Endostatin-Producing Cartilaginous Constructs Using Collagen Hydrogels and Pre-Formed Collagen Sponge-Like Scaffolds

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Statement of Purpose: The release of antiangiogenic agents, such as endostatin [1], from a tissue-engineered construct may be of benefit in the repair of naturally avascular tissues, like articular cartilage. Our objective was to examine strategies of nonviral transfection using collagen hydrogels and pre-formed collagen sponge-like scaffolds. We compared endostatin expression levels of monolayer-transfected cells seeded in gels and sponges. We also investigated direct transfection in plasmid-incorporating scaffolds, using collagen sponges additionally supplemented with heparan sulfate (HS) and chondroitin sulfate (CS), glycosaminoglycans (GAGs) found in native cartilage [2].

Methods: 0.2M passage 2 (P2) caprine marrow-derived mesenchymal stem cells (MSCs), which were transfected in monolayer with GenePORTER 2 (Genlantis, San Diego, CA) lipoplexes using 5 μg of human endostatin plasmid (pEndo) per 1M cells, were seeded into a 0.2% (w/v) type I collagen gel (BD Biosciences, San Jose, CA), 16 mm in diameter and 2 mm thick. Three scaffolds were fabricated by freeze-drying for *in situ* transfection: (1) 0.5% (w/v) type I/III collagen (CI) sponge-like scaffolds (Geistlich Biomaterials, Wolhusen, Switzerland), (2) 0.03% CS-0.5% CI, and (3) 0.03% HS-0.5% CI. GenePORTER 2 lipoplexes containing 20 μg of pEndo were added to scaffold discs, 8 mm in diameter and 1.5 mm thick, which were then seeded with 4M P2 goat MSCs for transfection. Endostatin was determined by ELISA and histochemical staining. Type II collagen and GAG content, measures of chondrogenesis, were determined by histochemical/Safranin-O staining.

Results: The highest endostatin level in the expended medium of gels seeded with monolayer-transfected MSCs was found to be 92 ng/ml for the 3-day collection period ending on day 5 (Fig. 1). This was almost an order of magnitude higher than the peak endostatin level ~12.5 ng/ml measured during the first week of culture of ~0.2M monolayer-transfected (10 μg pEndo; 50 μg pEndo per 1M cells) P2 MSCs grown in pre-formed type I/III collagen sponge-like scaffolds [3]. Of note was that the endostatin levels remained at 13 ng/ml or above throughout the 3-week period (Fig. 1), comparable to *in vitro* therapeutic levels [4]. For scaffold transfection, endostatin levels in the expended medium of MSC-seeded, pEndo-supplemented sponge constructs were found to increase to a peak of ~30 ng/ml in the first week of culture and then decrease to 5 ng/ml; the levels were significantly lower for the GAG-supplemented constructs than for the non-supplemented constructs at all collection periods (Fig. 2). Nontransfected MSCs did not express endostatin (Fig. 2). ANOVA revealed significant effects of scaffold material and collection period on endostatin levels. Fisher's post-hoc revealed a significant difference between CI scaffolds and both GAG-supplemented scaffolds. Constructs showed positive staining for endostatin for only a few cells throughout the 3-week culture period, suggesting that most of the protein was not retained in the engineered constructs. All constructs showed areas of positive staining for type II collagen and Safranin-O at 3 weeks post-seeding.

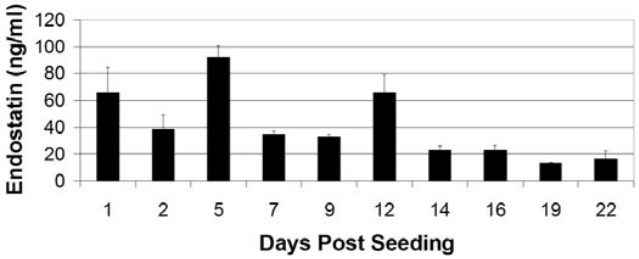

Figure 1. Endostatin in Expended Medium of Collagen Hydrogels (n=4; mean±SEM).

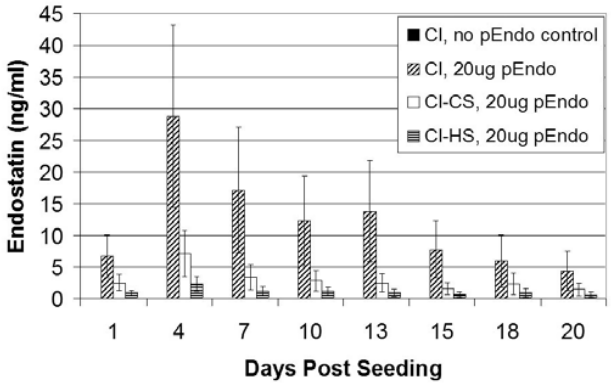

Figure 2. Endostatin in Expended Medium of Collagen Sponge-Like Scaffolds (n=2-4; mean±SEM).

Conclusions: Cartilaginous constructs using collagen gels and sponges and producing physiological levels (~30 ng/ml) of endostatin were engineered using small amounts of pEndo. Monolayer-transfected cells seeded in hydrogels resulted in much higher levels of endostatin in the expended medium compared to sponge scaffolds, suggesting that scaffold form may affect development of endostatin-producing cartilaginous constructs. A lipoplex-supplemented scaffold may provide the additional utility of a one-step off-the-shelf transfection construct, compared to a two-step process of monolayer transfection and subsequent scaffold seeding. The addition of HS and CS did not have a beneficial effect on the temporal profile of endostatin expression, possibly due to change in the physical properties of the scaffold.

Acknowledgements: Dept. of Veterans Affairs; NSF GRFP and Siebel Scholarship (LJ).

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