# Cell and Tissue Culture Technology for Cartilage Repair

**Shuichi Mizuno, PhD and Kara Johnson**
Brigham and Women's Hospital

## INTRODUCTION

Cartilage repair is of great interest for cell–based therapy using tissue engineering methods. Recently, cartilage tissue engineering has focused on construction of articular cartilage for resurfacing damaged articular cartilage. Autologous chondrocyte implantation (ACI) or transplantation (ACT) was the first tissue engineering procedure used clinically for the treatment of focal cartilage defects (1). That method uses processed cells that are isolated from the patient, expanded *in vitro*, and injected as a suspension into the lesion that has been prepared and covered with a periosteal graft. Current tissue engineering methods have focused on improving ACI and ACT. Many studies have been reported in the last decade and the same basic science questions remain on biomechanical aspects, cellular repair and optimization of constructs and culture conditions.

1. Material and Biomechanical Aspects: *How stiff is the engineered cell construct? Will the construct withstand weight bearing and joint loading?*

2. Cellular Repair: *How does an implanted, engineered cell construct integrate with the host tissue? Where are reparative cells coming from, surface, adjacent cartilage, or bone marrow? Would stem cells be a viable option for promoting cartilage repair?*

3. Optimization: *What options are there to improve cell viability, phenotype maintenance and chondrogenesis? What advantages do bioreactors confer?*

## MATERIAL AND BIOMECHANICAL ASPECTS

*How stiff is the engineered cell construct? Will the construct withstand weight bearing and joint loading?*

For articular cartilage engineering using cell constructs, it is thought that the constructs need to have "optimal" mechanical durability and rigidity. Because the construct is expected to replace the damaged tissue that is subjected to weight bearing and joint-loading, mechanical durability after transplantation needs to be considered. While mechanical durability and rigidity of the construct may be important criteria, it is important to also consider the biological ramifications of the chosen construct.

**Dr. Mizuno** is an Instructor, Department of Orthopaedic Surgery, Brigham and Women's Hospital and Harvard Medical School

Address correspondence to:

Brigham & Women's Hospital
75 Francis Street
Boston, MA 02115

Agarose (2), calcium alginate (3), hyaluronan gel (4), fibrin glue (5), PGA (6), PLGA-polyvinyl (7), PLGA-collagen mesh (8), collagen gels (9) or sponges (10), and combined hyaluronan-collagen sponge (11), peptides: glycine-hydroxyproline-glycine-glutamate-arginine (12), etc. have been used for cartilage constructs. However, none of these constructs have an equivalent rigidity to native cartilage. Agarose cell constructs are the most rigid cell construct, although agarose is still only1/8 the rigidity of native cartilage (2). If rigidity is the critical criteria for the cell construct, agarose is the best candidate.

Although native cartilage-like rigidity may seem desirable, the mechanical property of forming neo-cartilage may not be critical because non-weight bearing and joint-loading motion can be managed in a patient during rehabilitation (CPM, etc.). Therefore, the rigidity of the *in vitro* cell construct may not be a critical factor for optimal cell-based therapies. Engineered cartilage *in vitro* should focus more on optimizing the healing process after implantation. If optimizing the biological repair process is critical for successful implantation, agarose cell constructs might be inappropriate because cells are embedded in a non-bioresorbable scaffold that prevents cell adhesion.

## CELLULAR REPAIR

*How does an implanted, engineered cell construct integrate with the host tissue? Where are reparative cells coming from, surface, adjacent cartilage, or bone marrow?* (Figure 1)

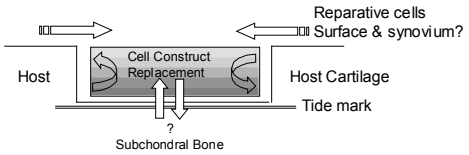


**Figure 1**

In an ideal situation, cell-based therapy would promote the native tissue to replace the engineered cell construct. However, it is not clear where reparative cells come from; whether they come from the surface layer of cartilage including the synovial membrane, adjacent cartilage, or bone marrow. Periosteum derived cells may also participate in resurfacing the defect. Cells derived from all of these sites have the potential to resurface, integrate, and repair cartilage defects. Cells derived from synovium (13), bone marrow (14), periosteum (15) all have the potential to produce extracellular cartilage matrix. The potential these cell types demonstrate indicate that they may all play a role in cartilage repair.

*Would stem cells be a viable option for promoting cartilage repair*?

The use of stem cells is of growing interest, however the chondrogenic potency of isolated stem cells needs to be assessed (16,17). Adipose-derived cells derived from adult humans *in vitro* showed chondrogenic nodule (18). Mouse inguinal fat-pad derived cells developed the chondrocyte phenotype *in vitro* (19). Purification methods have not been well established for heterogeneous stem cell populations such as adult tissue derived stem cells. Extensive research on cellular characterization and manipulation of the stem cells will be required for the use of multipotential cells *in vivo*.

## OPTIMIZATION

*What options are there to improve cell viability, phenotype maintenance and chondrogenesis? What advantages do bioreactors confer?*

Use of a bioreactor over the culture period is one option to improve cell viability and chondrogenesis of isolated target cells. Spinner flasks (20), perfusion (21), rotator vessels (22) and hydrostatic pressure systems (23) are available bioreactors for cartilage engineering with 3D scaffolds. Evaluation of engineered neo-cartilage has focused on synthesis and accumulation of cartilage ECM (sulfated glycosaminoglycans and collagen type II) as well as biomechanical properties. Each type of bioreactor has beneficial effects to achieve chondrogenic properties *in vitro* with optimal culture conditions.

Mass transfer between the growing construct and growth medium must ensure sufficient nutrient exchange and removal of waste products. Chondrocytes produce an extracellular matrix with relatively large molecular weight constituents, some of which leak out of the scaffold to the medium phase. Some multiple step culture procedures (24) promote algorithms for cell proliferation and metabolic functions separately to manipulate function at both the cellular and tissue level.

Mechanical loading, which is used to test tissue's properties, may also be applied to stimulate cellular functions by mimicking the physiological microenvironment of cartilage. Typical articular cartilage experiences tremendously high hydrostatic pressure, ~10 MPa. Our culture system was designed to apply hydrostatic pressure with constant or cyclic 2.8 MPa without deformation (23).

## CURRENT TECHNOLOGY

Several new methods improving ACI and ACT have been developed and are undergoing clinical evaluation.

Matrix-Induced Autologous Chondrocyte Implantation (MACI) from Verigen is a joint venture with Genzyme (Cambridge, MA). That method uses processed cells that are isolated from the patient and expanded *in vitro*. The chondrocytes were then seeded between layers of a bilaminate collagen membrane (25). The collagen membrane with chondrocytes is immediately implanted into the defect. That method appeared to result in a homogeneous distribution of cells in the membrane carrier, an advantage over ACI. Although complete clinical evaluation is underway, this method could result in a shorter surgical time because sutures and periosteal grafts are not needed.

3D amorphous carriers, such as a collagen gel, are useful for cell delivery and phenotype maintenance because they allow chondrocytes to maintain their round shape, seen *in vivo*, and to accumulate cartilage ECM around the cells. A chondrocyte/collagen gel construct clinical study, conducted by Japan Tissue Engineering Corporation, licensed by Dr. Ochi, Hiroshima University, Japan (26), is underway. In this study, they have seeded cells in a collagen gel and cultured the cell/gel construct in a regular culture dish for 4 weeks. The cells accumulated newly synthesized cartilage extracelluar matrix (ECM) in the gel. The cell/gel construct was implanted underneath a periosteum cover. This method is useful to reduce the risk of cells leaking out of any holes between the periosteum cover and the cartilage defect.

A two-step calcium alginate encapsulation method has been developed by Rush's group (24). In the first step chondrocytes were encapsulated in calcium alginate and incubated in a regular culture dish for 2 weeks. The cells accumulated new synthesized cartilage ECM in the alginate. In the second step, the alginate carrier was depolymerized by taking out $Ca^{2+}$ with EDTA. The cells were then removed from the depolymerized alginate. The cells and newly synthesized ECM were seeded onto a supporting membrane. The cells continued to produce cartilage ECM. Prior to implantation the supporting membrane was removed, so only the cells and ECM were implanted. This culture method is rather complicated but has the advantage that only endogenous material is implanted into the defect. Therefore, the patient is less likely to have immunological side effects and/or biological contamination than with other constructs. A good outcome with this method was demonstrated in an animal model.

We have jointly developed novel technology for cartilage engineering with BWH and Histogenics (Malden, MA). We are currently licensing this technology. It is designed to incubate an autologous chondrocytes/collagen gel/sponge construct cultured with treatment of hydrostatic pressure at 0.5 MPa, 0.5 Hz follow by static culture prior to implantation. This technology has three major phases: seed cells in gel/sponge scaffold, incubate cells under physical stimuli, and implant the cell construct with novel collagen/PEG based adhesive. The collagen construct is stiff enough to be handled with forceps. The cells accumulated cartilage ECM in the construct. The construct is implanted, according to the surgeon's decision, with either an adhesive or with sutures and an adhesive. A phase I clinical study is being conducted by Drs. Marc Safran (UCSF), Dilworth Cannon (UCSF), and Dennis Crawford (Oregon Health Science University). Data was partially presented at AAOS and ORS (27) this year.

## SUMMARY

The use of autologous cells has great advantages in avoiding immunological and ethical issues. However, because the amount of expendable, harvestable cartilage is limited, expansion of cell number is required prior to cell implantation. Restoration/maintenance of the phenotype of the expanded cells becomes critical to restore tissue histogenesis and the biomechanical properties needed for tissue regeneration.

The goal of cartilage repair is to produce a tissue that fills the defect, integrates with the adjacent articular cartilage, has the same viscoelastic mechanical properties, and maintains its matrix thereafter. Ideally it would restore articular function with a repair tissue that approaches regeneration. The technology currently available, and in development, are making steps toward achieving this goal.

## References


1. **Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Lsaksson O, Peterson L.** Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N Engl J Med 1994 Oct; 331(14):889-95.
2. **Hung C, Lima E, Mauck R, Taki E, LeRoux M, Lu H, Stark R, Guo X, Ateshian G.** Anatomically shaped osteochondral constructs for articular cartilage repair. J Biomech 2003 Dec; 36(12): 1853-6.
3. **Lee D, Reisler T, Bader D.** Expansion of chondrocytes for tissue engineering in alginate beads enhances chondrocytic phenotype cpmpared to conventional monolayer techniques. Acta Orthop Scand 2003 Feb; 74(1): 6-15.
4. **Grigolo B, Roseti L, Fiorini M, Fini M, Giavaresi G, Giardino R, Facchini A.** Transplantation of chondrocytes seeded on hyaluronan derivative (hyaff-11) into cartilage defects in rabbits. Biomaterials 2001 Sep; 22(17): 2417-24.
5. **Sims C, Butler P, Cao Y, Casanova R, Randolpf M, Black A, Vacanti C, Yaremchuk M.** Tissue engineered neocartilage using plasma derived polymer substrates and chondrocytes. Plast Reconstr Surg 1998 May; 101(6): 1580-5.
6. **Rahman M, Tsuchiya T.** Enhancement of chondrogenic differentiation of human articular chondrocytes by biodegradable polymers. Tissue Eng. 2001 Dec; 7(6): 781-90
7. **Gorti G, Lo J, Falsafi S, Kosek J, Quan Skhuu D, Koch R.** Cartilage tissue engineering using cryogenic chondrocytes. Arch Otolaryngol Head Neck Surg 2003 Aug; 129(8): 889-93.
8. **Chen G, Sato T, Ushida T, Hirochika R, Tateishi T.** Redifferentiation of dedifferentiated bovine chondrocytes when cultured in vitro in a PLGA-collagen hybrid mesh. FEBS Lett 2003 May; 542(1-3): 95-9.
9. **Wakitani S, Kimura T, Hirooka A, Ochi T, Toneda M, Yasui N, Owaki H, Ono K.** Repair of rabbit articular surfaces with allograft chondrocytes embedded in collagen gel. J Bone Joint Surg Br 1989 Jan; 71(1): 74-80.
10. **Mizuno S, Allemann F, Glowacki J.** Effects of medium perfusion on matrix production by bovine chondrocytes in three-dimensional collagen sponges. J Biomed Mat Res 2001 Sep; 56(3): 368-75.
11. **Allemann F, Mizuno S, Eid K, Yates K, Zaleske D, Glowacki J.** Effects of hyaluronan on engineered articular cartilage ECM gene expression in 3-dimensional collagen scaffolds. J Biomed Mat Res 2001 Apr; 55(1): 13-19.
12. **Reyes C, Garcia A.** Engineering integrin-specific surfaces with a triple-helical collagen-mimetic peptide. J Biomed Mater Res 2003 Jun; 65(4): 511-23.
13. **Nishimura K, Solchaga LA, Caplan AI, Yoo JU, Goldberg VM, Johnstone B.** Chondroprogenitor cells of synovial tissue. Arthritis Rheum, 1999 Dec; 42(12):2631-7.
14. **Zhou S, Eid K, Glowacki J.** Cooperation between TGF-beta and Wnt oathway during chondrocyte and adipocyte differentiation of human marrow stromal cells. J Bone Miner Res, 2004 Mar; 19(3):463-70.
15. **Brittberg M, Sjogren-Jansson E, Thornemo M, Faber B, Tarkowski A, Peterson L, Lindahl A.** Clonal growth of human articular cartilage and the functional role of the periosteum in chondrogenesis. Osteoarthritis Carilage 2005 Feb; 13(2): 146-53.
16. **Dragoo J, Samimi B, Zhu M, Hame S, Thomas B, Lieberman J, Hedrick M, Benhaim P.** Tissue-engineered cartilage and bone using stem cells from human infrapatellar fat pads. J Bone Joint Surg Br 2003 Jul; 36(5): 740-7.
17. **Wickham M, Erickson G, Gimble J, Vail T, Guilak F.** Multipotent stromal cells derived from the infrapatellar fat pad of the knee. Clin Orthop 2003 Jul; 412: 196-212.
19. **Ogawa R, Mizuno H, Watanabe A, Makita M, Shimada T, Hyakusoku H.** Osteogenic and chondrogenic differentiation by adipose-derived stem cells harvested from GFP transgenic mice. Biochem Biophys Res Commun 2004 Jan; 313(4): 871-7.
20. **Freed L, Vunjak-Novakovic G.** Microgravity tissue engineering. In Vitro Cell Dev Biol Animal. 1997 May; 33(5): 381-5.
21. **Sittinger M, Bujia J, Minuth W, Hammer C, Burmester G.** Engineering of cartilage tissue using bioresorbable polymer carriers in perfusion culture. Biomaterials 1996 Feb; 15(3): 451-61.
22. **Marlovits S, Tichy B, Truppe M, Gruber D, Schlegel W.** Collagen expression in tissue engineered cartilage of aged human articular chondrocytes in a rotating bioreactor. Int J Artif Organs 2003 Apr; 26(4): 319-30.
23. **Mizuno S, Ushida T, Tateishi T, Glowacki J.** Hydrostatic fluid pressure enhances matrix synthesis and accumulation by bovine chondrocytes in three-dimensional culture. J Cell Physiol 2002 Dec; 193(3): 319-27.
24. **Marlovits S, Striessnig G, Kutscha-Lissberg F, Resinger C, Aldrian SM, Vecsei V, Trattnig S.** Early postoperative adherence of matrix-induced autologous chonadrocyte implantation for the treatment of full-thoickness cartilage defects of the femoral condyle. Knee Surg Sports Traumatol Arthrosc. 2004 Oct (epub ahead of print).
25. **Masuda K, Sah R, Hejna M, Thonar E.** A novel two-step method for the formation of tissue-engineered cartilage by mature bovine chondrocytes: the alginate-recovered-chondrocyte (ARC) method. J Orthop Res 2003 Jan; 21(1): 139-48.
26. **Ochi M, Uchio Y, Kawasaki K, Wakitani S, Iwasa J.** Transplantation of cartilage-like tissue made by tissue engineering in the treatment of cartilage defect of the knee. J Bone Joint Surg Br. 2002 May; 84(4):571-578.
27. **Kusanagi A, Blahut EB, Johnson JM, Mascarehas AC, Murata T, Tsuchiya A, Mochizuki M, Mizuno S.** Cartilage repair with engineered chondrocyte constructs: a 6 month study in a swine model. Orthopedic Research Society Annual Meeting, #302, Washington DC, 2005.