A novel approach for encapsulation of cells to overcome cell protrusion.

Bhujbal S.V.[a,b], de Haan B.J.[a], Niclou S.P.[b*] and de Vos P.[a*]
a) Pathology & Med Biol, Univ Med C Groningen, The Netherlands (s.bhujbal@umcg.nl)
b) NorLux Neuro-Oncology Laboratory, Centre de Recherche Public de la Santé, Luxembourg.

INTRODUCTION AND OBJECTIVE

Cell encapsulation technology is an attractive approach for the delivery of biologically active compounds to treat various forms of disease. This strategy may involve the use of recombinant cells that secrete a protein with therapeutic potential. The cells are often encapsulated in alginate. A key engineering challenge in designing immunoisolating alginate-based microcapsules is maintaining unimpeded exchange of nutrients, oxygen, and therapeutic factors (released by the encapsulated cells), while simultaneously avoiding protrusion of cells from capsules, swelling and subsequent rupture of the microcapsules (Chang 2005). Protrusion means outgrowth of cells from capsules. Even a minor protrusion of cells can cause an strong immune response from the host and cause graft failure. A system in which protrusion of cells is prevented is mandatory for any cell-based therapy.

Most conventional encapsulation systems do not meet the prerequisite of prevention of protrusion, as protrusion of cells is more the rule than an exception (Figure 1). Also entrapment of cells, and growth of cells in alginate, weakens the alginate matrix, thereby causing capsule swelling and rupture. These are the major hurdles in bringing cell encapsulation technology to the clinic.

In the present study we present a novel "Perfect Trap" concept, which overcomes the problem of protrusion and may also prolongs the viability of the encapsulated cells.

Figure 1 : Protrusion of cells from alginate capsules.

MATERIALS AND METHODS

Concept of Perfect Trap

The Perfect Trap system is produced from fully biocompatible materials. The concept is based on applying alginates with different physiochemical properties. Every cell has its own threshold to withstand shear forces. In the present study we tested the survival and function of Baby Hamster Kidney (BHK) cells in different types of alginate (Intermediate G alginate and high G alginate). BHK control and BHK cells overexpressing soluble LRIG1 (Leucine rich repeats and immunoglobulin like domain) a negative regulator of growth factor signaling, will be applied in our future studies to treat brain tumors. In the inner capsule we apply intermediate G alginate, which facilitates the survival of cells and has a rigidity that allows for extension of the population of cells by replication. This is different in the outer high G alginate where a high crosslinking and very rigid network is accomplished. It provides rigidity to the system but also prevents cells from growing and expanding finally inducing cell death. Thus any cell which protrudes from the inner capsule to the outer rigid capsule, will eventually die, thus preventing protrusion (Figure 2).

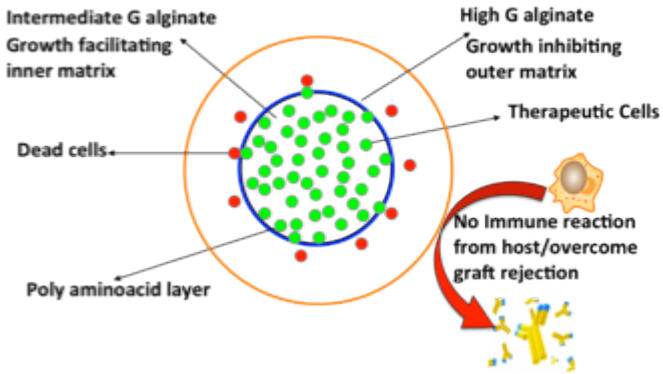


Figure 2 : Concept of the "Perfect Trap" system

Cell line and encapsulation

BHK and BHK-sLRIG cells at a cell concentration of $6x10^6$ cells per milliliter of ultra pure intermediate G alginate (3.4%w/v) and high G alginate (2%w/v) were encapsulated by electrostatic bead generator, using 100 mM $CaCl_2$ as gelling solution. Subsequently the Ca-alginate beads were coated with 0.05% poly L-lysine (PLL). Also a novel multilayer encapsulation technology (Perfect Trap) was applied to encapsulate cells. Briefly PLL-intermediate G alginate capsules were again enveloped with high G alginate by air driven droplet generator. The encapsulated cells were cultured in 25 cm^2 culture flasks containing 5 ml growth medium and kept in a standard tissue culture incubator conditions.

Viablity of encapsulated cells

Viability of encapsulated cells was determined by applying live dead staining kit of Invitrogen (Calcein AM-Ethidium homodimer-1) on day 1, 7, 14, 21, 28

and 35, and fluorescence measurement by confocal microscopy.

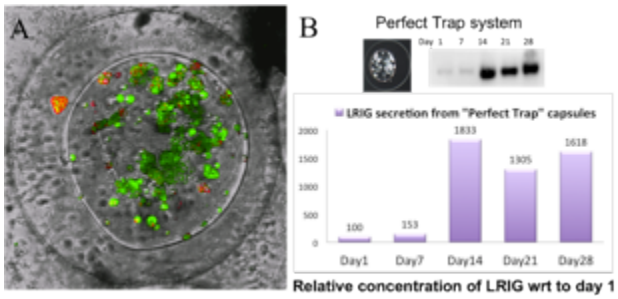
Quantification and detection of secreted sLRIG1

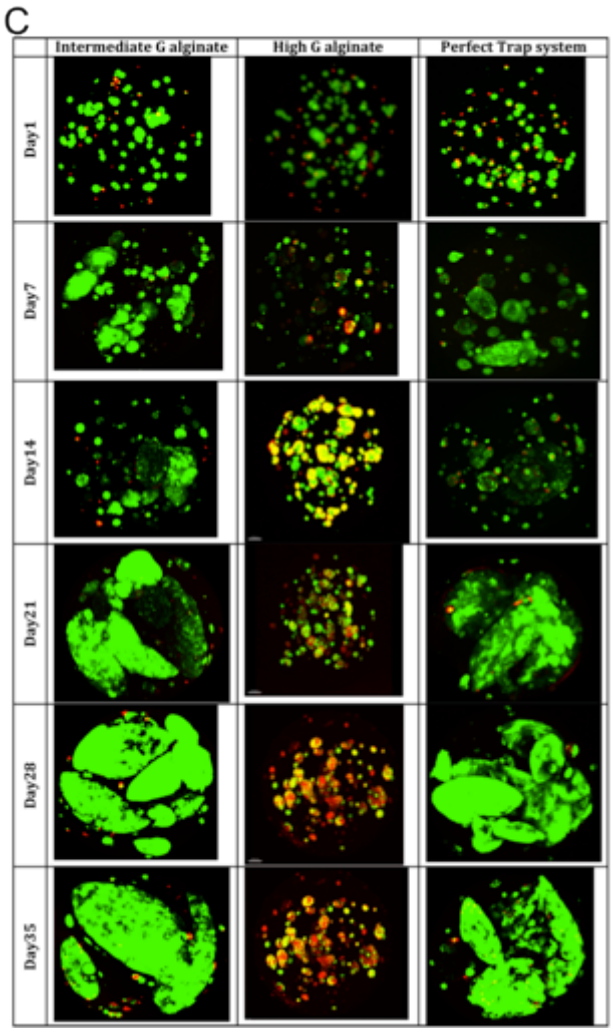
Quantification and detection of secreted sLRIG1 on day 1, 7, 14, 21, and 28 by 50 capsules was done by Western blot analysis.

Surface roughness of capsules by atomic force microscopy

Surface roughness of empty alginate capsules and cell containing capsules (day 30) were determined by atomic force microscopy

RESULTS AND DISCUSSION





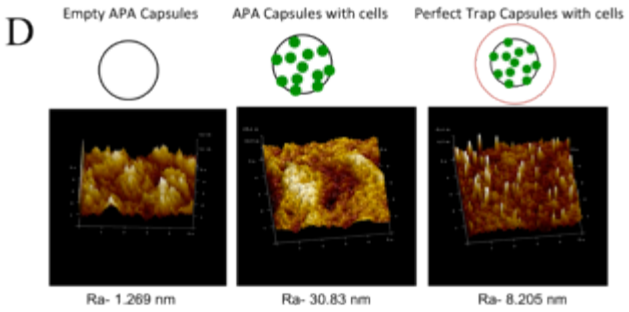


Figure 3: A: Localization of live and dead cells in "Perfect Trap" capsule. Gray scale overlay image of "Perfect Trap" capsule. The cells growing into the outer shell are killed (red) while the cells in the inner core grow and survive (green). B: Western blot result of secreted LRIG1 protein from "Perfect Trap" system. Cells in the Perfect Trap system keep on producing the anti-tumor protein sLRIG1 for prolong period of time. C: The cells survive in intermediate-G alginate (C, left) but are killed in high-G alginate within 35 days (C, middle). These different effects of the alginates are combined in the perfect trap system (C, right). D: Surface roughness (Ra) of alginate-PLL-alginate (APA) capsules with and without cells and in "Perfect Trap" system with cells. "Perfect Trap" capsule has lower surface roughness than APA capsules with cells.

CONCLUSION

BHK cells survive better in intermediate G alginate than in high G alginate. By using this property we can create capsules with versatile properties such as creating an outer layer in capsules where cells die when protruding from capsules. Surface roughness plays an important role in the attraction of cells involved in foreign body reactions. The degree of protrusion from the capsule is directly proportional to the surface roughness. As "Perfect Trap" overcomes protrusion of cells, it has lower surface roughness (8.205 nm) as compared to alginate capsules with cells (30.83 nm). Overall "Perfect Trap" system increases the biocompatibility and long-term stability of the capsules. This system will now be tested for mechanical stability in vitro and in vivo for its efficacy in killing brain tumor cells.

REFERENCES

- Chang TM. (2005) *Therapeutic applications of polymeric artificial cells.* Nat Rev Drug Discov 4(3) 221-35.

ACKNOWLEDGEMENT

We thank the Fonds National de la Recherche, Luxembourg, CRP Santé, Luxembourg, the University of Groningen, Netherlands for funding this project.