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Research Paper

Factors influencing the mechanical stability of alginate beads applicable for immunoisolation of mammalian cells

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ABSTRACT

Transplantation of microencapsulated cells has been proposed as a cure for many types of endocrine disorders. Alginate-based microcapsules have been used in many of the feasibility studied addressing cure of the endocrine disorders, and different cancer types. Despite years of intensive research it is still not completely understood which factors have to be controlled and documented for achieving adequate mechanical stability. Here we studied the strength and elasticity of microcapsules of different composition with and without cell load. We compared strength (force) versus elasticity (time) required to compress individual microcapsule to 60% deformation. It is demonstrated that the alginate viscosity, the size of the beads, the alginate type, the gelling time, the storage solution and the cell load are dominant factors in determining the final strength of alginate-based microcapsules while the type of gelling ion, the polyamino acid incubation time, the type of polyamino acid and the culturing time determines the elasticity of the alginate-based microcapsules.

Our data underpin the essence of documenting the above mentioned factors in studies on encapsulated cells as mechanical stability is an essential factor in the success and failure of encapsulated grafts.

1. Introduction

Microencapsulation of cells is a commonly applied procedure to protect cells from the host immune system in the absence of immunosuppression. The technology of microencapsulation is proposed as therapeutic option for diseases where a minute-to-minute regulation of metabolic processes is required and where pharmaceutical intervention is not precise enough

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(Orive et al., 2003). Alginate is the most commonly applied molecule for the core of the microcapsules. Alginate is a linear binary polysaccharide with blocks of (1–4)-linked β-D-mannuronic (M) and α-G-guluronic (G) residues of widely varying composition and sequence (Andersen et al., 2012). Based on the G-content alginates are classified as high-G alginate, intermediate-G alginate, and low-G alginate. Usually the cells are entrapped in a gel of alginate that is crosslinked with divalent cations such as Ca^{2+}, Ba^{2+}, and Sr^{2+}, with uronic acid residues in alginate (Mørch et al., 2006). After crosslinking with cations the matrix is referred to as bead. When the surface is crosslinked with a polyamino acid the system is usually named a capsule (Bunger et al., 2003; Ponce et al., 2006; De Vos et al., 2012).

The mechanical stability of the microcapsules is an essential factor in the success and failure of encapsulated cells. This already starts before implantation. Beads or capsules should be strong enough to withstand the shear forces associated with the implantation procedure (Thanos et al., 2007). Also they should be able to withstand the forces and changes in the microenvironment when brought into transport fluid. In this fluid but also after implantation beads are exposed to all types of substances such as phosphate, sodium and potassium ions that might destabilize the capsules (Mørch, 2008; De Vos et al., 2009). Also, the capsules may undergo serious damage by shear forces they are exposed at the transplantation site (Thanos et al., 2007). In spite of this knowledge quantification and documentation of the mechanical stability of capsules has gained not more than minor attention in publications in the field (Zhao and Zhang, 2004; Zhang et al., 1999).

The mechanical stability of capsules is determined by the alginate type, the alginate concentration, and the type of applied gelling cation (Mørch et al., 2006). For example, alginate with a high guluronic acid content has a higher affinity for cations than alginates with a high mannuronic acid content. In many applications the mechanical stability is reinforced by applying a polycation layer around the alginate core (Thu et al., 1996b, 1996a). Commonly applied examples are poly-L-lysine (PLL) (Thu et al., 1996a; De Vos et al., 2002), poly-D-lysine (PDL) (Strand et al., 2002), poly-L-arginine (PLA), and poly-L-ornithine (PLO) (Leung et al., 2008; Darrabie et al., 2005). These polyamino acids form stabilizing membranes on the surface and simultaneously decrease the pore size of the alginate beads which is mandatory for providing immunoprotection. Also factors such as the cell load and culture conditions can influence the mechanical stability of beads or capsules (Shoichet et al., 1996; Hunt et al., 2010; Rokstad et al., 2002). Surprisingly this has not been studied up to now in a systematic fashion.

The present study was undertaken to investigate and document the effect of commonly applied variations in the encapsulation procedures on the mechanical stability of capsules. To this end we defined two parameters we wished to distinguish. This is (i) the strength and (ii) the elasticity of beads or capsules. The strength (i) is measured by quantifying the force required to compress the bead or capsule. The elasticity (ii) is assessed by measuring the time required to compress the bead or capsules to a predefined value. Combined these values determine the success of beads or capsules in vivo.

2. Materials and methods

2.1. Alginates purification procedure

Crude alginates containing varying amounts of guluronic acid (G)-chains and of mannuronic acid (M)-chains-intermediate-G (44% G+56% M) (Keltone LV) and high-G (67% G+33% M), (Manugel) sodium alginates were obtained from ISP Alginates Ltd UK. The method of alginate purification has been described in detail elsewhere (De Vos et al., 1997). After purification both intermediate-G and high-G alginates were 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.

2.2. Polyamino acid

Poly-L-lysine hydrochloride (PLL) (product no. P2658), poly-D-lysine hydrobromide (PDL) (product no. P4408), poly-L-arginine hydrochloride (PLA) (product no. P7762), poly-L-ornithine hydrobromide (PLO) (product no. P0421) were purchased from Sigma-Aldrich (St. Louis, MO, USA). A solution at 0.05% (w/v) of each polyamino acid solution was prepared in Ca^{2+}-free KRH 310 mOsm (135.0 mM NaCl, 4.7 mM KCl, 25.0 mM Hepes, 1.2 mM KH_2PO_4, and 1.2 mM $MgSO_4$).

2.3. Encapsulation procedure

A 4% viscosity alginate concentration was used as stock and further diluted to a desired concentration in Ca^{2+}-free KRH 310 mOsm/L. Beads were produced using an air driven droplet generator as previously described (De Vos et al., 1997) using a 23 g needle. We routinely apply in our lab 3.4% intermediate-G and 2% high-G alginate to produce beads. The reason is that with these concentrations a viscosity of 4 cps is reached, which is required for the formation of spherical beads (Klokk and Melvik, 2002). This is the upper limit at which 0.2 μm filtration for sterilization is still possible. To study the effect of the type of gelling ions we used 100 mM $CaCl_2$, 10 mM $BaCl_2$, 50 mM $SrCl_2$ as gelling solution. Beads were gelled for 5 min after the last drop of alginate extruded into the gelation bath. To study effects of the gelling time we used 100 mM $CaCl_2$ as gelling solution. Beads were incubated in the gelling solution for 5, 10, 15, and 20 min. All beads were washed with KRH buffer (132.0 mM NaCl, 4.7 mM KCl, 1.2 mM $MgCl_2 \cdot 6H_2O$, 25 mM Hepes, and 2.52 mM $CaCl_2 \cdot 2H_2O$) 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 $CaCl_2 \cdot 2H_2O$) containing 2.5 mM/L $CaCl_2$ till further use. To study the effect of polyamino acid coating we used 100 mM $CaCl_2$ as gelling solution. Subsequently beads were gelled for 5 min, 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 min. To study the effect of polyamino acid coating time we used 100 mM $CaCl_2$ as gelling solution. Beads were gelled for 5 min, washed with KRH buffer containing 2.5 mM/L $CaCl_2$ and then incubated with PLL for 5 min and 10 min at room temperature. Non bounded polyamino acid was removed by washing with Ca^{2+}-free KRH