Yonsei Medical Journal
Vol. 41, No. 6, pp. 766−773, 2000

An Overview of Cartilage Tissue Engineering

Hyun Woo Kim and Chang Dong Han

Abstract

Articular cartilage regeneration refers to the formation of new tissue that is indistinguishable from the native articular cartilage with respect to zonal organization, biochemical composition, and mechanical properties. Due to a limited capacity to repair cartilage, scar tissue frequently has a poorly organized structure and lacks the functional characteristics of normal cartilage. The degree of success to date achieved using a purely cell- or biological-based approach has been modest. Potentially the development of a hybrid strategy, whereby, chondrocytes or chondrogenic stem cells are combined with a matrix, making cartilage *in vitro*, which is then subsequently transplanted, offers a route towards a new successful treatment modality. The success of this approach depends upon the material being biocompatible, processable into a suitable three-dimensional structure and eventually biodegradable without harmful effects. In addition, the material should have a sufficient porosity to facilitate high cell loading and tissue ingrowth, and it should be able to support cell proliferation, differentiation, and function. The cell-polymer-bioreactor system provides a basis for studying the structural and functional properties of the cartilaginous matrix during its development, because tissue concentrations of glycosaminoglycan and collagen can be modulated by altering the conditions of tissue cultivation.

Key Words: Cartilage, tissue engineering

INTRODUCTION

Normal knee function requires a smooth gliding articular cartilage surface on the ends of the bones. This surface is composed of a thin layer of slippery, tough tissue called hyaline cartilage, which derives its form and mechanical function from its matrix that consists of tissue fluid and a framework of structural macromolecules (collagens, proteoglycans, and noncollagenous proteins and glycoproteins). It is a well-known phenomenon that if cartilage lesions are confined to the substance of the articular tissue itself (partial thickness or superficial defects), they fail to heal spontaneously.[1-4] If they penetrate the underlying layer of subchondral bone (full thickness defects), then a limited spontaneous repair reaction ensues, with cells for this response originating from the bone marrow and vascular spaces.[2,4] However, the degree to which healing occurs is highly variable, with respect to both the quantity and quality of the tissue formed. Early attempts to surgically treat focal joint lesions were geared toward enhancing this spontaneous repair reaction. However, despite technological advances, the degree of success to date of a purely cell- or biologically-based approach has been modest, and there have been no reported instances of the structure and mechanical competence of native articular cartilage being restored. Presently, the opportunity exists to develop a hybrid strategy, whereby living cells are combined with an artificial support to provide a tissue-engineered approach for the successful repair or replacement of articular cartilage defects.

Tissue engineering approaches to cartilage repair offer potentially important advantages over the modern generation of metal-and-plastic joint prostheses. While total joint replacement using such prostheses has been enormously successful in providing pain relief and joint function for many individuals with debilitating arthritis, non-living prostheses wear and generate problematic debris, and they are not sufficiently durable for physically active individuals. Currently emerging cartilage tissue engineering therapies are directed toward the treatment of focal cartilage defects. These therapies involve the delivery of osteo-

Received November 17, 2000

Department of Orthopedic Surgery, Yonsei University College of Medicine, Seoul, Korea.

This work was supported by a grant from the Ministry of Health and Welfare (#HMP-99-E-05-0001).

Reprint address: request to Dr. C. D. Han, Department of Orthopedic Surgery, Yonsei University College of Medicine, C.P.O. Box 8044, Seoul 120-752, Korea. Tel: 82-2-361-5640, Fax: 82-2-363-1139, E-mail: cdhan@yumc.yonsei.ac.kr

chondral grafts, cells, or cell-laden tissue constructs (grown *in vitro*) to the defect site. Groups of researchers and companies have succeeded in producing viable cartilage tissue grown on bioabsorbable artificial scaffolds seeded with cells derived from humans, rabbits and calves.[5-12] Functional tissue equivalents of native cartilage can be grown *in vitro* using isolated chondrocytes, biodegradable polymer scaffolds, and bioreactors and then implanted *in vivo* to form subcutaneous cartilage or promote joint repair. This article will discuss the basic concepts of cartilage tissue engineering, and will describe the requisite properties of tissue constructs in terms of cells and artificial matrices.

CELL SOURCES FOR TISSUE CONSTRUCTS

Cells are necessary for cartilage repair, however, questions remain to be answered about the optimal sources of the cells. For example, one could use chondrocytes or cells with a chondrogenic differentiation capacity as part of a transplantable implant. If a limited amount of hyaline cartilage of non-weight bearing portion is available for harvest, the cells can be expanded *in vitro* before being delivered back to the patient. Because sites for articular cartilage harvest are limited, the use of cells with chondrogenic potential, such as the periosteum, perichondrium and bone marrow have also been advocated for isolation, culture expansion, and cell delivery. However, clinical and experimental data showing the benefits of particular cell types in cartilage repair is lacking.

Chondrocytes are very easy to obtain, but it has been found that when the cells are cultured for extended periods, the cartilage formed becomes increasingly fibrous in nature.[2,13] Certainly, the issue of cell age is a major concern, and there exists a weight of evidence that older chondrocytes may be less responsive to anabolic cytokines and other bioactive or physical stimuli. Currently, the only cell based method for articular cartilage repair approved by the Food and Drug Administration (FDA), involves expanding autologous articular chondrocytes from the patient in culture, and re-injecting them under a periosteal flap sutured over the defect as a barrier.[14-17] Although initially the procedure described above caused cartilage regeneration in canine knees, it proved not permanent, and 12−18 months after surgery, the cartilage began to degrade.[14,15] In another study, at 12−18 months, no difference was observed between the healing of control defects, those with the addition of a periosteal flap alone, or that of a flap and autologous chondrocytes.[17] Instead of using tissue flaps, highly porous scaffolds were used to maintain differentiated cells in a given area.[6,8,10-12,18,19] This design is an improvement because it significantly reduces donor site morbidity, and in addition to simply providing a boundary for cell retention, the scaffold also acts as a substrate for the anchorage-dependent chondrocytes. When cultured two-dimensionally, chondrocytes de-differentiate over time, however, when grown in three-dimensions, they maintain their differentiated phenotype and functions. In this way, scaffolds can encourage the proliferation of chondrocytes without sacrificing important functions to de-differentiation.

Bone marrow stromal cells (BMSCs) have been studied extensively because of their known chondrogenesis capabilities and because they can repair subchondral bone and articular cartilage. BMSCs are involved in the natural repair of skeletal tissues and can be differentiated into several mesenchymal lineages; osteoblasts, chondrocytes, adipocytes, and myocytes, depending on local environmental conditions.[20,21] Potential advantages of using BMSCs include: the low number of cells required initially, because they can be readily expanded in monolayers and still maintain their differentiation potential in contrast to chondrocytes; the relative simplicity of the bone marrow harvesting procedure; the higher biosynthetic activity of bone marrow stromal cells obtained from older individuals, despite the age-dependent decline in the concentration of these cells in the marrow; and the possibility of engineering composites of both bone and cartilage for the repair of osteochondral defects[20,22-28] The use of BMSCs in scaffolds has been studied extensively and promising results have been reported. In addition to offering the possibility of repairing large defects, these highly proliferative cells also present an attractive proposition in gene therapy with retrovial vectors, which require actively dividing cells for integration into the host cell genome. Thus, it may be possible to alter these reparative cells genetically to change the expressions of proteins that facilitate the reparative response.[25]

MATRICES

Irrespective of whether the technology used to promote articular cartilage repair is cell, gene or growth factor based, it is essential that matrix material be implanted within the defect void.[1] The reason for this is that the mesenchymal cells recruited from the synovium for repair purposes lack the organizational ability to populate the lesion void. Thus, the treatment of chondral defects with cell transplants requires a method of delivering, and in most circumstances, at least temporarily stabilizing cells in the defect. In such cases, a matrix may stimulate the ingrowth of host cells, matrix formation, and the binding of new cells and matrix to host tissue.[2,6,9,11]

Various scaffold materials have been tested, including naturally derived and synthetic polymers.[27-38] Although preliminary results are promising for naturally derived polymers, such as collagen and hyaluronic acid-based carriers, there are practical concerns about the availability and quality of these materials, particularly in terms of possible pathogen contamination. These reservations have prompted researchers to investigate the use of synthetic polymers. A more promising approach involves the use of matrices *in vitro* as three-dimensional scaffolds that allow seeded chondrocytes or cells with chondrogenic potential to establish a three-dimensional cartilage-like matrix, which can then be used as a graft tissue to repair cartilage lesions. A lack of available data makes it difficult to compare the relative merits of different types of matrices or to evaluate the possibility that some implanted materials may cause synovitis.[39] However, available evidence indicates that artificial matrices, such as, polyglycolic (PGA), polylactic (PLA), and polylactic-coglycolic acid (PLGA) scaffolds can significantly contribute to the restoration of the articular surface.

MATRIX REQUIREMENTS AND THE MODULATION OF CONSTRUCT STRUCTURE AND FUNCTION

The design and construction of an ideal matrix requires careful consideration.[7,9] Scientific questions about the optimal approach include; which material or materials should be used for the matrices to optimally stimulate cartilage repair?; should the material be biodegradable?; what is the optimal diameter, fiber packing density and pore size of the matrix?; should the fibers of the matrices be coated with bioactive species, such as growth factors? All these questions must be addressed, as they affect the composition, micro-organization and biomechanical properties of the repair tissue. Other important requirements include, the mechanical stability, bonding to the host tissue, biocompatibility, and internal cohesiveness.

Obviously, the first requirement of an implant is that it allows cell loading, and provides a scaffold seeding for the formation of functional cartilage. This can be as simple as mixing cells with a liquid form of a matrix that can be induced to gel or harden in some manner, which does not affect the cells. However, as surface tension can prevent cell borne liquid media from entering synthetic scaffolds of, for example, PGA and polylactic acid (PLA), these matrices must be pre-wetted with alcohol, which can then be displaced by media to produce seeding deep within the construct.[29,30,38,40] Other techniques to improve seeding include dipping the scaffolds in poly L-lysine or type-II collagen to improve the initial cell attchment.[41] If the pore size is too large, other important attributes such as internal cohesiveness and/or stiffness may be compromised. Also, if cells are seeded too sparsely the scaffold may be incompletely filled, which may result in fibrous ingrowths and in turn adversely affect the properties of the tissue generated.[41,42] High cell seeding densities (4 to 10 million chondrocytes per 10 mm diameter, 5 mm thick, or 5 million chondrocytes per 5 mm diameter, 2 mm thick) are required to form cartilaginous constructs for PGA scaffolds. Cells should not also be permitted to adhere so tightly that their subsequent attempts to detach themselves are hindered, because detachment is also necessary for the rounding up process required for their transformation into chondrocytes from BMSCs. The selections of an appropriate scaffold depend on the cell seeding density as well as the sources of the cells. For example, fibrous PGA meshes initiated with high densities of mammalian chondrocytes[29,32] and avian BMSCs,[22,26] supported chondrogenesis. Whereas, a scaffold with greater structural stability was required for chondrogensis when starting from mammalian BMSCs.

In addition to sufficient porosity to permit the cell lodgment, once as matrix carrying cells has been

deposited within the defect void, its volume should remain stable. If the material shrinks or swells, thereby, causing its volume to reduce or exceed that of the surrounding articular cartilage, then the surface of the repair tissue subsequently laid down will not be flush with that of the native compartment. Restoration of a smooth surface contour is crucial for optimal functioning of articular cartilage in joint physiology, and collagen gels are an example of such a matrix. There is good cell adherence to such matrices, however, with time, the cells cause the matrix to contract and the implant size becomes much reduced. This is a problem if the implant is being culture for any length of time *in vitro* before implantation, because one must allow for this considerable size reduction, which may vary between implants. This phenomenon may also be a problem when implants that are positioned immediately after cell loading because they will shrink within the defect site.

Once the matrix has been deposited within the defect void, it should permit subsequent egress of transplanted cells, or allow the ingress of native cells mobilized by chemo-attractants. To do so, the matrix must possess an internal cohesiveness, and it should be composed of a material that will bond well to the walls of the defect from the time when it is first introduced. This will not be achieved unless it is also biocompatible. The inherent stiffness of the matrix should be sufficient to assure its integrity in all conceivable anatomic positions adopted by the patient, even those opposed to the forces of gravity. If uninterrupted continuity between the matrix and native articular cartilage is not established from the onset and subsequently maintained, then the repair and native tissue will never become integrated. If discontinuities occur at any point along the interface, then forces generated during loading cannot be transmitted smoothly, and this circumstance cause stress zones to become established, which are particularly vulnerable to enzymatic degradation.

As normal osteochondral repair involves numerous bioactive factors, and utilization of these factors to stimulate or promote cartilage repair presents an attractive approach. These factors are mediated by cell-surface receptors (integrins) on chondrocytes, and they may also directly modify the extracellular matrix and thus, modulate such signals as, stresses, strains, and fluid pressures and flows, transmitted to the cells from the surrounding extracellular matrices. Growth factors are generally required to engineer cartilaginous tissues starting from BMSCs.[27,43-45] In contrast to avian BMSCs, which undergo chondrogenic differentiation in media supplemented only with serum and ascorbic acid,[28] the regeneration of cartilaginous tissues starting from mammalian BMSCs required additional supplementation with TGF-β, insulin, and dexamethasone.[27,44] The simplest solution would be to incorporate the growth factor directly into the matrix during the manufacturing process. Alternatively, the matrix construct should be able to hold free suspensions of these substances, for immediate release, or to accommodate microsphere or nanosphere particles within which such agents are entrapped for delayed release. At the same time, the properties of the matrix should not hinder the liberation of these growth factors from their delivery system. In addition to the growth factors, several extracellular matrix components (fibronectin, laminin)[46] and cell adhesion molecules (N-cadherin)[47] are used for coating the matrices in order to enhance cell-to-cell contact. One of future challenges will be to develop scaffolds in which specific regulatory molecules are incorporated and released in their active form with well-defined kinetic rates.

Oxygen seems to play an important role during the cultivation of engineered cartilage. *In vivo*, oxygen tensions range from 45 to 57 mm of Hg at the articular surface to less than 7.6 mm of Hg in the deep zone.[1]

Constructs grown aerobically for 5 weeks were superior to those grown anaerobically with respect to wet weight, total cell number, wet weight fractions of glycosaminoglycan (GAG) and total collagen, and the rates of cartilaginous matrix biosynthesis. These findings indicate that hypoxic conditions comparable with those present *in vivo* are not optimal for *in vitro* chondrogensis.

Matrices should biodegrade at a controlled rate, which matches that of chondrogenesis, particularly during the course of the first few weeks.[7,9] This property may be dictated by the source and the seeding density of cells used in the matrices. If a non-biodegradable material is used, it will persist for several or many years, but probably will not remain biologically inert. For biodegradable matrices, the transport of the degradation products by diffusion and convection is problematic. Such mass transport processes depend on the molecular size and formation of

the polymeric end product and on the fine structure of the repair tissue. Without effective removal of the degraded material from the repair tissue, deleterious toxic effects may be produced. Much current research has focused on chondrocyte interaction with the FDA approved biodegradable polymers PGA, PLA and their copolymer, PLGA.[29-32,38,40,48] These polymers are both poly-α-hydroxy esters that are degraded by hydrolysis. PLA is more hydrophobic and less crystalline than PGA and degrades at a slower rate. In the majority of experiments, the scaffold was made of PGA formed as a 97% porous non-woven mesh of 13m diameter fibers. The mass of this scaffold decreases by approximately 40% to 63% during the first 4 or 8 weeks of cultivation. Other experiments have used scaffolds made of PLGA and polyethylene glycol (PEG) in the form of porous sponges. Firm hyaline-like cartilage were observed six weeks after undifferentiated perichondrial cells were seeded onto PLA meshes and implanted in the femoral condyles.[30,31,38] Similar cartilage morphology was found using PGA porous non-woven scaffolds seeded with bovine chondrocytes and cultured *in vitro* for 12 weeks. Both types of degradable polyester tended to increase proteoglycan synthesis more so than the collagen. PLA has been found to be less toxic to human chondrocytes than PGA, in studies at constant pH over 12 days. Constructs based on mammalian BMSCs and PGA meshes first contracted and then collapsed, whereas, the more structurally stable PLGA-PEG (polyethylene glycol) sponges, based on the same cells, maintained their original dimensions. Mammalian BMSCs appear to require different scaffolds than either chondrocytes from the same species or avian BMSCs.

Because operations are required for implantation, developments have been undertaken on polymers that can be injected with cells and cross-linked in situ to form matrices. Several investigators are also exploring the option of combining fibrinogen and thrombin to form a degradable fibrin mesh that can be used to support chondrocytes.[18] When the cell-fibrinogen-thrombin mixture was injected into defects, more GAGs, aggrecan and type II collagen were found in the new tissue at eight months than in defects that were left untreated.

BIOREACTORS FOR CARTILAGE TISSUE GROWTH

Matrix scaffolds permit chondrocytes to maintain their differentiated phenotype and provide a three-dimensional framework for tissue regeneration. Bioreactors provide control over the conditions of cell seeding and tissue cultivation, which affect construct structures and compositions. Ideally, bioreactors for tissue engineering should provide efficient, spatially uniform initial distributions of cells throughout the three-dimensional scaffolds, and for the efficient transport of biochemical species within the culture medium. High-density cell cultures can be used to prevent the chondrocytic de-differentiation that often occurs in two-dimensional cultures, moreover, bioreactors present numerous advantages for growing large amounts of tissue quickly.[49,50] Bioreactors provide uniform mixing and precise control over mass transfer rates, facilitating the maintenance of nutrient levels and pH. For large animal and clinical studies, a scale-up in the size and quantity of constructs is also needed. In which case, constructs should be produced with uniform physical shapes and dimensions, and with the required *in vivo*-like biochemical and biomechanical properties. Reaching this goal depends upon our being able to achieve a better level of understanding of the design and parameters that affect construct growth, and in particular of the kinematics of chondrocyte proliferation and matrix deposition within constructs cultured in the recirculating bioreactor.

Based on the general physiology of the human articular cartilage, a final product with uniform and substantial dimensions (>2 mm) is likely to be required. Also, it is estimated that to resurface an entire joint would require an engineered cartilage implant of 5 cm or so in diameter. Currently laboratory techniques only achieve 5 mm diameters (1–5 mm thick).[1] Three design features of bioreactor culture vessels, at least, can be exploited to improve the structure and function of engineered cartilage as compared with conventional Petri dish culture[49-51]; mixing patterns can result in efficient and spatially uniform cell seeding of the three-dimensional scaffolds, enhance the transport of chemical species through the medium and to the construct surfaces, and allow the hydrodynamic stimulation of the construct during its development.

Physical stimuli (pressure, shear) that mimic those normally present during *in vivo* chondrogenesis,[51-55] can be induced in a dynamically stirred system, and have shown to increase construct dimensions significantly and promote matrix accumulation of proteoglycans and collagens, when primary articular chondrocytes are cultured on PGA scaffolds. In addition, shear stress can be easily monitored in many types of reaction vessels, and this has been found to have a significant impact on the morphology and the mechanical properties of cartilage produced, therefore, the regulation of this parameter is essential for product quality control. Using process control techniques, flow rates in bioreactors can be modulated to maintain important parameters (i.e. nutrient concentrations) over time, as the construct becomes less permeable due to matrix deposition and increased chondrocyte numbers. A system that combines different methods of operation within one unit may also be advantageous if different phases of the culture period have different requirements. For example, a convection-flow system may be optimal for achieving uniform and efficient cell seeding of the porous materials, subsequently a shear flow system may be preferential for the culture phase to promote matrix synthesis.

There are three basic designs of bioreactors according to the operational hydrodynamics[56]: the static flask (constructs fixed in place, static medium), the mixed flask (constructs fixed in place, with unidirectional turbulent mixing), and the rotating vessel (constructs dynamically suspended in a laminar flow pattern). In the static flasks, low diffusion rates result in small constructs with low collagen and high water content and GAG accumulation only at the periphery. In mixed flasks, magnetic stirring generates turbulence at a level below that reported to cause cell damage. This induces the formation of fibrous capsules at the construct surfaces and increases the tissue component fractions, in particular it increases the collagen level, as compared with constructs grown in the static system. Currently, the rotating vessel is viewed as a promising design that can be used with scaffolds, and addresses the concerns about mechanical mixing. It enhances the mass transfer rates and the hydrodynamic effects associated with dynamic laminar flow patterns. In rotating vessels, fluid mixing is generated by settling the discoid constructs in the tumble-slide regimen, as observed visually and predicted on the basis of the estimated values of inertia and drag.[28,49] The associated fluctuations in fluid velocity appear to permit chondrocytes to maintain their differentiated phenotype and to form large cartilaginous constructs with GAG fractions, which are not significantly different from those observed in natural cartilage explants. When the rate of oxygen exchange was varied, it was discovered that, although cartilage is generally regarded as a hypoxic tissue, higher rates of oxygen tension resulted in larger tissue constructs from pre-seeded polymer scaffolds. These types of bioreactor constructs also showed higher levels of extracellular matrix synthesis than those grown at lower oxygen concentrations.[57]

SUMMARY

Tissue engineering offers the possibility of creating functional cartilaginous equivalents for joint repair. Cartilage-like tissue can be regenerated *in vitro* on biomaterials using chondrocytes or cells with chondrogenic differentiation capacity. This approach has the potential advantage of transplanting a preformed functional tissue or cells only. However, cells tend to retain their differentiated phenotype *in vitro* only if cultured under conditions that resemble their natural *in vivo* environment. The mechanical and biochemical signals that affect *in vivo* tissue development, maintenance, and remodeling are likely to play similar roles during the *in vivo* cultivation of engineered tissue.

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