Oxidation for Enhanced Degradation of Hydrogels Formed From Photocrosslinkable Alginate

Oju Jeon,[1] Shaoly M. Ahmed,[1] Daniel Alt,[1] Caitlin Powell,[1] Eben Alsberg.[1,2]
Departments of [1]Biomedical Engineering and [2]Orthopaedic Surgery, Case Western Reserve University

Statement of Purpose: Recently, we reported on the development of a new alginate macromer that can be photopolymerized to form hydrogels with controllable mechanical properties, swelling ratios, and degradation rates *in vitro*.[1] The photocrosslinked alginate hydrogels exhibit excellent cytocompatibility, and are promising as cell carriers for tissue engineering applications since macromer solutions containing cells can be injected minimally invasively into the target tissue defect, and undergo *in situ* gelation. Hydrolysis of the ester bonds is controlled by altering the degree of methacrylation on the polymer backbone. However, the degradation rate of the hydrogels is slower *in vivo* than *in vitro*. The purpose of this study was to develop photocrosslinked alginate hydrogels with an increased range of biodegradation rates for more rapid *in vivo* biodegradation in tissue engineering applications. Therefore, we oxidized alginate prior to methacrylation to change the uronate residue conformations to an open-chain adduct, which could be more prone to hydrolysis.[2] Here, we demonstrate that the swelling behavior, storage moduli, and degradation profiles of photocrosslinked hydrogels formed from oxidized, methacrylated alginates (OMAs) are tunable by varying the degree of alginate oxidation.

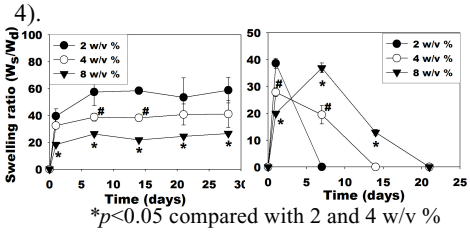
Methods: OMAs were prepared by reacting sodium alginate (Mw=245.8 kDa) with sodium periodate.[3] The oxidized alginate was then methacrylated (14 % actual) using carbodiimide chemistry, and resultant OMAs were photocrosslinked using ultraviolet light with a photoinitiator.[1] The swelling behavior, storage moduli, and degradation rates of hydrogels formed with alginates oxidized to various degrees were evaluated in DMEM. Human bone marrow-derived mesenchymal stem cells (hMSCs) were photoencapsulated into OMA-9 hydrogels (2 w/v %). After 14 days of culture, the viability and proliferation of hMSCs photoencapsulated in hydrogels were evaluated using Live/Dead and Picogreen® DNA assays, respectively. All quantitative data is expressed as mean ± standard deviation. Statistical analysis was performed with one-way analysis of variance (ANOVA) with Tukey significant difference post hoc test using Origin software (OriginLab Co., Northampton, MA). A value of $p<0.05$ was considered statistically significant.

Results: Hydrogels were prepared with different degrees of alginate oxidation and polymer concentrations. Swelling ratios of these hydrogels in DMEM are shown in Fig 1. The swelling of OMA-9 (9 % actual oxidation of alginate) hydrogels decreased as the weight percentage of polymer in the hydrogels increased. All OMA-9 hydrogels reached equilibrium swelling within 7 days. The swelling of OMA-14 reached a maximum by 1 day or 1 week and then decreased as they degraded.

The % mass loss of OMA hydrogels over time was determined as a measure of degradation (Fig 2). OMA-9 hydrogels with different weight percentages of alginate had relatively similar degradation rates. Compared to OMA-9, OMA-14 exhibited the faster degradation. As weight percentage of alginate in OMA-14 hydrogels increased, their degradation rate decreased (Fig 2).

Rheological measurements were performed on the OMA-9 hydrogels (2 w/v %) during the degradation study in order to determine their storage moduli. The storage modulus (G') of OMA-9 hydrogels significantly decreased with time (Fig 3) as expected.

hMSCs encapsulated in OMA-9 hydrogels exhibited high cell viability and proliferated over the course of 14 days (Fig 4).



*$p<0.05$ compared with 2 and 4 w/v %
#$p<0.05$ compared with 2 w/v %

Fig 1. Swelling behavior of OMA-9 (left) and -14 (right) hydrogels.

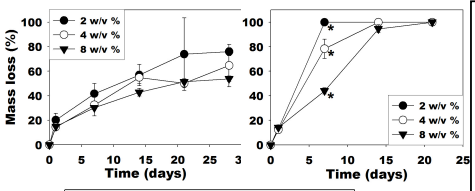


Fig 2. Degradation profiles of OMA-9 (left) and -14 (right) hydrogels. *$p<0.05$

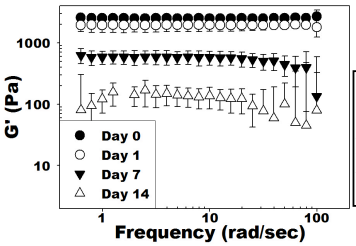


Fig 3. Storage moduli change of OMA-9 hydrogels (2 w/v %) over time.

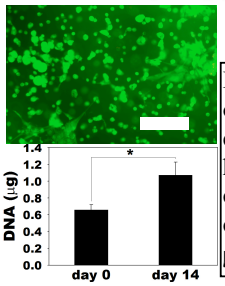


Fig. 4. Fluorescence micrograph (left) of live (green) and dead (orange-red) encapsulated hMSCs in an OMA-9 hydrogel at day 14 and construct DNA content (right) over 14 days in *in vitro* culture. The scale bar indicates 200 μm. *$p<0.05$

Conclusions: In this study we have engineered biodegradable hydrogels using oxidized and methacrylated alginate with controllable swelling ratio, storage moduli, and degradation rates. hMSCs photoencapsulated into the hydrogels were viable and proliferated over 7 days. It is anticipated that these hydrogels formed with oxidized, methacrylated alginate may have accelerated degradation profiles *in vivo* compared to unoxidized material for biomedical applications such as matrices for regenerative medicine and drug delivery.

Reference: 1. Jeon O. Biomaterials. 2009;30:2724-2734.
2. Bouhadir KH. Biotechnol Prog. 2001;17:945-950.
3. Gomez CZ. Carbohydr Polym. 2007;67:296-304.