surface of the patellofemoral joint after MPFL reconstruction by second-look arthroscopy.

Several papers have been reported for cartilage lesions of the patellofemoral joint in recurrent patellar dislocation [7, 26]. Nomura et al. [7] reported that the continuation of patellar dislocation made the patellar cartilage lesions worse. The mechanism for cartilage damage is thought to involve the shear stress produced as the patellar dislocates and reduces, and the medial facet and the central ridge of the patella engage the lateral femoral condyle. The main reason for the healing cartilage lesion on the central ridge after MPFL reconstruction seems to be avoidance of further dislocation by MPFL reconstruction. Before MPFL reconstruction, the patella was laterally shifted in all patients. After MPFL reconstruction, the patella was medialized to the center of the trochlear groove. However, such medialization of the patella by tightening of the medial structure might generate increased joint pressure at the patellofemoral joint, especially with high-graded trochlear dysplasia. In this series, three of six patients with improvement of chondral lesions had low-grade dysplasia, and the ratio was greater when compared to the entire cohort. The present results suggest that MPFL reconstruction could change the natural course of patellofemoral osteoarthritis for patients with recurrent patellar subluxation. However, fibrous cartilage tissue might cover the region of chondral damage in most cases. Because fibrous cartilage might be mechanically and biologically different from native hyaline cartilage tissue, longer follow-up with regard to the development of patellofemoral osteoarthritis is necessary.

On the other hand, slight deterioration in the femoral groove was observed in six knees without significance. This fact indicates that MPFL reconstruction might generate a slight increases in joint pressure at the patellofemoral joint, particularly in the femoral groove, by reducing the patella to the center on the femoral groove. Effects of MPFL reconstruction on patellofemoral contact pressure and kinematics have been investigated by several authors [3, 22, 27–29]. While Bicos et al. [3] reported that overload of the medial femoral trochlea was not noted with reconstruction of the MPFL, Elias et al. [27] reported that small errors in graft length and position could dramatically increase the force and pressure applied to the medial patellofemoral cartilage in vitro. Servien et al. [30] reported that only 65 % of femoral tunnels, the location of which had been identified by visual inspection during surgery, were located in a proper position after MPFL reconstruction. In the current case series, femoral tunnels were created using intraoperative anatomical landmarks alone, and non-anatomical placement of femoral tunnel might lead to cases with a deteriorated chondral surface in the femoral groove. Currently, all femoral drill positions are checked on intraoperative lateral radiography to achieve anatomical placement of the femoral drill hole [28, 31]. Moreover, increased patellofemoral contact pressure could be avoided by applying low loads to the graft [29], checking graft isometricity [32], or adding lateral release, although further examination of this issue is needed before such recommendations can be applied clinically.

Several limitations in this study must be considered. First, not all patients who underwent MPFL reconstruction were examined in this series. Some potential for bias in patient selection may thus exist, and the 32 knees investigated in the present study might not have been representative of all 81 patients. Second, the stability and repeatability of the ICRS score have been reported as satisfactory, and internal consistency is adequate [33]. However, such a subjective evaluation method might influence the results, and objective evaluation methods such as magnetic resonance imaging should therefore be adopted. Third, a period of 1 year from initial surgery to second-look arthroscopy might be too short to detect the effects of MPFL reconstruction on the patellofemoral cartilage. Longer follow-up with regard to the development of patellofemoral osteoarthritis is necessary, and further examination is needed for the establishment of an optimal operation in order to prevent the onset of further osteoarthritis.

In summary, according to short-term results, patellofemoral chondral status after isolated anatomical medial patellofemoral ligament reconstruction was not altered at second-look arthroscopy in most part of patellofemoral joint. At the central ridge of the patella, significant improvement of ICRS grading was observed. Chondral injuries in general might not worsen after MPFL reconstruction.

Conflict of interest The authors report no conflict of interest.

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Osteochondral Tissue Engineering with Biphasic Scaffold: Current Strategies and Techniques

Kazunori Shimomura, MD, PhD,[1,2] Yu Moriguchi, MD, PhD,[1] Christopher D. Murawski, BS,[3] Hideki Yoshikawa, MD, PhD,[1] and Norimasa Nakamura, MD, PhD[4,5]

The management of osteoarthritis (OA) remains challenging and controversial. Although several clinical options exist for the treatment of OA, regeneration of the damaged articular cartilage has proved difficult due to the limited healing capacity. With the advancements in tissue engineering and cell-based technologies over the past decade, new therapeutic options for patients with osteochondral lesions potentially exist. This review will focus on the feasibility of tissue-engineered biphasic scaffolds, which can mimic the native osteochondral complex, for osteochondral repair and highlight the recent development of these techniques toward tissue regeneration. Moreover, basic anatomy, strategy for osteochondral repair, the design and fabrication methods of scaffolds, as well as the choice of cells, growth factor, and materials will be discussed. Specifically, we focus on the latest preclinical animal studies using large animals and clinical trials with high clinical relevance. In turn, this will facilitate an understanding of the latest trends in osteochondral repair and contribute to the future application of such clinical therapies in patients with OA.

Introduction

OSTEOARTHRITIS (OA) is a common disease causing joint pain, joint deformity, and functional disability. Overall, as many as 40% of patients aged 65 years and older may have symptomatic OA in large joints, consequently affecting the quality of life of elderly populations.[1–3] Current treatment strategies can be divided into nonsurgical (conservative) and surgical therapies according to the severity of OA.[4–6] In the early stage of OA, pharmacologic and/or physical therapies as conservative treatments are typically selected for the purpose of reducing pain, and, in some cases, attempting to delay the progressive structural deterioration in affected joints. Surgical therapies such as joint replacement and osteotomy are available for patients who fail to respond to more conservative measures. These treatments are well established and effective for reducing pain and improving quality of life. Regardless of the available therapeutic options, however, there is no method available that facilitates complete healing of the articular cartilage.[7–12] Recently, several biological approaches, such as the use of tissue-engineered materials, have been tested to overcome such potential problems. This review will focus on the feasibility of employing tissue-engineered materials in osteochondral repair and highlight recent advances in the biological repair of osteochondral lesions.

Anatomy of Cartilage and Subchondral Bone

The osteochondral complex consists of both the articular cartilage and underlying subchondral bone. Biochemically, cartilage tissue largely comprises water, chondrocytes, type II collagen, and proteoglycan.[13–15] Cartilage can be differentiated into four distinct zones: the superficial, middle, deep, and calcified cartilage zones (Fig. 1).[16] Each zone is defined by a particular composition and organization of cells and extracellular matrix (ECM) molecules. The differential proportions in ECM composition influence the mechanical properties of each zone of the cartilage. For example, the superficial zone is strong in tension along the alignment of its collagen fibrils, thereby assisting in the resistance of shear forces at the surface. By comparison, the deep zone has a more compressive strain.

Bone is a complex tissue consisting of water, collagen type I, and hydroxyapatite (HA), with the two latter components providing the tissue's stiffness and compressive strength.[13,14,17] The compressive modulus of the subchondral bone is higher than that of cartilage. The different

[1]Department of Orthopaedics, Osaka University Graduate School of Medicine, Osaka, Japan.
[2]Center for Cellular and Molecular Engineering, Department of Orthopaedic Surgery, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.
[3]Department of Orthopaedic Surgery, University of Pittsburgh, Pittsburgh, Pennsylvania.
[4]Institute for Medical Science in Sports, Osaka Health Science University, Osaka, Japan.
[5]Center for Advanced Medical Engineering and Informatics, Osaka University, Osaka, Japan.

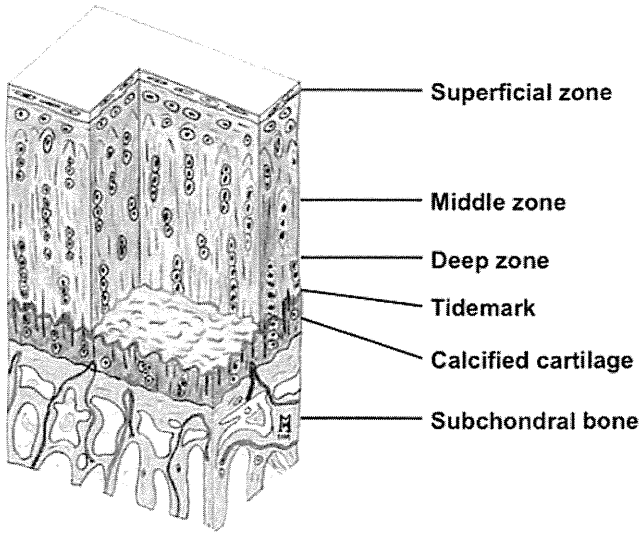

FIG. 1. Schematic drawing of the different zones of articular cartilage and subchondral bone (Quoted and modified from ref #16). Color images available online at www.liebertpub.com/teb

morphological compositions and mechanical properties of bone and cartilage indicate the complexity of the tissue interface.

The osteochondral interface is described by the interaction of calcified cartilage and the underlying subchondral bone.[18] Structurally, collagen fibers extend from the deep zone to the calcified cartilage through a wavy tidemark, which enables the dispersal of force through the vertical orientation of collagen fibrils.[19] However, despite the fact that calcified cartilage is mineralized tissue, its mechanical strength is lower than that of the subchondral bone.[20] Calcified cartilage is interdigitated with subchondral bone, but fibers do not extend across the zone into the bone.[19,21] The wavy tidemark and vertically oriented fibers at the tidemark, as well as interdigitations present at the interface, may enable a reduction in stress concentrations, as well as a better integration with the underlying subchondral bone.[14,19]

An osteoarthritic joint is characterized by degenerative changes, such as articular cartilage loss, subchondral bone thickening, and osteophyte formation.[22–26] The primary morphologic changes include thinning, fissuring, and fragmentation of articular cartilage. With progression of the disease comes a continuous loss of articular cartilage, accompanied with a decrease of collagen type-II and aggrecan,[27,28] leading to exposure of subchondral bone. Secondary changes are frequently seen in the underlying bone, such as fibrosis, cystic change, and new bone formation. These changes are considered to be triggered by a multitude of factors, including aging, trauma, obesity, mechanical overload, congenital disorder, and infection, which do not heal spontaneously once damaged.

Strategy for Osteochondral Repair

For an ideal repair of osteochondral lesions, it is important to regenerate subchondral bone and to facilitate zonal restoration of cartilage and subchondral bone, layer by layer, mimicking the natural articular structure.[29–35] As a strategy to regenerate these structures in a layer-by-layer fashion, biphasic or triphasic constructs have been developed due to both mechanical and biological reasons, including the acquisition of initial mechanical strength, mimicking a natural articulate structure, a uniform tidemark at the osteochondral junction, and integration of the biphasic implant with host tissue to sustain biological function.[7,36–44] For satisfying the biological requirements, an osteochondral implant should ideally have a rigid osseous layer (to support the overlying cartilage and integrate with the native bone) and a chondral layer (to enable the seeding and proliferation of chondrocytes or mesenchymal stem cells (MSCs) and subsequent deposition of cartilaginous ECM).

Design and Fabrication of Biphasic Scaffold

A successful tissue engineering approach for osteochondral repair involves the design of a biphasic scaffold with the potential to regenerate both cartilage and subchondral bone. The fabrication of the majority of scaffolds is performed through independent processes, by which different scaffolds for the two sides are created and then combined, or via a simultaneous process through which a single scaffold is created and cultured simultaneously for both sides.[14,45] A biphasic construct developed independently enables the cultivation of both chondrogenic and osteogenic cells in separate media and environmental conditions. However, these constructs should be hybridized into a single composite graft by connecting the two layers together. The potential disadvantage of this approach might be the difficulty of achieving a secure biological and mechanical integration between the two layers.[45] On the other hand, when the two layers are hybridized before culture, a complicated system will be required to promote osteo- and chondral differentiation separately in each layer. Due to the difficulty of two different cell cultures simultaneously, such predeveloped biphasic constructs are mainly used as a cell-free scaffold.[31]

Some research groups have raised the importance of an intermediate layer between the cartilage and subchondral bone layers to represent the tidemark or calcified cartilage; triphasic scaffolds were, therefore, developed.[31,37] However, the intermediate layer has unique osteochondral characteristics owing to the infiltration of blood vessels, and, thus, it may be difficult to mimic the unique structure with currently available biomaterial technologies. In fact, the superiority of triphasic scaffolds over biphasic ones for osteochondral repair has not yet been demonstrated and requires further investigation.

Choice of Cells and Growth Factors

The most direct cell source may be the biopsy specimens taken from the patients from whom mature osteoblasts and chondrocytes may be obtained. However, since the number of cells obtained is usually limited, it is typically not enough to enable seeding onto the scaffolds. In addition, the expansion of primary cells may result in a loss of differentiation capacity; for example, the expansion of articular chondrocytes can lead to de-differentiation into fibroblasts.[46–48] To overcome such potential problems with regard to de-differentiation, a three-dimensional (3D) culture can be used to retain the cellular phenotype and avoid de-differentiation.[49] The most common method is the use of various scaffolds to produce a 3D culture condition,[50,51] and it may be combined with the supplementation of growth

factors,[52] the use of bioreactors,[53] mechanical stimulation of the cells,[54,55] and the use of low oxygen tension[56] during cultivation. In addition, even if chondrocytes lose their differentiated phenotype, de-differentiated chondrocytes can regain their differentiated phenotype through the re-differentiation process of cultivation in a 3D scaffold combined with growth factors.[57,58]

As an additional option, stem cells may represent promising alternatives.[59] Specifically, MSCs have the capability to differentiate into a variety of connective tissue cell types, including bone, cartilage, tendon, muscle, and adipose tissue.[8,60] These cells may be isolated from various tissues, such as bone marrow, skeletal muscle, synovial membrane, adipose tissue, and umbilical cord blood.[11,12,60–63] In addition, the use of a growth factor or its cocktail (combination), including insulin-like growth factor-1 (IGF-1), transforming growth factor beta-1 (TGF-β1), fibroblast growth factor-2 (FGF-2), and bone morphogenetic proteins (BMP-2, BMP-7), may support tissue maturation for cartilage.[64–67] Similar to cartilage, the bone also possesses a large variety of growth factors that are involved in the regenerative process, including TGF-β, BMP-2, -4, -6, and -7, IGF-1 and -2, and platelet-derived growth factor (PDGF).[68–70]

On the other hand, some researchers have tested an acellular approach using a scaffold alone.[31,71] Considering the time and cost effectiveness, as well as safety issues associated with cell culture, this approach could represent a reasonable strategy in tissue engineering. Scaffolds should be developed to meet requirements such as the recruitment of enough tissue progenitor cells from the host tissue.

Materials of Cartilage Layer Scaffold

Several methods have been proposed to develop biphasic scaffolds with the hybridization of two distinct biomaterials, each of which is adequate to integrate with the respective surrounding tissue.[45] Many specific material types have been developed for both cartilage and bone regeneration, which are typically made of biocompatible and biodegradable polymers. For the cartilage layer, natural or synthetic polymer based scaffolds are commonly used. More recently, scaffold-free implants have been developed and their potential feasibility has been tested.

Natural polymers

The materials of natural-derived polymers could provide a naturally occurring environment for cells and tissues, thereby potentially facilitating cell proliferation and differentiation.[72,73] Moreover, natural polymers usually contain specific molecular domains that can support and guide cells at various stages of their development[14,45]; thus, biological interactions of the scaffold with the host tissue can be enhanced. However, they are, in general, biomechanically weak and less stiff than other materials.[14] As a source of materials, collagen, gelatin, glycosaminoglycan, chitosan, starch, hyaluronic acid, alginate, and bacterial-sourced polymers (hydroxyalkanoates) are commonly used.

Synthetic polymers

Biodegradable synthetic polymers offer several advantages compared with other materials for developing scaffolds in tissue engineering. The main advantages are being able to control mechanical properties (i.e., strength and stiffness) and degradation speed.[74] Synthetic polymers are also attractive, because they can be fabricated into various shapes with a desired pore according to the speed of cell migration or tissue in-growth.[75] Moreover, the progression of current techniques such as electrospinning methods and the 3D printer have enabled the simple design and fabrication of scaffolds.[76–78] On the other hand, synthetic polymers have limitations in bioactivity due to their hydrophobic surface not supporting cell attachment and proliferation.[79–82] Surface treatment with chondroitin sulfate,[83] silicate,[84] and alkaline[81] could increase hydrophilicity and provide a suitable scaffold for tissue engineering. In addition, these polymers, incorporated with growth factors such as TGF-β and BMP, would be helpful and convenient to support cell proliferation and differentiation, stimulating the repair of damaged tissue.[85,86] As a source of biodegradable synthetic polymers, poly(glycolic acid), poly(D,L-lactic-co-glycolic acid), poly(L-lactic acid), poly(caprolactone), and poly(ethylene glycol) have been commonly used.

Scaffold-free biomaterials

Polymer-based scaffolds have been reported to contribute to good osteochondral repair *in vivo*.[36–41] Despite this, there remain several concerns associated with the long-term safety of these constructs due to the involvement of chemical- or animal-derived materials. To overcome such potential problems, we have developed a scaffold-free 3D tissue-engineered construct (TEC) composed of MSCs derived from the synovium and ECMs synthesized by the cells.[11,12] The feasibility of the resultant TEC to facilitate cartilage repair was demonstrated in a large animal model.[8,11,87] These TECs are developed without an artificial scaffold, and, thus, their implantation could eliminate or minimize the risk of potential side effects induced by extrinsic chemical or biological materials. Furthermore, such TEC are highly adherent to cartilage matrix and secure integration of the TEC until adjacent cartilage tissue is observed after implantation. Therefore, combined constructs of TEC and several materials for the subchondral bone layer may effectively repair an osteochondal lesion with zonal restoration, and TEC could be considered one of the strong candidates for a cartilage bioimplant. In our animal study, we have demonstrated that the combined bioimplant of TEC and ceramic-based artificial bone significantly accelerated and improved osteochondral repair. (*in submission*).

Materials of Subchondral Bone Scaffold

For a scaffold of the subchondral bone layer, it is important to choose materials with initial mechanical strength, good bone ingrowth, and integration of native surrounding bone. Ceramics, glasses, and metallic materials are commonly used as follows. In addition, natural or synthetic polymers, mentioned earlier, could be used alone or combined with ceramics.[41,43,88–91]

Ceramics and glasses

Ceramics, such as HA or other calcium phosphates, such as tricalcium phosphate (TCP) and bioactive glasses, such as

Bioglass®, are widely used for bone tissue engineering.[92–95] These materials promote the formation of a bone-like tissue and enhance integration of the scaffold to the host tissue due to excellent osteoconductivity and osteoinductivity. The inclusion of growth factors in the scaffolds may be an interesting concept to explore and contribute to the maturation of bone tissue. Notably, the inclusion of BMP-2 in an HA-based scaffold was reported to promote subchondral bone repair as well as cartilage.[92] On the other hand, these scaffolds have low structural integrity due to being brittle and unsuitable for applications under mechanical stress, although they exhibit suitable stiffness.[14] The degradation behavior of these scaffolds can be controlled by changes in the porous structures, which can be tailored in terms of their degradation kinetics appropriate for bone tissue engineering. It is also well known that increasing porosity further impairs the mechanical properties of bioceramic scaffolds. This problem can be solved by modifying any porous scaffolds with infiltration or coating by biodegradable polymers.[96–98]

Metallic materials

Metals are widely used in orthopedic implants such as titanium, titanium alloys, stainless steels, and cobalt-chromium alloy. As an application of osteochondral bone repair, metallic materials withhold the capability of withstanding mechanical loading when used in the subchondral bone layer. On the other hand, the lack of degradation over time and the possibility of wear particle release or corrosion are disadvantages. As one such example, porous tantalum was reported to induce subchondral bone growth and showed integration to adjacent host bone in an *in vivo* rabbit study.[99]

Preclinical Study and Clinical Trial

Many therapeutic procedures have been investigated that biologically repair damaged cartilage, some of which are already at the stage of clinical application. On the contrary, considering the higher incidence of OA, which involves subchondral bone pathology, by comparison to isolated chondral injury,[1,5,100–103] there is an urgent need to develop novel therapeutic methods for osteochondral repair with clinical relevance. In this regard, the number of animal experiments and clinical trials to treat osteochondral lesions has been recently increased. In Table 1, we outline the latest preclinical animal studies using large animal and clinical studies.

Marquass *et al.* used an MSC-seeded combined implant with a collagen I hydrogel and β-TCP in an ovine osteochondral defect model and showed comparable repair quality to osteochondral autografts in terms of histology and biomechanical testing.[37] Miot *et al.* prepared engineered cartilage, which was generated from autologous chondrocytes cultured in hyaluronic acid scaffolds of different preculture periods, and implanted the engineered cartilage above HA/hyaluronic acid sponges into goat osteochondral defects. They concluded that 2 weeks of preculture of engineered cartilage achieved a suitable compromise between tissue maturity and structural/integrative properties of the repair tissue. These data demonstrate that the stage of development of engineered cartilage is an important parameter to be considered in designing cartilage repair strategies.[104] Kon *et al.* used an aragonite/hyaluronate biphasic scaffold for osteochondral defects in a goat model and showed that mechanical modification with drilled channels in the cartilage phase and impregnation of HA within the coral pores enhanced the scaffold's cartilage regenerative potential.[105] Schleicher *et al.* compared two biphasic scaffolds of either hydroxylapatite/collagen or allogenous sterilized bone/collagen and tested their integration in a sheep model. They showed that the latter scaffold proved to be stable and sufficiently integrated in the short term.[106] Kon *et al.* developed an acellular three-gradient multilayer scaffold made of collagen type I and nano-particles of HA, and tested the scaffold with or without autologous chondrocytes in sheep osteochondral defect model. They concluded that the scaffold contributed to the process of bone and hyaline-like cartilage regeneration, regardless of the use of chondrocytes.[91] They also treated 27 patients with chondral or osteochondral lesions using an acellular scaffold,[31,107,108] and demonstrated the safety and potential clinical benefit of the graded biomimetic osteochondral scaffold in promoting bone and cartilage tissue with good clinical and magnetic resonance imaging results until the 5-year follow up. Dhollander *et al.* treated 27 patients for cartilage lesions with an acellular osteochondral plug, which is composed of polylactide-co-glycolide copolymer, calcium-sulfate, polyglycolide fibers, and surfactant (TruFit plug; Smith & Nephew, Andover, MA).[71] In this clinical pilot study, a modest clinical improvement became apparent at 12 months of follow up. In addition, MRI data showed no deterioration of the repair tissue. However, 20% of the patients had persistent clinical symptoms after surgery, and had an additional surgery such as removal of the osteochondral plug remnants. The two latter studies were Level IV study, and further studies, which would be compared with conventional treatment such as bone marrow stimulation and osteochondral transplantation, are necessary. In contrast with cell-free scaffolds, no clinical trial using cell-seeded scaffolds has been reported, and these studies should be expected in the near future.

Summarizing recent animal studies (Table 1), the work has been focused on not only investigating the effectiveness of materials or cells, but also on applying several new concepts and techniques such as mechanical,[105] microstructural,[75] and local microenvironment modification[86] for the design and fabrication of scaffolds. In addition, the most suitable biomaterials for the cartilage or subchondral bone layers have not been fully investigated, while there are many biomaterials available for osteochondral repair. Therefore, a comparison of these materials should be performed to ultimately determine the ideal material.

Future Directions

The management of OA remains challenging and controversial. Considering the steady progression of tissue engineering and cell-based technologies over the past decade, we may have new therapeutic options for osteochondral repair in clinical practice. In this review, we have focused on biphasic implants for osteochondral repair, including the concept, scaffold fabrication, in addition to the selection of cells and materials. There have been many promising scaffolds developed, some of which contribute to good osteochondral repair *in vivo*. Moreover, some of them are already

TABLE 1. SUMMARY OF *IN VIVO* STUDY USING BIPHASIC SCAFFOLD

		Cartilage layer			*Subchondral bone layer*					
Authors	*Year*	*Material*	*Cells*	*Chondrogenesis*	*Material*	*Cells*	*Osteogenesis*	*Intermidiate layer*	*Animal*	*Ref. number*
Gao *et al.*	2002	Hyaluronic acid	Bone marrow MSC	–	CP	—	N/A	–	Rabbit	42
Alhadlaq *et al.*	2005	PEG hydrogel	Bone marrow MSC	+	PEG hydrogel	Bone marrow MSC	+	+	Rat	41
Kandel *et al.*	2006	CPP	Chondrocyte	N/A	CPP	—	N/A	–	Sheep	7
Ahn *et al.*	2009	Hyaluronic acid/ atelocollagen	Chondrocyte	N/A	HA/βTCP	—	N/A	–	Rabbit	40
Marquass *et al.*	2010	Collagen hydrogel	Bone marrow MSC	+	βTCP	Bone marrow MSC	–	Autologous plasma	Sheep	37
Kon *et al.*	2010	Collagen	Chondrocyte	N/A	Collagen/HA	—	N/A	Collagen/HA	Sheep	91
Chen *et al.*	2011	Chitosan/gelatin	Bone marrow MSC	+	HA/chitosan/gelatin	Bone marrow MSC	+	–	Rabbit	43
Kon *et al.*	2011, 2013	Collagen	—	N/A	Collagen/HA	—	N/A	Collagen/HA	Human	31, 108
Reyes *et al.*	2012	Alginate	—	N/A	PLGA	—	N/A	–	Rabbit	85
Deng *et al.*	2012	Gelatin/CS/SH	Chondrocyte	N/A	Gelatin/Ceramic bovine bone	Bone marrow MSC	+	–	Rabbit	88
Miot *et al.*	2012	Hyaluronic acid	Chondrocyte	N/A	HA/Hyaluronic acid	—	N/A	–	Goat	104
Dhollander *et al.*	2012	PLG/calcium-sulfate/PGA	—	N/A	PLG/calcium-sulfate/PGA	—	N/A	–	Human	71
Zhang *et al.*	2013	Collagen	—	N/A	PLLA	—	N/A	–	Rabbit	89
Zhang *et al.*	2013	Collagen	—	N/A	Silk/HA	—	N/A	–	Rabbit	90
Reyes *et al.*	2013	Polyurethane	—	N/A	PLGA	—	N/A	–	Rabbit	86
Duan *et al.*	2013	PLGA	Bone marrow MSC	—	PLGA	—	N/A	–	Rabbit	75
Schleicher *et al.*	2013	Collagen	Chondrocyte	N/A	Collagen/HA	—	N/A	–	Sheep	106
		Collagen	Chondrocyte	N/A	Allogenous bone	—	N/A	–		
Kon *et al.*	2013	Hyaluronic acid/aragonite	—	N/A	Aragonite	—	N/A	–	Goat	105

MSC, mesenchymal stem cell; PEG, polyethylene glycol; HA, hydroxyapatite; CP, calcium phosphate; CPP, calcium polyphosphate; βTCP, beta-tricalcium phosphate; PLGA, polylactic-co-glycolic acid; CS, chondroitin sulfate; SH, sodium hyaluronate; PLG, polylactide-co glycolide; PGA, polyglycolide; PLLA, poly-L-lactic acid; N/A, not applicable.

at the stage of preclinical, large animal studies, as well as clinical trials. Therefore, the application of additional new implants to osteochondral lesions could be expected in the near future. On the other hand, the optimization of and selection of biomaterials and their fabrication methods have not been fully investigated. Thus, the ideal structure and composition of bioimplants that repair osteochondral lesions have not been elucidated. Further studies will be needed and should be conducted in a methodologically rigorous fashion.

Finally, in order to evaluate the feasibility and safety of new implants with clinical relevance, the selection of appropriate animal models is important. Due to the differences in matrix structure and composition, as well as in the natural osteochondral healing response and technical difficulty in creating the lesions of consistent size and location, the use of small animals such as rabbits, rats, and mice may not be appropriate.[109–111] Rather, in consideration of clinical relevance, it is preferable to utilize larger animal models, such as pigs, sheep, goats, and horses.

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Disclosure Statement

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Address correspondence to:
Norimasa Nakamura, MD, PhD
Institute for Medical Science in Sports
Osaka Health Science University
1-9-27, Tenma, Kita-ku, Osaka City
Osaka 530-0043
Japan

E-mail: norimasa.nakamura@ohsu.ac.jp

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間葉系幹細胞由来組織再生材料と人工骨補填材による軟骨修復
—ナノスケール摩擦特性—

中村　亮介[※1]　望月　翔太[※1]　中村　憲正[※2]　藤江　裕道[※1]

The use of a scaffold-free tissue-engineered construct (TEC) bio-synthesized from synovium-derived mesenchymal stem cells with porous synthetic bones for cartilage repair : Nanoscale mechanical properties.

Ryosuke NAKAMURA, B.S., Shota MOCHIZUKI, B.S., Norimasa NAKAMURA, M.D., Ph.D., Hiromichi FUJIE, Ph.D.

Abstract

Important biomechanical functions of articular cartilage are lubrication properties. Articular cartilage bears numerous of cyclic load applications for a long period while keeping the frictional coefficient at a negligible level. However, once a degenerative disease or physical damage occurs in articular cartilage, such functions immediately deteriorate and cannot be restored due to the limitation of healing capacity. To solve the problem, we have been developing a new tissue-engineering technique using a scaffold-free tissue-engineered construct (TEC) bio-synthesized from allogenic or autogenic synovial-derived mesenchymal stem cells (MSCs) as a potential MSC-based therapeutic method. The objective of the present study was to perform a nanoscale friction test using an atomic force microscope (AFM) for cartilage-like tissues repaired with the TEC and combined with porous synthetic bones in a rabbit model.

Synovium-derived MSCs were obtained from the synovial membrane of rabbit knee joints. When the cell density reached 4.0×10^5 cells/cm^2 (6-cm dish), cells were allowed to undergo active contraction for 8 hours to develop a TEC specimen. A cylindrically shaped osteochondral defect of 6 mm in diameter and 5 mm in depth was created in the articular surface of the femoral groove of a 24-week-old rabbit. A composite of hydroxyapatite (HA) or β-TCP with or without the TEC was allografted into the defect. Nanoscale friction tests were performed for the specimens at friction speeds was of 10, 20, 50, or 100 μm/s, and contact force was 15.90-27.04 nN.

From the results of the friction test, in each specimen, there was no significant change of friction speed and contact force in the coefficient of friction. The coefficient of friction was significantly higher in HA and β-TCP groups than in the normal group.

It is considered from the result of the coefficient of friction that the TEC/HA group or TEC/β-TCP group is similar to normal cartilage, and recovery of the boundary lubrication properties and the shape of the cartilage surface is premature using the TEC.

Key words : Articular cartilage, Mesenchymal stem cells, Friction, Atomic force microscpe.

※ 1　首都大学東京大学院システムデザイン研究科　〒191-0065　東京都日野市旭ヶ丘6-6
※ 2　大阪大学整形外科　〒565-0871　大阪府吹田市山田丘2-2

Corresponding Author : Hiromichi FUJIE, PhD. Faculty of System Design, Tokyo Metropolitan University.
6-6 Asahigaoka, Hino, Tokyo 191-0065, Japan.
Tel & Fax : 042-585-8628　E-mail address : fujie@sd.tmu.ac.jp

緒　　言

関節軟骨の優れた摩擦，潤滑特性により，私たちは日常動作をスムーズに行うことが出来る．しかし，関節軟骨は自己修復機能が乏しいため，一度損傷すると本来備わっている機能などが失われてしまう[1]．我々は滑膜由来間葉系幹細胞に細胞外マトリックスを自己生成させて作製した組織再生材料（Tissue Engineered Construct, TEC）を用いることで損傷した軟骨を修復する研究を行っている[1),5),9)]．正常な関節軟骨表面にはナノスケールの微小な凹凸やうねりが存在しており，これが関節潤滑に寄与する影響は大きいと考えられる．一方TECによる修復軟骨では表面構造が異なる観察結果が得られており，正常軟骨とは異なる摩擦，潤滑特性を有していると予想される．過去の研究において軟骨や修復軟骨の潤滑特性，摩擦特性が様々な方法により調べられている[2),3)]．しかし軟骨表面の微小な構造の影響を強く受けるのは境界潤滑特性であり，その特性を調査するためには軟骨内の流体移動の影響を極力排除した摩擦試験を行わなくてはならない．そこで，本研究では正常，修復軟骨に対しナノスケール摩擦試験を行い，関節潤滑の解明および修復度合いの検討を行うことを目的とした．

実験方法

試験片作成

ウサギ膝関節の滑膜より滑膜細胞を採取し，in vitroで培養した．継代培養は4から7回行った．培養液（DMEM, 10% Fetal Bovine Serum, 1 % Penicillin-Streptomycin）で満たされた培養皿に，初期細胞密度4.0×10^5 cells/cm^2で細胞を播種した．その後，アスコルビン酸2リン酸を0.2 mMの濃度で添加し，7日間培養することで，基質の生成を促した．培養後，産生したマトリックス－細胞の複合体を培養皿底面から剥がし，8時間自己収縮させ，TECを生成した（図1）．6-well plateで作成したTEC 1枚を丸め，直径5 mm，高さ4 mmの骨補填材の上面に貼り付け，TEC/骨補填材複合体とした（図2）．骨補填材には気孔率約75%を有する，ハイドロキシアパタイト（HA）（NEOBONE, コバレントマテリアル株式会社）とβリン酸三カルシウム（β-TCP）（OSferion, オリンパス株式会社）を用いた[1),7),9)]．24週齢以上の成熟ウサギ膝関節の膝蓋大腿関節面に，直径5 mm，深さ6 mmの全層骨軟骨欠損を作製し，TEC/骨補填材複合体を埋入した．術後24週で屠殺し，修復部の関節面から直径4 mmの円柱状に切り出した組織を試験片とした．Normal群として，非手術側（正常軟骨部分）の膝蓋大腿関節面より同様に採取した（図3）．

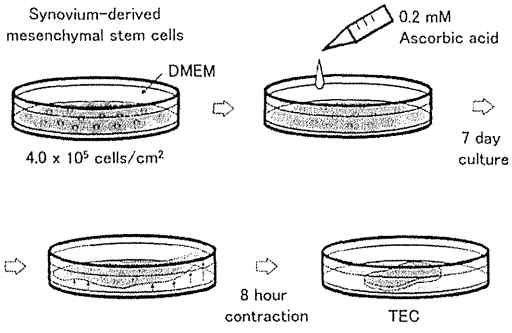

図1．Production procedure of TEC.

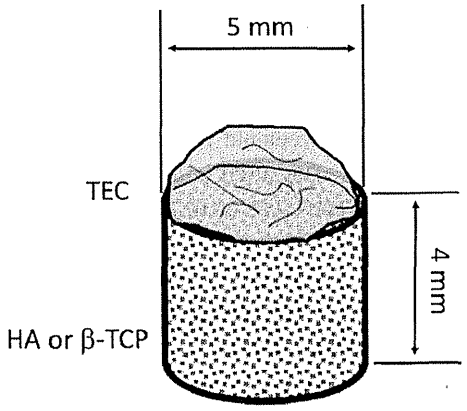

図2．Composite of TEC with porous synthetic bones.

ナノスケール摩擦試験

予備実験として原子間力顕微鏡（AFM）（Nanoscope Ⅲa，Veeco Instruments）を用いた摩擦試験における，カンチレバーで走査することによる軟骨表面形状への影響を観察するた

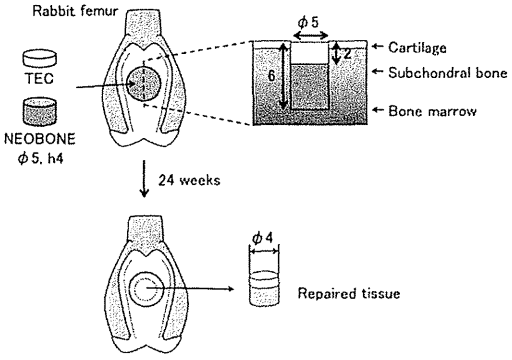

図3．Preparation of repaired cartilage specimen.

め，試験片にNormal群を用いて同一の測定領域で4回表面形状測定を行った．測定条件は，測定領域10×10 μm，走査速度20 μm/s，接触力18.7 nN（平均接触圧4.27 MPa）とした．試験片をホルダーに固定した後，軟骨表面の表面張力や凝着の影響を極力除くため，生理食塩水で満たし，AFMピエゾスキャナ上部のステージに乗せた．液中測定用セルに測定用カンチレバー（バネ定数0.06 N/m，曲率半径10 nm）（DNP-S10, BRUKER）を固定し，AFMヘッドに取り付けた．摩擦走査を4回行う過程の表面形状の変化を観察した．

TEC/骨補填材複合体を用いた修復軟骨の摩擦特性を調べるため，それぞれの試験片に対して，表面形状測定と同様のカンチレバー，雰囲気条件で摩擦試験を行った．摩擦試験は，摩擦距離を10 μm，接触力（荷重）を15.90～27.04 nN（平均接触圧4.02～4.79 MPa），摩擦速度を10, 20, 50, 100 μm/sで行った．摩擦係数は，試料表面と探針との摩擦力を走査時の接触力で除した値とした．

結　　果

図4に表面形状測定結果を示す．測定1回目では，先行研究の報告と同様に，今回の測定条件においても軟骨表面の微小な突起やうねりが観察された．測定2回目では，1回目と突起の数に大きな変化は見られないが，3回目では減少し，突起が大きくなった．測定4回目では，3回目に出来上がった突起が平滑化され，低くなった．表面の算術平均粗さは測定1回目では370.95 nm，2回目では338.29 nm，3回目では366.87 nm，4回目では314.83 nmであった．同一面上の複数回摩擦走査により軟骨表面の凹凸の破壊が見られたため，摩擦試験においては，一回のみの走査で摩擦係数を求めることとした．

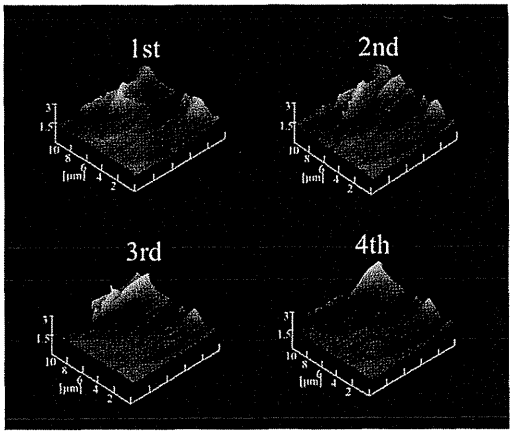

図4．Atomic force microscopic observation of the surface of normal articular cartilage at 1st, 2nd, 3rd and 4th probe scan.

図5は摩擦速度20 μm/s時の摩擦係数を荷重別に比較した結果である．それぞれの試験群は荷重の増加に対して右肩上がりの応答を示したが，分散分析（ANOVA，$P<0.05$）を行った結果，荷重依存性はどの試験群においても現れなかった．また，有意差検定の結果，TECを用いていないHA群とβ-TCP群において摩擦係数が正常軟骨に比べて有意に増大することが分かった．一方，TECを用いた両群の摩擦係数は正常軟骨に比べてわずかに高い傾向を示したものの，有意差は見られなかった．図6は荷重21.5 nN時の摩擦係数を摩擦速度別に比較した結果である．それぞれの試験群に対し分散分析を行った結果，速度依存性はなかった．TECを用いていないHA群とβ-TCP群において，摩擦係数が正常軟骨に比べて高い傾向が見られ，速度によっては有意差も見られた．一方，TECを用いた両群の摩擦係数は正常軟骨に比べてわずかに高い傾向を示したものの，有意差

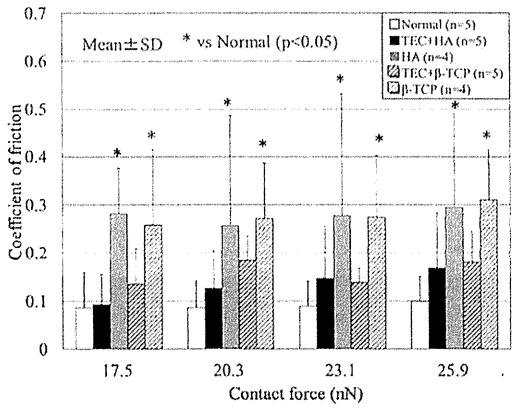

図5．Coefficient of friction of repaired cartilage as a functional of contact force at a friction speed of 20 μm/s.

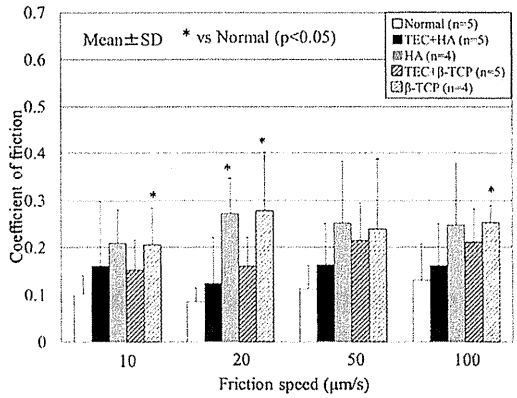

図6．Coefficient of friction of repaired cartilage as a functional of friction speed at a contact force of 21.5 nN.

は見られなかった．

考　　察

表面形状測定の結果，図4のように形状測定の回数が増すにつれて表面の算術平均粗さが370.95 nmから314.83 nmまで減少した．表面形状測定に用いた荷重条件は摩擦試験条件のおおよそ中央値に当たるが，今回の条件では非生理的に大きい接触圧が生じたと考えられる．平面状弾性体（弾性率1.5 MPa[7]，ポアソン比0.35[8]）である軟骨と，球状剛体であるプローブのヘルツ接触[9]と考え，接触部中央の最大接触圧力を算出したところ4.73 MPaとなった．この値は，生理的環境において軟骨が受ける圧力に比べ，はるかに大きいため，軟骨表面の微小な突起が破壊され，測定領域内が平坦になったためと考えられる．AFMによる一般的な摩擦試験では摩擦運動差分から摩擦力を求めることが多いが，この結果より，摩擦試験は複数走査を行わずに完了させる必要があることが分かった．

図5，6より今回の条件においては，荷重依存特性および速度依存性はないことが分かった．今出らの行った軟骨の摩擦応答解析[4]や，望月らのマクロスケールの摩擦試験[6]の結果より，摩擦係数は摩擦速度と荷重に依存して変化することが示されている．この結果は，Mowらによって提唱された二相性理論[7]で理解できる．つまり，摩擦速度や荷重が増加した時，軟骨内部の流体が荷重を支持する割合が増加し，固体部分に作用する荷重が減少して摩擦係数が低下したと考えられる．そもそも関節軟骨はコラーゲン線維とプロテオグリカンからなる固体部分の中に，液体である水分を80%も含んだ二相性構造をとっている．そのような二相性材料が圧縮，摩擦されると，固体と液体の振る舞いが相和されて，軟骨全体としての挙動となって現れると考えられる．しかし，本研究では，荷重や速度の変化が摩擦係数に影響を及ぼさなかった．ナノスケールの摩擦試験では軟骨内部の流体は影響を受けず，摩擦係数は，表面の境界潤滑特性のみが反映されたためと考えられる．

先行研究で行われた表面形状測定の結果より，TECを用いて修復された軟骨は正常軟骨と同様の摩擦特性が得られることが分かっている[10]．本研究において，TECと骨補填材を用いて修復された軟骨が正常軟骨と同程度の摩擦係数を示したことより，修復軟骨の境界潤滑特性の回復向上のためには，骨補填材による下骨修復だけでは不十分であり，TECを併用することで効果が得られることが分かった．

結　　言

家兎骨軟骨全層欠損モデルに対し，HAまたはβ-TCPを骨修復材料に，TECを軟骨修復材料に用いた軟骨修復実験を行い，ナノスケールの摩擦特性に関して以下のことを明らかにした．

・骨補填材だけでは摩擦係数は回復しない
・TECを併用することで境界潤滑特性が回復する

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組織再生材料(TEC)/コラーゲンシート複合体の引張特性

首都大学東京大学院システムデザイン研究科
池谷　基志・藤江　裕道

東海大学工学部機械工学科
大家　　渓

札幌医科大学生体工学・運動器治療開発講座
鈴木　大輔

株式会社ニッピ　バイオマトリックス研究所
小倉　孝之・小山　洋一

大阪大学整形外科
杉田　憲彦・中村　憲正

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組織再生材料(TEC)/コラーゲンシート複合体の引張特性

池谷　基志[※1]　大家　渓[※2]　鈴木　大輔[※3]　小倉　孝之[※4]
小山　洋一[※4]　杉田　憲彦[※5]　中村　憲正[※5]　藤江　裕道[※1]

Tensile properties of stem cell-based tissue engineered construct (TEC) cultured on a collagen sheet.

Motoshi IKEYA, B.S., Kei OYA, Ph.D., Daisuke SUZUKI, Ph.D.,
Takayuki OGURA, Youichi KOYAMA, Norihiko SUGITA, M.D., Ph.D.,
Norimasa NAKAMURA, M.D., Ph.D., Hiromichi FUJIE, Ph.D.

Abstract

We have been developing a novel tissue-engineering technique for the repair of cartilage, ligaments, and tendons, which involves a stem cell-based tissue engineered construct (TEC). However, cartilage-like tissues repaired with pure TEC are inferior to normal cartilage in terms of permeability, compressive properties, and friction properties. To solve these problems, we developed TEC combined with a collagen sheet (CS) in the present study. Mesenchymal stem cells (MSCs) were obtained from synovial membranes of a human knee joint. The cells were plated to develop TEC on the CS for 7, 14, and 28 days. Scanning electron microscopy indicated that there are more MSC in the upper layer of TEC on CS at 28 days. The tangent modulus and strength of TEC significantly increased over the culture period, and were higher than those of CS soaked in culture medium at 28 days.

Key words : Stem cell-based tissue engineered construct (TEC), Tissue engineering, Collagen sheet, Tensile property.

※1　首都大学東京大学院システムデザイン研究科　〒191-0065　東京都日野市旭ヶ丘6-6
※2　東海大学工学部機械工学科　〒259-1292　神奈川県平塚市北金目4-1-1
※3　札幌医科大学生体工学・運動器治療開発講座　〒060-0061　北海道札幌市中央区南1条西17
※4　株式会社ニッピ　バイオマトリックス研究所　〒302-0017　茨城県取手市桑原520-11
※5　大阪大学整形外科　〒565-0871　大阪府吹田市山田丘2-2

Corresponding Author : Hiromichi FUJIE, PhD. Faculty of System Design, Tokyo Metropolitan University.
6-6 Asahigaoka, Hino, Tokyo, 191-0065, Japan.
Tel & Fax : 042-585-8628　E-mail address : fujie@sd.tmu.ac.jp