Photo-cross-linkable chitosan-lactide hydrogels for growth factor delivery: Development and *in vitro* characterization

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Statement of Purpose: Photo-cross-linkable hydrogels have been widely studied for many biomedical applications such as drug delivery, cell therapy, and tissue engineering scaffold. The delivery targets include drugs, growth factors, and cells which are loaded into the hydrogel formulation, which is then directly applied to the targets in a minimally invasive manner, remaining structural integrity in the site of interest by UV exposure. Moreover, the precise structure of the cross-linked hydrogel networks can be also achieved by photopatterning, or rapid prototyping (RP) techniques [1, 2]. However, there is still a great need for optimizing swelling behaviors, degradation kinetics, and mechanical properties of the cross-linked hydrogels for improved efficacy and efficiency of drug and cell delivery [2]. In the present study, we have developed and characterized photo-cross-linkable chitosan-lactide hydrogels with tunable swelling behavior, degradation rates, mechanical properties, and drug release kinetics. Our hypothesis in this study was that physicochemical properties of the hydrogels can be tuned by varying the ratio of chitosan to lactide and cross-linking density of hydrogels. More specifically, the novel chitosan-lactide copolymer is comprised of hydrolytically degradable amide, ester linkages and methacrylates. The gelation of cross-linked hydrogel networks were initiated and formed by a radical polymerization upon application of UV light. We further investigated the efficacy of the delivery system by measuring *in vitro* bioactivity of BMP-2 using W-20-17 preosteoblast mouse bone marrow stromal cells and C2C12 mouse myoblast cells.

Methods: The lactide-chitosan polymers were synthesized based on different mass ratios (8:1, 4:1, or 1:1). Briefly, tin (II) 2-ethylhexanoate and triethylamine (TEA) were added dropwise to the mixture of lactide and chitosan, then the reaction occurred at 80 °C with magnetic stirring for 20 hours in nitrogen atmosphere. The mixture was dialyzed in distilled water using dialysis tubing (molecular weight cut off: 14,000) for 1day. 2.5 % (w/v) methacrylic anhydride was added into the dialyzed mixture dropwise, and the reaction was allowed to proceed for 8 hours at 60 °C. The photoinitiator solution was then added to the prepolymer solutions to make a final concentration of 0.5 % (w/v). Finally, the prepolymer solutions were exposed to 6.9 mW/cm^2 UV light to allow for free radical polymerization by photo-cross-linking. The chemical and structural changes of the hydrogels were investigated using ^{1}H-NMR, FTIR, and SEM. The chitosan-lactide hydrogels were further examined regarding degradation rates, compressive modulus, and release kinetics of BSA. Subsequently, we also investigated the cellular response to the hydrogels including cytotoxcity and BMP-2 induced osteoblast differentiation and mineralization using W-20-17 and C2C12 cells.

Results/Discussion: ^{1}H-NMR and FTIR results show that novel chitosan-lactide hydrogels were formed via the amidation and esterification between chitosan and lactide, and methacrylation for further photo-cross-linkable networks. Addition of hydrophobic moiety of lactide increased swellability, softness, and degradation rate of the hydrogels. Cross-linking density by UV exposure exhibited considerably greater effect on physicochemical properties of the hydrogels than the ratio of hydrophobicity to hydrophilicity. Cells can grow well and mineralize in presence of hydrogels regardless of the composition of the polymers and UV cross-linking, indicating non-cytotoxicity of the spectra of hydrogels. Higher ALP activities of both W-20-17 and C2C12 cells were observed in the presence of the hydrogels via UV 30s at day 5 (data not shown). In Figure 1(a), the BMP-2 containing hydrogels via UV 30s resulted in greater mineralization in W-20-17 cells compared to those via UV 300s, suggesting a beneficial effect of burst release. On the other hand, in Figure 1(b), C2C12 cells accumulated higher calcium content over time and the hydrogels with longer exposure time exhibited greater mineralization compared to those with shorter exposure time and direct addition of BMP-2 into medium, suggesting delayed response of C2C12 or independence of the BMP-2 release profile.

Conclusions: The present study shows that initial mechanical strength and release kinetics of bioactive factors are tunable via the ratio of chitosan to lactide and cross-linking density via UV irradiation intensity. Our study also demonstrates that the novel chitosan-lactide hydrogels affect BMP-2 induced osteoblastic differentiation and mineralization *in vitro* though the cell response is probably cell or species dependent.

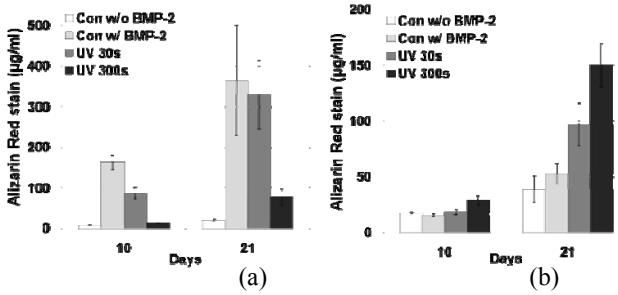

Figure 1. The calcium mineral contents quantitatively determined by Alizarin Red S staining extracts from the cell layers of (a) W-20-17 and (b) C2C12 cells at day 10 and 21.

References:

1. Jeon O, Bouhadir KH, Mansour JM, Alsberg E. Biomaterials 2009;30:2724-34.
2. Vo TN, Kasper FK, Mikos AG. Adv Drug Deliv Rev 2012:64;1292-309.