Photocrosslinkable Fibronectin-Hyaluronic Acid Hydrogels as 3D Scaffolds

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Statement of Purpose:

Hyaluronic acid (HA)-based biomaterials have shown promise as promoters of wound healing and angiogenesis.[1] HA mediates endothelial cell interactions via CD44 and the receptor for HA-mediated motility (RHAMM) cell surface receptors.[2] These interactions regulate various processes vital to angiogenesis. However, HA biomaterials are non-adhesive to cells.

Thus, we aim to modify HA hydrogels with fibronectin (FN) to create 3D scaffold supports for integrin-mediated adhesion of human umbilical vein endothelial cells (HUVECs). We plan to use this system to develop an in vitro, 3D experimental model to study the effects of extracellular matrix on angiogenesis. Specifically, these materials can be utilized to investigate the effects of varying both FN and HA concentration on HUVEC expression of CD44, RHAMM, and integrins.

Methods:

HA (MW~$2x10^6$) (Biochimika) and FN were modified with methacrylate groups (GMHA) or acryloyl groups via an N-hydroxysuccinimide-poly(ethylene glycol)-acryloyl (NHS-PEG-Ac) (MW 3400) (FNAc) (Nektar Therapeutics), respectively, similar to previously reported methods.[1,3] The FNAc reaction was first optimized by substituting an N-hydroxysuccinimide-PEG-fluorescein (NHS-PEG-Fl) (Nektar Therapeutics) in lieu of NHS-PEG-Ac such that the absorbance of fluorescein correlates to the degree of labeling. Upon UV exposure, mixtures of GMHA and FNAc were photocrosslinked into hydrogels in the presence of Irgacure2959 (Ciba Specialty Chemicals) and N-vinylpyrrolidinone (VP) (Sigma), a reaction accelerant. Enzymatic degradation of the hydrogels by bovine testicular hyaluronidase (Sigma) was investigated as previously described.[1,3]

Optimal conditions for photoencapsulating HUVECs (Cambrex) inside the GMHA-FNAc hydrogels were determined by varying several parameters: HUVEC concentration, photoinitiator concentration, gelation time, GMHA concentration, FNAc concentration, and degree of acrylate modification of GMHA and FNAc. Viability was evaluated after approximately 24 hrs in culture with a live/dead stain assay (Molecular Probes).

Results / Discussion:

Modification of FN with NHS-PEG-Ac was optimized for reaction time and pH. Concentration was not a factor since NHS-PEG-Ac was added in excess. In order to analyze the degree of labeling, NHS-PEG-Fl was substituted for NHS-PEG-Ac. After purification by dialysis, the absorbance peak of the reaction product was measured around 488 nm to indicate degree of conjugation. Reaction times of 2 and 24 hrs were evaluated, but no additional labeling was apparent after 2 hrs. Reaction pH was varied between 7.5 and 8.5 and pH 8.5 was found to be optimal. However, preliminary data indicate some degree of non-covalent interaction between the FN and NHS-PEG-Fl that may account for up to 25% of apparent labeling. Nevertheless, apparent labeling in optimal conditions is still around 9 molecules of fluorescein per molecule of FN.

GMHA-FNAc hydrogels were successfully crosslinked and swelled in PBS, pH 7.4. After 24 hrs, the surrounding media was collected and analyzed for protein content using a bicinchoninic acid (Pierce) assay. No detectable amount of protein was observed in the excess media. The gels were enzymatically degraded in 50 U/mL hyaluronidase and their weight measured over a period of 24 hrs (Figure 1). By 2 hrs, the gels had lost around 75% of their original swollen weight.

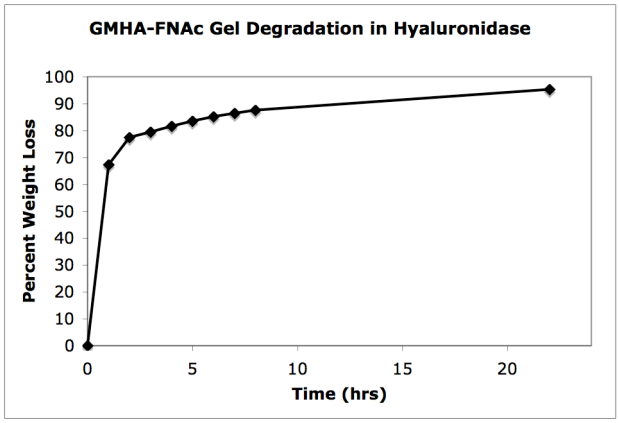

Figure 1. Degradation of GMHA-FNAc gels by hyaluronidase.

We evaluated the viability of HUVECs photoencapsulated within GMHA-FNAc gels during UV crosslinking. Initially, we determined the minimal amount of potentially toxic conditions (i.e., photoinitiator, UV exposure) required to support gelation. From these results, the following gel parameters were tested for HUVEC encapsulation: 0.5% (w/v) or 0.1% (w/v) Irgacure2959, 0.1% (v/v) VP, 2% (w/v) GMHA, 0.025-0.075% (w/v) FN-PEG-Ac, and 1.5 or 3 min UV exposure. Current studies are investigating the effects of HUVEC encapsulation concentration on viability.

Conclusions:

We have functionalized FN with acryolyl groups and then photocrosslinked FNAc into hybrid hydrogels with GMHA. Furthermore, these materials have potential to photoencapsulate HUVECs into 3D scaffolds to support proliferation, adhesion, and viability.

References:

1. Baier Leach et al., Biotech Bioeng 2003; 82.
2. Savani et al. J Biol Chem 2001; 276(39).
3. Leach et al., J Biomed Mater Res 2004; 70A (74).