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.

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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 only 1/8 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)

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 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 \textit{in vitro} showed chondrogenic nodule (18). Mouse inguinal fat-pad derived cells developed the chondrocyte phenotype \textit{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 \textit{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 \textit{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, $\sim 10$ 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 \textit{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 \textit{in vivo}, and to accumulate cartilage ECM around the cells. A chondrocyte/collagen gel construct clinical study, conducted by Japanese 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 extracellular 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 scaffold. 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


