Purpose: To determine the superiority of chondrogenic potential from BMSCs using an ultra-purified alginate gel over a commercial grade one and to assess the reparative tissues treated with BMSCs and a purified alginate gel.

Methods: Ultra-purified alginate (Sea Matrix®, Mochida Pharma., Japan, endotoxin level of 5.76 EU/g) and commercial grade alginate (Sodium Alginate 500, Wako, Japan, endotoxin level of 75,950 EU/g) were prepared. Cytotoxicity of CaCl₂: BMSCs in alginate were exposed to 50, 100, 200, and 400 mM CaCl₂. The living cells were counted with Cell Counting Kit-8 (CCK-8). Proliferation assay: BMSCs encapsulated in purified and commercial grade alginate beads were cultured in DMEM with 10% FBS. At 0, 1, 2, 3, and 7 days, the viable cells were counted with CCK-8. Alginates beads culture: Alginates beads containing BMSCs were cultured in chondrogenic medium containing TGF-β3. At 7 and 14 days, rabbit models were analyzed by real-time RT-PCR. At 14 and 21 days, each alginate bead was histologically evaluated. Rabbit model: The defects (Ø5mm) were created in the patella groove and divided into five groups: defect (no treatment); CAIg, commercial grade alginate alone; CAIgC, commercial grade alginate with BMSCs; PAig, purified alginate alone; PAigC, purified alginate with BMSCs. To avoid excessive exposure to cytotoxic CaCl₂, CaCl₂ was injected to the surface of alginate. It needed no any fixation. In vivo evaluation: At 4 and 12 weeks, the macroscopic and histological findings were scored with a grading scale, and the mechanical properties were measured by indentation test. Cadaver model: The stained alginate gel was injected into the defect with our novel technique. After the operation, manual mobilization was intermittently performed. Statistical Analysis: Comparison was assessed by an ANOVA followed by multiple-comparison post hoc tests. P < 0.05 was statistically considered significant.

Results: Cytotoxicity of CaCl₂: The cell number in the 200 and 400 mM groups significantly decreased, compared to the other groups (p < 0.05). Proliferation assay: From 1 to 7 days, the number of living cells in the purified alginate gel was significantly higher (p < 0.05). Alginates beads culture: The levels of aggrecan and sox 9 genes in the purified alginate at 14 days were significantly higher (p < 0.05). The proliferated BMSCs and a newly synthesized matrix were observed within pore spaces in the both alginate. Rabbit model: The surface of the defect group was rough and depressed. In the PAig and PAigC groups, the defects were substituted with firm and white cartilage-like tissue. The macroscopic score at 12 weeks in the PAigC group was significantly superior to the CAIg and CAIgC groups (p < 0.05). Histological findings: In the CAIg and CAIgC groups, residual alginate gels were found, and inflammatory reactions which were found in subchondral bone lesion. The PAigC group exhibited the normal cartilaginous structures. The histological scores at 12 weeks were significantly higher in the PAig and PAigC groups than in the defect and CAIgC groups (p < 0.05). Mechanical properties: From 4 to 12 weeks, the modulus of the CAIgC and PAigC groups significantly improved (p < 0.05) and approximately reached to 80% of normal. Cadaver model: After mobilization, the alginate gel was not detached and maintained the initial shape and hardness.

Conclusions: We confirm that the purified alginate gel has a great promise as a cell-carrier material for a clinical immunosolated implantation of cells into cartilage defects. This study showed a clinical possibility of arthroscopic cartilage implantation using the current injectable system to the articular cartilaginous lesions.

Purpose: Articular cartilage shows poor intrinsic repair, leading to progressive joint damage. Therapies like marrow stimulation or tissue engineering of cartilage depend on the chondrogenic differentiation of progenitor cells. However, this chondrogenic differentiation needs to take place in a diseased joint with an altered environment. We postulate that catabolic factors in this environment will inhibit chondrogenesis of progenitor cells.

Methods: We examined the effect of interleukin-1 (IL-1) and tumour necrosis factor alpha (TNFalpha) on human mesenchymal stem cells undergoing chondrogenic differentiation. In addition, osteoarthritis synovium derived conditioned medium was added to human mesenchymal stem cells undergoing chondrogenic differentiation. Chondrogenesis was examined by determining the mRNA levels of the chondrogenic markers collagen type II and aggrecan. Additionally, the hypertrophy marker collagen type X was examined. Proteoglycan deposition was analyzed by safranin O staining of histological sections.

Results: Chondrogenesis of human mesenchymal stem cells, as determined by safranin O proteoglycan staining, was completely abolished at day 14 by adding IL-1 (10 ng/mL) from day 0, 3, 7 and 10 onwards. Under these conditions the expression of type II collagen and aggrecan mRNA as well as the hypertrophy marker collagen type X were also blocked completely, except by late addition of IL-1 (day 10), resulting in only a partial inhibition of these mRNA markers. IL-1 appears to be a very potent inhibitor of chondrogenesis, not only at the onset, but throughout the process of chondrogenesis. IL-1 as well as TNFα inhibited chondrogenesis in a dose-dependent manner. Even at the lowest dose tested (0.1 ng/mL) IL-1 and TNFalpha downregulated the mRNA levels of collagen II, aggrecan and collagen type X. However, TNFα showed less potent effects on this downregulation than IL-1. Moreover, TNFα did not inhibit safranin O staining completely even at the highest concentration applied (10 ng/mL). In contrast, safranin O staining was already fully blocked by 1.0 ng/mL IL-1, indicating the greater potency of IL-1 in inhibiting chondrogenesis. Addition of osteoarthritic conditioned medium, containing an unknown mixture of anabolic and catabolic factors, strongly inhibited chondrogenesis of differentiating mesenchymal stem cells. Conditioned medium of synovia of five different patients inhibited proteoglycan deposition. In addition, chondrogenic mRNA markers were inhibited strongly in all patients but one, in which they were slightly stimulated. These results show that chondrogenesis of differentiating mesenchymal stem cells is inhibited by factors produced by OA synovium.

Conclusions: Our study shows for the first time that chondrogenesis of human mesenchymal stem cells is blocked by single catabolic factors as well as factors produced by osteoarthritic synovium. Our findings have major implications for therapies that are currently being applied or developed. Therapies such as tissue engineering or marrow stimulation techniques depend on tissue repair and chondrogenesis by mesenchymal stem cells. In addition, the intrinsic repair response will be blocked by catabolic factors present in disease joints. Successful cartilage regeneration can be expected to fail if the catabolic environment of a damaged joint is not altered. Our study shows that transplantation of mesenchymal stem cells is insufficient and needs to be combined with inhibition of catabolism.
THE PRESENCE OF A CALCIFIED INTERFACE
THE EFFECT OF LOW-INTENSITY PULSED ULTRASOUND
BONE-MARROW-DERIVED CELLS (BMDCS) ONE STEP

After the cells had attached to the Transwell® membranes, 0.6 ml culture
was added to each membrane in the lower compartment. Following 4 h of incubation at the same tem-
perature, the cells on the upper side of the membrane were removed with a cotton swab, and the remaining migrated cells on the lower side
of the filter were fixed with 4% formaldehyde and stained with crystal
violet. Control wells with only DMEM in the bottom well were included in
the experiment. All measurements were from at least six independent
experiments performed in triplicate.

The number of migrated cells in the control and stimulated wells was counted for 10 random fields per well at 100× magnification. Results
were expressed as a chemotactic index (CI). This index was determined as
the average number of migrated cells in control wells. Data were statistically
analysed with the Mann-Whitney U test for comparison of the two groups.

Results: The chemotaxis index for the control group was CI: 5.13 ± 0.42,
while that for the stimulated group was CI: 9.13 ± 0.61 (p < 0.05) was considered statistically
significant.

Results: rhPDGF-BB (1 ng/ml) added to the bottom compartment of the Transwell insert caused a significant increase in migrating MSCs from
patients with OA compared to controls (CI: 5.13 ± 1.19 vs. 3.35 ± 0.42, p = 0.043). When identical amounts of rhPDGF-BB were simultaneously
added to the both upper and lower compartments, the cell migration of MSC remained unchanged.

Conclusions: These data could indicate an activation of MSCs from bone
marrow in OA in response to permanent signals sent by the bone and
cartilage damage characteristic of this disease.

486 THE PRESENCE OF A CALCIFIED INTERFACE
STRENGTHENS TISSUE ENGINEERED
CARTILAGE-SUBSTRATE INTERFACIAL SHEAR STRENGTH

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Purpose: To design tissue engineered cartilage with improved attachment
to a porous calcium polyphosphate (CPP) bone substitute (biphasic
construct) by the formation of a zone of calcified cartilage (ZCC) at
the cartilage-CPP interface zone.

Methods: Cartilage-CPP biphasic implants were formed by seeding chondrocytes isolated from the deep-zone of bovine articular cartilage
onto the top surface of porous CPP substrates coated with a thin sol-
gel-formed hydroxyapatite film. The cells were grown in Ham’s F12 containing 10% FBS in the absence or presence of -glycerophosphate
supplementation to induce mineralization. After 8 weeks, tissues were
harvested and examined histologically, biochemically, by RT-PCR for gene
expression and for the shear properties of the interface between cartilage
and the substrate using a specifically designed shear test apparatus.

Results: Histological sections stained with toluidine blue and von
Kossa demonstrated cartilage tissue with a thin ZCC when cultured
in mineralization-inducing media. The ZCC was localized in the tissue
directly above the substrate, mimicking osteochondral zonal organization.
The mineral formed in cartilage in vitro under these conditions has similar
composition and crystal size to that present in the ZCC of native cartilage.
Consequently, new mineralization was observed in cartilage formed in the ab-
ence of -glycerophosphate. Collagen and glycosaminoglycan contents
were similar for tissues with and without a calcified zone. Expression
levels of cartilage extracellular matrix genes including collagen type II
and type X were not affected by the generation of a ZCC. Interestingly,
the expression of the pro-collagen I gene was increased by 2- and 3-fold,
respectively, in the tissue engineered cartilage with a ZCC compared to cartilage without a ZCC.

This was associated with 240% improvement in the peak shear load and in 340% increase in energy to failure measurements. The maximal
displacement prior to failure was similar in both tissues.

Conclusions: This study demonstrates a significant increase in interfacial shear strength of biphasic cartilage constructs by mimicking the zonal
organization of articular cartilage by generating a ZCC at the tissue/bone
substrate interface. Generating biphasic cartilage constructs with a cal-
cified cartilage interface will be critical for the clinical success of biphasic
constructs used to repair larger joint defects.

487 THE EFFECT OF LOW-INTENSITY PULSED ULTRASOUND
FOR FORMING OF SCAFFOLD-FREE CARTILAGE TISSUE
IN VITRO

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Purpose: The aim of this study is to evaluate the effect of low-intensity pulsed ultrasound (LIPUS) for forming of scaffold-free cartilage in vitro.

Methods: Chondrocytes were collected from articular cartilage of Wis-
star rats. For acquisition the number of cells, the collected primary chondrocytes (passage 0) were cultured up to subconfluent in 500 cm²
square dishes. The cells were then condensed to the density at 10⁷
cells/cm² (passage 1) on synthetic membranes. The LIPUS application group was stimulated by LIPUS for 20 min/day. The mode of the applied ultrasound is a 200 µs sine wave of 1.5 MHz repeating at 1 kHz with an
intensity of 30 mW/cm². To investigate effect LIPUS stimulation on the matrix-synthesis of the tissues, mRNA expression of type II collagen
(col2), aggrecan and type I collagen (col1) was studied using real-time polymerase chain reaction. Synthesis of type II collagen and proteoglycan
was also assessed histochemically in 4 weeks culture.

Results: In our previous study, we presented that high-density culture with P1 chondrocytes more than 10⁶ cells/cm² could form a cell mass as
scaffold-free cartilage tissue under existing cell-cell interactions.

In this way, the chondrocytes prepared at 10⁷ cells/cm² automatically
detached from the membranes to form a mass of chondrocytes around
the third day of starting P1 culture. After forming the mass, they were
moved to simple 6 well dishes for oxygen and nutrition diffusion. The
expression of col2 and aggrecan mRNA was significantly higher in the
group by stimulation of LIPUS than the group by no stimulation.

The resultant scaffold-free cartilage like tissues formed via cell conden-
sation at 10⁷ cells/cm² grew in thickness until the 7th day of P1 culture.
They grew in size until the 14th day of P1 culture. The tissues were stained
with safranin O, and there were partially columnar pattern. Immunohistochemical analysis revealed that type II collagen
labeled was abundantly deposited in the tissues. Moreover with toluidine
blue staining, there was a metachromatic matrix in them. Respectively, LIPUS stimulation group was more strongly stained than the control
group.

Conclusions: The chondrocytes of P1 cells prepared at 10⁷ cells/cm²
developed into scaffold-free cartilage like tissue under existing cell-cell
interactions. As a result of application of LIPUS, we could form scaffold-
free cartilage like tissues which were similar to native cartilage.

488 BONE-MARROW-DERIVED CELLS (BMDCS) ONE STEP
REPAIR PROCEDURE (“ONE STEP”) VERSUS AUTOLOGOUS
CHONDROCYTE IMPLANTATION (ACI) IN SURGICAL
TREATMENT OF OSTEOCHONDRAL LESIONS OF THE
TALUS: A COMPARATIVE HISTOLOGICAL STUDY

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Purpose: Osteochondral lesions of the talus are frequent occurrences in
young active patients. Various surgical options have been proposed
derto these lesions in order to restore a continuous and adequate cartilaginous
layer at the lesion site. Among them, only mosaicplasty and Autologous
Chondrocyte Implantation (ACI) were capable, up to now, to restore
hyaline cartilage at the lesion site, although major disadvantages of these
techniques have been widely described. These considerations led in
search of new methods for cartilage repair and recently the use of Bone
Marrow-Derived Cells (BMDCs) was indicated as a reliable alternative
for the treatment of articular cartilage defects. The aim of this study was
to verify the ability of an original technique based upon BMDCs to
regenerate hyaline articular cartilage, and to compare the clinical and
histological results with them obtained after ACI.

Methods: From October 2005 to April 2008, 50 patients affected by
osteochondral lesions of the talus >1.5 cm² underwent a new arthroscopic
“One Step” procedure by using bone marrow harvested from the iliac
crest, directly concentrated in Operatory Room and seeded on a scaffold
with the addition of Platelet-Rich-Fibrin (PRF).

Patien evaluation included clinical AOFAS score, X-Rays, MRI preop-
and at different established follow-up. A series of 46 patients
operated by ACI procedure and comparable for age and lesion types
was used as control group. At 12 months follow up after “One Step” procedure,
an arthroscopic second look and a biopsy of the regenerated cartilage