In Vivo Stability and Encapsulation of Subcutaneously Implanted Alginate Disks

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Statement of Purpose: Alginate is a commonly used biomedical hydrogel whose *in vivo* behavior is only poorly understood. The goal of this study was to compare the rheological behavior and level of encapsulation of alginate hydrogels over time. The effects due to *in situ* gelling versus diffusion gelling and a Poly-L-lysine coating were investigated.

Methods: *Alginate Preparation:* Hydrogels were prepared by reacting 1.95% 43 mPas UPLVG alginate (Novamatrix) with either 680 mM $CaCl_2$ or 100 mM $CaCO_3$ and 80 mM glucono-δ-lactone [1, 2]. To test the effect of a coating, half of the $CaCl_2$ gels were additionally coated with poly-L-lysine ($CaCl_2$/PLL) as described [3]. Gels were made in two volumes: 2 ml for rheology and 500 ul for histology. All gels used for rheology were weighed prior to implantation. *Surgery:* All rats were anesthetized with a ketamine mixture as described [4]. Fifty Sprague Dawley rats were subcutaneously implanted with 4 gels (rheology study) or 6 gels (histology study). Rats were euthanized at predetermined time points (1, 3, 5, 7, 14, and 21 days) for analysis. Explants were weighed before rheological measurements were taken. *Rheology:* Hydrogels were tested with a parallel plate rheometer (AR 550, TA Instruments) using a 25 mm plate at 37 ˚C as described [1, 2]. Shear modulus (G^*) and loss angle (δ) were calculated as described [1, 2]. For all time points, shear modulus was normalized to day 0 measurements. *Histology:* Two gels per condition were inserted subcutaneously in each animal for examination of the reactive tissue response. The gels were explanted at 7, 14, and 21 days, fixed in Bouin's solution, and processed for paraffin embedding. Three 8 um-thick sections per implant were stained with Masson's trichrome. Cell infiltration was analyzed on a subjective scale (0-3) similar to published reports [5], while collagen encapsulation thickness was measured as described [6]. *Statistics:* All results were analyzed with ANOVA and an appropriate post-hoc test where significant differences were noted.

Results / Discussion: Hydrogel weights varied with duration of implant. $CaCl_2$ and $CaCl_2$/PLL gels both demonstrated swelling behavior up to 7 days. Both initially decreased in weight on day 1, but increased 3% ($CaCl_2$/PLL) and 10% ($CaCl_2$) above initial weights by day 7. $CaCO_3$, however, trended towards continual degradation, decreasing in weight consistently over the entire time course. After day 7, all hydrogels decreased in weight.

G^* modulus varied with both experimental condition and time. A large decrease (>80%) in G^* was determined for all alginate conditions for 1 day post implant ($p<0.001$). This significant decrease was exhibited by all conditions for all time points (1, 3, 5, 7, 14, and 21 day). $CaCO_3$ was significantly more stable than $CaCl_2$ and $CaCl_2$/PLL hydrogels up to 7 day ($p<0.01$). At day 7, $CaCl_2$/PLL gels were more stable than $CaCl_2$ gels ($p<0.01$).

Hydrogel δ varied with both time and experimental condition. All three conditions varied significantly with time ($p<0.001$). Loss angle decreased for all conditions up to 3 days post implant and then slowly increased up to 21 days, never exceeding initial δ measurements. On day 1, $CaCO_3$ loss angle was significantly less than that for $CaCl_2$ and $CaCl_2$/PLL ($p<0.001$).

Collagen encapsulation was low initially, then increased significantly at 14 days for $CaCl_2$ and $CaCl_2$/PLL implants and 21 days for $CaCO_3$ implants ($p<0.05$). $CaCO_3$ gels had thicker encapsulation than other conditions, and this was significant at the 21 day time point as compared to $CaCl_2$ implants ($p<0.05$). Encapsulation thickness was in the range of 15-30 um for all conditions at 14 and 21 days post-implantation. Cell infiltration was high initially (in the 0.9-1.5 range at 7 days), and then significantly dropped thereafter for all conditions ($p<0.05$). In general, $CaCO_3$ and PLL-coated implants were more intact than uncoated $CaCl_2$ implants at 7 days. Additionally, the reaction to $CaCl_2$ implants was highly variable as compared to $CaCO_3$ and $CaCl_2$/PLL implants.

Conclusions: It has been shown in the literature that prolonged exposure to sodium ions will decrease both compressive and shear stiffness of alginate, indicating that physiological conditions will soften the gel over a time period of up to 7 days after gelation [7]. This study by Leroux et al., also showed that alginate gels retained significant solid-like behavior after the 7 day period. Our *in vivo* results for G^* and δ support the findings from the previous *in vitro* studies. Although controversial, PLL-coatings are believed to enhance the mechanical stability of alginate, which is reinforced by the present data at the 7 day time point [8].

Overall, all of the implants elicited a mild but sustained foreign body response that followed the same general time course (cellular infiltration followed by mild collagen encapsulation). This time course is similar to published reports [6, 9], and the encapsulation thickness at 21 days (15-35 um) is similar to that reported for other alginate implants at 28 days (21 um) [6]. Histology also revealed that $CaCO_3$ and PLL-coated implants seemed to be more intact than $CaCl_2$ implants, which corroborated the mechanical testing data.

References: [1] Nunamaker, EA. *Biomaterials*, in prep. [2] Nunamaker, EA. *Biomaterials*, in sub. [3] Strand, BL. *J Microencapsul*, vol. 19, pp. 615-30, 2002. [4] Vetter, RJ. *IEEE Trans on Biomedical Engineering*, vol. 51, pp. 896-904, 2004. [5] King, A. *J Biomed Mater Res*, vol. 57, pp. 374-83, 2001. [6] Zhang, H. *J Biomed Mater Res A*, 2005. [7] LeRoux, MA. *J of Biomed Mater Res*, vol. 47, pp. 46-53, 1999. [8] Gugerli, R. *J Microencapsul*, vol. 19, pp. 571-90, 2002. [9] Ratner, BD. *J Control Release*, vol. 78, pp. 211-8, 2002.