Extracellular matrix molecules incorporated into bioinert hydrogels enhance matrix deposition and retention

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Statement of Purpose: Poly(ethylene glycol) (PEG) hydrogels offer numerous advantages in designing controlled 3D environments for cartilage regeneration, but offer little biorecognition for the cells. Additionally, significant fractions of the newly synthesized matrix are lost to the surrounding medium and this loss is further enhanced by the application of dynamic loading. In an effort to better design 3D hyrogels for regenerating cartilage under dynamic mechanical stimulation, we hypothesized that incorporating extracellular matrix (ECM) analogs into bioinert PEG hydrogels would not only improve matrix synthesis, but would also aide in matrix retention thus enhancing overall tissue accumulation within the construct. Specifically, we examined two ECM molecules: i) an oligopeptide derivative of link protein, which is invovled in stabilizing hyaluronic acid (HA) and aggrecan in the native tissue and which has been shown to have positive biological effects on cartilage cells[1] and ii) hyaluronan for its known positive effects on cartilage cells[2].

Methods: Poly(ethylene glycol) (PEG) hydrogels were fabricated from 10%w/w PEG dimethacrylate in phosphate buffer saline and either 0.05%w/w photoinitiator (I2959, Ciba Specialty Chemical) exposed to 365 nm light at ~6 mW/cm^2 for 10 min or via redox initiation using 0.05 M ammonium persulfate and 0.05 M TEMED at room temperature for 15 min. An oligopeptide derived from link protein[1], DHLSDNYTLDHDRAIH, was synthesized (Link-N, Applied Biosystem 433A Peptide Synthesizer), purified by HPLC, confirmed by MALDI-TOF mass spectrometry, and coupled to monoacryloyl PEG-NHS (MW 3400, Nektar Therapeutics). 1mg/ml Link-N, hyaluronic acid or fluoresceinamine-labeled hyaluronic acid (f-HA, MW 130 kDa) was incorporated in PEG gels. Freshly isolated immature bovine articular chondrocytes were encapsulated in PEG gels at 4x10^{6}cells/construct (5x5mm, cylinders). The constructs were culutured under free swelling or subjected to dynamic compressive strains (0.3 or 1 Hz, 15% amplitude strains). Release of f-HA was quantified by fluorescence (495 nm excitation/520 nm emission) with known standards. Matrix synthesis and deposition were examined by glycosaminoglycan content in the constructs and in the culture medium using dimethylmethylene blue dye method and by immunohistochemistry for aggrecan, collagen II. Data were analyzed by ANOVA with $p<0.05$ significant. Error bars are standard deviation of the mean.

Results: Initially, we examined the ability of Link-N to retain model ECM proteins by entrapping hyaluronic acid into the PEG gels (fig 1a). PEG hydrogels alone resulted in a significant loss of the entrapped HA by ~40% with the application of loading (in comparison to only ~4% released under free swelling conditions). However, the addition and tethering of Link-N into the PEG hydrogel resulted in ~15% release of HA, a 39% reduction after 140 hrs of dynamic loading. These findings indicate that Link-N is capable of retaining HA, a molecule that is synthesized by cartilage cells and which together make up aggrecan macromolecules.

Next, we investigated whether these matrix analogs were capable of retaining matrix molecules synthesized by cartilage cells (fig 1b). Release of glycosaminoglycans (GAGs), building blocks of aggrecan, into the culture medium was assessed over 25d for cell-laden PEG gels subjected to loading. Interestingly, simple entrapment of HA into PEG gels significantly reduced the loss of GAGs reaching values, which were indistinguishable from their free swelling counterparts. Link-N alone was able to reduce the mean release, but was not statistically different from PEG gels that did not contain ECM analogs.

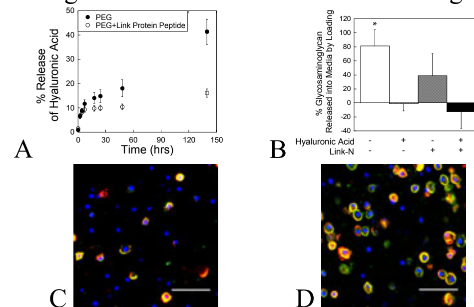

Figure 1. A) Incorporation of Link-N reduced release of hyaluronic acid (HA) from PEG gels under loading. (B) Cummulative GAG release due to loading in PEG gels without and with matrix analogs after 25d. Cartilage specific matrix production in cell-laden PEG (C) and PEG+HA+Link-N (D) gels after 25d under loading, green=collagen II, red=aggrecan, blue=nuclei.

Immunohistochemistry was performed to evaluate secretion of specific cartilage matrix molecules, namely collagen II and aggrecan (fig1c,d). In PEG gels without any matrix analogs, ~50% cells strained positive for cartilage matrix molecules, but this we reduced to ~30% with loading (Fig 1c) suggesting a negative effect with loading. However, the addition of both HA and Link-N resulted in ~75% of cells staining positive for matrix and exhibited a more robust pericellular matrix after 25 d.

Conclusions: With loading being a known stimulator of cartilage cells, we demonstrate that the incorporation matrix analogs, specifically Link-N and HA were capable of minimizing load-induced matrix loss. However, Link-N did not appear to have a stimulatory effect on cartilage matix production, as expected based on prior work, while HA dramatically enhanced cartilage matrix deposition, which was maintained under loading. These findings support the idea that cells receive insoluble biochemical cues from their ECM and that these cues may be important for not only tissue homeostasis, but also in supporting neo-tissue secretion and deposition.

References: 1. Mwale F. J. Cell Biochem. 2003;88,1202.
2. Knudsen W. Arthrits Rhem. 2000;43(5):1165-74.