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Development and remodeling of engineered cartilage-explant composites *in vitro* and *in vivo*[1]

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Summary

Objective: Development and remodeling of engineered cartilage-explant composites were studied *in vitro* and *in vivo*.

Design: Individual and interactive effects of cell chondrogenic potential (primary or fifth passage bovine calf chondrocytes), scaffold degradation rate (hyaluronan benzyl ester or polyglycolic acid), and adjacent tissue cell activity and architecture (vital trabecular bone (VB), articular cartilage (AC), devitalized bone (DB) or digested cartilage (DC)) were evaluated over 8 weeks *in vitro* (bioreactor cultures) and *in vivo* (ectopic implants).

Results: *In vitro*, significant effects of cell type on construct adhesive strength ($P < 0.001$) and scaffold type on adhesive strength ($P < 0.001$), modulus ($P = 0.014$), glycosaminoglycans (GAG) ($P < 0.001$), and collagen ($P = 0.039$) were observed. Chondrogenesis was best when the scaffold degradation rate matched the extracellular matrix deposition rate. *In vivo*, adjacent tissue type affected adhesive strength ($P < 0.001$), modulus ($P < 0.001$), and GAG ($P < 0.001$) such that 8-week values obtained for bone (VB and DB) were higher than for cartilage (AC). In the AC/construct group, chondrogenesis appeared attenuated in the region of the construct close to the AC. In contrast, in the VB/construct group, a 500 μm thick zone of mature hyaline-like cartilage formed at the interface, and signs of active remodeling were present in the bone that included osteoclastic and osteoblastic activity and trabecular rebuttressing; these features were not present in the DB group or *in vitro*.

Conclusions: Development and remodeling of composites based on engineered cartilage were mediated *in vitro* by cell chondrogenic potential and scaffold degradation rate, and *in vivo* by type of adjacent tissue and time.

Key words: Tissue engineered cartilage, Bone remodeling, Biomaterial, Bioreactor.

Introduction

Osteochondral defect repair remains an important, unsolved clinical problem, and a number of tissue engineering approaches involving cells and biomaterial scaffolds have been studied in an attempt to promote articular cartilage (AC) regeneration[1,2]. However, the most commonly used animal model, orthotopic implants in rabbit knee joints, is complicated by high variability and a fundamentally different biological situation from that existing in human joint lesions[2,3]. To address the problem of biological complexity, controlled *in vitro* studies have been done in petri dishes[4–7] and bioreactors[8,9]. Complementary *in vivo* data have also been obtained using ectopic (subcutaneous) implants in nude mice[10–15].

In the present study, *in vitro* (bioreactor) and *in vivo* (ectopic implant) models were utilized to explore the hypotheses that development and remodeling in composites made of engineered cartilage constructs and bone or cartilage explants depended on: (i) chondrogenic potential of the cell in the construct, (ii) scaffold degradation rate, (iii) adjacent tissue cell activity and architecture, and (iv) time (Fig. 1). We compared two cell types: primary (P0) calf chondrocytes that were expected to undergo rapid chondrogenesis[16,17] and fifth passage (P5) chondrocytes that were expected be dedifferentiated by serial expansion in monolayers using media containing 10% serum[18,19]. We compared two scaffolds: hyaluronan benzyl ester (Hyaff®11) that was expected to degrade only minimally over 8 weeks[9,20] and a structurally indistinguishable scaffold made of polyglycolic acid (PGA) that was expected to be almost completely degraded over 8 weeks[21,22].

We compared four different adjacent tissues: AC and vital trabecular bone, (VB), to represent the range of tissues involved in osteochondral defect repair[1], devitalized bone (DB) to explore whether bone cells or architecture influenced integration[9], and digested cartilage (DC) in an

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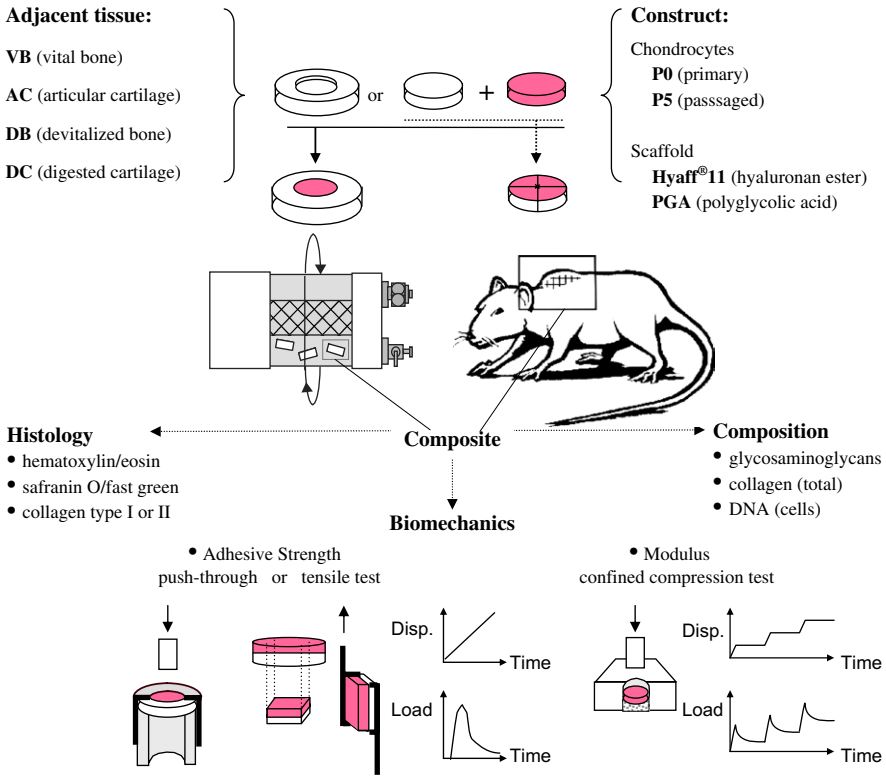

Fig. 1. Experimental design. Composites were generated using explanted adjacent tissues (VB, AC, DB, or DC) and constructs made of chondrocytes (P0 or P5) and biomaterial scaffolds (Hyaff®11 or PGA). Disc-in-ring composites made by press-fitting discs (5 × 2 mm) into rings (10/5 mm × 2 mm) were cultured in rotating bioreactors. Sandwich-like composites made by suturing together two discs (8 × 2 mm) were implanted subcutaneously in nude mice. Histology, biomechanics, and biochemical composition were evaluated after 4 and 8 weeks.

attempt to enhance integration by depletion of surface glycosaminoglycans (GAG)[8,23] in the explants. We evaluated composite histological structure, biomechanical properties, and biochemical composition after 4 and 8 weeks.

Methods

EXPERIMENTAL DESIGN

Twelve experimental groups were studied, each of which provided specific controls for other groups as follows. Based on our previous study[9], three basic groups were evaluated *in vitro* and *in vivo*; AC/P0/Hyaff®11, VB/P0/Hyaff®11, and DB/P0/Hyaff®11. *In vitro* we also studied AC/P5/Hyaff®11 composites, where dedifferentiated P5 cells provided a control for more chondrogenic P0 cells, and AC/P0/PGA composites, where quickly degrading PGA provided a control for more slowly degrading Hyaff®11. *In vitro* we also studied individually cultured specimens of VB and DB that, in contrast to AC[24], had not previously been cultured in rotating bioreactors. *In vivo*, we also studied DC/P0/Hyaff®11 composites, where DC was expected to provide a more adhesive substrate than AC[25] and VB/AC composites which provided an explant/explant control group.

CONSTRUCTS

Chondrocytes were obtained from full thickness cartilage harvested from the femoropatellar groove (FPG) of 2–4-week-old bovine calves within 8 h of slaughter[21]. The P0 cells were seeded onto scaffolds immediately after isolation, whereas the P5 cells were seeded onto scaffolds after five serial passages in monolayers plated at low initial density (10,000 cells/cm^2). All cells were cultured in Dulbecco's modified Eagle medium (DMEM) containing 4.5 g/L glucose, 10% fetal bovine serum (FBS), 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) 0.1 mM nonessential amino acids, 0.4 mM proline, 50 mg/L L-ascorbic acid, 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.5 μg/mL fungizone[21].

The Hyaff®11 and PGA scaffolds, respectively obtained from Fidia Advanced Biopolymers (Abano Terme, IT) and Smith & Nephew (York, UK), were formed as non-woven meshes that were indistinguishable with respect to three-dimensional structure[17]. The Hyaff®11 had a weight averaged molecular weight (M_w) of 168 kDa and was minimally degraded over 8 weeks[9,20,26], whereas the PGA had a M_w of 69 kDa and was essentially degraded over 8 weeks[22]. The scaffolds were wetted in FBS for 1–2 h, vacuum-dried, die-punched into discs, and seeded with cells for a period of 3 days in spinner flasks[9], using 60 million cells, 10 scaffolds (6 × 2 mm discs), and 150 mL per flask for *in vitro* studies, or 80 million cells, 10 scaffolds (8 × 2 mm discs), and 180 mL per flask for *in vivo* studies.

EXPLANTS

Osteochondral cores (10 and 8 mm in diameter for *in vitro* and *in vivo* studies, respectively) were obtained from the