Improvement of Alginate Microbeads by Enzymatic Modification

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Introduction

Alginate microcapsules are widely used as immune barriers for cell transplantation. To improve the stability of such microcapsules, a polycation layer is often added to the alginate gel core. The polycation seems, however, to be responsible for the fibrotic overgrowth often seen on implanted microcapsules. New procedures omitting the polycation treatment have given good results both in allo- and xenograft transplantation (Omer et al. 2003). However, limited stability of gel beads *in vivo* is still one of the major challenges in cell immobilization systems.

Alginate is a linear copolymer of guluronic (G) and mannuronic (M) acid. In nature, the monomers are arranged in blocks of MM, GG and MG along the chain. Biopolymer engineering of the alginate using enzymes (epimerases) converting M to G in the polymer chain, results in polymers with novel properties. In the present study, new alginate materials completely free of M-blocks were made by introducing G-blocks into polyalternating alginate (polyMG) using the epimerases AlgE1 and AlgE6.

Materials and Methods

Alginates: Mannuronan (polyM) isolated from an epimerase-negative mutant of *Pseudomonas fluorescens* (Gimmestad et al. 2003) was epimerized with the AlgE4 epimerase giving a strictly polyalternating alginate (polyMG) (Ertesvåg et al. 1994).The resulting polyMG was further epimerized with AlgE1 or AlgE6 in order to form G-blocks in various amount. High-G alginate from *Laminaria hyperborea* stipe (66% G) and high-M alginate from *Macrocystis pyrifera* (40% G) were used as controls.
Beads: Inhomogenous gel beads of approx. 500 μm in diameter were made by dripping alginate (1.8% w/v) in a solution containing 50 mM $CaCl_2$ using an electrostatic bead generator.
Stability: Initial size and swelling potential of beads in 0.9% NaCl was determined by measuring the bead diameter. The saline solution was exchanged every 60 min.
Alginate distribution: Beads were made from fluorescein-labelled alginate and the alginate distribution in the gel was visualized in a confocal laser scanning microscope taking micrographs of optical sections through the equator of the beads.
Permeability: The permeability of IgG into beads was studied by monitoring the binding of radiolabelled (^{125}I) anti-mouse IgG to encapsulated Dynabeads coated with IgG antibodies (Kulseng et al 1997).
Gel strength: Gel strength was monitored by compression measurements on gel cylinders to rupture point. Gels were made by internal gelling with calcium (Donati et al. 2005) using 15 mM $CaCO_3$, 30 mM GDL and 0.8% (w/v) alginate.

Results and Discussion

The swelling of alginate beads in saline is drastically reduced upon epimerization compared to a natural high-G alginate.

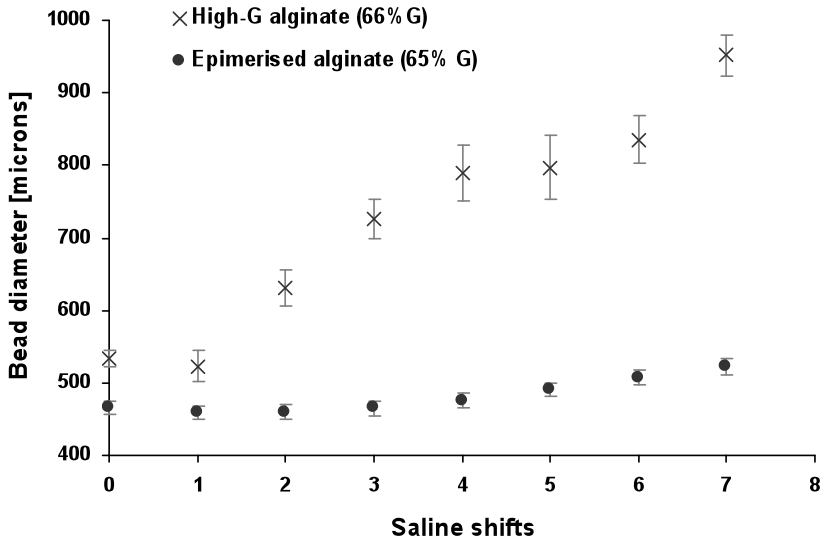

Figure 1: Swelling of alginate gel beads in saline solution measured as the increase in bead diameter. The NaCl solution was exchanged every hour. The epimerized alginate is an AlgE1 epimerized polyMG. Results are presented as means ± SD of 30 beads.

The swelling of beads is highly dependent on the degree of epimerization. A minimum in swelling is reached at approx. 60% total G content, which in this case corresponds to about 40% G-blocks and 60% alternating sequences.

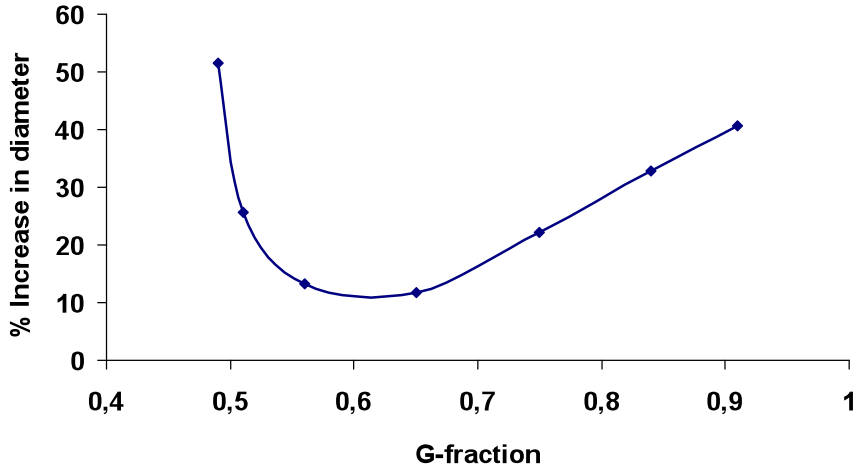

Figure 2: Percentage increase of bead diameter after seven shifts of saline solution as a function of the total G-content in AlgE1 epimerized polyMG alginate.

Ca-alginate beads are less permeable to immunoglobulin G after epimerization. The permeability increases with increasing G-content.

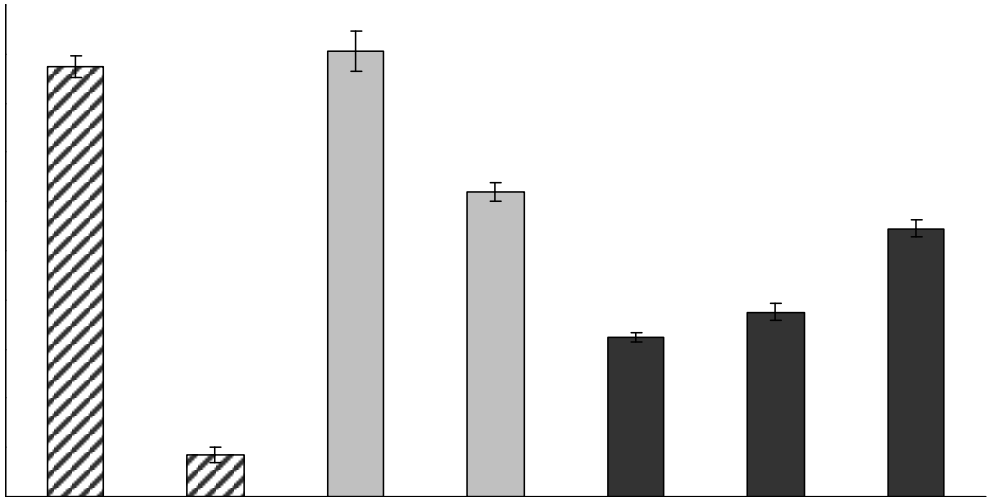

Figure 3: Binding of ^{125}I-IgG to encapsulated Dynabeads after twenty hours incubation with IgG. The Dynabeads were immobilized in alginate gel beads of two natural alginates (high-G and high-M; shown in grey) and AlgE6 epimerized polyMG alginates with various G content (shown in black). Positive control (max. binding) is Dynabeads in solution, negative control (min. binding) is alginate beads without Dynabeads. Results are means ± SD of 3 parallels.

Rupture strength of gel cylinders increases after epimerization and increases with increasing G-content.

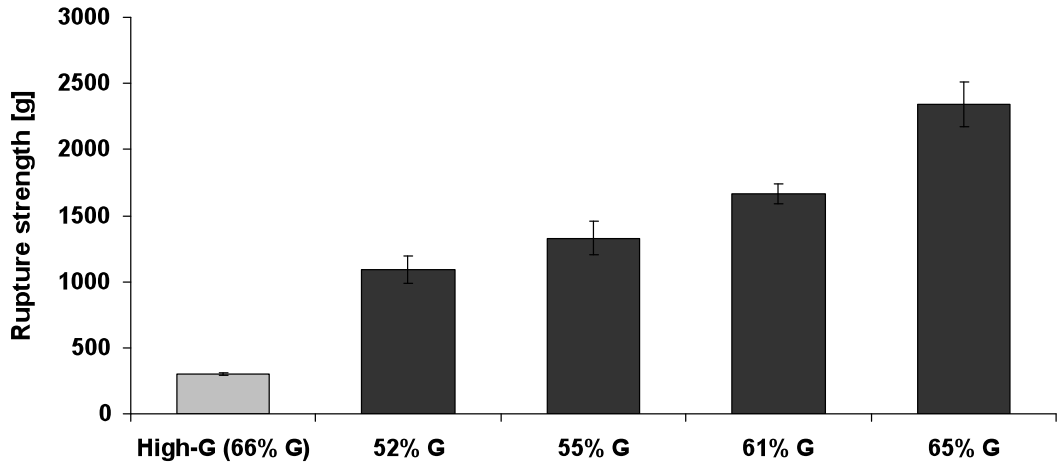

Figure 4: Rupture strength of alginate gel cylinders made by internal gelling with Ca^{2+}. Grey: Natural high-G alginate. Black: AlgE6 epimerized polyMG alginate. Results are means ± SD of 5 parallels.

The concentration of alginate at the bead surface increases upon epimerization. The higher concentration of polymer at the surface will probably contribute to lower permeability and higher stability of the gel beads.

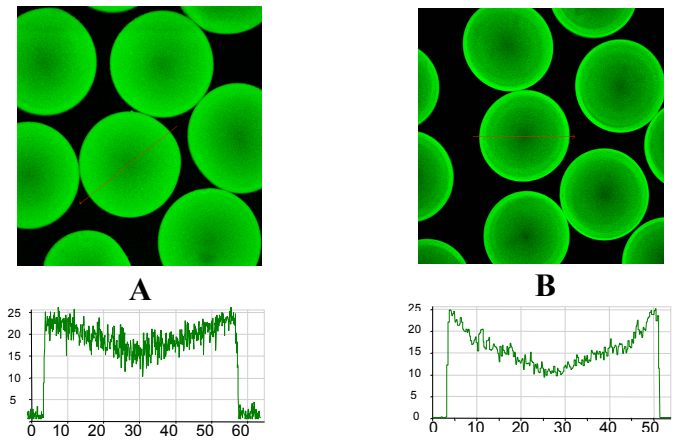

Figure 5**:** Alginate was labelled with fluorescein (green) to visualize the distribution of alginate in the gel beads. Images are taken as an optical slice in the bead equator. The profiles beneath the images are intensity profiles of the signal from alginate. **A:** Natural high-G alginate (66% G). **B:** AlgE1 epimerized polyMG alginate (65% G).

Conclusions

Enzymatic modification of alginate, removing all M-blocks in the polysaccharide chain can give gels with highly improved properties for cell immobilization purposes. The stability of beads made from these epimerized alginates was greatly increased, the beads being almost non-swelling in saline solution. Also, the permeability of IgG into gel beads was highly reduced and the gel strength increased for these novel alginates.

References

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