Photocrosslinkable Collagen-Polyethylene Glycol Hybrid Hydrogels for *in situ* Modification of Scaffold Stiffness

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Statement of Purpose: Hybrid materials consisting of natural biomaterials combined with synthetic components may be useful as tissue engineering scaffolds. Type-I collagen is attractive due to its cytocompatibility and its ability to self assemble into a fibrillar network. Here, we use collagen as a base material, modify it by making it photosensitive, and modulate its material properties by introducing both autocrosslinks and synthetic polyethylene glycol crosslinks. Using the directed application of light to control the crosslinking, we can fine tune the substrate's local stiffness. Recent work has shown that scaffold mechanics have a profound effect on cellular behavior such as migration and differentiation[1,2]. As actual tissues are comprised of complex mixtures of cell types and matrices, this material may allow for highly controllable scaffolds capable of directing encapsulated primary cellular behavior or guiding a single stem cell population into differentiation along multiple lineage pathways simultaneously.

Methods:

Collagen Methacrylation: Type I bovine collagen is modified by reacting the free amines with methacrylate groups. Briefly, a solution of collagen (EPC products) in 0.02N acetic acid is reacted with methacrylic acid (MA). 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, (EDC) and N-hydroxysuccinimide (NHS) in MES buffer are used to activate the carboxyl group of MA for 10 minutes at 37°C, then added to the collagen and reacted for 24 hours at 4°C. CMA is then dialyzed in 0.02N acetic acid to remove excess reagents, lyophilized, and resuspended in 0.02N acetic acid for use in forming hydrogels. Substitution efficiency is verified using H^1 NMR.

Photoinitiating System: The water soluble photoinitiator Irgacure 2959 (I2959, Ciba Pharmaceuticals), is used as the photosensitizing agent. UVA (365nm) from a UVL-21 long wave UV light source at 5-8 mW/cm^2 is used to activate the I2959.

Hydrogel Formation and Photocrosslinking: CMA gels are made in 1mL batches on ice, in which 677µL CMA (3.75 mg/mL) is added to 20µL HEPES, 140µL 0.15N NaOH, 100µL 10X MEM, 42µL Vehicle (PBS or DMEM), 10µL L-Glutamine, and 1µL pen/strep. Polyethylene glycol diacrylate (PEGDA, Sigma-Aldrich) is added. After thorough mixing, the alkalinized CMA solution is plated into PDMS molds or multi-well plates and allowed to gel at 37°C for 1 hour in a humidified incubator with 5% CO_2. Immediately following gelation, gels are exposed to UV light to crosslink, and then PBS or culture medium is added.

Mechanical Testing: 800µl cylindrical gels of alkalinized CMA are formed in PDMS molds, allowed to self assemble into stable gels, crosslinked with I2959 and UV light, and loaded on to a parallel plate rotational rheometer. The storage and loss moduli are used to characterize the mechanical properties of the material at various strain rates.

Cytotoxicity Testing: NIH-3t3 fibroblasts are encapsulated within CMA gels, and allowed to culture for 24 hours. Afterwards, I2959 is added and exposed to UV light. Medium is then changed and cells are cultured for 24 hours, after which viability is visually assessed by staining with Hoechst 33342, Calcein-AM, and ethidium homodimer.

Results: Rotational rheometry results showed that autocrosslinking CMA more than doubled the stiffness of the material. Including 0.1% (w/v) PEGDA increased by almost four-fold after UV exposure (Figure 1, left). Moreover, the crosslinking process could be performed in the presence of NIH-3t3 fibroblasts within a usable photoinitiator concentration with significant post-crosslinking viability (Figure 1, right) although I2959 concentrations above 0.04% resulted in the majority of cells dying.

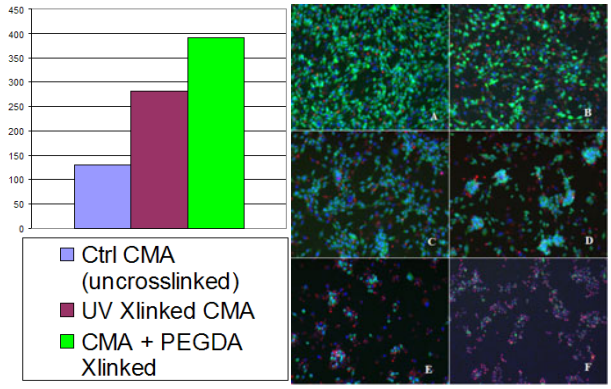

Figure 1. Left: Storage Modulus (Pa) of CMA gels (Blue), autocrosslinked CMA Gels (Maroon), 0.01% PEGDA crosslinked CMA (Green). Right: Cytotoxicity of I2959; A: 0%, B: 0.01%, C: 0.02%, D: 0.04%, E: 0.05%, F: 0.1%

Conclusions: CMA-PEGDA hydrogels present a powerful tool for allowing *in situ* modification of the local mechanical properties of a self-assembling tissue engineering scaffold. After further optimization, this material can allow for defects of abritrary size and shape to be filled with a liquid mixture of polymer and cells, quickly gel into a stable matrix, and then be modified to introduce desired mechanical heterogeneity that may be useful towards guiding cell migration within the injury site or behavior or controlling stem cell differentiation.

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References: 1.) Evans ND. Eur Cell Mater. 2009 Sep 21;18:1-13; 2.) Leipzig ND. Biomaterials. 2009 Sep 22. [Epub ahead of print]