Factors influencing the mechanical stability of alginate beads

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INTRODUCTION AND OBJECTIVE

Stability of the alginate beads/capsules and prevention of its rupture are important determinants for long-term proper function of encapsulated cells. Under physiological conditions capsules are exposed to combinations of physiological forces like compression. Force required to resist compression represents the strength of capsules and time required to resist compression represents elasticity of capsules. Together these values determine the stability of the beads/capsules. For in vivo application both strength and elasticity are essential factors for the functional survival of cells.

Stability of alginate beads/capsule is dependent on alginate composition, viscosity, size, type of gelling ion, time for gelling, type of polycation coating, time for coating, type of storage medium and culturing. These factors act in combination to determine the final stability of beads/capsules. To our best knowledge there are no studies available in which all these factors have been effectively documented and measured with respect to strength and elasticity. We aim to investigate how the above mentioned factors determine the stability (force and time) of bead/capsule to resist 60% compression.

MATERIALS AND METHODS

Alginates purification procedure

Crude alginates containing various amounts of guluronic acid (G)-chains and of mannuronic acid (M)-chains- intermediate-G (Keltone LV), and highG (Manugel) sodium alginates were obtained from ISP Alginates Ltd UK. The method of alginate purification has been described in detail elsewhere (De Vos, De Haan, Wolters, Strubbe, & Van Schilfgaarde, 1997). After purification both intermediate-G and high G alginate at a concentration of 4% (w/v) of was dissolved in 220 mOsm Ca^{2+} -free Krebs-Ringer-Hepes (KRH) solution consisting of 90.0 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, and 25.0 mM Hepes.

Poly amino acid

Poly-L-lysine hydrochloride (PLL), Poly-D-lysine hydrobromide (PDL), Poly-L-arginine hydrochloride (PLA), Poly-DL-ornithine hydrobromide (PLO) were purchased from Sigma–Aldrich (St. Louis, MO, USA). 0.05% of each poly amino acid solution was prepared in Ca^{2+}-free KRH 310mOsm and stored in 4°C till further use.

Encapsulation procedure

4% viscosity alginate concentration was further diluted to 3.4%, 3%, 2.5%, and 2% with Ca^{2+}-free KRH 310 mOsm/L to produce capsules using 500µl alginate, by air driven droplet generator, using 23-G needle, and 100mM $CaCl_2$ as gelling solution. We routinely in our lab use 3.4% intermediate-G and 2% high G alginate to make capsule. Hereafter all studies were carried out with 3.4% intermediate-G and 2% high G alginate. To study effect of gelling ion we used 100mM $CaCl_2$, 10mM $BaCl_2$, 50mM $SrCl_2$ as gelling solution, capsules were gelled to 5 minutes after the last drop of alginate extruded the needle. To study effect of gelling time we used 100mM $CaCl_2$ as gelling solution, capsules were incubated in gelling solution for 5, 10, 15, 20, 30 minutes. All beads were washed with KRH buffer (132.0 mM NaCl, 4.7 mM KCl, 1.2 mM $MgCl_2.6H_2O$, 25 mM Hepes, and 2.52 mM $CaCl_2$ $.2H_2O$) containing 2.5 mM/l $CaCl_2$ and stored in KRH solution (133.0 mM NaCl, 4.69 mM KCl, 25 mM Hepes, 1.18 mM KH_2PO_4, 1.18 mM $MgSO_4$, and 2.52 mM $CaCl_2$ $.2H_2O$) containing 2.5 mM/l $CaCl_2$ till further use. To study the effect of polyamino acid coating we used 100mM $CaCl_2$ as gelling solution. Subsequently beads were gelled for 5 minutes, washed with KRH buffer containing 2.5 mM/l $CaCl_2$, and incubated with 0.05% of PLL, PDL, PLA, or PLO at room temperature for 5 minutes. To study the effect of polyamino acid coating time we used 100mM $CaCl_2$ as gelling solution. Beads were gelled for 5 minutes, washed with KRH buffer containing 2.5 mM/l $CaCl_2$ and then incubated with PLL for 5 minutes and 10 minutes at room temperature. Non bounded polyamino acid was removed by washing with Ca^{2+}-free KRH 310 mOsm/l. Polyamino acid coated beads of intermediate-G alginate and high-G alginate were further immersed in 10X diluted solution of 3.4% intermediate-G alginate and 2% high-G alginate respectively in Ca^{2+}-free KRH 310 mOsm/L, for 5 minutes to form alginate-polyamino acid-alginate capsule. All capsules were stored in KRH solution till further use.

Mechanical properties of beads

The mechanical properties of microcapsules were tested on a Texture Analyzer TA-2Xi (Stable Micro Systems,) equipped with a mobile probe (P/25L) of resolution 1mN. The mechanical stability of beads/capsules was measured by compressing the individual bead/capsule (n=10) to 60% deformation with pretest speed of 0.5 mm/sec, test speed of 0.01 mm/sec, and post test speed of 2 mm/ sec. The force

exerted by the probe to compress the beads/capsules was recorded as a function of the time.

Statistical analysis

Anova and Tukey tests were carried out using R software package. All values were expressed as mean ± standard deviation, differences were considered significant if $p<0.05$.

RESULTS AND DISCUSSION

The viscosity of an alginate solution increases with higher concentrations of alginate. Viscosity of alginate determines shape and size of alginate bead. Alginate bead size and type determines strength of beads (figure 1). Prolong gelling time reduces strength of bead (figure 2a). Stability of beads is dependent on type of gelling ion (figure 2b).

(Due to space constraint we are unable to include all our results)

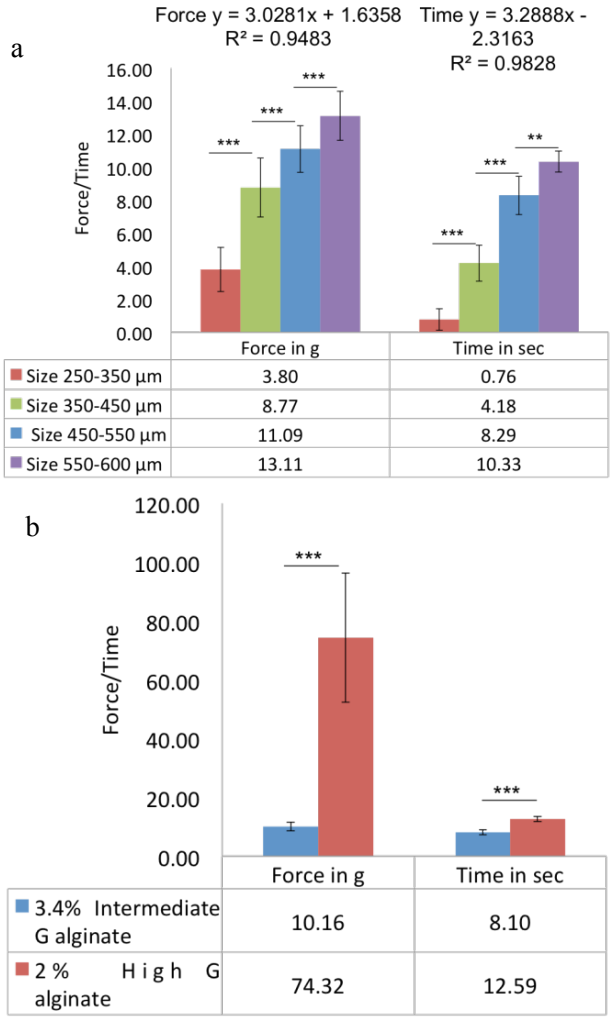

Figure 1: (a) Effect of size on 3.4%intermediate-G alginate beads. Time for gelling with 100mM $CaCl_2$ was kept constant for 5 minutes. Both force and time required to compress beads (n=15) by 60% increases significantly ($p<0.001$) and shows a linear relationship. (b) Effect of alginate type on strength of beads (n=10). Time for gelling with 100mM $CaCl_2$ and size of bead was kept constant for 5 minutes and 500 µm respectively.

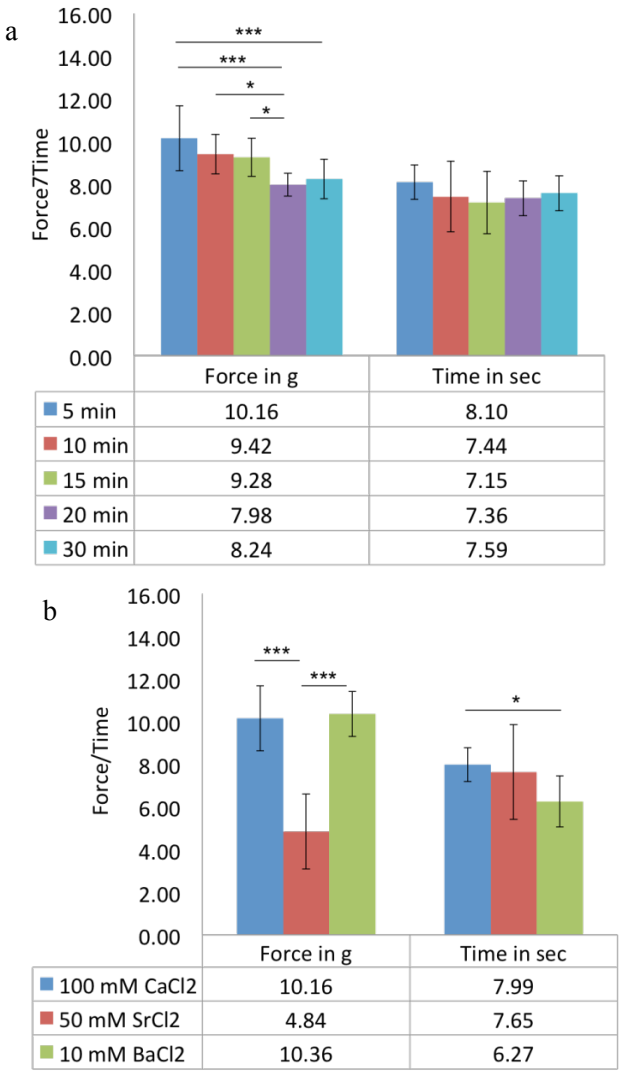

Figure 2: (a) Effect of prolong gelling time with 100mM $CaCl_2$ on 3.4% intermediate-G alginate. (n=10). (b) Effect of gelling solution 100mM $CaCl_2$, 10mM $BaCl_2$, 50mM $SrCl_2$ on a 3.4% intermediate-G alginate (n=10). Time for gelling was kept constant for 5 minutes. Size of the beads was kept constant at 500 µm.

CONCLUSION

Our study shows that the size of the bead, the alginate type, the gelling time, the storage solution, are dominant factors in determining the final strength of alginate-based capsules while the type of gelling ion, the polyamino acid incubation time, and the type of polyamino acid determine the elasticity of the alginate-based capsules.

REFRENCES

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