COLLAGEN AND HYDROXYAPATITE INCORPORATION INTO POLY(ETHYLENE GLYCOL) HYDRGELS FOR ORTHOPEDIC APPLICATIONS

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BACKGROUND:

By providing a substrate for cell adhesion and a matrix for cell proliferation, polymers have become a focus for orthopedic tissue engineered scaffolds. Hydrogels, crosslinked polymeric structures, are of particular interest for such scaffolds because of their ability to absorb and retain water [1]. Based on previous research, hydrogels can potentially act as orthopedic adhesives, via their swelling ability, and encourage bone infiltration and formation through its mesh structure. One polymer for such a scaffold is poly(ethylene glycol) (PEG), which has previously been investigated as a tissue engineering scaffold [2]. However, PEG has no natural signaling factors for bone growth.

Therefore, a PEG-bioactive molecule composite hydrogel is being investigated to increase the proliferation of the osteoblasts in these hydrogels. Separately, PEG-collagen and collagen-hydroxyapatite (HA) composites have been examined as bone substitutes [3,4,5]. Since collagen, collagen-HA, and PEG-collagen have been previously investigated individually and each had a positive osteoblast response, it is of interest to investigate a poly(ethylene glycol)-collagen-HA composite hydrogel for orthopedic applications. The objectives of this on going study include:

- conjugation of collagen and PEG,
- formation of a PEG-collagen hydrogel, and
- characterization of hydrogels vs. controls via cell studies and mechanical evaluation

Upcoming work includes incorporation of hydroxyapatite into PEG-collagen hydrogels, determination of cell growth and function on the gels, characterization of mechanical properties, and comparison of the results for all gels studied.

METHODS:

Conjugation of PEG-collagen:

N-hydroxysuccinimide (NHS) ester modifications to PEG have previously been successful with conjugation of monomethoxy-PEG (mPEG) to collagen. The NHS group on mPEG-SPA, which is formed form a PEG-propionic acid intermediate, is known to covalently bind to lysine groups of proteins (Figure 1) [5]. Type I collagen (from rat tail tendon) in 0.02M acetic acid was neutralized to physiological pH with 0.1 M PBS. A 4x molar excess of mPEG-SPA to the lysine groups in collagen was diluted in water and added to the neutralized collagen. After 15 hrs at 25C, the solution was centrifuged and washed with PBS 3x. The pellet was then dried for 24 hrs on a lyophilizer and the weight of the sample was noted.

Analysis of this reaction was conducted via H-NMR and FTIR in order to indicate the presence of the NHS group in the mPEG-SPA and its absence after conjugation with collagen [6]. The mPEG-SPA, collagen, and the PEG-collagen conjugate were diluted in D_2O in order to obtain a spectra for H-NMR. For analysis via FTIR, mPEG-SPA and the conjugate were diluted in PBS.

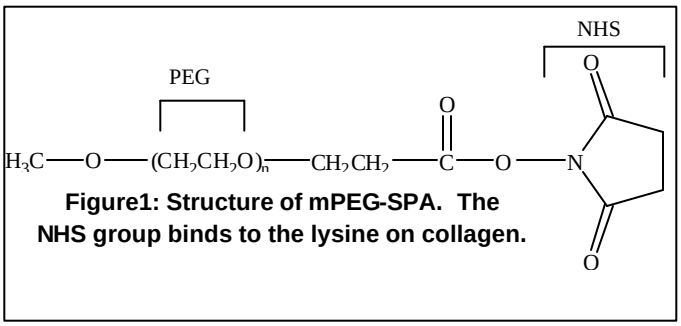

Figure1: Structure of mPEG-SPA. The NHS group binds to the lysine on collagen.

Fabrication of PEG-collagen hydrogel:

Once data confirmed the bonding of collagen and PEG, a PEG-collagen hydrogel was formed (Figure 2). This procedure included production of the collagen and PEG conjugate as described above, but replacing mPEG-SPA with acrylated-PEG-NHS (Shearwater, Birmingham, AL). Gels were fabricated at conjugate concentrations of 1%, 2.5%, and 5% (w/w). Appropriate amounts of a photoinitiator (Igacure 2959, Ciba, Tarrytown, NY), PEG-diacrylate (Shearwater, Birmingham, AL), water, and PEG-collagen conjugate will mixed and then placed under a 4mW/cm2 UV light for 20 minutes to crosslink the PEG-diacrylate and acrylated-PEG-collagen [7]. In order to prove

the presence of collagen in the hydrogel, the gel were sectioned and analyzed with Accustain Trichrome (Sigma, St Louis).

Fabrication of a PEG-collagen-hydroxyapatite hydrogel:

Finally, HA was incorporated into the hydrogel. First, collagen was brought to physiological pH using 0.1M PBS and mixed with HA at a ratio of 35:65, which has previously been shown to be optimal [3]. Next, the acrylated-PEG-NHS was mixed in the solution and, finally, crosslinked via UV light. Analysis of the presence of HA in the hydrogel was conducted via histological staining with Alizarin Red (Sigma, St Louis).

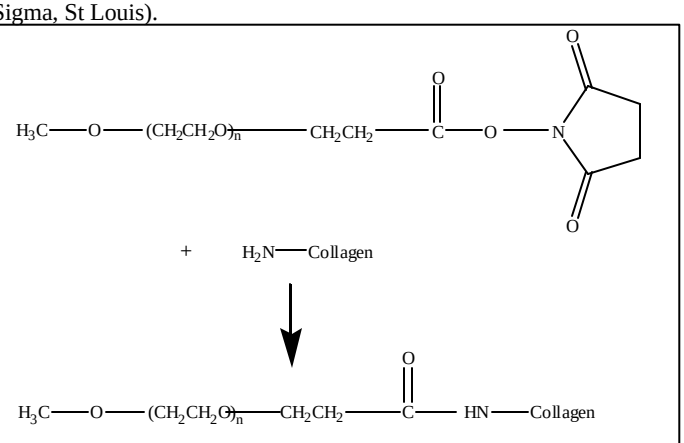

Figure 2: Reaction between acrylated PEG-NHS and collagen

Cell Culture:

UMR-106 cells were cultured according to ATCC protocol, in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA) [8]. Once near confluent, the cells will be split and seeded onto a hydrogel at $1.35x10^5$ cells/mL. Media was changed on the gels approximately every other day.

Analysis of hydrogels:

At time points of 2 hr, 1wk, and 2 wk, the hydrogels were washed 3x in PBS and lysed using 0.2% Triton X-100 (Sigma, St Louis) for 30 minutes on a vortex. A 20 µL sample was placed into an alkaline phosphatate reagent (Sigma, St Louis) and the absorbance was measured. Hoechst 33258 (Sigma, St Louis), a stain for DNA, is used to measure the fluorescent intensity of the samples.

Analysis of Physical Properties of Hydrogels:

The water incorporation into the hydrogels was investigated via swelling tests, which involve placing the hydrogel in water and recording its weight every hour for up to 5 hrs and 24 hrs later. Then, the overall water incorporation into each gel was calculated using the ratio of the weight change from 24 hrs (w_{24}) and the initial weight of the gel (w_i) to the weight at 24 hrs ($(w_{24}-w_i)/w_{24}$). The weights recorded in the first 5 hrs of the swelling tests indicate the time needed for the gels to reach their swelling equilibrium. Finally, tensile properties were analyzed by determining the elastic modulus and ultimate strength of the gels.

RESULTS/DISCUSSION:

Results for control gels (containing no collagen or HA) indicated that the number of osteoblasts on 15% (w/w) PEG gels was four times higher than the number seeded after 2 wks, with significant growth between 2 days and 1 wk. A Live Dead stain (Molecular Probes, Eugene, OR) suggested that cells remained viable for then entire 2 weeks of study. Finally, the average percent water incorporation for the 15% PEG gels was calculated to approximately 88%, with the gels reaching swelling equilibrium after 3 hours.

The conjugation of collagen to PEG has been completed and the composite has been incorporated into the hydrogel. Preliminary results from H-NMR demonstrate the presence of the NHS group in mPEG-SPA, which is located at 2.8 ppm; no peak is present at 2.8 ppm for the spectra of the PEG-collagen conjugate. Similar conclusions have been suggested from FTIR analysis. Also, hydrogels containing the PEG-collagen conjugate stained positive for collagen even after extensive washing, although more studies need to be completed to confirm this finding.

This continuing study will evaluate the effects of integration of bioactive molecules such as collagen and hydroxyapatite on cell growth, function and physical properties. Similar assays and tests will be completed to compare the composite hydrogels to the control PEG gels. It is expected that the composite hydrogels will enhance cell proliferation and function, thus improving the use of these hydrogels for bone integration.

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