Osteoarthritis and Cartilage (2009) **17,** 714–722

doi:10.1016/j.joca.2008.11.017

Osteoarthritis and Cartilage

International Cartilage Repair Society

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Repair of full-thickness femoral condyle cartilage defects using allogeneic synovial cell-engineered tissue constructs[1]

M. Pei†‡*, F. He†‡, B. M. Boyce† and V. L. Kish†

† *Tissue Engineering Laboratory, Department of Orthopaedics, West Virginia University, Morgantown, WV 26506, USA*

‡ *Division of Exercise Physiology, West Virginia University, Morgantown, WV 26506, USA*

Summary

Objective: Synovium-derived stem cells (SDSCs) have proven to be superior in cartilage regeneration compared with other sources of mesenchymal stem cells. We hypothesized that conventionally passaged SDSCs can be engineered *in vitro* into cartilage tissue constructs and the engineered premature tissue can be implanted to repair allogeneic full-thickness femoral condyle cartilage defects without immune rejection.

Methods: Synovial tissue was harvested from rabbit knee joints. Passage 3 SDSCs were mixed with fibrin glue and seeded into non-woven polyglycolic acid (PGA) mesh. After 1-month incubation with growth factor cocktails, the premature tissue was implanted into rabbit knees to repair osteochondral defects with Collagraft® as a bone substitute in the Construct group. Fibrin glue-saturated PGA/Collagraft® composites were used as a Scaffold group. The defect was left untreated as an Empty group.

Results: SDSCs were engineered in rotating bioreactor systems into premature cartilage, which displayed the expression of sulfated glycosaminoglycan (GAG), collagen II, collagen I, and macrophages. Six months after implantation with premature tissue, cartilage defects were full of smooth hyaline-like cartilage with no detectable collagen I and macrophages but a high expression of collagen II and GAG, which were also integrated with the surrounding native cartilage. The Scaffold and Empty groups were resurfaced with fibrous-like and fibrocartilage tissue, respectively.

Conclusion: Allogeneic SDSC-based premature tissue constructs are a promising stem cell-based approach for cartilage defects. Although *in vitro* data suggest that contaminated macrophages affected the quality of SDSC-based premature cartilage, effects of macrophages on *in vivo* tissue regeneration and integration necessitate further investigation.

Key words: Synovium, Mesenchymal stem cell, Tissue engineering, Cartilage defect, Repair, Allograft.

Introduction

Once damaged, articular cartilage has only limited intrinsic capacity for self-repair[1,2]. Although autologous chondrocyte implantation (ACI) has some advantages, a general problem with this chondrocyte-based procedure in cartilage repair is the quality of newly formed cartilage. For example, implanted chondrocytes undergo hypertrophic differentiation with subsequent ossification[3,4] and poor integration to host tissue[5,6]. In contrast, it has been shown that immature constructs using mesenchymal stem cells (MSCs) integrate better and are more durable[7–9]. MSCs can regenerate not only cartilage but also the underlying subchondral bone[10] and are therefore able to resurface osteochondral defects as well.

MSCs are characterized by their multipotentiality and capacity for self-renewal[11]. The hypoimmunogenic nature implies that MSCs can be used in allogeneic cell-based therapy[12,13]. MSCs from different sources have exhibited different properties in expansion capacity and multi-lineage differentiation[14]. Synovium-derived stem cells (SDSCs) are a promising source of stem cells for cartilage tissue engineering because they display greater chondrogenic and less osteogenic potential than MSCs derived from bone marrow or periosteum[15]. They are also proven to be superior to other sources of MSCs such as adipose tissue and muscle[16–18]. Under appropriate stimulation conditions, they are able to migrate into articular cartilage defects and subsequently undergo chondrogenic differentiation[10,19]. Studies also show that the molecular profile of SDSCs is stable during *in vitro* expansion from passage 3 up to at least passage 10[20,21].

Imitating the involvement of growth factors in cartilage development, our previous study characterized the properties of SDSCs and defined growth factor cocktails for maximal cell proliferation and chondrogenic differentiation[22]. However, it is unknown whether SDSCs can be engineered into cartilage-like tissue in bioreactor systems supplemented with growth factor cocktails. We also question if allogeneic SDSC-engineered premature tissue can be implanted to repair full-thickness femoral condyle cartilage defects without immune rejection. Although MSCs have immune "privilege" and immunomodulatory capacity, the implanted tissue constructs derived from allogeneic SDSCs

[1]Supported by a grant from the Musculoskeletal Transplant Foundation (MTF) to M.P.

*Address correspondence and reprint requests to: M. Pei, Tissue Engineering Laboratory, Department of Orthopaedics, West Virginia University, PO Box 9196, One Medical Center Drive, Morgantown, WV 26506-9196, USA. Tel: 1-304-293-1072; Fax: 1-304-293-7070; E-mail: mpei@hsc.wvu.edu

Received 30 June 2008; revision accepted 28 November 2008.

having partially differentiated into chondrocytes. Therefore, we are unsure whether the differentiated MSCs still have immunomodulatory properties.

We hypothesized that SDSCs can be engineered *in vitro* into cartilage tissue constructs and allogeneic SDSC-engineered premature tissue can be implanted to repair full-thickness femoral condyle cartilage defects without immune rejection. Our long-term goal is to engineer high-quality cartilage constructs using allogeneic SDSCs for the repair of cartilage defects resulting from trauma and osteoarthritis.

Materials and methods

SDSC ISOLATION AND CULTURE

Random biopsies of synovial tissue were obtained aseptically from the knees of two 8-month-old New Zealand white rabbits and pooled together [Fig. 1(A)]. The synovial tissue was finely minced and digested at 37°C for 30 min in phosphate-buffered saline (PBS) containing 0.1% trypsin and then for 2 h in 0.1% solution of collagenase P in DMEM/10% fetal bovine serum (FBS). After passing through a 70-μm nylon filter, the cells were collected from the filtrate by centrifugation. Cells were plated and cultured for 4 days in complete medium (DMEM/F12/10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin). Non-adherent cells were removed by a PBS wash on days 2 and 4. After 90% confluence, primary cells were trypsinized and replated as passage 1. Passage 3 SDSCs were collected for this study [Fig. 1(B)].

IN VITRO ENGINEERED SDSC-BASED TISSUE CONSTRUCTS

Degradable polyglycolic acid (PGA) scaffolds (a mesh of 15-μm fibers and 97% void volume; Synthecon, Houston, TX) were punched into 5-mm-diameter × 2-mm-thick discs and sterilized with ethylene oxide, then immersed in 100% ethanol, 70% ethanol, and PBS (without Ca^{2+} and Mg^{2+}). In a centrifuge tube, 150 μL fibrinogen (100 mg/mL in PBS, Sigma, St. Louis, MO) [Fig. 1(C)], 140 μL PBS with cells, 5 μL thrombin (0.1 U/μL, Sigma), and 5 μL $CaCl_2$ (50 mM) were sequentially added. Then, 26 μL of SDSC-gel mixture was pipetted onto a PGA disc [Fig. 1(D)] in a Petri dish. This procedure resulted in a total of 56 fibrin-PGA composites containing 2.6×10^6 cells per scaffold [Fig. 1(E)], corresponding to an initial seeding density of 100×10^6 cells/mL. The dish with constructs was transferred into an incubator for 10 min. Complete medium was then added to cover the constructs.

After 1 h, the medium was replaced by chemically defined medium (high-glucose DMEM, 40 μg/mL proline, 100 nmol/L dexamethasone, 0.1 mmol/L ascorbic acid 2-phosphate, 100 U/mL penicillin, 100 μg/mL streptomycin, and 1 × ITS™ Premix) supplemented with a proliferative growth factor cocktail (10 ng/mL transforming growth factor β1 [TGF-β1], 50 ng/mL basic fibroblast growth factor [FGF-2], and 500 ng/mL insulin-like growth factor I [IGF-I]) for 3 days [Fig. 1(F)][22]. The cell-fibrin-PGA constructs were transferred to a rotating bioreactor (Rotary Cell Culture System-4 (RCCS-4); Synthecon) filled with chemically defined medium supplemented by a differentiative growth factor cocktail (10 ng/mL TGF-β1 and 500 ng/mL IGF-I) for 28 days [Fig. 1(G)][22] The bioreactor rotation speed was adjusted to maintain the growing constructs freely suspended in the rotating flow. Forty-four tissue constructs were harvested [Fig. 1(H)] for the analyses at days 0, 3, 15, and 31. Another 12 1-month constructs were used for *in vivo* implantation.

IN VIVO IMPLANTATION OF PREMATURE TISSUE FOR CARTILAGE REPAIR

This project was approved by the Institutional Animal Care and Use Committee (IACUC) and conducted in compliance with the National Advisory Committee for Laboratory Animal Research Guidelines. Eighteen New Zealand white rabbits (8-month-old males weighing 3.5–4.0 kg) (Covance, Denver, PA) were used in this study. The rabbits were anesthetized with 5 mg/kg xylazine (Phoenix Pharmaceutical, St. Joseph, MO) and 35 mg/kg ketamine (Phoenix Pharmaceutical) intramuscularly and maintained with isofluorane. The knee joints were approached through medial parapatellar incisions and the articular surfaces were exposed by lateral dislocation of the patellae. Full-thickness osteochondral defects, 4 mm diameter × 5 mm deep, were

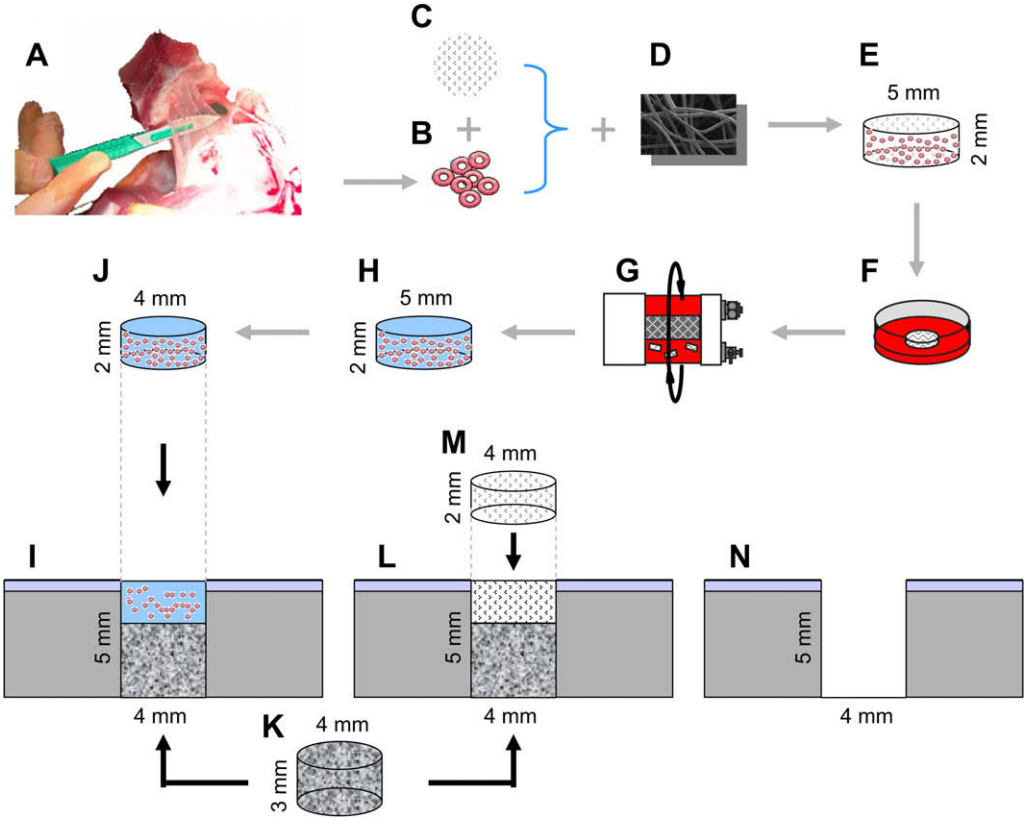

Fig. 1. Diagram illustrating research design. (A): Synovial tissue harvesting. (B): SDSCs. (C): Fibrin glue. (D): PGA disc. (E): Cell-fibrin glue-PGA construct. (F): Static culture (3 days). (G): Bioreactor culture (4 weeks). (H): Premature tissue construct. (I): Construct group. (J): Punched tissue construct. (K): Fibrin glue-saturated Collagraft®. (L): Scaffold group. (M): Fibrin glue-saturated PGA scaffold. (N): Empty group.