Effect of Multiple Confined Compression/Stress Relaxation Cycles on Cross-linked Alginate Pore Size.

Sindhu Kolar Venkat, Erik Risa, Bergren Antell, Maryam Mobed-Miremadi
Santa Clara University, CA

Introduction: Alginate is a biopolymer and hydrogel comprised of (1,4)-linked β-D-mannuronic (M) and (1,3)-α-L-guluronic (G) acid residues. The average pore size associated with crosslinked high MW 1-2 (w/v%) alginate structures has been reported to be 3-5.2 nm for proteins. In this effort, the experiments were designed to meet a single research objective of assessing the effect of confined compression/stress relaxation cycles on extending the upper limit of size exclusion of the cross-linked hydrogel membrane

Materials and Methods: Solutions of 0.5 mg/ml FITC-Dextran MW markers (66 kDa/5.8 nm ,500 kDa/14.5 nm) dissolved in 0.9% (w/v) NaCl (saline) were prepared. Microcaspules (MC)s made of 2% (w/v) alginate formed by polyelectrolyte complexation were extruded from a 32 G needle into a 1% (w/v) calcium chloride solution and cross-linked for 60 min. After saline washes, the 3.5 mm MCs embedded in 2 mls of fluorescent marker were subjected to up to five consecutive confined compression/stress relaxation cycles under a 12.7 mm flat indenter using the Mach I mechanical testing systems (Biomomentum Inc.) for correlating the effect of mechanical treatment to fluorescent marker convection/diffusion under the following protocol: 28% compression under a load of 10 kg at a constant strain rate of 0.5 mm/s, hold time of 5 s, and then drop to preload. Image acquisition was conducted under the microscope at seven radial positions along the perimeter of the capsule using an FITC/Acridine Orange filter followed by processing using ImageJ (version 1.49). The average radial fluorescent (R) distance was used as a metric to monitor the progress of diffusion after each compression. Empty uncompressed control microcapsules were incubated in the MW markers and a two sample t-test was used at the 95% confidence interval using MATLAB (v2016) to test the difference in radial diffusion between the compressed (experimental) and control MCs.

Results and Discussion: As indicated by the p-values (<0.05) presented in Table 1, there is a significant difference at the 95% confidence interval between the experimental MCs and the controls after the third mechanical cycle. A sampled difference is illustrated graphically for the 500 kDa marker in Figure 1a where the radial distance traveled is significantly higher for the experimental capsule [$R_{experimental}$= 467 μm± 33 μm] as compared to control [$R_{control}$=324 μm ± 11 μm]. After the third compression, there is also a marked decrease in the Young's Modulus (E) (E_{N2}= 57 kPa and E_{N3}= 16 kPa) suggesting mechanical weakening due to breaking of the cross-links with no leakage in the supernatant as shown in Figure 1b. As a result of this decrease in elasticity, the membrane has become more permeable to the 500 kDa/ 14.5 nm marker.

Table 1: P values obtained Student t-test results comparing mechanical treatment to passive diffusion for the radial distance traveled for consecutive mechanical cycles.

Compression	66 kDa	500 kDa
n=3	P = 0.0187	P = 0.0042
n = 4	P = 0.0000	P = 0.0000
n=5	P = 0.0000	P = 0.0000

Figure 1a: Comparison of radial distance traveled for the 500kDa marker by passive diffusion (Control) and after the fifth mechanical cycle (Experimental).

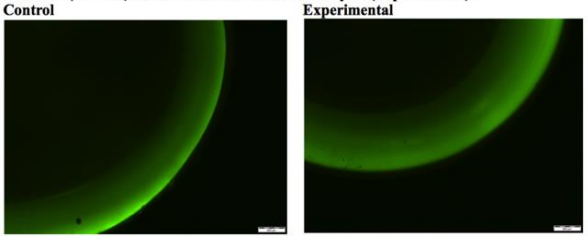
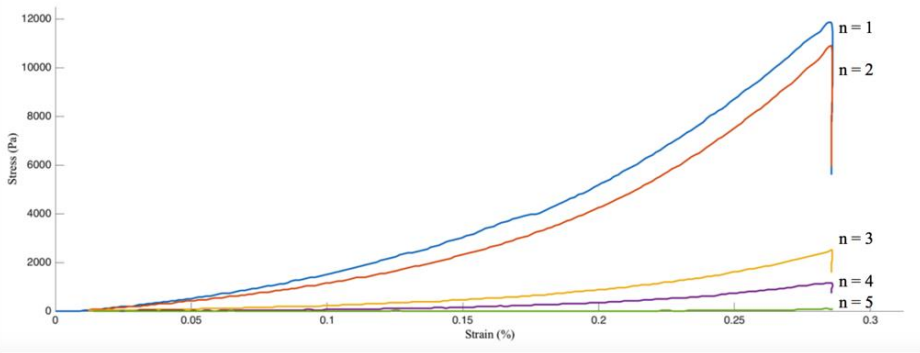


Figure 1b: Compression and stress relaxation profiles for the 500 kDa marker for consecutive mechanical cycles.



Conclusions: Mechanical treatment is an effective method for increasing the alginate hydrogel pore size. Future studies will investigate the validity of these observations for depolymerized steam sterilized alginate solutions and marker specific mechanical characterization post relaxation recovery.