# Hyaluronan-Based Multi-Phasic Scaffolds for Osteochondral Tissue Regeneration

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**Statement of Purpose:**
Osteoarthritis (OA) is the predominant form of arthritis in our aging population. With individuals living increasingly longer lives, we face new problems relating to the degradation of tissues within the body. Regenerative tissue engineering utilizing bio-polymer scaffolds allows treatment to begin at the first sign of OA, alleviating pain as well as returning them to a pre-osteoarthritic condition. As the osteochondral tissues display a gradient of properties and structure, an integrated multi-phasic scaffold made of extracellular matrix (ECM) components was designed to mimic natural tissue. Our goal is to fabricate an integrated multi-phasic scaffold in the presence of drug-encapsulated microspheres to direct osteochondral tissue regeneration and to possibly aid in disease prevention.

**Methods:**
Multi-phasic scaffold fabrication: Methacrylation of hyaluronan (HA) was performed through a novel method in dimethyl sulfoxide by ion exchange with an ammonium salt. The solution was reacted with methacrylic anhydride for 24 hours, hydrolyzed, lyophilized, and characterized by $^{1}$H-NMR.[1] Cytocompatibility of the HA-MA was previously verified with primary human mesenchymal stem cells (hMSCs). HA-MA (2-4% w/v) blends containing varying concentrations of collagen IV, laminin, and heparan sulfate were photocrosslinked in the presence of a photoinitiator (Irgacure 2959). Integrated multi-phasic scaffolds were formed by successive layering and polymerization of varied polymer blends in a custom mold.
Microsphere Fabrication: In addition, scaffolds were formed in the presence of PEG-modified alginate (AA-g-PEG) microspheres for the purpose of controlled drug release. Briefly, AA was modified with methoxy-terminated PEG amine using EDC/NHS chemistry and characterized by $^{1}$H-NMR. Toluidine Blue O (TBO) encapsulated AA-g-PEG microspheres were fabricated using a water-in-oil emulsion and calcium-ion crosslinking. Microsphere morphology and diameter were characterized by scanning electron microscopy (SEM).

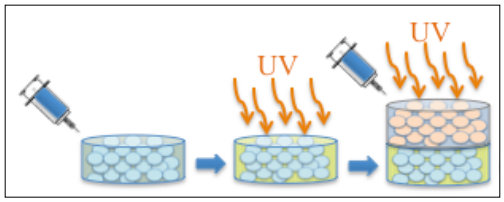


**Fig 1.** Schematic of scaffold fabrication process. Photo-crosslinking of multiple layers of HA-MA and ECM component blend solutions, from top to bottom, each incorporated with AA-g-PEG microspheres.

**Results:**
A layer-by-layer polymerization of HA-MA and ECM component blends was successful in fabricating an integrated multi-phasic scaffold. The individual layers of the scaffold were in-separable (upon tensile or shear loading) yet distinguishable in the presence of colorimetric dyes (such as TBO). AA-g-PEG microspheres with nominal diameters of 500nm-1µm were homogenously dispersed through-out the polymerized scaffold. More importantly, the microspheres were retained within their respective layer within the scaffold. AA-g-PEG microspheres were uniform in size (per batch) and spherical in shape.

**Fig 2.** Tri-phasic HA-MA-based scaffold; yellow layer tinted with TBO (pre-photocrosslinking) for comparison.

**Conclusions:**
The HA-MA-based scaffolds demonstrate efficacy of incorporating ECM components and AA-g-PEG microspheres into the hydrogel network. Multi-phasic scaffolds may be utilized to spatially and temporally control the release of cell signaling molecules to aid in the regeneration of complex tissues, such as osteochondral tissue. Future work will include the incorporation of 100-200 µm AA-g-PEG microspheres into the hydrogel solution prior to photocrosslinking and subsequent dissolution of the microspheres to create a macroporous scaffold. Mechanical testing will be performed to optimize properties of each scaffold layer. Differentiation and proliferation of hMSCs within our scaffold will be investigated to determine the optimal concentration of ECM components and drug release profiles within each layer of the scaffold.

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**References:**
[1] Galperin and Oldinski *et al.* Advanced Healthcare Materials, *in press*, 2013.