

ENGINEERING A BIOLOGICAL JOINT

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INTRODUCTION

Because of the poor reparative capacity of injured articular cartilage in the mature skeleton, many patients with arthropathies would benefit from innovative biological approaches to stimulate repair or to provide replacement tissue. Currently, many laboratories are directed towards novel materials with biologic signals, delivery of growth factors or genes, or cell-based composites to aid in repair of articular lesions.

The physeal bar is a corollary of the focal articular cartilage lesion in proliferating cartilage. If small, the physeal bar is a tether that interferes with the kinetic program of the surrounding intact growth plate. Resection and replacement with inert fillers are the present solution, limited by the requirement that at least fifty percent of the growth plate must be intact to allow acceptable growth. Focal replacement of the resected physeal region with cartilage has been attempted experimentally, but it is unclear whether such transplanted cartilage provides cells that participate in growth. Furthermore, even if the transplanted cartilage had kinetic activity, it may not match that of the remaining physeal cartilage following untethering.

An even larger challenge would be the engineering of an entire joint that was congenitally absent or severely damaged. Repair of congenital or acquired joint deformities requires the creation of compound structures with complex anatomical and functional features. Progress in musculoskeletal cell, developmental, and molecular biology, advances in *in vitro* histogenesis, and innovations in materials and manufacturing processes have not yet resulted in widespread clinical solutions to such devastating problems.

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RESEARCH PROGRAM

The central hypothesis of our program of tissue engineering of growing joints is that a developing joint or skeletal anlage from one species may serve as a morphogenic scaffold for chondrocytes of another species. The developing joints or skeletal anlagen are rendered devoid of innate chondrocytes and are seeded with chondrocytes from another organism for culture. The devitalized joint is intended to serve as an architectural scaffold with a microenvironment favorable for chondrogenesis, morphogenesis, and growth. The source of the viable chondrocytes, and thus the cell surface antigens within this engineered growing joint or anlage, could therefore be selected for isogenicity or compatibility with the ultimate recipient. Neonatal bovine chondrocytes have been used for these preliminary studies because they are easily isolated in large numbers from freshly butchered shoulders obtained from a local abattoir. The chondrocytes grow and maintain the differentiated phenotype *in vitro*. They have been used for numerous studies on cartilage cell biology and tissue engineering.^{1,2}

Several means for delivering the viable chondrocytes were evaluated. Aliquots of a suspension of bovine articular chondrocytes (bACs) were inoculated into the distal femoral and proximal tibial devitalized chick chondroepiphyses with a dissecting microscope and a micromanipulator. Small foci of viable chondrocytes were identifiable with this method but not with the desired widespread penetration. Bovine chondrocytes were precultured with braided polyglactic acid suture for 5-28 days and were found to adhere to and permeate the interstices of that braided material. The suture was then passed into devitalized chick knees. Histologic analysis showed that viable bovine chondrocytes were present, predominantly at the outer surface of the suture where it interfaced with the devitalized chick matrix. In the next test, canals were created in devitalized chick chondroepiphyses either with a 28½-gauge needle or with an Er:YAG laser with an average of 15 canals per joint surface. A suspension of cells was placed onto the prepared surface for culture. Viable chondrocytes were found in the canals made either way. There was filling of the canal with some invasion of the devitalized matrix. This became more extensive with longer time in culture. In another series, canals were created by the physical means described. The chondroepiphyses were further treated with lytic enzymes (2.5 mg/ml trypsin, 1 mg/ml hyaluronidase and 1 mg/ml collagenase) for only 10 minutes and re-lyophilized. The

treated joint surfaces were rehydrated with a suspension of cells and were cultured for 7 and 21 days. There was enhanced infiltration of viable chondrocytes with the addition of enzyme pre-treatment. This was interpreted as a consequence of the enzymes' rendering the matrix more penetrable.

IN VITRO AND IN VIVO CHARACTERISTICS OF KNEE CONSTRUCTS

The most successful model we have developed to date uses porous collagen sponges to deliver the viable chondrocytes to the anlage (Figure 1). This phase of the program uses an experimental construct composed of an embryonic chick devitalized by lyophilization and viable chondrocytes isolated from neonatal bovine articular cartilage and delivered to the joint in porous collagen sponges (Figure 1). The sponges are meant to contain the cells in the desired location. These collagen sponges have been shown to support chondrogenesis¹, chondroinduction^{2,3}, osteogenesis⁴, and *in vivo* compatibility^{1,5}.

Constructs were engineered *in vitro* and their ectopic *in vivo* fate was examined.⁶ Chimeric joints were made by affixing porous collagen sponges that contained bACs to opposing shaved articular femoral and tibial surfaces of devitalized embryonic (19 d) chick knees. Membranes of expanded polytetrafluoroethylene (ePTFE) were positioned between the femoral and tibial sponges. The constructs were cultured and subsequently transplanted into heterotopic sites in severe-combined-immunodeficiency-disease (SCID) mice, chosen to avoid immune rejection of the bovine cells. The constructs were analyzed by histology at intervals *in vitro* and *in vivo* and by gene expression analyses for bovine markers.

After 1 week *in vitro*, collagen sponges with bACs were adherent to the shaved articular surfaces of the devitalized chick joints. Subsequently, metachromatic neocartilage accumulated in the sponges. The bovine chondrocytes were the source of the neocartilage, as demonstrated by RT-PCR analysis of extracted RNA. Molecular analysis showed expression of bovine cartilage genes (collagen type II, aggrecan core protein, and aggrecan link protein) for the 3-week observation period. The ePTFE membranes were successful in maintaining a joint space between opposing sponges.

Some constructs were cultured for 1 week before transplantation into dorsal subcutaneous pouches of 5-week-old immunodeficient mice. Chimeric joints exhibited dramatic changes *in vivo*. The bACs invaded the articular matrix of the devitalized knees. At 6 weeks, bovine neocartilage replaced much of the chick cartilage. At 8 weeks, bAC proliferation and neocartilage formation transgressed the scaffolds and created a synchondrosis around the joint. This duration is considered to be excessive in this unloaded site. Seeded bACs repopulated the devitalized chick scaffold and ePTFE membranes maintained knee joint spaces.

ENABLING TECHNOLOGIES

This construct consisted of elements contributing to its success in repopulating a devitalized knee with viable chondrocytes. The porous collagen sponges supported the

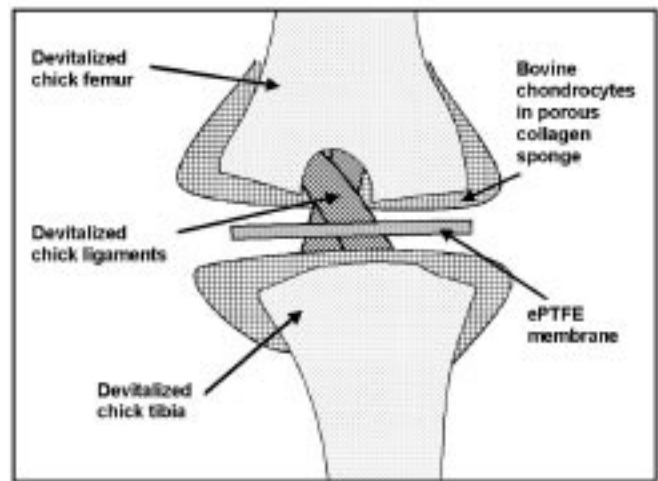


Figure 1. Composite biological joint. Two porous collagen sponges containing bovine chondrocytes are positioned against shaved articular surfaces of femur and tibia of a lyophilized, devitalized embryonic chick knee. An ePTFE membrane is inserted to maintain the joint space.

viability and delivery of bACs into the joint constructs. In themselves, collagen sponges are adherent to tissues.

Proliferation and matrix production were shown to be exuberant and, in this immobilized, unloaded system, often resulted in synchondrosis across the two sponges. This was effectively prevented by insertion of ePTFE membranes between the sponges, but may not be needed in mobile and loaded sites. Bovine neocartilage penetrated into the devitalized chick articular cartilage *in vitro* and *in vivo*. In SCID mice, the bovine neocartilage replaced the chick scaffold at 6 weeks, but transgressed the construct at 8 weeks; this duration is considered to be excessive in this unloaded site. The *in vivo* subcutaneous environment in SCID mice was conducive to cartilage formation in joint constructs. Because of the immunodeficiency of these test animals, it is possible, albeit unlikely, that host responses to bioengineered joints may involve an inflammatory element that would require modulation.

Recently, chondrogenesis has been enhanced in collagen sponges *in vitro* by several means. A small amount of hyaluronan (2% of dry weight) that had been incorporated into three-dimensional collagen scaffolds enhanced chondrogenesis, but a greater amount was inhibitory, apparently due to excessive filling of the porous network.⁷ The results with the composite hyaluronan/collagen sponge show the potential for modifying scaffolds to improve production of engineered cartilage for *in vivo* applications. Mechanical forces have also been shown to modify chondrogenesis *in vitro*. With a novel fluid pressure culture system, 2.8 MPa fluid pressure applied in either a constant or cyclic manner stimulated synthesis of cartilage specific proteoglycans by chondrocytes cultured in 3-dimensional collagen sponges.⁸ Optimal matrix production may be achieved in bioengineered joints using hyaluronan/collagen sponges, preculture with fluid pressure, as well as with supplemental growth factors.

CLINICAL RELEVANCE

The overall goal of this research program is to engineer a biological joint using a devitalized joint from one species and chondrocytes from another species and to apply it in a small animal model. The functional small animal experiment will be to repopulate a devitalized chick knee with murine chondrocytes and to use the construct to replace a knee in a syngeneic mouse. An example of a conceivable clinical model would employ a devitalized, cleansed joint from a pig that has been repopulated with chondrocytes or marrow-derived chondroprogenitors from the patient who will receive the construct. In the case of pediatric applications, it would be necessary for the construct to grow with the child.

SUMMARY

These results indicate the feasibility of repopulating a devitalized joint with cells from a different species and support the possibility of this approach for construction of autogenous joints. An engineered biological joint would enable reconstruction of articular loss resulting from congenital, traumatic, neoplastic, and degenerative conditions.

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