Controlled release of insulin-like growth factor-1 from hydrogels improves integration with cartilage

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Statement of Purpose: Poly(vinyl alcohol) (PVA) hydrogels have long been studied for replacement of damaged articular cartilage because of their similar mechanical properties to articular cartilage *(1)*. However, their hydrophilic nature prevents cell adhesion and protein adsorption, so implanted PVA hydrogels fail to integrate with surrounding cartilage *(2)*. We have fabricated PVA hydrogels with an interspersed degradable phase of poly(lactic-co-gylcolic acid) (PLGA), which causes a high degree of porosity and increased cell adhesiveness, while still retaining the desirable material properties of PVA hydrogels *(3)*. We aimed to control the release of insulin-like growth factor-1 (IGF-1) from the hydrogels through encapsulation in the PLGA phase. We hypothesized that the release of IGF-1 would enhance intgeration between cartilage and the PVA hydrogels. As a model, the effects on integration between tissue engineered cartilage and the hydrogels were examined *in vivo* after subcutaneous implantation in nude mice.

Methods: IGF-1 was encapsulated into the PLGA phase of semi-degradable hydrogels based on PVA through a modified double-emulsion technique as previously described *(3)*, except that a solution of IGF-1 (high dose, 27µg; low dose, 3µg; and no dose, 0µg, per resultant hydrogel) in acetic acid was used as the internal aqueous phase. The hydrogels were physically cross-linked through 2 cycles of freezing and thawing. The resultant hydrogels were dried through critical point drying and wrapped in unwoven poly(glycolic acid) (PGA) fibers to facilitate cell seeding and cause the formation of cartilage layers around the hydrogels. Release of IGF-1 from the hydrogels was measured over 6 weeks of swelling in PBS. Primary porcine chondrocytes in the amount of 10 million cells in a 100µl suspension were added to the hydrogels, cultured in vitro for 1 week, and implanted subcutaneously into nude mice. After 6 weeks, the cartilage-hydrogel constructs were removed and analyzed grossly and histologically for integration with the hydrogels, biochemically with reaction with dimethylmethylene blue dye for the quantity of sulfated glycosaminoglycans (GAGs), and mechanically in unconfined, uniaxial compression.

Results: The release of insulin, a model for IGF-1, was controlled over 6 weeks, with less than 3% released in the first 10 days, and most released in the following 30 days (Fig. 1).

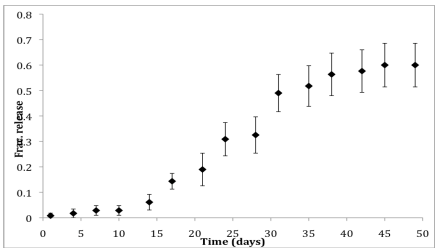

Figure 1. Cumulative release of insulin.

Gross observation of the cross-sections of the cartilage-hydrogel constructs showed that cartilage layers surrounded the hydrogels (Fig. 2). The thickness of the cartilage was proportional to the dose of IGF-1. The cartilage layers were slightly integrated with hydrogels with a low dose and were well-integrated with a high dose of IGF-1. Hydrogels without IGF-1 showed no integration and could be easily separated from the cartilage.

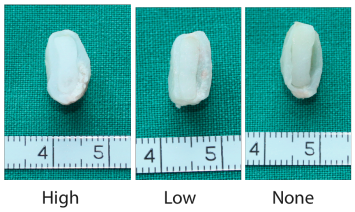

Figure 2. Gross examination of integration.

The wet weights of the hydrogels and the quantity of GAGs were higher for hydrogels that released IGF-1 than those that did not, though the dose did not appear to have a large effect (Table 1). The same trend was observed in compressive moduli ($p<0.001$). Interestingly, hydrogels without IGF-1 were no stiffer than the control hydrogels that did not have any cells and thus no cartilage layers.

Table 1. Properties of hydrogel-cartilage constructs.

Dose of IGF1	Wet mass (g)	Compressive modulus (kPa)
High	0.689±0.066	169.5±40.11
Low	0.643±0.843	200.5±120.0
None	0.487±0.445	71.25±23.44
No cell control	0.233±0.020	39.58±27.42

Conclusions: To our knowledge, this is the first study that has shown integration between PVA hydrogels and cartilage. Controlled release of IGF-1 was required for integration. The spatial control over delivery from inside the hydrogels outwards directed cell behavior in response to the concentration gradient and enhanced integration. The extended release of IGF-1 was controlled over a period of 6 weeks. Most of the encapsulated protein was released between 10 and 40 days, indicating that the mechanism of release was primarily degradation of the PLGA followed by diffusion through the PVA phase. The quality of integration between tissue engineered cartilage and the hydrogels was proportional to the dose of IGF-1. However, the wet weights, GAG content, and compressive modulus were unaffected by an increase in dose of IGF-1. Future studies will examine the effects of a pre-culture period to increase cell infiltration into the hydrogels, since the deposition of ECM components will be slower than *in vivo*, as well as the strength of integration between the layers, measured in tension.

References: 1. Bray and Merrill, 1973. JBMR 7: p.431-443. 2. Maher et al, 2007. JBMR 83A: p. 145-155. 3. Spiller et al, 2008. Acta Biomaterialia 4: p. 17-25.