Hyaluronic Acid-Based Microgel Systems with Tunable Viscoelasticity and Therapeutic Potentials for Soft Tissue Regeneration

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Statement of Purpose: Hyaluronic acid **(**HA) is a natural polysaccharide consisting of 200-10,000 repeating disaccharide units based on β-1,4-linked D-glucuronic acid (GlcUA) and β-1,3 N-acetyl-D-glucosamine disaccharide (GlcNAc). It is an essential ECM component that not only modulates cellular adhesion, signaling and mobility, but also plays a key role in the natural wound healing process.[1] However, natural HA lacks mechanical integrity and has a very limited in vivo lifetime. Thus, chemically crosslinked HA hydrogels have been evaluated for their potentials as injectable materials for soft tissue regeneration.[2] Unfortunately, these bulk gels do not exhibit hierarchical structures that are necessary to facilitate tissue infiltration and neovascularization. Their degradation profile and mechanical properties can not be adjusted without adversely affecting each other. We are interested in developing HA based microgel systems that overcomes these limitations and can be used as injectable materials for soft tissue regeneration.

Methods: HA carrying aldehyde (HAALD) and adipic acid hydrazide (HAADH) functionalities were synthesized following established procedures.[3] HA microgels were synthesized using an inverse emulsion crosslinking technique. The inverse emulsion was prepared by homogenizing HAADH (1% in H_2O) in mineral oil containing Span 80. HAALD (1t% in H_2O) was added to the emulsion which was further homogenized for 5 min. The aqueous phase was allowed to evaporate overnight at 40°C with constant stirring. Microgels were isolated by precipitation in large access of isopropyl alcohol followed by centrifugation to remove the oil phase. The resulting microgels (~15 μm in diameter, Figure 1, left) were thoroughly washed with isopropyl alcohol, hexane and acetone before drying under vacuum at room temperature. Alternatively, HA microgels of smaller dimensions were synthesized using a water-in-oil micro-emulsion system. Thus, HA (4% in 0.2 M NaOH) was dispersed in an AOT solution containing 1-heptanol. The mixture was vortexed until clear suspension is obtained. Slow addition of pre-determined amount of divinyl sulfone imitated the crosslinking reaction in situ. The reaction was proceeded 12 at ambient temperature. The reaction mixture was filtered and precipitated in acetone. Microgels were subjected to the same washing and drying process described above. The resulting HA microgels have an average particle size of 2 μm (Figure 1, right).

R**esults/Discussion:** Two approaches have been developed for the preparation of HA microgels with different particle sizes. Taking advantages of the built-in functionalities, microgels with average diameter of ~15 μm were obtained via in situ crosslinking of two mutually reactive HA derivatives (HAALD and HAADH) within the inverse emulsion droplets that were stabilized by Span 80. Alternatively, smaller particles were prepared via in situ crosslinking of HA with DVS using AOT reverse micelle as the template. In vitro cytotoxicity studies using

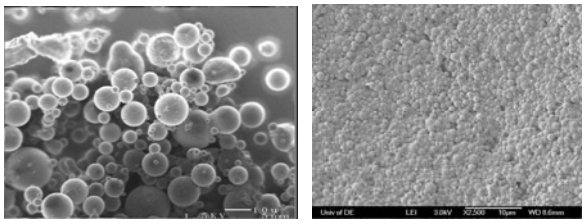

Figure 1. SEM of HA microgels.

vocal fold fibroblasts indicate that these HA-based microgels are essentially non-toxic. These microgels are enzymatically more stable than their corresponding macroscopic counterparts. HA microgels prepared from HAADH and HAALD exhibit residual functional groups on their surfaces that allows for further elaboration of their biological and materials properties. On the other hand, HA microgel prepared from HA and DVS were subjected to post-modification process to impart aldehyde functionality. Spatial and temporal release of therapeutic reagemts was accomplished by covalent conjugation of drug molecules to the microgel surfaces or their physical encapsulation inside the microgels. The presence of residual functional groups allows for subsequent crosslinking of the microgels with polyethylene glycol dihydrazide, giving rise to doubly crosslinked networks (DXN) with tunable viscoelasticity. In our crosslinked microgel networks, the individual microgels can be highly crosslinked, making them resistant to degradation, while macro-scale mechanical properties can be independently tunable by adjusting microgel dimensions or inter-microgel crosslinking. Moreover, the microgels have a relatively large surface area, which might improve tissue integration and facilitate controlled delivery of therapeutics. Finally, the presence of two levels of crosslinking (within and between individual mcirogels) may offer potential for rapid recovery from mechanical stress. These HA-based microgel systems are promising candidates for the use as injectable materials in wound healing, adhesion prevention and soft tissue engineering.

Conclusions: we have created a new class of biocompatible materials based on HA microgels that exhibit controlled particles sizes, improved enzymatic stability, defined surface functionalities and tunable mechanical properties. These microgel systems are promising candidates as injectable materials for soft tissue regeneration.

References:

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