P-026	**Perfect TRAP to prevent malignant cell outgrowth in transplant recipients.**	

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INTRODUCTION AND OBJECTIVES

Cell encapsulation involves the entrapment of living cells within semi permeable membranes which allow influx of nutrients and oxygen and outflux of therapeutic molecules and metabolic waste products. These membranes should at the same time protect the cells from the hostile effects of the host immune system. The main motive for application of cell encapsulation is to overcome graft rejection in the absence of immunosuppression. Alginate is the most commonly applied material for cell encapsulation. Alginate is a linear binary polysaccharide of α (1-4) glycosidically linked a- L-guluronic acid (G) and its C-5 epimere, ß-D-mannuronic acid (M). The composition and sequential structure of alginate is a key functional parameter, and variations in the composition and/or the sequential structure may cause differences in physico-chemical properties like viscosity, stiffness, ion binding strength which determines the biocompatibility for cells for a particular end use. For example stiffness of the alginate chain blocks has been found to increase in the order MG<MM<GG.

Major challenges for large scale application of cell encapsulation are protruding cells (outgrowth of cells from alginate beads), destabilizing forces (swelling and shrinkage during encapsulation), capsule rupture, capsule cell-loads (beads do not sediment down if loaded with higher concentration of cells). A system in which outgrowth of cells is prevented is mandatory when application of cell encapsulation is considered in immunocompromised patients or in immune privileged sites such as in the brain where tumor growth may occur. In the present study we present a novel capsule concept, the socalled TRAP system in which an inner capsule is applied that facilitates growth and function of therapeutic cells and an outer shell that is deleterious for the cells.

MATERIALS AND METHODS

Concept

It is a known phenomenon that the rigidity and chemical properties of alginate is an essential factor in the survival of encapsulated cells. In the present study we tested the survival and function of Bovine hamster kidney (BHK) cells in different types of alginate. BHK cells will be applied in our future studies as carrier system for therapeutic molecules to treat brain tumors (i.e. transplantation in an immunological privileged site). To this end we exposed BHK cells to alginates with 2.7 % intermediate G alginate (40-45% G-content), and 2% high G alginate (> 53% G -content). Subsequently we again encapsulated intermediate G beads (inner sphere) with 2% high G alginate (outer sphere).

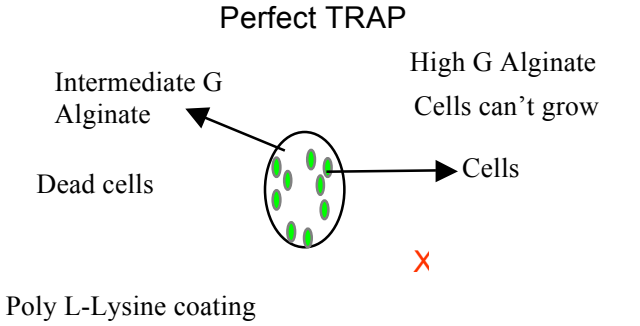

Figure 1: Concept of the Perfect Trap

Alginate Purification

Alginates of different composition were obtained from ISP Alginates Ltd UK. Intermediate-G (40-45% G-content) and high-G (greater than 53% G-content) sodium alginates were purified with our own procedure (De Vos. P. 1997). Purified alginates at concentration of 2.7% (intermediate G alginate, 40-45% G content) and 2 %(high G alginate, G content higher then 53%) were dissolved at 4^0C in sterile filtered Krebs-Ringer-Hepes (KRH 220 Osmolarity).Before application the solutions were sterilized by 0.2 µm filtration.

Cell culture

The BHK cells (passage 5-20) were cultured in flasks (75 cm^2, Ventcap, Corning Inc, New York) containing DMEM (Lonza, Bio Whittaker, Walkersville, MD) supplemented with L-glutamine, 10% FCS, gentamycine.

Cell encapsulation

BHK cells were trypsinized, washed and brought in suspension with a defined volume of alginate to reach a concentration of $3x10^6$ cells per milliliter alginate. For producing beads, the alginate/BHK mixture was converted into droplets using an electrostatic bead generator. The alginate droplets were transformed to alginate beads by gelling in a 100 mM $CaCl_2$ (10 mM HEPES, 2 mM KCl) solution for at least 5 min. Subsequently, the Ca-alginate beads were suspended for 1 min in Krebs-Ringer Hepes (KRH) buffer containing 2.5 mmol/l $CaCl_2$. Also a novel multilayer encapsulation technology (Perfect TRAP) was applied to encapsulate cells. Briefly cells are first entrapped in 2.7 % intermediate G alginate by applying an electrostatic bead generator to form beads, followed by

0.05 % Poly-L- Lysine (PLL) coating (3 minutes on ice and 4 minutes at room temperature) to obtain immunoprotection. The capsules were washed with 310 osmolar Ca-free KRH buffer and enveloped with 2% high G alginate. The encapsulated cells were cultured in 25 cm^2 culture flasks containing 5 ml growth medium and kept in a standard tissue culture incubator at 37°C, 100% humidity, 95% air, and 5% CO_2. The diameters of the beads and capsules were measured with a dissection microscope equipped with an ocular micrometer with an accuracy of 25 µm. The beads had a diameter between (200-300 µm) and multilayer capsules had a diameter between (400-550 µm).

Live dead staining

Viability of encapsulated cells was determined by live dead staining (DAPI-AO) on day 1, 7, 14, 21, 30 days by fluorescence microscopy.

RESULTS AND DISCUSSION

An alginate matrix of high G alginate with a G content larger than 53% is more viscous, more rigid and more durable than intermediate G alginate matrixes with a G content in range of 40-45%. Some cells types such as BHK cells have difficulties to survive in rigid gels such as high-G matrixes. Most of the BHK cells died within 21 days after encapsulation in >53% G alginate. This was not observed in 40-45% G alginate. The cells grew effectively and formed clusters after day 14 in 40-45% G alginate. When applied in the multilayer capsules we observed the same phenomenon. In the first 14 days after encapsulation the BHK cells were growing mainly in the core i.e. the growth facilitating matrix. As of day 21 we observed the first outgrowth of BHK cells towards the outer layer. These cells however did not have a chance to leak out of the capsule as they die in > 53% G alginate. (See figure 2).

The risk of protrusion of cells in patients suffering from brain tumors is twofold. First the protruding cells may leak and form malignancies in immunocompromised patients. Although this argument is very hypothetical and never demonstrated it is always an item that is brought up by medical ethical commissions to decline a clinical trial. The second risk is that even minor amounts of protruding cells can cause an enormous inflammatory response and induce graft failure. The multilayer capsule system which we call the perfect TRAP technology solves both issues. BHK cells encapsulated in 2.7% intermediate G alginate with a G content of 40-45% (inner sphere) grow actively but will die instantly when leaking into the > 53% G alginate. It might be argued that the additional >53% G alginate layer interferes with diffusion of nutrients and may not be beneficial for survival and function of the therapeutic cells. This however is not the case as high G alginates have a more open Ca-alginate network than alginates with a higher proportion of M blocks. This is confirmed by the survival and growth rates of the cells in the inner capsule when compared to the beads.

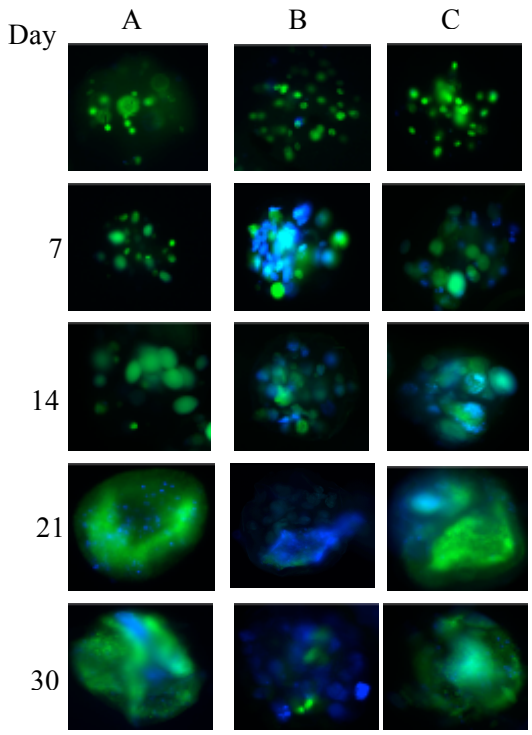

Figure 2 : Fluorescent images of A:- Beads in 2.7 % Inter G Alginate, B:-Beads in 2.0% High G Alginate, C: Multilayer capsules, with BHK cells ($3x10^6$ cells/ml alginate). The cells are stained with acridine orange (green) or DAPI (blue). Viable cells emit green fluorescence, dead cells emit blue fluorescence. Viability staining of cells was done on day 1, 7, 14, 21, 30 after encapsulation. Cells survive better in intermediate G alginate then in high G alginate.

CONCLUSIONS

Physiochemical properties of alginate play a vital role in determining biocompatibility for cells in the capsule. In the present study we tested survival of BHK cells in different types of alginate. BHK cells will be applied for the treatment of brain tumors in our future studies. BHK cells survive better in 2.7% intermediate G alginate with G content in range of 40-45% than in 2% high G alginate with G content greater than 53%. This knowledge was applied to construct a new multilayer capsule, the so called perfect TRAP. The inner capsule contains the alginate that facilitates growth of BHK cells while the outer shell does prevent outgrowth of the cells into the host.

REFERENCES

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