SURVIVAL AND ADAPTATION OF BIPHASIC CARTILAGE-CALCIUM POLYPHOSPHATE IMPLANTS IN A SHEEP MODEL

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INTRODUCTION

Cartilage defects in the knee can create significant disability leading to economic and lifestyle changes for patients. Current treatments include osteochondral grafts, enhancement of extrinsic repair with marrow stimulation and implantation of chondrocytes, periosteum or scaffolds to promote development of repair tissue. The quality, attachment and retention of this tissue are highly variable and so implantation of more mature, fully differentiated cartilaginous tissue would be preferred. A tissue-engineered construct that could address subchondral bone deficits and resulting incongruities would be ideal. This study was designed to demonstrate that biphasic grafts composed of cartilaginous tissue grown in vitro on porous calcium polypophosphate (CPP) substrates can survive and adapt after implantation.

METHODS

The porous CPP component was made by sintering CPP powders to form truncated conical structures of 65% density with interconnected pores of approximately 75 micron average pore size. Straight cylindrical performs were machined to form shapes 6mm long with a straight 4 mm diameter cylindrical region over the superior-most 0.5 mm length and a 5° taper over the remaining length. Autologous chondrocytes were obtained by a sequential enzymatic digestion of cartilage harvested from the left knee of sheep. Chondrocytes (160,000/mm³) were contained on top of the porous CPP shapes, and held in Ham’s F12 medium supplemented with up to 20% autologous serum and ascorbic acid (100 µg/ml) for eight weeks before implantation. This resulting tissue was 200 µm thick before implantation. Surgical implantation was achieved using general anesthesia and aseptic technique to expose the femoropatellar joint in 18 sheep. Two or three biphasic constructs were press-fitted into tapered drill holes in the middle third of the trochlear ridges of the right knee in 11 sheep. Control plugs consisting of CPP alone were also implanted in 7 sheep. Detailed surgical records and photographs were made to document the location and congruence of the grafts and no attempt was made to limit postoperative weight bearing. Sheep were sacrificed 3 months (n=8) and 9 months (n=3) postoperatively. Assessment of the cartilaginous portion of the grafts included undecalcified histology, collagen (hydroxy-proline), DNA and proteoglycan concentration, and biomechanical testing. Integration of the CPP with the recipient site was assessed by radiography, histology and backscattered scanning electron imaging. Biochemical and mechanical characterization results were determined and the data evaluated for significant differences using the Mann–Whitney U test. Significance was assigned at p-values < 0.05.

RESULTS

At 3 and 9 months all the implants were stable within defect and but 5 were covered by tissue. The 5 biphasic implants without cartilage were due to placing the grafts in a recessed, tipped or protruding position, allowing invasion of fibrous tissue or mechanical damage to the cartilage. Of 14 grafts evaluated histologically at 3 months, 9 grafts had intact neocartilage that integrated with the adjacent cartilage when in contact. There was focal separation of the neocartilage from the CPP where the graft had been handled for press-fitting. Ingrowth of bone into the CPP cylinders was present. At nine months the implanted cartilage was still present and where the graft had been handled for press-fitting. Ingrowth of bone into the implant interface (toluidine blue x 50) . Arrows indicate grafts.

DISCUSSION

These data show that the biphasic implants were integrated to the adjacent cartilage and bone by 3 months and they were still intact at 9 months. The implanted cartilage tissue was superior in all assessments to repair tissue formed in defects receiving CPP cylinders alone. The progressive improvement of implanted cartilage properties with time demonstrates that maturation of neocartilage can occur in an ambulatory patient. However, 5 of 19 constructs at 3 months had lost the cartilage surface due to technical errors during implantation. It may be possible to avoid these failures by controlling early weight bearing and improving the accuracy of implantation. Specifically, in joint surfaces such as the sheep knee where the cartilage is thin (~1 mm) there is little room for error with respect to the height of the construct. Tipped or recessed grafts are overgrown by fibrous tissue and the cartilage eroded from the grafts left protruding. Delivering such grafts to human patients would be less demanding because the cartilage in the knee is twice as thick, and controlled postoperative weight bearing or continuous passive motion would promote cartilage maturation before full ambulation. Overall using biphasic implants to resurface joint defects appears feasible and further study is required to determine whether implants of clinically relevant size will function as well.


<table>
<thead>
<tr>
<th>Tissue</th>
<th>DNA (µg/mg dry weight)</th>
<th>Proteoglycan (µg/mg dry weight)</th>
<th>OH-proline (µg/mg dry weight)</th>
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<tr>
<td>Biphasic</td>
<td>3.0±0.4</td>
<td>220±19.7</td>
<td>129±5.9</td>
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<tr>
<td>Control</td>
<td>5.0±1.7</td>
<td>69±19.9</td>
<td>248±50.5</td>
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<tr>
<td>Native</td>
<td>2.0±0.1</td>
<td>267±24.9</td>
<td>149±8.1</td>
</tr>
</tbody>
</table>

**Table 1: Biochemical Evaluation of Implants at 3 months**

**Table 2: Characteristics of three and six month cartilaginous tissues.**

References: 1) Pilliar R et al. Biomaterials 22 :963, 20002. Acknowledgements: This work was supported by NSERC and CIHR. Departments of Pathology and Laboratory Medicine* and Orthopedic Surgery**. Mount Sinai Hospital, Institute of Biomaterials and Biomedical Engineering, University of Toronto, Department of Chemical Engineering, Queen’s University, Kingston.