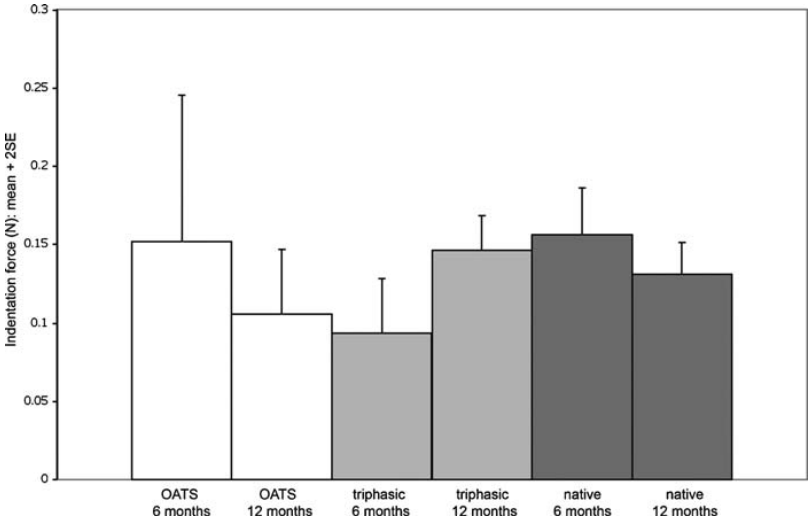

Figure 9. Ball indentation test showing 2× standard error (2SE).

of the cartilage matrix and subchondral cavity formation. In response to this, an MSC-seeded osseous phase was added to provide subchondral stability and an intermediate activated plasma layer was used for fixation. In addition, an experimental study showed superior results with in vivo cartilage repair when prior in vitro chondrogenic differentiation was induced.[37] Based on these findings, this study describes a novel triphasic construct for repair of deep osteochondral defects using a collagen I scaffold seeded with chondrogenically differentiated MSCs, an intermediate layer of autologous plasma and a porous, seeded β-TCP cylinder. This implant was compared to the clinically established OATS technique using a validated large animal model for degenerated critical-size cartilage defects.[22] In contrast to our first hypothesis, the OATS group did appear to have superior cartilage matrix morphology. However, histological analysis showed generally similar results between the osteochondral autograft and triphasic groups, as no significant differences were observed in the total sum of any of the 3 histological scores used for analysis. The superior cartilage bonding to adjacent native tissue seen in the 6-month triphasic group was a notable finding given the well-known issue of incomplete cartilage bonding in osteochondral autografts.[10–12] It further demonstrates the capacity of MSCs to differentiate into various tissues[38] and the ability of MSCs to achieve good bonding to surrounding native cartilage.[39] Additional longer-term studies are required here to chart the course of repair provided by these implants.

Our second hypothesis was that the autografts and β-TCP implants would both show good bone integration. For the β-TCP implants, we even noted significant resorption of the implant and ingrowth of trabecular bone. This supports the good biocompatibility of the implants, a concern presented by a previous study examining non-porous calcium phosphate scaffolds.[40] On the other hand, sinking of the β-TCP cylinders more than 3 mm into the underlying bone was noted in 4 of the 10 samples with accompanying new formation of a subchondral bone layer above the cylinder, a phenomenon that was not observed in the autograft groups. MSCs have osteogenic potential, particularly in combination with osteosupportive material, such as hydroxyapatite/β-TCP.[41] If this bony overgrowth were derived from the MSCs seeded onto the β-TCP implant, one would assume a more polydirectional growth pattern originating from the β-TCP. Instead of this, we observed overgrowth from the cylinder's rim. Thus the new bone formation does not appear to originate within the β-TCP implant, but rather appears to grow from the subchondral bone at both sides of the defect zone until the implant is covered. This is in accordance with a study performed by Petersen et al.,[40] which showed sinking of an implant with accompanying bone overgrowth. The regeneration of the subchondral layer over time is in line with similar long-term studies.[40,42] In another study, Guo et al.[43] published results of cartilage repair using MSC-seeded β-TCP scaffolds, but did not report any sinking or dislocation of the implants. One possible explanation is that a defect depth of 4 mm measured from the cartilage surface was used. Assuming a cartilage thickness of 1.5 mm and a further 1–2 mm for the calcified layer and subchondral bone plate,[44] the implants might not have been as deep inside the cancellous bone, allowing for superior fixation. Given the relative softness of the cancellous femoral condyle in comparison to the rigid subchondral bone plate, one might assume that under weight-bearing, the rigid β-TCP implant is pushed deeper into the cancellous bone. Comparable mean sinking values after 6 and 12 months indicate that this process most probably occurs in the early post-implantation phase. Pulliainen et al.[45] reported sinking of PLDLA scaffolds and attributed this to impact on the joint surface causing cracks in the subchondral bone. This effect might also be related to the implantation technique used for the triphasic implants, which may not have provided the same immediate retention force as the press-fit implantation of the OATS cylinders. Further studies in which postoperative weight-bearing can be limited are needed to confirm this and MRI imaging of gross joints with in situ implants could provide more precise, quantifiable information.

Some possible shortcomings of the OATS method were also seen in our research. In 7 of the 10 autografts, subchondral peri-implant cyst formation was seen in histological analysis (Fig. 5A). This did not always correlate with the surface appearance of the cartilage, and the functional significance of this finding is unclear. However, reports of this in other animal model studies of osteochondral autografts lead us to believe that this is not an isolated occurrence.[46,47] We also noticed severe degeneration at the donor sites with incomplete tissue filling and substantial bone exposure (Fig. 3C). Although the significance of donor site morbidity remains a controversial topic, the poor healing response noted in this study would seem to correlate well with previous findings of poor tissue quality and postoperative pain. Use of a more extensive arthrotomy including patellar luxation for the OATS method is one factor that requires discussion. After plug transfer, the medial retinaculum was reconstructed and patellar tracking was tested to ensure normal alignment. Harvesting the osteochondral plug from the medial trochlea is a commonly used method, but this requires patellar luxation. Although we cannot be certain that this did not influence our final outcome, we feel that the following points make this unlikely. First, the animals were clinically observed two times a day in the early postoperative period with regard to wound healing and effusion. We found no differences between both knees, indicating that no additional hematoma or bleeding was present in the OATS-treated knees. Furthermore, no postoperative patellar luxation occurred. At the time of harvest, a normal gait pattern was observed in all animals. Third, the defect on the medial condyle was in the load-bearing area influenced by vertical load. Even if instability or lateralization of

the patella would have been present, an effect on plug healing would be unlikely. Another point to discuss in this regard is the potential for different load-bearing in the hind limbs. We did not measure the load-bearing, which is a limitation of the study design. However the defects were created and later treated in the same size and location on both sides. By operating on both knees at the same time, unloading of one leg was difficult to obtain for the animal, thus ensuring similar load-bearing of both knees. Further limitations of this study included the small sample size used, which was limited by experimental board approval. The data obtained from this study will allow for accurate power analysis in future studies and provide support for larger sample groups. We did observe a high level of variability in the quality of repair tissue, as can be seen in the standard deviations of histological scores. Further studies should also include control groups such as microfracture or untreated defects for comparison with more widespread treatment modalities. Additional endpoints such as in vivo radiographs or MRI imaging, gait analysis and ROM testing could lend clinical relevance to this treatment modality.

In general, these findings indicate that the MSC-seeded triphasic implants used here cannot be considered as a superior treatment option due to the observed sinking phenomenon of triphasic implants and high variability in the results of the repair tissue. However, good osseointegration of the MSC-seeded β-TCP implants and superior bonding of MSC-assisted cartilage repair were observed. Based on these results, further studies should investigate superior implant fixation into subchondral bone. More basic research regarding the integration of the chondral and osseous phases as well as animal studies with a larger sample size will also be necessary before clinical applications can be developed.

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APPENDIX A: MATERIALS AND METHODS

Isolation and Cultivation of Ovine MSCs

Prior to the first surgery, bone marrow aspirates of 20–40 mL were obtained from the iliac crest of each sheep. Mononuclear cells were isolated from the heparinized aspirates (500 IU [3.125 μg/mL] per mL; Ratiopharm, Ulm, Germany) by Ficoll density gradient centrifugation (density 1.077 g/mL; Biochrom, Berlin, Germany) and plated at 2×10^4 cells/cm^2 in tissue culture flasks with Dulbecco's modified Eagle's medium (DMEM; Gibco, Karlsruhe, Germany) supplemented with 10% autologous serum, 100 U/mL penicillin, and 100 μg/mL streptomycin (both Biochrom), referred to below as autologous expansion medium. Cultures were maintained at 37°C in a humidified atmosphere containing 95% air and 5% O_2 to 5% CO_2 balanced with N_2 in a tri-gas incubator (Thermo Fisher Scientific, Dreieich, Germany). Medium was changed twice weekly. After 10–14 days, the cells were detached using trypsin/EDTA (0.25%/0.05 mM; Biochrom), passaged at 5,000 cells/cm^2 and cultured to reach 80–90% confluence of passage one (P1) before collagen I gel and β-TCP-cylinder preparation.

Isolation of Autologous Serum

Venous blood (100 mL) was collected from each sheep by puncturing the veins of both ears using S-Monovette blood withdrawal systems containing coagulation activator (Sarstedt, Nümbrecht, Germany). The monovettes were centrifuged for 10 min at 2,500*g* and 20°C. The serum phase was harvested and inactivated by heat (56°C, 30 min).

Isolation of Autologous Plasma

For autogenic plasma and fibrin glue production, 18 mL of venous blood was obtained aseptically from each animal using S-monovettes containing EDTA (Sarstedt). The collected EDTA/whole blood mixture was processed to plasma by centrifuging at 2,500*g* for 10 min at 20°C and removing the plasma fraction. The prepared plasma was transferred into 2 mL cryo tubes and stored at −80°C until seeding of the β-TCP cylinder with MSCs and the implantation procedure.

Implant Preparation (Fig. 1)

Preparation and Cultivation of the Chondral Phase

For preparation of the chondral phase, 4.0×10^5 MSCs/mL were mixed with a clinically approved collagen type I hydrogel (CaReS®, Arthro Kinetics Biotechnology) according to the manufacturer's instructions. For each construct, an aliquot of 1.8 mL cell–gel-suspension were transferred to 12-well plates (BD Falcon, Heidelberg, Germany) and cultured 14 days with serum-free, chondrogenic medium (Chondrogenic Differentiation BulletKit®) supplemented with 10 ng/mL TGF-β_3 (both Lonza, Wuppertal, Germany). The differentiation was performed at 20% pO_2 (Thermo Fisher Scientific). The chondrogenic medium was changed twice weekly (Fig. 1).

Preparation and Cultivation of the Osseous Phase

Resorbable pure-phase β-TCP CERASORB® cylinders with a diameter of 6 mm and length of 10 mm were supplied as a gift from Curasan. The cylinders were seeded with 1×10^6 MSCs of P1 and cultured for 14 days in autologous expansion medium. Per cylinder, 1×10^6 cells were mixed in 100 μL thawed autologous plasma and 1 μL of 0.8 M $CaCl_2$ was added to achieve fibrin polymerization with a clotting reaction. The β-TCP ceramic cylinders were then soaked in a 96-well plate

Table A1. Primer Used for RT-PCR

Genes	Primer Sequences	From Species	Fragment Size (bp)	Annealing Temperature (°C)	Cycle Number
Aggrecan	5′-ACGCCATCTGCTACACAGG-3′ 5′-AAAGGCTCCTCAGGTTCTGG-3′	Ovis aries	219	56	30
Chondroadherin	5′-GATACCTGGAGACGCTCTGG-3′ 5′-GGCTTTCTTGGACCTCTTGG-3′	Bos taurus	337	56	30
Collagen 1A1	5′-CCAGTCACCTGCGTACAGAACG-3′ 5′-GCCAGTGTCTCCTTTGGGTCC-3′	Ovis aries	245	67	28
Collagen 2A1 transcription variant 1	5′-CAGGATGTCCAGGAGGCTGG-3′ 5′-CCCAGGAGGTCCTTTGGGTC-3′	Homo sapiens	289	62	30
Collagen 2A1 transcription variant 2	5′-CCAGGATGTCCGGCAACCAGG-3′ 5′-GGAGGTCCTCGAGGTCCCATG-3′	Homo sapiens	353	62	30
GAPDH	5′-CCACTGGGGTCTTCACTACC-3′ 5′-AAGCAGGGATGATGTTCTGG-3′	Ovis aries	335	62	30
Link protein	5′-TCAGGAACTACGGGTTTTGG-3′ 5′-TTGGCCTAGAGATGGGGTAG-3′	Bos taurus	260	62	32
Sox9	5′-AGGTGCTCAAAGGCTACGAC-3′ 5′-GCGGCTGGTACTTGTAATCC-3′	Homo sapiens	294	62	28

with this mixture, transferred into separate wells of a 24-well plate filled with 1 mL of autologous expansion medium (see above), and incubated at 37°C, 95% relative humidity in an air/5% CO_2 atmosphere (Thermo Fisher Scientific). Medium was changed twice weekly. Each culture condition was carried out in triplicate.

Determination of Cell Viability

The viability of the MSCs in both composites was determined following culture by a fluorometric live/dead staining method (Mobitec, Goettingen, Germany), which allowed for quantification of the cellular uptake of calcein AM and ethidiumhomodimer. After taking out both scaffolds from the well plates, the constructs were washed with 5 mL PBS for 2 h each and afterwards incubated with fluorometric assay components for 30 min. The excitation and detection wavelengths were 515 and 635 nm respectively.

Total RNA Extraction, cDNA Synthesis, and RT-PCR

Gene expression profiles of chondral constructs were evaluated by using RT-PCR in a Primus 96 Plus PCR machine (MWG Biotech, Ebersberg, Germany). Two to three gels were digested with 2 mg/mL collagenase A solution (Roche, Basel, Switzerland) for 2 h at 37°C and resuspended in RLT lysis buffer (Qiagen, Hilden, Germany). Total RNA was purified using the RNeasy Mini Kit (Qiagen). Digestion of DNA was performed by applying 2 U RQ1-DNAse (Promega, Mannheim, Germany) at 37°C for 30 min. RNA concentration and purity were measured with a NanoDrop spectrophotometer (Peqlab, Erlangen, Germany) at 260 and 280 nm. Single-strand cDNA copies were generated from 1 μg samples of total purified RNA by using random primers and M-MLV reverse transcriptase (both Promega) according to the manufacturer's protocol.

The prepared MasterMIX contained 10× PCR buffer, 25 mM $MgCl_2$, 0.5 U Taq polymerase (Qiagen), 8 mm dNTPmix (Fermentas, St. Leon-Rot, Germany) and 10 mm of sequence specific primers (Table A1; Operon Biotechnologies, Cologne, Germany). PCR reactions condition were as follows: 95°C for 5 min/95°C for 30 s, annealing for 30 s, 72°C for 30 s, and final extension at 72°C for 5 min. All samples were normalized with the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Scanning Electron Microscopy

Constructs for scanning electron microscopy (SEM) were rinsed with Sorenson's buffer (pH 7.2) and immersed and fixed at 4°C in 2% glutaraldehyde prepared in Sorenson's buffer for 2 h. Scaffolds were rinsed in Sorenson's buffer and postfixed in 1% osmium tetroxide (Plano, Marburg, Germany) for 2 h. Scaffolds were rinsed again and dehydrated in acetone series, dried in Critical Point Dryer CPD30 (Baltec, Tucson, AZ) sputter coated with platinum and examined in a Hitachi S4500 scanning electron microscope (Finchampstead, UK).

Immunohistochemistry

Immunohistochemistry of cryosections was performed using the two-step indirect method. The samples were embedded in Tissue Tek® (Sakura, Zoeterwoude, the Netherlands) sectioned at a thickness of 8 μm by a Leica CM3050S cryotome (Leica Microsystems, Nussloch, Deutschland). The sections were fixed for 5 min in acetone, blocked for 30 min (sheep serum 1:10 diluted in PBS) and incubated with the primary antibody (collagen type II: mouse monoclonal antibody [Clone: II-4C11; MP Biomedicals, Solon, OH], diluted 1:2,000 in PBS; aggrecan: monoclonal mouse antibody [Acris Antibodies, Herford, Germany], diluted 1:50 in PBS). After washing with PBS, the secondary antibody of peroxidase-conjugated goat-anti-mouse IgG (Jackson ImmunoResearch, Cambridgeshire, UK; diluted 1:50 in PBS) was added for 1 h at 37°C. Immunostaining was developed by 3-amino-9-ethyl-carbazol substrate. Cell nuclei were counterstained with Meyer's hematoxylin (Lillie's Modification; DakoCytomation, Hamburg, Germany).

Surgical Techniques

Defect Application

Following bone marrow aspiration and blood draw, an initial operation was performed to create bilateral full-thickness osteochondral defects in the medial femoral condyles of the sheep. Preoperative sedation, orotracheal intubation, and anesthesia were performed under supervision of a veterinarian. Arthrotomy of the hind knee joints was performed using a medial arthrotomy under standard sterile conditions. The defects were created with a diameter of 4 mm and a depth of 2 mm below the calcified layer using a custom depth-limited drill (Aesculap, Tuttlingen, Germany). The sheep were then returned to the herd for degeneration of the defects. Regular post-operative wound checks were performed.

Triphasic Construct Implantation

After 6 weeks, the animals were returned to the laboratory for implantation of the constructs. The primary arthrotomy was reopened in standard fashion to expose the chronic lesions, all of which were noted to be grade 4 on the Brittberg ICRS Visual Scale. The initial defect was cored out using a 6.4 mm harvesting drill to a depth of 12 mm and the defect cylinder was removed using a graft harvester (Zimmer, Winterthur, Switzerland). The MSC-seeded β-TCP cylinder was inserted using a press-fit technique. The intermediate autologous plasma phase was then activated using $CaCl_2$ and applied to the surface of the cylinder. The seeded collagen I hydrogel phase was affixed onto the plasma layer and fitted to the condyle surface using an impactor (Zimmer). The joint capsule and wound were then closed in standard layer-by-layer fashion.

Osteochondral Autograft Transplantation

Surgery continued on the contralateral side and the defect was removed with a coring drill as previously described. The patella was released medially and laterally subluxated using an extended parapatellar incision. A 6.6 mm diameter and 12 mm deep osteochondral cylinder was then harvested from the medial facet of the trochlea. The cylinder was implanted with a press-fit technique into the condylar defect crater using SDS instruments. After plug transfer, the medial retinaculum was reconstructed using Vicryl 2-0 sutures and patellar tracking was tested to ensure normal alignment.

Explantation

The hind knees were then dissected at 6 or 12 months for analysis of regeneration. A diamond band saw (E310, Exakt Apparatebau GmbH, Norderstedt, Germany) was used to cut blocks containing each defect region of approximately 1 cm in diameter and 3 cm in depth from the femurs of the sheep. The blocks were cut perpendicular to the articular surface. All samples were initially placed in PBS prior to biomechanical testing and transferred to formaldehyde at 24 h post-explantation.

Macroscopic Analysis

Prior to further evaluation, each sample was visually graded by a specialist orthopedic surgeon according to the Brittberg ICRS Visual Scale (Table A2).[29,30] This

Table A2. ICRS Visual Scale[29,30]

	Points
I: Degree of defect repair	
In level with surrounding cartilage	4
75% repair of defect depth	3
50% repair of defect depth	2
25% repair of defect depth	1
0% repair of defect depth	0
II: Integration to border zone	
Complete integration with surrounding cartilage	4
Demarcating border <1 mm	3
3/4th of graft integrated, 1/4th with a notable border >1 mm width	2
1/2 of graft integrated with surrounding cartilage, 1/2 with a notable border >1 mm	1
From no contact to 1/4th of graft integrated with surrounding cartilage	0
III: Macroscopic appearance	
Intact smooth surface	4
Fibrillated surface	3
Small, scattered fissures, or cracks	2
Several, small, or few but large fissures	1
Total degeneration of grafted area	0
Overall repair assessment (max. 12 points)	
Grade I: normal	12
Grade II: nearly normal	11–8
Grade III: abnormal	7–4
Grade IV: severely abnormal	3–1

Table A3. ICRS Visual Histological Scale[34]

	Points
I: Surface	
Smooth/continuous	3
Discontinuities/irregularities	0
II: Matrix	
Hyaline	3
Mixture: hyaline/fibrocartilage	2
Fibrocartilage	1
Fibrous tissue	0
III: Cell distribution	
Columnar	3
Mixed/columnar-clusters	2
Clusters	1
Individual cells/disorganized	0
IV: Cell population viability	
Predominantly viable	3
Partially viable	1
<10% viable	0
V: Subchondral bone	
Normal	3
Increased remodeling	2
Bone necrosis/granulation tissue	1
Detached/fracture/callus at base	0
VI: Cartilage mineralization (calcified cartilage)	
Normal	3
Abnormal/inappropriate location	0
Sum of points (max. 18 points)	

system assesses (I) degree of defect repair (defect filling), (II) integration to border zone and (III) macroscopic surface appearance.

Histological Analysis

The samples were dehydrated using progressive alcohol concentrations and placed into light-curing embedding resin (Technovit 7200 VLC, Heraeus Kulzer, Hanau, Germany) for 2 days. Following this, infiltration with embedding resin was performed for an additional 13 days with agitation under vacuum conditions. The samples were then placed into molds, which were filled with embedding resin. Polymerization was performed in two steps using a commercial polymerization device (Histolux, Exakt Apparatebau GmbH). Treatment with low light intensity was followed by a second stage of high light intensity to complete the polymerization process over a total time of 10 h. The samples were kept overnight in an incubator (Memmert, Schwabach, Germany) before being cut and mounted onto slides.

The slides were then treated with toluidine blue[31] and Levai–Laczko[32,33] stains and finally graded independently by three reviewers including a specialist orthopedic surgeon and a musculoskeletal pathologist using the ICRS Visual Histological Visual Scale (Table A3),[34] the O'Driscoll Scale (Table A4)[35] and the semiquantitative Siebert score (Table A5).[36]

Table A4. O'Driscoll Score[35]

	Points
I: Nature of predominant tissue	
(A) Cellular morphology	
Hyaline articular cartilage	4
Incompletely differentiated mesenchyme	2
Fibrous tissue or bone	0
(B) Safranin-O staining of the matrix	
Normal or nearly normal	3
Moderate	2
Slight	1
None	0
II: Structural characteristics	
(A) Surface regularity	
Smooth and intact	3
Superficial horizontal lamination	2
Fissures: 25–100% of the thickness	1
Severe disruption, including fibrillation	0
(B) Structural integrity	
Normal	2
Slight disruption, including cysts	1
Severe disintegration	0
(C) Thickness	
100% of normal adjacent cartilage	2
50–100% of normal cartilage	1
0–50% of normal cartilage	0
(D) Bonding to the adjacent cartilage	
Bonded at both ends of graft	2
Bonded at one end, or partially at both ends	1
Not bonded	0
III: Freedom from cellular changes of degeneration	
(A) Hypocellularity	
Normal cellularity	3
Slight hypocellularity	2
Moderate hypocellularity	1
Severe hypocellularity	0
(B) Chondrocyte clustering	
No clusters	2
<25% of the cells	1
25–100% of the cells	0
IV: Freedom from degenerative changes in adjacent cartilage	
Normal cellularity, no clusters, normal staining	3
Normal cellularity, mild clusters, moderate staining	2
Mild or moderate hypocellularity, slight staining	1
Severe hypocellularity, poor or no staining	0
Sum of points (max. 24 points)	

Table A5. Semiquantitative Siebert Score[36]

	Points
(A) Surface characteristics	
Smooth	2
Somewhat irregular	1
Irregular	0
(B) Cartilage thickness	
76–100% of the surrounding cartilage	4
51–75%	3
26–50%	2
<25%	1
0% (defect)	0
(C) Bonding	
Apposition of both contact surfaces	2
One surface	1
No bonding	0
(D) Subchondral reconstruction	
Even with surrounding bone	4
Slightly offset (25%)	3
Moderately offset (50%)	2
Significantly offset (75%)	1
No reconstruction/formation	0
(E) Cartilage gap	
No gap	4
Gap up to 25% of the transplanted cartilage thickness	3
26–50%	2
51–75%	1
76–100%	0
Sum of points (max. 16 points)	

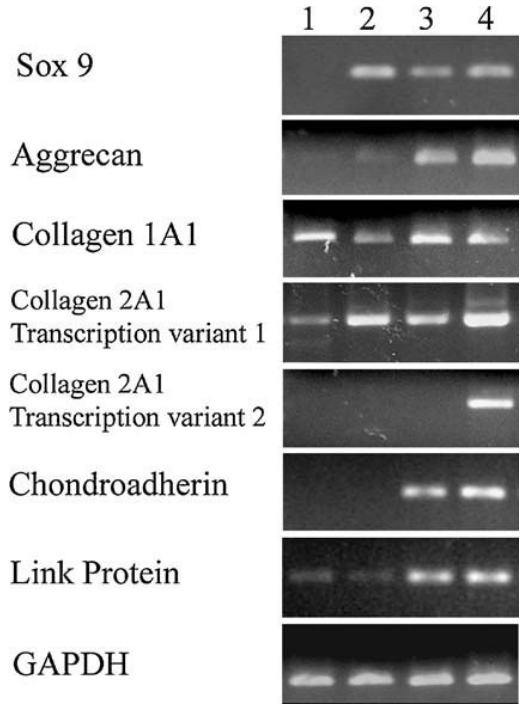

Figure B2. Time course of gene expression of Sox9, aggrecan, collagen 2A1 (transcription variants 1 and 2), chondroadherin and link protein in collagen-I-hydrogel cultures measured by RT-PCR. Expression levels were normalized to the housekeeping gene GAPDH. Lane 1: monolayer culture of P1; lanes 2–4: gel cultures of days 1, 7, and 14.

Biomechanical Testing

Prior to histological analysis, the cut sample cylinders underwent biomechanical analysis with a ball indentation test. An area near the center of the defect was used for testing to avoid a boundary effect. In addition, samples of native cartilage from the lateral condyle of the right femur of each animal were tested for comparison. The samples were held uniaxially in a ring clamp. To obtain near-physiological conditions, the clamp was mounted in a transparent PMMA tank filled with PBS at a pH of 7.4. The tank was mounted onto a two-dimensional miniature electromechanical goniometer stage, which was in turn mounted onto a linear X, Y stage. This setup allowed for a maximum load of 40 N. To evaluate the mechanical properties of the samples, a ball indentation test was used with a ISO 14577-2 compatible setup. A steel ball (X105CrMo17) with a diameter of 1 mm and a Young's modulus of 215×10^3 N/mm^2 was used. A displacement-controlled regime was performed with a loading/unloading velocity of 10 μm/s and a maximum depth indentation of 200 μm. The indentation depths of 200 μm were determined in a preliminary test on untreated native knee joins as the optimum between substrate influence from the bone tissue and the contact problem.

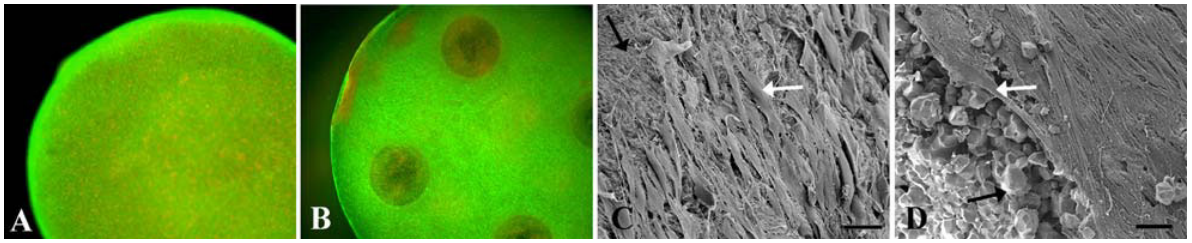

Figure B1. Viability, distribution, morphology of MSCs in the chondral (A and C) and osseous (B and D) phases after 14 days in vitro. Fluorescence images of live (green) and dead (red) MSCs in the respective scaffolds as determined by fluorometric viability assay (A and B 1,000 μm bar). Scanning electron microscopy images showing typical cell distribution and morphology of MSCs (white arrows) in the chondral and osseous material (black arrows) (C and D 30 μm bar).

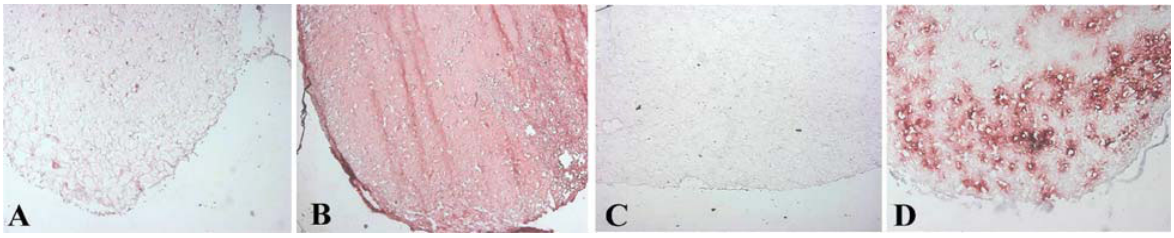

Figure B3. Immunohistochemical detection of aggrecan (A and B) and collagen type II (C and D) in collagen-I hydrogel cultures (50×, 200 μm bar). One day after gel preparation no aggrecan staining was visible (A). Fourteen days after chondrogenic differentiation strongly positive staining of aggrecan through the entire construct was evident (B). Similar to this result, no collagen type II was evident at day 1 (C) but was observed at day 14 (D).

Pre-testing contact force was 2 mN. During the way-controlled experiment the force and travel distance were measured independently by a load cell and a inductive displacement sensor.

In relation to the relative varied morphology and thickness of different tissues layer the measured force values were influenced by these and represent a mixed/averaged value.

APPENDIX B

Viability, Distribution, and Morphology of Embedded Cells

Primary ovine MSCs from P1 were mixed with both the collagen I hydrogel for the chondral phase and the autologous plasma for seeding onto the β-TCP-cylinder for the osseous phase. Both constructs were cultivated for 2 weeks, combined with autologous plasma and later implanted into the osteochondral defect site. Overall cell viability, distribution and morphology were determined. As represented in Figure B1A and B, both types of scaffolds showed a high cell viability of ~95% after 2 weeks of 3D cultivation. Fluorescence microscopy of the MSC collagen gels (Fig. B1A) demonstrated a high density of living MSCs and few dead cells within the gel. Fluorescence staining with a live/dead kit was also performed 14 days after seeding and cultivation of the MSC-plasma suspension onto β-TCP cylinders. This showed similar results to microscopy with a homogeneous distribution of living MSCs and few dead cells. Cross-sectional imaging of both MSC-seeded matrices by SEM analysis prior to implantation showed the characteristic microstructure of both biomaterials, the compact collagen fibers (Fig. B1C) and the fibrin fibrillar structure (Fig. B1D). Both images illustrate the integration of MSCs into the respective framework with a typical dense cell occurrence in the peripheral zones. Horizontally oriented cell layers of MSCs with fibroblastic morphology were seen covering both biomaterials.

Accumulation and Expression of Cartilage-Specific Extracellular Matrix Markers

RT-PCR

Chondrogenic differentiation of the cartilage phase was analyzed using reverse transcriptase polymerase chain reaction (RT-PCR) (Fig. B2) and immunohistochemistry (Fig. B3). In the monolayer culture (Fig. B2, lane 1) there was no significant expression of the analyzed genes, except for weak expression of the early, immature splice variant of collagen 2A1, the transcription variant 1. Collagen 1A1, which is expressed constitutively by MSCs, was found at constant levels during 3D cultivation. In contrast, at the time of implantation (day 14), the MSC gels were demonstrated to express mRNA of the genes Sox9, aggrecan, chondroadherin, and link protein, which represent important markers of chondrogenesis (Fig. B2, lane 4).

The constructs also showed expression of the mature splice variant of collagen 2A1, transcription variant 2, which is known to be expressed in a later stage of chondrogenesis and which is encoded for the major solid component matrix protein of hyaline cartilage. Thus, these results suggest progression of chondrogenic differentiation.

Immunohistochemistry

Chondrogenesis of MSCs at the protein level was examined by immunostaining for proteoglycan aggrecan and hyaline cartilage specific collagen type II after gel preparation (Fig. B3A and B) and prior to implantation (Fig. B3C and D). After 1 day the cryosections of the gels showed no evidence of these major cartilage matrix markers. In contrast, the accumulation of aggrecan and collagen type II were evident after 14 days in the MSC hydrogels cultured under chondrogenic conditions, as demonstrated by the strong positive immunostaining. The immunohistochemical results showed a pronounced, strongly positive interterritorial staining of aggrecan throughout the entire construct, whereas collagen type II showed a distinct but not uniform distribution of staining within the hydrogels. These findings at the protein level in the MSC gels provide additional evidence of chondrogesis.

REFERENCES

1. Shelbourne KD, Jari S, Gray T. 2003. Outcome of untreated traumatic articular cartilage defects of the knee: A natural history study. J Bone Joint Surg Am 85-A(Suppl. 2):8–16.
2. Gudas R, Kalesinskas RJ, Kimtys V, et al. 2005. A prospective randomized clinical study of mosaic osteochondral autologous transplantation versus microfracture for the treatment of osteochondral defects in the knee joint in young athletes. Arthroscopy 21:1066–1075.

3. Hangody L, Füles P. 2003. Autologous osteochondral mosaicplasty for the treatment of full-thickness defects of weight-bearing joints: Ten years of experimental and clinical experience. J Bone Joint Surg Am 85-A (Suppl. 2):25–32.
4. Hangody L, Vásárhelyi G, Hangody LR, et al. 2008. Autologous osteochondral grafting—Technique and long-term results. Injury 39 (Suppl. 1):S32–S39.
5. LaPrade RF, Botker JC. 2004. Donor-site morbidity after osteochondral autograft transfer procedures. Arthroscopy 20:69–73.
6. Reddy S, Pedowitz DI, Parekh SG, et al. 2007. The morbidity associated with osteochondral harvest from asymptomatic knees for the treatment of osteochondral lesions of the talus. Am J Sports Med 35:80–85.
7. Paul J, Sagstetter A, Kriner M, et al. 2009. Donor-site morbidity after osteochondral autologous transplantation for lesions of the talus. J Bone Joint Surg Am 91:1683–1688.
8. Iwasaki N, Kato H, Kamishima T, et al. . Donor site evaluation after autologous osteochondral mosaicplasty for cartilaginous lesions of the elbow joint. Am J Sports Med 35:2096–2100.
9. Jackson DW, Scheer MJ, Simon TM. 2001. Cartilage substitutes: Overview of basic science and treatment options. J Am Acad Orthop Surg 9:37–52.
10. Rose T, Craatz S, Hepp P, et al. 2005. The autologous osteochondral transplantation of the knee: Clinical results, radiographic findings and histological aspects. Arch Orthop Trauma Surg 125:628–637.
11. Horas U, Pelinkovic D, Herr G, et al. 2003. Autologous chondrocyte implantation and osteochondral cylinder transplantation in cartilage repair of the knee joint. A prospective, comparative trial. J Bone Joint Surg Am 85-A:185–192.
12. Lane J, Massie J, Ball S, et al. 2004. Follow-up of osteochondral plug transfers in a goat model: A 6-month study. Am J Sports Med 32:1440–1450.
13. Keeney M, Pandit A. 2009. The osteochondral junction and its repair via bi-phasic tissue engineering scaffolds. Tissue Eng Part B Rev 15:55–73.
14. Hung CT, Lima EG, Mauck RL, et al. 2003. Anatomically shaped osteochondral constructs for articular cartilage repair. J Biomech 36:1853–1864.
15. Oliveira JM, Rodrigues MT, Silva SS, et al. 2006. Novel hydroxyapatite/chitosan bilayered scaffold for osteochondral tissue-engineering applications: Scaffold design and its performance when seeded with goat bone marrow stromal cells. Biomaterials 27:6123–6137.
16. Sherwood JK, Riley SL, Palazzolo R, et al. 2002. A three-dimensional osteochondral composite scaffold for articular cartilage repair. Biomaterials 23:4739–4751.
17. Ahn J-H, Lee T-H, Oh J-S, et al. 2009. A novel hyaluronate-atelocollagen/beta-TCP-hydroxyapatite biphasic scaffold for the repair of osteochondral defects in rabbits. Tissue Eng Part A 15:2596–2604.
18. Alhadlaq A, Mao JJ. 2005. Tissue-engineered osteochondral constructs in the shape of an articular condyle. J Bone Joint Surg Am 87:936–944.
19. Frenkel SR, Toolan B, Menche D, et al. 1997. Chondrocyte transplantation using a collagen bilayer matrix for cartilage repair. J Bone Joint Surg Br 79:831–836.
20. Gao J, Dennis JE, Solchaga LA, et al. 2002. Repair of osteochondral defect with tissue-engineered two-phase composite material of injectable calcium phosphate and hyaluronan sponge. Tissue Eng 8:827–837.
21. Kandel R, Grynpas M, Pilliar R, et al. 2006. Repair of osteochondral defects with biphasic cartilage-calcium polyphosphate constructs in a sheep model. Biomaterials 27:4120–4131.
22. Hepp P, Osterhoff G, Niederhagen M, et al. 2009. Perilesional changes of focal osteochondral defects in an ovine model and their relevance to human osteochondral injuries. J Bone Joint Surg Br 91:1110–1119.
23. Marquass B, Hepp P, Schmidt S, et al. 2009. Chondrogenic predifferentiation of ovine mesenchymal stem cells leads to a better histological repair of chronic osteochondral defects after one year in a sheep model. In: Beger H, editor. Proceedings of the 13th Annual Meeting on Surgical Research in Langenbecks Arch Surg, Vol. 394. Munich, Germany: Springer Berlin/Heidelberg; p 915–970.
24. Alhadlaq A, Elisseeff JH, Hong L, et al. 2004. Adult stem cell driven genesis of human-shaped articular condyle. Ann Biomed Eng 32:911–923.
25. Cao T, Ho K-H, Teoh S-H. 2003. Scaffold design and in vitro study of osteochondral coculture in a three-dimensional porous polycaprolactone scaffold fabricated by fused deposition modeling. Tissue Eng 9 (Suppl. 1):S103–S112.
26. Chen G, Sato T, Tanaka J, et al. 2006. Preparation of a biphasic scaffold for osteochondral tissue engineering. Mater Sci Eng C26:118–123.
27. Chen G, Tanaka J, Tateishi T. 2006. Osteochondral tissue engineering using a PLGA-collagen hybrid mesh. Mater Sci Eng C26:124–129.
28. Shao XX, Hutmacher DW, Ho ST, et al. 2006. Evaluation of a hybrid scaffold/cell construct in repair of high-load-bearing osteochondral defects in rabbits. Biomaterials 27:1071–1080.
29. Brittberg M, Peterson L. 1998. Introduction to an articular cartilage classification. ICRS Newsl 1:8.
30. Peterson L, Minas T, Brittberg M, et al. 2000. Two- to 9-year outcome after autologous chondrocyte transplantation of the knee. Clin Orthop Relat Res 374:212–234.
31. Feder N, Wolf M. 1965. Studies on nucleic acid metachromasy: II. Metachromatic and orthochromatic staining by toluidine blue of nucleic acids in tissue sections. J Cell Biol 27:327.
32. Laczko J, Levai G. 1975. A simple differential staining method for semi-thin sections of ossifying cartilage and bone tissues embedded in epoxy resin. Mikroskopie 31:4.
33. Donath K. 1988. Die trenn-dünnschliff-technik zur herstellung histologischer präparate von nicht schneidbaren geweben und materialien. Der Präparator 34:197–206.
34. Mainil-Varlet P, Aigner T, Brittberg M, et al. 2003. Histological assessment of cartilage repair: A report by the Histology Endpoint Committee of the International Cartilage Repair Society (ICRS). J Bone Joint Surg Am 85-A (Suppl. 2):45–57.
35. O'Driscoll SW, Keeley FW, Salter RB. 1988. 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 70:595–606.
36. Siebert CH, Miltner O, Weber M, et al. 2003. Healing of osteochondral grafts in an ovine model under the influence of bFGF. Arthroscopy 19:182–187.
37. Zscharnack M, Hepp P, Richter R, et al. 2010. Repair of chronic osteochondral defects using pre-differentiated mesenchymal stem cells in an ovine model. Am J Sports Med (in press).
38. Pei M, He F, Boyce B, et al. 2009. Repair of full-thickness femoral condyle cartilage defects using allogeneic synovial cell-engineered tissue constructs. Osteoarthritis Cartilage 17:714–722.
39. Uematsu K, Hattori K, Ishimoto Y, et al. 2005. Cartilage regeneration using mesenchymal stem cells and a three-dimensional poly-lactic-glycolic acid (PLGA) scaffold. Biomaterials 26:4273–4279.

40. Petersen JP, Ueblacker P, Goepfert C, et al. 2008. Long term results after implantation of tissue engineered cartilage for the treatment of osteochondral lesions in a minipig model. J Mater Sci Mater Med 19:2029–2038.
41. Bruder SP, Kurth AA, Shea M, et al. 1998. Bone regeneration by implantation of purified, culture-expanded human mesenchymal stem cells. J Orthop Res 16:155–162.
42. Frosch KH, Drengk A, Krause P, et al. 2006. Stem cell-coated titanium implants for the partial joint resurfacing of the knee. Biomaterials 27:2542–2549.
43. Guo X, Wang C, Zhang Y, et al. 2004. Repair of large articular cartilage defects with implants of autologous mesenchymal stem cells seeded into beta-tricalcium phosphate in a sheep model. Tissue Eng 10:1818–1829.
44. Stockwell RA. 1971. The interrelationship of cell density and cartilage thickness in mammalian articular cartilage. J Anat 109:411–421.
45. Pulliainen O, Vasara A, Hyttinen M, et al. 2007. Poly-LD-lactic acid scaffold in the repair of porcine knee cartilage lesions. Tissue Eng 13:1347–1355.
46. Benazzo F, Cadossi M, Cavani F, et al. 2008. Cartilage repair with osteochondral autografts in sheep: Effect of biophysical stimulation with pulsed electromagnetic fields. J Orthop Res 26:631–642.
47. von Rechenberg B, Akens MK, Nadler D, et al. 2003. Changes in subchondral bone in cartilage resurfacing—An experimental study in sheep using different types of osteochondral grafts. Osteoarthritis Cartilage 11:265–277.

3. Summary

Cumulative dissertation for obtaining the academic title Dr. med.

Mesenchymal Stem Cell Constructs for Repair of Focal Cartilage Defects in an Ovine Model

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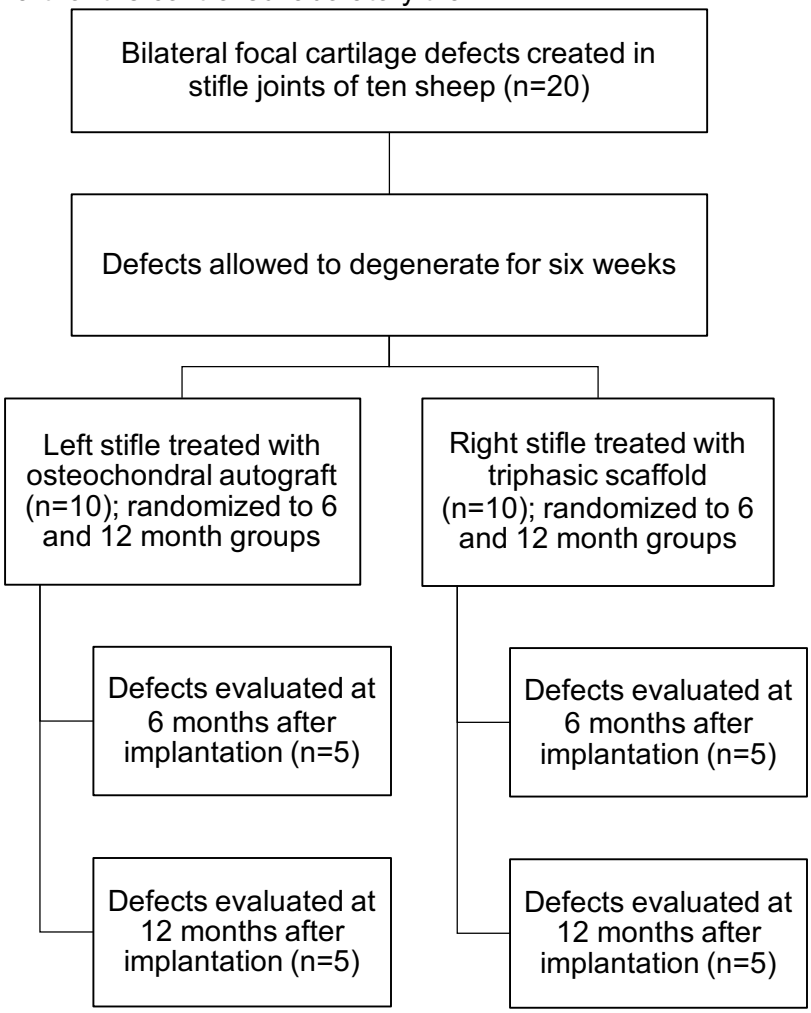
Submitted in: December 2015

Background: The treatment of FCDs remains a problem for orthopaedic surgeons given the disadvantages of current methods. In the absence of treatment, focal lesions will continue to degenerate and result in OA. Techniques for transplanting native osteochondral tissue into defect areas have been described in the literature under the names osteochondral autograft transfer (OAT) or mosaicplasty. These methods have been successful, but result in morbidity at the donor site that limits the amount of tissue that can be harvested. Further, bonding of the grafts to surrounding tissue has been inadequate in some cases based on clinical and animal testing. Use of a synthetic multiphasic implant seeded with multipotent MSCs has the promise to provide similar repair qualities without the limitations of donor site morbidity.

Aims: The goal of this work was to determine whether repair of critical-size chronic FCDs using MSC-seeded triphasic constructs would provide similar repair qualities as OAT in a large animal model at six and twelve months.

Method and Results: Ten female merino sheep were randomized into groups of 6-month and 12-month follow-up at the beginning of this trial (see Figure 4). Each sheep underwent a primary surgery in which both stifle joints were arthrotomized and a 4-mm diameter defect extending 2 mm below the calcified layer was created in the medial femoral condyles.

Figure 4: Flow chart for the controlled laboratory trial.



At the time of surgery, bone marrow was aspirated for culture of MSCs. The animals were then given a six-week period to allow the defects to degenerate. Following this, each animal underwent a second surgery in which the defects were cored out using a 6.4-mm cylindrical drill. The right stifle joint was treated with an osteochondral autograft taken from the non-weight-bearing portion of the trochlea, which was press fit into the defect zone. The left stifle joint was treated with a synthetic, tissue-engineered construct. The osseous layer was made using a commercially-available TCP cylinder (CERASORB, Curasan, Kleinostheim, Germany) that was seeded with 1 x 10^6 MSCs. The cartilage layer was created by seeding a collagen type I hydrogel (CaReS, Arthro Kinetics Biotechnology, Krems, Austria) with 4 x 10^5 MSCs and culturing the construct in a chondrogenic medium mixed with 10 ng/ml transforming growth factor-beta. The osseous cylinders were implanted into the defect, followed by an autologous activated plasma phase and the cartilage phase.

Sheep were sacrificed at 6 or 12 months and the tissue was assessed using macroscopic evaluation (ICRS Brittberg score), histological evaluation (ICRS-1 Visual Histological Scale, O'Driscoll score and Siebert Semiquantitative Score). Biomechanical testing was performed to determine the force required to make a standardized indentation in each group. Micro-CT scans were performed to evaluate the resorption and placement of the triphasic implants at latest follow-up.

Macroscopic evaluation of the defects at 6 and 12 months showed no significant differences based on the ICRS Brittberg score. Microscopic analysis using the ICRS-1 Visual Histological Scale (ICRS-VHS1), the O'Driscoll score and the Siebert Semiquantitative Score also showed no differences at 6 or 12 months in overall score. A significantly better score was seen in the subcategories for "matrix composition", "cell distribution" and "cell population" in the OAT group at 12 months using the ICRS-VHS1 score, while the "bonding to adjacent cartilage" O'Driscoll subscore was higher in the triphasic group at 6 months. Biomechanical indentation testing showed no significant differences in softness between the two test groups or native cartilage at 12 months. Radiographic testing showed that four of the ten osseous phase implants subsided to 3 mm or more below the subchondral bone plate.

Discussion: Treatment with a tissue-engineered triphasic implant showed comparable histological repair qualities to osteochondral allograft transfer in a sheep model of chronic FCDs at 6 and 12 months. The evidence of superior bonding to adjacent cartilage tissue in the 6 month groups was encouraging. However, the sinking of 4 of 10 of the osseous phase implants below the subchondral surface is cause for concern. The authors surmised that the sinking phenomenon was due to the relative softness of the femoral cancellous bone relative to the harder TCP cylinders, as has been reported in other studies. Although the proof of concept is considered successful, given the differentiated growth of cartilage and bone layers from an MSC-seeded graft, further study is needed to investigate superior fixation of the osseous phase into bone.

3.1 References

Ahn J-H, Lee T-H, Oh J-S, Kim S-Y, Kim H-J, Park I-K, Choi B-S, Im G-I. 2009. Novel hyaluronate-atelocollagen/beta-TCP-hydroxyapatite biphasic scaffold for the repair of osteochondral defects in rabbits. Tissue Eng. Part A, 15(9):2595–2604 DOI: 10.1089/ten.TEA.2008.0511.

Alhadlaq A, Mao JJ. 2005. Tissue-Engineered Osteochondral Constructs in the Shape of an Articular Condyle. J Bone Jt. Surg Am, 87(5):936–944 DOI: 10.2106/JBJS.D.02104.

Artzi Z, Weinreb M, Givol N, Rohrer MD, Nemcovsky CE, Prasad HS, Tal H. 2004. Biomaterial resorption rate and healing site morphology of inorganic bovine bone and beta-tricalcium phosphate in the canine: a 24-month longitudinal histologic study and morphometric analysis. Int. J. Oral Maxillofac. Implants, 19(3):357–368.

Bartlett W, Gooding CR, Carrington RWJ, Skinner JA, Briggs TWR, Bentley G. 2005. Autologous chondrocyte implantation at the knee using a bilayer collagen membrane with bone graft A PRELIMINARY REPORT. J. Bone Joint Surg. Br., 87-B(3):330–332 DOI: 10.1302/0301-620X.87B3.15552.

Baustian C, Hanley S, Ceredig R. 2015. Isolation, selection and culture methods to enhance clonogenicity of mouse bone marrow derived mesenchymal stromal cell precursors. Stem Cell Res. Ther., 6:151 DOI: 10.1186/s13287-015-0139-5.

Behrens DP. 2005. Matrixgekoppelte Mikrofrakturierung. Arthroskopie, 18(3):193–197 DOI: 10.1007/s00142-005-0316-0.

Bentley G, Biant LC, Vijayan S, Macmull S, Skinner JA, Carrington RWJ. 2012. Minimum ten-year results of a prospective randomised study of autologous chondrocyte implantation versus mosaicplasty for symptomatic articular cartilage lesions of the knee. J. Bone Joint Surg. Br., 94-B(4):504–509 DOI: 10.1302/0301-620X.94B4.27495.

Beris AE, Lykissas MG, Kostas-Agnantis I, Manoudis GN. 2012. Treatment of full-thickness chondral defects of the knee with autologous chondrocyte implantation: a functional evaluation with long-term follow-up. Am. J. Sports Med., 40(3):562–567 DOI: 10.1177/0363546511428778.

Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. 1994. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N. Engl. J. Med., 331(14):889–895 DOI: 10.1056/NEJM199410063311401.

Caminal M, Peris D, Fonseca C, Barrachina J, Codina D, Rabanal RM, Moll X, Morist A, García F, Cairó JJ, Gòdia F, Pla A, Vives J. 2015. Cartilage resurfacing potential of PLGA scaffolds loaded with autologous cells from cartilage, fat, and bone marrow in an ovine model of osteochondral focal defect. Cytotechnology DOI: 10.1007/s10616-015-9842-4.

Caplan AI, Goldberg VM. 1999. Principles of tissue engineered regeneration of skeletal tissues. Clin. Orthop., (367 Suppl):S12–16.

Chen J, Chen H, Li P, Diao H, Zhu S, Dong L, Wang R, Guo T, Zhao J, Zhang J. 2011. Simultaneous regeneration of articular cartilage and subchondral bone in vivo using MSCs induced by a spatially controlled gene delivery system in bilayered integrated scaffolds. Biomaterials, 32(21):4793–4805 DOI: 10.1016/j.biomaterials.2011.03.041.

Cicuttini F, Ding C, Wluka A, Davis S, Ebeling PR, Jones G. 2005. Association of cartilage defects with loss of knee cartilage in healthy, middle-age adults: A prospective study. Arthritis Rheum., 52(7):2033–2039 DOI: 10.1002/art.21148.

Curl WW, Krome J, Gordon ES, Rushing J, Smith BP, Poehling GG. 1997. Cartilage injuries: a review of 31,516 knee arthroscopies. Arthrosc. J. Arthrosc. Relat. Surg. Off. Publ. Arthrosc. Assoc. N. Am. Int. Arthrosc. Assoc., 13(4):456–460.

Deng T, Lv J, Pang J, Liu B, Ke J. 2014. Construction of tissue-engineered osteochondral composites and repair of large joint defects in rabbit. J. Tissue Eng. Regen. Med., 8(7):546–556 DOI: 10.1002/term.1556.

Gao J, Dennis JE, Solchaga LA, Goldberg VM, Caplan AI. 2002. Repair of Osteochondral Defect with Tissue-Engineered Two-Phase Composite Material of Injectable Calcium Phosphate and Hyaluronan Sponge. Tissue Eng., 8(5):827–837 DOI: 10.1089/10763270260424187.

Gooding CR, Bartlett W, Bentley G, Skinner JA, Carrington R, Flanagan A. 2006. A prospective, ranomised study comparing two techniques of autologous chondrocyte implantation for osteochondral defects in the knee: Periosteum covered versus type I/III collagen covered. The Knee, 13(3):203–210 DOI: 10.1016/j.knee.2006.02.011.

Goyal D, Keyhani S, Lee EH, Hui JHP. 2013. Evidence-Based Status of Microfracture Technique: A Systematic Review of Level I and II Studies. Arthrosc. J. Arthrosc. Relat. Surg., 29(9):1579–1588 DOI: 10.1016/j.arthro.2013.05.027.

Hangody L, Füles P. 2003. Autologous Osteochondral Mosaicplasty for the Treatment of Full-Thickness Defects of Weight-Bearing Joints. J Bone Jt. Surg Am, 85(suppl 2):25–32.

Hangody L, Kish G, Kárpáti Z, Szerb I, Udvarhelyi I. 1997. Arthroscopic autogenous osteochondral mosaicplasty for the treatment of femoral condylar articular defects A preliminary report. Knee Surg. Sports Traumatol. Arthrosc., 5(4):262–267 DOI: 10.1007/s001670050061.

Heir S, Nerhus TK, Røtterud JH, Løken S, Ekeland A, Engebretsen L, Årøen A. 2010. Focal Cartilage Defects in the Knee Impair Quality of Life as Much as Severe Osteoarthritis A Comparison of Knee Injury and Osteoarthritis Outcome Score in 4 Patient Categories Scheduled for Knee Surgery. Am. J. Sports Med., 38(2):231–237 DOI: 10.1177/0363546509352157.

Hoemann CD, Tran-Khanh N, Chevrier A, Chen G, Lascau-Coman V, Mathieu C, Changoor A, Yaroshinsky A, McCormack RG, Stanish WD, Buschmann MD. 2015. Chondroinduction Is the Main Cartilage Repair Response to Microfracture and Microfracture With BST-CarGel: Results as Shown by ICRS-II Histological Scoring and a Novel Zonal Collagen Type Scoring Method of Human Clinical Biopsy Specimens. Am. J. Sports Med., 43(10):2469–2480 DOI: 10.1177/0363546515593943.

Hurtig MB, Buschmann MD, Fortier LA, Hoemann CD, Hunziker EB, Jurvelin JS, Mainil-Varlet P, McIlwraith CW, Sah RL, Whiteside RA. 2011. Preclinical Studies for Cartilage Repair Recommendations from the International Cartilage Repair Society. Cartilage, 2(2):137–152.

Kandel RA, Grynpas M, Pilliar R, Lee J, Wang J, Waldman S, Zalzal P, Hurtig M, CIHR-Bioengineering of Skeletal Tissues Team. 2006. Repair of osteochondral defects with biphasic cartilage-calcium polyphosphate constructs in a sheep model. Biomaterials, 27(22):4120–4131 DOI: 10.1016/j.biomaterials.2006.03.005.

Kim HK, Moran ME, Salter RB. 1991. The potential for regeneration of articular cartilage in defects created by chondral shaving and subchondral abrasion. An experimental investigation in rabbits. J. Bone Joint Surg. Am., 73(9):1301–1315.

Kock NB, Hannink G, van Kampen A, Verdonschot N, van Susante JLC, Buma P. 2011. Evaluation of subsidence, chondrocyte survival and graft incorporation following autologous osteochondral transplantation. Knee Surg. Sports Traumatol. Arthrosc., 19(11):1962–1970 DOI: 10.1007/s00167-011-1650-6.

Kon E, Delcogliano M, Filardo G, Fini M, Giavaresi G, Francioli S, Martin I, Pressato D, Arcangeli E, Quarto R, Sandri M, Marcacci M. 2010. Orderly osteochondral regeneration in a sheep model using a novel nano-composite multilayered biomaterial. J. Orthop. Res., 28(1):116–124 DOI: 10.1002/jor.20958.

Kon E, Filardo G, Robinson D, Eisman JA, Levy A, Zaslav K, Shani J, Altschuler N. 2014. Osteochondral regeneration using a novel aragonite-hyaluronate bi-phasic scaffold in a goat model. Knee Surg. Sports Traumatol. Arthrosc. Off. J. ESSKA, 22(6):1452–1464 DOI: 10.1007/s00167-013-2467-2.

Lam J, Lu S, Lee EJ, Trachtenberg JE, Meretoja VV, Dahlin RL, van den Beucken JJJP, Tabata Y, Wong ME, Jansen JA, Mikos AG, Kasper FK. 2014. Osteochondral defect repair using bilayered hydrogels encapsulating both chondrogenically and osteogenically pre-differentiated mesenchymal stem cells in a rabbit model. Osteoarthr. Cartil. OARS Osteoarthr. Res. Soc., 22(9):1291–1300 DOI: 10.1016/j.joca.2014.06.035.

LaPrade RF, Botker JC. 2004. Donor-site morbidity after osteochondral autograft transfer procedures. Arthrosc. J. Arthrosc. Relat. Surg. Off. Publ. Arthrosc. Assoc. N. Am. Int. Arthrosc. Assoc., 20(7):e69–73 DOI: 10.1016/j.arthro.2004.06.022.

Lee WD, Hurtig MB, Pilliar RM, Stanford WL, Kandel RA. 2015. Engineering of hyaline cartilage with a calcified zone using bone marrow stromal cells. Osteoarthr. Cartil. OARS Osteoarthr. Res. Soc., 23(8):1307–1315 DOI: 10.1016/j.joca.2015.04.010.

Lindahl A, Brittberg M, Peterson L. 2014. Health economics benefits following autologous chondrocyte transplantation for patients with focal chondral lesions of the knee. Knee Surg. Sports Traumatol. Arthrosc., 9(6):358–363 DOI: 10.1007/s001670100209.

Marquass B, Schulz R, Hepp P, Zscharnack M, Aigner T, Schmidt S, Stein F, Richter R, Osterhoff G, Aust G, Josten C, Bader A. 2011. Matrix-associated implantation of predifferentiated mesenchymal stem cells versus articular chondrocytes: in vivo results of cartilage repair after 1 year. Am. J. Sports Med., 39(7):1401–1412 DOI: 10.1177/0363546511398646.

Mellor LF, Mohiti-Asli M, Williams J, Kannan A, Dent MR, Guilak F, Loboa EG. 2015. Extracellular Calcium Modulates Chondrogenic and Osteogenic Differentiation of Human Adipose-Derived Stem Cells: A Novel Approach for Osteochondral Tissue Engineering Using a Single Stem Cell Source. Tissue Eng. Part A, 21(17-18):2323–2333 DOI: 10.1089/ten.TEA.2014.0572.

Meng F, He A, Zhang Z, Zhang Z, Lin Z, Yang Z, Long Y, Wu G, Kang Y, Liao W. 2014. Chondrogenic differentiation of ATDC5 and hMSCs could be induced by a novel scaffold-tricalcium phosphate-collagen-hyaluronan without any exogenous growth factors in vitro. J. Biomed. Mater. Res. A, 102(8):2725–2735 DOI: 10.1002/jbm.a.34948.

Miller DJ, Smith MV, Matava MJ, Wright RW, Brophy RH. 2015. Microfracture and Osteochondral Autograft Transplantation Are Cost-effective Treatments for Articular Cartilage Lesions of the Distal Femur. Am. J. Sports Med., 43(9):2175–2181 DOI: 10.1177/0363546515591261.

Minas T, Keudell AV, Bryant T, Gomoll AH. 2013. The John Insall Award: A Minimum 10-year Outcome Study of Autologous Chondrocyte Implantation. Clin. Orthop. Relat. Res., 472(1):41–51 DOI: 10.1007/s11999-013-3146-9.

Miot S, Brehm W, Dickinson S, Sims T, Wixmerten A, Longinotti C, Hollander AP, Mainil-Varlet P, Martin I. 2012. Influence of in vitro maturation of engineered cartilage on the outcome of osteochondral repair in a goat model. Eur. Cell. Mater., 23:222–236.

Mithoefer K, Peterson L, Saris DBF, Mandelbaum BR. 2012. Evolution and Current Role of Autologous Chondrocyte Implantation for Treatment of Articular Cartilage Defects in the Football (Soccer) Player. Cartilage, 3(1 Suppl):31S–36S DOI: 10.1177/1947603511406532.

Mithoefer K, Williams RJ, Warren RF, Potter HG, Spock CR, Jones EC, Wickiewicz TL, Marx RG. 2006. Chondral resurfacing of articular cartilage defects in the knee with the microfracture technique. Surgical technique. J. Bone Joint Surg. Am., 88 Suppl 1 Pt 2:294–304 DOI: 10.2106/JBJS.F.00292.

Niemeyer P, Andereya S, Angele P, Ateschrang A, Aurich M, Baumann M, Behrens P, Bosch U, Erggelet C, Fickert S, Fritz J, Gebhard H, Gelse K, Günther D, Hoburg A, Kasten P, Kolombe T, Madry H, Marlovits S, Meenen NM, Müller PE, Nöth U, Petersen JP, Pietschmann M, Richter W, Rolauffs B, Rhunau K, Schewe B, Steinert A, Steinwachs MR, Welsch GH, Zinser W, Albrecht D. 2013. [Autologous chondrocyte implantation (ACI) for cartilage defects of the knee: a guideline by the working group “Tissue Regeneration” of the German Society of Orthopaedic Surgery and Traumatology (DGOU)]. Z. Für Orthop. Unfallchirurgie, 151(1):38–47 DOI: 10.1055/s-0032-1328207.

O’Driscoll SW. 1998. Current Concepts Review - The Healing and Regeneration of Articular Cartilage*. J Bone Jt. Surg Am, 80(12):1795–1812.

Peterson L, Vasiliadis HS, Brittberg M, Lindahl A. 2010. Autologous Chondrocyte Implantation A Long-term Follow-up. Am. J. Sports Med., 38(6):1117–1124 DOI: 10.1177/0363546509357915.

Rackwitz L, Djouad F, Janjanin S, Nöth U, Tuan RS. 2014. Functional cartilage repair capacity of de-differentiated, chondrocyte- and mesenchymal stem cell-laden hydrogels in vitro. Osteoarthritis Cartilage, 22(8):1148–1157 DOI: 10.1016/j.joca.2014.05.019.

Richter DL, Schenck RC, Wascher DC, Treme G. 2015. Knee Articular Cartilage Repair and Restoration Techniques A Review of the Literature. Sports Health Multidiscip. Approach:1941738115611350 DOI: 10.1177/1941738115611350.

Schleicher I, Lips KS, Sommer U, Schappat I, Martin AP, Szalay G, Hartmann S, Schnettler R. 2013. Biphasic scaffolds for repair of deep osteochondral defects in a sheep model. J. Surg. Res., 183(1):184–192 DOI: 10.1016/j.jss.2012.11.036.

Seo J-P, Kambayashi Y, Itho M, Haneda S, Yamada K, Furuoka H, Tabata Y, Sasaki N. 2015. Effects of a synovial flap and gelatin/β-tricalcium phosphate sponges loaded with mesenchymal stem cells, bone morphogenetic protein-2, and platelet rich plasma on equine osteochondral defects. Res. Vet. Sci., 101:140–143 DOI: 10.1016/j.rvsc.2015.06.014.

Shimomura K, Moriguchi Y, Murawski CD, Yoshikawa H, Nakamura N. 2014. Osteochondral Tissue Engineering with Biphasic Scaffold: Current Strategies and Techniques. Tissue Eng. Part B Rev., 20(5):468–476 DOI: 10.1089/ten.teb.2013.0543.

Shive MS, Stanish WD, McCormack R, Forriol F, Mohtadi N, Pelet S, Desnoyers J, Méthot S, Vehik K, Restrepo A. 2015. BST-CarGel® Treatment Maintains Cartilage Repair Superiority over Microfracture at 5 Years in a Multicenter Randomized Controlled Trial. Cartilage, 6(2):62–72 DOI: 10.1177/1947603514562064.

Smith B, Sigal IR, Grande DA. 2015. Immunology and cartilage regeneration. Immunol. Res. DOI: 10.1007/s12026-015-8720-7.

Spaas JH, Broeckx SY, Chiers K, Ferguson SJ, Casarosa M, Van Bruaene N, Forsyth R, Duchateau L, Franco-Obregón A, Wuertz-Kozak K. 2015. Chondrogenic Priming at Reduced Cell Density Enhances Cartilage Adhesion of Equine Allogeneic MSCs - a Loading Sensitive Phenomenon in an Organ Culture Study with 180 Explants. Cell. Physiol. Biochem. Int. J. Exp. Cell. Physiol. Biochem. Pharmacol., 37(2):651–665 DOI: 10.1159/000430384.

Stanish WD, McCormack R, Forriol F, Mohtadi N, Pelet S, Desnoyers J, Restrepo A, Shive MS. 2013. Novel Scaffold-Based BST-CarGel Treatment Results in Superior Cartilage Repair Compared with Microfracture in a Randomized Controlled Trial. J Bone Jt. Surg Am, 95(18):1640–1650 DOI: 10.2106/JBJS.L.01345.

Steadman JR, Rodkey WG, Singleton SB, Briggs KK. 1997. Microfracture technique forfull-thickness chondral defects: Technique and clinical results. Oper. Tech. Orthop., 7(4):300–304 DOI: 10.1016/S1048-6666(97)80033-X.

Tang C, Jin C, Xu Y, Wei B, Wang L. 2015. Chondrogenic differentiation could be induced by autologous bone marrow mesenchymal stem cell-derived extracellular matrix scaffolds without exogenous growth factor. Tissue Eng. Part A DOI: 10.1089/ten.TEA.2014.0491.

Vachon A, Bramlage LR, Gabel AA, Weisbrode S. 1986. Evaluation of the repair process of cartilage defects of the equine third carpal bone with and without subchondral bone perforation. Am. J. Vet. Res., 47(12):2637–2645.

Vangsness CT, Farr J, Boyd J, Dellaero DT, Mills CR, LeRoux-Williams M. 2014. Adult human mesenchymal stem cells delivered via intra-articular injection to the knee following partial medial meniscectomy: a randomized, double-blind, controlled study. J. Bone Joint Surg. Am., 96(2):90–98 DOI: 10.2106/JBJS.M.00058.

Zeifang F, Oberle D, Nierhoff C, Richter W, Moradi B, Schmitt H. 2010. Autologous Chondrocyte Implantation Using the Original Periosteum-Cover Technique Versus Matrix-Associated Autologous Chondrocyte Implantation A Randomized Clinical Trial. Am. J. Sports Med., 38(5):924–933 DOI: 10.1177/0363546509351499.

Zhang W, Chen J, Tao J, Jiang Y, Hu C, Huang L, Ji J, Ouyang HW. 2013. The use of type 1 collagen scaffold containing stromal cell-derived factor-1 to create a matrix environment conducive to partial-thickness cartilage defects repair. Biomaterials, 34(3):713–723 DOI: 10.1016/j.biomaterials.2012.10.027.

Zscharnack M, Hepp P, Richter R, Aigner T, Schulz R, Somerson J, Josten C, Bader A, Marquass B. 2010. Repair of chronic osteochondral defects using predifferentiated mesenchymal stem cells in an ovine model. Am. J. Sports Med., 38(9):1857–1869 DOI: 10.1177/0363546510365296.

3.2 List of figures

3.3 Publications related to this work

Poster Presentations

Schulz RM, **Somerson JS**, Zscharnack M, Hepp P, Richter R, Josten C, Bader A, Marquass B. Outcomes of pre-differentiated mesenchymal stem cell-seeded hydrogel implants in cartilage lesions. Biomedical Engineering Society Annual Meeting, Austin, TX: 6-9 October 2010.

Somerson JS, Marquaß B, Hepp P, Richter R, Aigner T, Josten C, Bader A, Zscharnack M, Schulz RM. Pre-differentiation of mesenchymal stem cells improves cartilage regeneration for treatment of chronic osteochondral lesions. 9th World Congress of the International Cartilage Repair Society; Barcelona, Spain: 26 – 29 September 2010.

Marquass B, Hepp P, **Somerson JS**, Osterhoff G, Schulz R, Josten C, Zscharnack M. Are biphasic constructs on basis of predifferentiated MSC inferior to autologous osteochondral grafts? 26th Conference of the German-speaking Association of Arthroscopy (AGA). Leipzig, Germany: 17-19 September 2009.

Marquass B, Schmidt S, Richter R, Stein F, **Somerson JS**, Hepp P, Schulz RM, Bader A, Josten C, Zscharnack M. Therapy of osteochondral defects using biphasic constructs from autologous mesenchymal stem cells in comparison to the OATS technique in a large animal model. 6th Research Festival for Life Sciences. Leipzig, Germany: 2007.

Publication

Marquass B[1], **Somerson JS**[1], Hepp P, Aigner T, Schwan S, Bader A, Josten C., Zscharnack M, Schulz RM. A novel MSC-seeded triphasic construct for the repair of osteochondral defects. Journal of Orthopaedic Research. 2010 Dec; 28(12): 1586-99.

[1]Both authors contributed equally to this work.

IV. Declaration of Independent Work

I formally declare that I have written the submitted dissertation independently and that I did not use any outside support except for the quoted literature and other sources mentioned in the paper. I herewith assure that no third parties have received either direct or indirect financial benefits from me for work connected with the submitted dissertation. The dissertation was not used in the same or in a similar version to achieve an academic grading and was not submitted or published so far of other authorities neither in Germany nor abroad. I have clearly identified and separately listed all of the literature which I employed when producing the dissertation, either literally or in content. All persons are named who were themselves directly involved or have contributed to the work. I also herewith declare that I have carried out my scientific work according to the principles of good scientific practice in accordance with the current rules of the University of Leipzig [Vorlesung zur “Guten Wissenschaftlichen Praxis”].

Leipzig 01.12.2015  Jeremy Samuel Somerson

V. Statement of Scientific Involvement of the Candidate

The publication “A Novel MSC-Seeded Triphasic Construct for the Repair of Osteochondral Defects” was the result of a team effort including multiple scientific researchers.

The work performed by the Candidate Jeremy Somerson in relation to this project is as follows:

1. Pre-study planning including power analysis and study operating procedures.
2. Assistance with study operations including surgical assisting for implantation, post-mortem dissection and preparation of specimens and performance of specimen imaging studies including micro-CT.
3. Histological analysis of specimens including macroscopic and microscopic cartilage evaluation with the International Cartilage Research Society scales.
4. Computer analysis of micro-CT data using ImageJ (NIH, Bethesda).
5. Statistical analysis of all data using SPSS (SPSS, Inc, Chicago).
6. Composition and submission of the final manuscript.

We can confirm these statements regarding the scientific work performed by the Candidate regarding the above-named publication.

The named first and second authors (Marquass B and Somerson JS) contributed equally to this work.

Leipzig, 01.12.2015

PD Dr. med. Bastian Marquass

Prof. Dr. med. Pierre Hepp

Dr. rer. nat. Ronny Schulz

Dr. rer. nat. Matthias Zscharnack

Prof. Dr. med. Christoph Josten

VI. Curriculum vitae

Jeremy Samuel Somerson

postgraduate training and fellowship appointments

Resident, Department of Orthopaedics (2010-2014)
University of Texas Health Science Center San Antonio (San Antonio, TX)
Director: Robert Quinn

Administrative Chief Resident (2014-2015)
University of Texas Health Science Center San Antonio (San Antonio, TX)

Visiting Resident, Department of Orthopaedics (September 2014)
Mayo Clinic (Rochester, MN)
Elective in Shoulder and Elbow Reconstruction
Director: Bernard F. Morrey

Fellow, Shoulder and Elbow Surgery (2015-2016)
University of Washington Medical Center (Seattle, WA)
Directors: Frederick Matsen and Winston Warme

education

University of Dayton (2001-2005)
Dayton, OH
B.S. in Premedical Studies, magna cum laude

University of Leipzig (2003-2009)
Leipzig, Germany
Course of Study: M.D.

licensure

Washington State, USA: Full Physician and Surgeon license (MD60462688)

awards, honors and memberships

2001	University of Dayton Presidential Scholar
2004	German Academic Exchange edu.de Scholar
2010-present	American Academy of Orthopaedic Surgeons resident member
2013	San Antonio Orthopaedic Society Resident Research Award
2014	J. Bradley Aust Resident Manuscript Award
2014	American Orthopaedic Association Resident Leadership Forum
2015	J. Bradley Aust Resident Manuscript Award
2015	Texas Orthopaedic Association Resident Research Award

grant funding

OMeGA Residency Grant (2013-2014)
Received $10,000 grant for the project “Microsurgery training for orthopaedic surgery residents”

research

Peer-reviewed journal publications

1. **Somerson JS**, Morrey ME, Sanchez-Sotelo J, Morrey BF. Current concepts: Diagnosis and management of periprosthetic elbow infection. *J Bone Joint Surg (Am)* [Accepted June 2015].

2. **Somerson JS**, Morrey ME, Morrey BF. Direct suture repair of unstable osteochondritis dissecans lesions of the capitellum: Surgical technique. *Techniques in Shoulder and Elbow Surgery* [Accepted June 2015].

3. Bois AJ, Whitney IJ, **Somerson JS**, Wirth MA. Humeral head arthroplasty and meniscal allograft resurfacing of the glenoid: A concise follow-up of a previous report and survivorship analysis. *J Bone Joint Surg (Am)* [Accepted April 2015].

4. **Somerson JS**, Wirth MA. Self-assessed and radiographic outcomes of humeral head replacement with nonprosthetic glenoid arthroplasty. *J Shoulder Elbow Surg*. 2015 July; 24(7): 1041-8.

5. Zscharnack M, Krause C, Aust G, Thummler C, Peinemann F, Keller T, Smink JJ, Holland H, **Somerson JS**, Knauer J, Schulz RM, Lehmann J. Preclinical good laboratory practice-compliant safety study to evaluate biodistribution and tumorigenicity of a cartilage advanced therapy medicinal product (ATMP). *J Transl Med*. 2015 May 20; 13(1): 160 [Epub ahead of print].

6. **Somerson JS**, Fletcher DJ, Srinivasan RC, Green DP. Compression screw fixation without bone grafting for scaphoid fibrous nonunion. *Hand*. 2015 Feb 18 [Epub ahead of print].

7. Holliday EB, Mitra HS, **Somerson JS**, Rhines LD, Mahajan A, Brown PD, Grosshans DR. Post-operative proton therapy for chordomas and chondrosarcomas of the spine: Adjuvant vs.salvage radiation therapy. *Spine (Phila Pa 1976).* 2015 Jan 23 [Epub ahead of print].

8. **Somerson JS**, Heiney JP, Battula S, Schoenfeld AJ. Hybrid plating shows equivalent biomechanical bending strength to unicortical locked plating. *J Mech Med Bio*. 2014 Oct; 14(5): 1450071.

9. **Somerson JS**, Rowley D, Kennedy C, Buttacavoli F, Agarwal A. Electromagnetic navigation reduces surgical time and radiation exposure for proximal interlocking in retrograde femoral nailing. *J Orthop Trauma*. 2014 Jul; 28(7): 417-21.

10. Schulz RM, Haberhauer M, Zernia G, Pösel C, Thümmler C, **Somerson JS**, Huster D. Comprehensive characterization of chondrocyte cultures in plasma and whole blood biomatrices for cartilage tissue engineering. *J Tissue Eng Regen Med*. 2014 Jul; 8(7):566-77.

11. **Somerson JS**, Bhandari M, Vaughan CT, Smith CS, Zelle BA. Lack of diversity in orthopaedic trials conducted in the United States. *J Bone Joint Surg (Am)*. 2014 Apr; 96(7):e56.

12. Malek F, **Somerson JS**, Mitchel S, Williams RP. Does limb-salvage surgery offer patients better quality of life and functional capacity than amputation? *Clinical Orthopaedics and Related Research*. 2012 Jul; 470(7): 2000-6.

13. Marquass B, **Somerson JS**, Hepp P, Aigner T, Schwan S, Bader A, Josten C., Zscharnack M, Schulz RM. A novel MSC-seeded triphasic construct for the repair of osteochondral defects. *Journal of Orthopaedic Research*. 2010 Dec; 28(12): 1586-99.

14. Zscharnack M, Hepp P, Richter R, Aigner T, Schulz R, **Somerson J**, Josten C, Bader A, Marquass B. Repair of chronic osteochondral defects using predifferentiated mesenchymal stem cells in an ovine model. *Am J Sports Med*. 2010 Sep; 38(9): 1857-69.

Invited review articles and book chapters

1. **Somerson JS** and Sanchez-Sotelo J. Deep infection after elbow arthroplasty: Diagnosis. In: Morrey BF, editor. Morrey's The Elbow and Its Disorders, 5th Ed. Philadelphia: Saunders [In process].

2. **Somerson JS**, Bois AJ and Wirth MA. Interposition shoulder arthroplasty. In: Armstrong AD and Murthi AM, editors. Anatomic Shoulder Arthroplasty: Strategies for Clinical Management. New York: Springer [In process].

3. Roy M, **Somerson JS**, Kerr K, Conroy J. Osteomyelitis: Pathophysiology and pathogenesis. In: Baptista MS, editor. Osteomyelitis. Rijeka: InTech Press; 2012.

4. Kupersmith L and **Somerson JS**. Amputation versus salvage for lower limb tumors. *Lower Extremity Review*. 2012 Jun.

Podium presentations

1. **Somerson JS**, Stein BA, Wirth MA. Distribution of high-volume shoulder replacement surgeons in United States metropolitan areas: Data from the 2014 Medicare Provider Utilization and Payment release. Texas Orthopaedic Association Annual Meeting 2015, Austin, TX: 17-19 April 2015.

2. Holliday EB, Mitra HS, **Somerson JS**, Rhines LD, Grosshans D. Post-operative proton radiation therapy for chordomas and chondrosarcomas of the spine: Timing of radiation initiation. American Academy of Orthopaedic Surgeons Annual Meeting 2015, Las Vegas, NV: 24-28 March 2015.

3. **Somerson JS**, Bhandari M, Vaughn C, Smith CS, Zelle BA. Lack of diversity in orthopaedic trials conducted in the United States. J. Bradley Aust Surgical Society Annual Meeting. San Antonio, TX: 6 June 2014.

4. **Somerson JS**, Bois A, Whitney IJ, Wirth MA. Meniscal allograft interposition versus non-prosthetic glenoid arthroplasty. Texas Orthopaedic Association Annual Meeting. San Antonio, TX: 10-12 April 2014.

5. **Somerson JS**, Bois A, Whitney IJ, Wirth MA. Humeral Head Replacement Combined With Meniscal Allograft Interposition Versus Non-Prosthetic Glenoid Arthroplasty: Preliminary Data From A Prospective Randomized Clinical Trial. American Academy of Orthopaedic Surgeons/American Shoulder Elbow Society Specialty Day, New Orleans, LA: 15 March 2014.

6. **Somerson JS**, Srinivasan RC, Fletcher DJ, Bagg MA, Pederson WC, Person D, Leversedge FJ, Green DP. Compression screw fixation without bone grafting for scaphoid fibrous nonunion. American Association for Hand Surgery Annual Meeting, Kauai, HI: 7-11 January 2014.

7. Rowley D, **Somerson JS**, Kennedy C, Buttacavoli F, Agarwal A. Electromagnetic Navigation Reduces Surgical Time and Radiation Exposure for Proximal Interlocking in Retrograde Femoral Nailing. Alamo Orthopaedic Society Biannual Meeting, San Antonio, TX: 19-21 April 2013.

8. **Somerson JS**, Baldwin M, Schmidt DR. Positions played by female basketball players presenting with ACL rupture. Texas Orthopaedic Association Annual Meeting, Austin, TX: 18-20 April 2013.

Abstract poster presentations

1. **Somerson JS**, Morrey ME, Morrey BF. Predictors of early reoperation after internal fixation of distal humerus fractures with articular involvement. American Shoulder and Elbow Surgeons 2015 Closed Meeting. Asheville, NC: 8-11 October 2015.

2. Waetjen E, **Somerson JS**, Girling R, Cromack D, Toohey J, Srinivasan R. Efficacy of Using Surgical Loupes versus Microscope Magnification to Train Orthopedic Residents in Microsurgery. 2014 American Orthopaedic Association Annual Meeting. Montreal, Canada: 18-21 Jun 2014.

3. **Somerson JS**, Kim I, Rajani R. Ethnic and gender disparities among limb-salvage rates in pediatric sarcoma patients. American Radium Society Annual Meeting 2014. St. Thomas, USVI: 22-26 Apr 2014.

4. **Somerson JS**, Bhandari M, Vaughan CT, Smith CS, Zelle BA. Lack of diversity in orthopaedic trials conducted in the United States. American Academy of Orthopaedic Surgeons Annual Meeting 2014, New Orleans, LA: 10-15 March 2014.

5. Schulz RM, **Somerson JS**, Zscharnack M, Josten C, Marquass B. Two-year outcomes in a randomized, blinded large animal model after mesenchymal stem cell transplantation for chronic focal cartilage defects. Tissue Engineering and Regenerative Medicine International Society North America Annual Conference and Meeting, Houston, TX: 11-14 December 2011.

6. Schulz RM, **Somerson JS**, Zscharnack M, Hepp P, Richter R, Josten C, Bader A, Marquass B. Outcomes of pre-differentiated mesenchymal stem cell-seeded hydrogel implants in cartilage lesions. Biomedical Engineering Society Annual Meeting, Austin, TX: 6-9 October 2010.

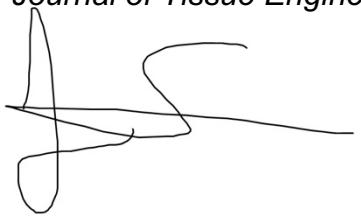
7. **Somerson JS**, Marquaß B, Hepp P, Richter R, Aigner T, Josten C, Bader A, Zscharnack M, Schulz RM. Pre-differentiation of mesenchymal stem cells improves cartilage regeneration for treatment of chronic osteochondral lesions. 9th World Congress of the International Cartilage Repair Society; Barcelona, Spain: 26 – 29 September 2010.

8. Marquass B, Hepp P, **Somerson J**, Osterhoff G, Schulz R, Josten C, Zscharnack M. Are biphasic constructs on basis of predifferentiated MSC inferior to autologous osteochondral grafts? 26th Conference of the German-speaking Association of Arthroscopy (AGA). Leipzig, Germany: 17-19 September 2009.

9. Marquass B, Schmidt S, Richter R, Stein F, **Somerson JS**, Hepp P, Schulz RM, Bader A, Josten C, Zscharnack M. Therapy of osteochondral defects using biphasic constructs from autologous mesenchymal stem cells in comparison to the OATS technique in a large animal model. 6th Research Festival for Life Sciences. Leipzig, Germany: 2007.

editorial positions

2010-present Reviewer: *Journal of Tissue Engineering and Regenerative Medicine*

Leipzig 01.12.2015  Jeremy Samuel Somerson

VII. Acknowledgements

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