Decellularized Stem Cell Matrix Rejuvenates Synovium-Derived Stem Cells for Cartilage Regeneration and Repair of Partial-Thickness Cartilage Defects

Introduction
Currently available treatments for partial-thickness cartilage defects do not have satisfactory clinical results. Recently, investigations demonstrated that intra-articular injection of MSCs were a feasible approach and less invasive for patients. A sufficient number of stem cells is a prerequisite for successful transplantation; however, in vitro expansion predisposes cells to lose their chondrogenic potential. Our previous studies indicated that extracellular matrix (ECM) deposited by synovium-derived stem cells (SDSCs) could provide a three-dimensional (3D) stem cell expansion system in which a large quantity of high-quality stem cells could be acquired [1,2]. In this study, our goal was to (1) compare the effect of transforming growth-factor beta (TGF-β1) and TGF-β3 on the chondrogenic induction of SDSCs pre-expanded on either ECM or Plastic; and (2) demonstrate that ECM-expanded SDSCs exhibit comparable chondrogenic ability in an animal model. Therefore, we hypothesized that TGF-β isotypes had comparable in vitro chondrogenic induction effects, an allogeneic source of SDSCs could repair partial-thickness cartilage defects, and ECM-expanded SDSCs could enhance cartilage defect regeneration. Intra-articular injection may provide a promising and minimally invasive non-surgical approach for future clinical treatment of cartilage defects. Our long-term goal is to develop an allogeneic stem cell-based minimally invasive approach for the treatment of cartilage defects to be used in the clinical setting.

Methods
Minipig SDSCs were isolated from synovial tissue. Passage 3 SDSCs were used to prepare decellularized stem cell matrix. Passage 2 SDSCs were expanded under two conditions: conventional plastic flasks (Plastic) and flasks coated with SDSC-deposited ECM (ECM). The expanded SDSCs were detached and incubated in a pellet culture system containing serum-free chondrogenic medium supplemented with either TGF-β1 or TGF-β3 at a concentration of 10 ng/mL. The pellets were harvested at day 14 for in vitro chondrogenic analysis. Analytical methods included (1) histology, Alcian blue (AB) staining for sulfated glycosaminoglycans (GAGs); immunostaining for collagens I, II, and X; (2) biochemistry for the DNA and GAG amount per pellet; (3) real-time PCR for Sox9, collagens I, II, and X, aggrecan, and alkaline phosphatase (ALP); (4) western blot was used to detect cell protein expression and signaling pathway; and (5) semi-quantitative histological scoring.

Chondrogenic potential of ECM-expanded SDSCs was also evaluated in miniature pig partial-thickness cartilage defect model. Thirteen miniature pigs (2 year-old females) were used for this in vivo study. Partial-thickness cartilage defects, 8 mm diameter x 1 mm deep, were created in the medial femoral condyles of both knees using a customized tube osteotome with a depth-limiter. After seven days, expanded cells were injected into knee joints with cartilage defects. Our experimental design included three groups: “ECM-expanded SDSCs in saline” (five pigs, 10 knees), “Plastic-expanded SDSCs in saline” (five pigs, 10 knees), and “saline alone” (three pigs, 6 knees). All minipigs were returned to their cages after the operation and allowed to move freely. Animals were euthanized by an intracardiac puncture of Euthasol® euthanasia solution (Fort Dodge Animal Health, Fort Dodge, IA) 3 months after the operation. The femoral condyles were isolated for histomorphological analysis.

Results
(1) ECM-expanded SDSCs were tiny with a fibroblast-like shape and grew quickly in the 3-D format with concomitant up-regulation of phospho-cyclin D1 (data not shown).

(2) Despite the fact that there was no significant difference in chondrogenic induction from TGF-β1 and TGF-β3, ECM-expanded SDSCs exhibited enhanced chondrogenic potential, evidenced by histology (data not shown), biochemistry (Figure 1), and real-time PCR (data not shown). Interestingly, we also found ECM-expanded SDSCs had an enhanced expression of TGF-β receptor II (data not shown), which might be responsible for promoting chondrogenic potential in SDSCs expanded on ECM.

Conclusion
Our study suggested that ECM expansion promoted SDSC proliferation by the up-regulation of cyclin D and enhanced SDSC chondrogenic potential by the up-regulation of TGF-β receptor II. TGF-β1 and TGF-β3 were similar in chondrogenic induction of expanded SDSCs on either ECM or Plastic; ECM-expanded SDSCs were superior to Flask-expanded SDSCs in the regeneration of partial-thickness cartilage defects after intra-articular injection, indicative of the feasibility of the minimally invasive approach using allogeneic SDSCs expanded on commercially available ECM in cartilage defect repair. Chondrogenic capacity of ECM-expanded SDSCs has been demonstrated in both in vitro and in vivo studies. The underlying mechanism needs to be determined before this technology can be applied in clinics.

Significance
Our study, for the first time, demonstrated that decellularized stem cell matrix is a novel and promising cell expansion system that not only promoted cell proliferation but also enhancing expanded cell chondrogenic potential in both in vitro and in vivo.

Reference

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