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# Repair of osteochondral defects with biphasic cartilage-calcium polyphosphate constructs in a Sheep model

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**Abstract**

There has been interest in developing novel biological treatments to repair focal cartilage defects. We have developed a method of forming biphasic constructs (''osteochondral''-type plug) in vitro consisting of cartilaginous tissue, formed on and anchored to the intended articulation surface of a porous ceramic substrate. The purpose of this study was to evaluate the biochemical and biomechanical properties and morphology of in vitro-formed biphasic constructs 3 and 9 months after implantation into 4 mm diameter full thickness osteochondral defects in the trochlear groove of sheep stifles. The implants withstood loading in vivo up to 9 months with evidence of fusion to adjacent native cartilage and fixation by bone ingrowth into the ceramic substrate. The cartilage layer was eroded from those implants that were proud to the joint surface. Control implants (ceramic only) had fibrous tissue on the articulating surface after implantation for 3–4 months. Neither the cellularity nor proteoglycan content of the implanted cartilage, when it remained, changed significantly between 3 and 9 months and the collagen content increased slightly. The elastic equilibrium modulus of the cartilage improved with time with the greatest improvement (10-fold) occurring early during the first 3–4 months after implantation. This study suggests that biphasic constructs may be suitable to repair joint defects as the implants were maintained up to 9 months in sheep. Importantly the mechanical properties of the implanted cartilage improved significantly after implantation suggesting that cartilage can mature in vivo after implantation. The results indicate that further study of this treatment approach is warranted to attempt to overcome the technical surgical difficulties identified in this study.


*Keywords:* Articular cartilage; Transplantation; Tisssue engineering

## 1. Introduction

Articular cartilage is present at the ends of the articulating bones in synovial joints and acts as a load-bearing cushion that distributes forces transmitted through the joint to the underlying subchondral bone [1]. Damage to articular cartilage, either by trauma or disease, can affect joint function as adult articular cartilage has a limited capacity for repair [2–4]. A variety of reasons for this lack of reparative response have been postulated including the inability of chondrocytes to migrate into the site of injury, the avascular nature of cartilage, and the absence of a fibrin clot scaffold into which the cells can migrate [3,5]. However other factors may also influence the repair process as chondrocytes have been shown to be able to reconstitute at least part of their extracellular matrix in vitro [6] as well as in vivo with the appropriate treatment [7]. Currently replacement of skeletal joints with a synthetic prosthesis represents the optimal treatment for end-stage disease [8].

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Primary hip replacements placed since the late 1980s have shown reasonable success rates. However, even these apparently successful implants have their limitations as failure rates of up to 20% have been reported after 10 years [9–12]. For this reason there has been interest in developing biological treatments for focal cartilage defects [4]. Currently several biological treatment options are being used [2,4,5]. One of these is autologous chondrocyte implantation which involves harvesting the patient's cartilage, and isolating and expanding the number of chondrocytes, which are then transplanted into the cartilage defect under a periosteal flap (to confine cells in the defect) [13–15]. There is even some question as to whether these cells regenerate articular hyaline cartilage as histological studies have documented the presence of fibrocartilage repair in some defects [5,13,16]. More recently it was shown, in a goat model, that there was a loss of bone underlying the implant, which could negatively impact on implant survival and have long-term effects on joint function [17]. Another approach involves the transplantation of osteochondral plugs into a defect site (osteochondral transfer/mosaicplasty) that have been obtained from donor sites within the same joint [4,18]. Several plugs are usually required to repair a single defect that may be several centimeters in diameter. The major disadvantages of this approach are the resulting donor site morbidity and difficulty in placing the plugs congruent with the joint surface [18]. Alternatively large articular shell grafts (articular cartilage with subjacent bone at least 1 cm in thickness) have been utilized with success but these allografts are limited in supply and can be associated with disease transmission [19]. Microfracture (perforation) of the subchondral bone has also been used to induce cartilage repair however this results in fibrocartilage rather than hyaline cartilage and as it does not have the necessary load bearing capability it degenerates over time [18,20]. As all of these treatments have limitations recent efforts have focused on developing methods to bioengineer articular cartilage constructs which include a bone-interfacing component (biphasic constructs) [4].

We have developed a method of forming biphasic constructs (''osteochondral''-type plug) in vitro [21,22]. These consist of cartilaginous tissue formed on the intended articulation surface of a porous ceramic substrate (calcium polyphosphate, CPP) by chondrocytes isolated from articular cartilage. As the CPP is porous chondrocytes can enter into the pores of the immediate subsurface and the cartilage that forms in this region anchors the tissue to the CPP. The cartilage tissue has been characterized previously and has been shown to resemble hyaline cartilage and to contain type II collagen and large proteoglycans similar to native cartilage [21]. There are several potential benefits to using these biphasic constructs to repair focal cartilage defects. As the cartilage is already formed, lateral integration to the adjacent native cartilage is possible immediately upon implant insertion. As the substrate CPP is porous, bone grows into pores not filled by cartilage after implantation, resulting in secure implant fixation [23,24]. The CPP is biodegradable and will ultimately be replaced by bone. The degradation products are calcium and phosphate and so do not incite an inflammatory reaction [24,25]. The purpose of this study was to determine whether an in vitro-formed biphasic construct could be used to repair a full thickness osteochondral defect in a sheep knee (stifle) and the change in the biochemical and biomechanical properties of the cartilage up to 9 months following implantation.

## 2. Materials and methods

### 2.1. CPP substrates

Porous CPP substrates were produced by sintering calcium polyphosphate glass powders as described previously [24]. The cylinders (4 mm in diameter) $\times$ 6 mm in length had an average density of $63.1 \pm 0.3\%$ (mean $\pm$ SEM, $n = 45$) of theoretical full density (approximate volume porosity of 37%). The substrates were then machined to have a 5° taper starting 1 mm from the top of the CPP. The resulting CPP tapered shapes were inserted in tubing (3.8 mm diameter, Thermoplastics Processor Inc., San Jose, CA) that formed a tight seal around the top cylindrical 1 mm length of the sample and protruded 3 mm above the CPP surface. This prevented chondrocytes from spilling over the edge of the substrate. The substrates with tubing were sterilized by $\gamma$-irradiation (3.5 MRad) prior to use.

### 2.2. Formation of biphasic constructs

To generate cartilaginous tissue in vitro, chondrocytes were isolated from the articular cartilage harvested from the knees of sheep (6–9 months old) by sequential enzymatic digestion, as described previously [21]. The cells obtained from each animal were re-suspended in Ham's F12 supplemented with 25 mM HEPES and 5% autologous (sheep) serum (heat-inactivated at 60 °C) and seeded on the top surface of the CPP substrates at a density of 160,000 cells/mm$^2$. The cultures were grown in an incubator maintained at 37 °C and 95% relative humidity supplemented with 5% $CO_2$. The serum concentration was increased to 20% at day 5 and on day 7, ascorbic acid (100 μg/mL final concentration) was added to the medium. These conditions were identified previously as optimal for cartilage tissue formation. The cells from each sheep were grown in autologous serum to ensure that the constructs were not exposed to any foreign proteins. The medium was changed every 2–3 days and fresh ascorbic acid was added with each change. Biphasic constructs were grown in culture for 8 weeks and then implanted into sheep.

### 2.3. Sheep model

All animal studies were approved by the University of Guelph Animal Care Committee. To harvest the cartilage from which chondrocytes were isolated and used to form the biphasic constructs, the sheep were anesthetised using ketamine and isoflurane. The right knee was exposed using a parapatellar incision. Articular cartilage was harvested from the trochlear ridge and groove of the distal femur over an area approximately 4 cm$^2$ using a scalpel, placed in sterile culture media and transported to the lab. This amount of tissue was sufficient to generate between 2 and 4 biphasic constructs from each sheep. The joint capsule and adjacent muscle and skin were sutured closed. The animals were fully ambulated and appeared to suffer no musculoskeletal consequences as they walked normally after 48 h.

After 8 weeks, the time required to grow the biphasic constructs in vitro, the sheep were again anaesthetized and the knee not used initially to provide chondrocytes exposed. Full thickness defects (4 mm diame-