Comparison of Silk Hydrogel and Porous Scaffolds for Cartilage Tissue Engineering

INTRODUCTION:

The clinical need for improved treatment options for patients with cartilage injuries has motivated tissue engineering studies aimed at the in vitro generation of functional replacement tissues. A scaffolding material is often required as a carrier of cells and could provide chemical, mechanical, and topographical cues in addition to structural support for the graft. In cartilage tissue engineering, studies have utilized a number of materials, including porous scaffolds (PLGA, chitosan) and hydrogels (agarose, alginate, PEG). Silk fibroin protein provides processing flexibility and can be processed into hydrogels as well as porous scaffolds. In the current report, we demonstrated cartilage development in both formations of silk material, hydrogel and porous scaffolds. Chondrocytes seeded in silk scaffolds showed excellent viability, proteoglycan accumulation and functional development. The ability to modify silk fibroin formation permits further optimization of engineered cartilage.

METHODS:

Preparation of Silk Hydrogel Construct: Silk fibroin protein was extracted as previously described. The sonicated silk solution was mixed with an equal volume of cell suspension (40x10^6 cells/ml). The silk/cell mixture was poured into sterile molds, and 4 mm diameter x 2.5 mm thick discs were cored out using a biopsy punch.

Preparation of Porous Silk Constructs: Silk solution and NaCl particles (500-600 µm in diameter) were mixed and incubated in Teflon cylinder container at room temperature for 24 hrs. Porous scaffolds were obtained after leaching NaCl particles in distilled water for 2 days. Primary chondrocytes (6.2x10^6 cells) in 25 µl DMEM were loaded into the DMEM-wetted scaffold and incubated at 37°C for 2 hrs.

Construct Cultivation: All constructs were maintained for up to 42 days in chondrogenic growth medium (hDMEM, 0.1 µM dexamethasone, 50 µg/ml ascorbate 2-phosphate, 50 µg/ml `-proline, 100 µg/ml sodium pyruvate, 1xITS+ (BD)) supplemented with 10 ng/ml TGF-β3 for the first 2 weeks (S). Medium was changed twice weekly.

Mechanical Testing: Compressive properties were measured in unconfined compression using a custom computer-controlled testing system [6]. After equilibration under a tare load of 0.5 gram, the stress-relaxation test was performed by applying 10% strain at a ramp rate of 1 µm/s and equilibrium Young’s modulus (E50) was calculated.

Biochemical and Histology Analysis: DNA was measured by using PicoGreen assay. GAG was measured using the dimethylmethylene blue dye binding (DMMB) assay. GAG and collagen distributions were evaluated histologically. Live-dead staining was conducted on Day 42.

Statistical Analysis: ANOVA with post-hoc tests were performed using the STATISTICA software (Statsoft).

RESULTS:

Both silk hydrogels and porous silk scaffolds supported cell proliferation and matrix elaboration (Fig.1). On day 42, silk hydrogel constructs maintained the original cylindrical shape, and the porous silk constructs developed irregular shape (Fig.2A). Chondrocytes encapsulated in silk hydrogel exhibited spherical morphology, while those in porous scaffolds demonstrated spindle-like morphology (Fig.2B). Histological staining of collagen and GAG revealed more uniform matrix distribution with lacunae formation in hydrogel than in porous constructs (Fig.3). Also, the total GAG content was higher in hydrogels than in porous silk constructs (Fig.1B), a result consistent with greater mechanical properties of hydrogels than porous constructs (Fig.1C).

DISCUSSION:

Silk protein demonstrates flexibility for processing into hydrogels, films, fibers or porous scaffolds with tunable properties. In the current study, we tested silk hydrogels and porous scaffolds for their capability for cartilage tissue engineering. Both materials supported chondrocyte proliferation and matrix elaboration. Interestingly, Biochemical content and mechanical properties of the porous scaffold group reached a plateau by 4 weeks, while silk hydrogels supported continuous growth for 6 weeks. Silk hydrogels could thus provide a more suitable microenvironment for chondrocytic phenotype. In addition to supporting the spherical cell morphology, silk hydrogels may possess more appropriate intrinsic properties. We have demonstrated similar functionalities of constructs made from silk and agarose hydrogels, which has generated engineered cartilage with physiological properties [7]. One particular silk hydrogel formulation resulted in similar compositions and mechanical properties of engineered cartilage as agarose gel. This raises a possibility to use silk hydrogels to investigate scaffold related regulatory factors of cartilage formation, and optimize the hydrogel properties for rapid formation of functional cartilage. The versatility of silk fibroin allows systematic investigation of physical parameters that impact construct growth by comparison of the same biomaterial in different structural forms, thus a valuable tool for material optimization.

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REFERENCES: