Bioencapsulation Research Group

Bioencapsulation Innovations

October 2012

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EDITORIAL

TISSUE ENGINEERING AND ENCAPSULATION

The first attempts to encapsulate tissues to protect them against harmful effects of the immune system dates back to 1923. At that time researchers were interested in behavior of tissues in the absence of vascular access. Varying types of tissues were packed in amnion sheets and studied. Most tissues functioned for long periods of time which may some researchers conclude that vascular access is not required for tissue survival. It took however till the 80' before the first researchers started to recognize the potentials of encapsulating tissues for curing human diseases. By then it was understood that encapsulation and absence of vascular access will only work for small cellular aggregates and never for whole organs. Since then the proof of principle for treating disorder by implantation of encapsulated tissues has been shown for many different types of disorders including Hemophilia B, anemia, dwarfism, kidney and liver failure, pituitary and central nervous system insufficiencies, and diabetes mellitus.

An alternative name for encapsulation in the medical area is immunoisolation. The word isolation refers to the fact that the islets are protected within the membrane for the harmful effects of the immune system of the host. Notably, it does not mean that the membrane prevents an immune response! During recent years many have shown that specific responses occur in the host that are directed to reject the tissue in the capsules. However, an adequate membrane protects the tissue for this rejection response for the life span of the host.

How to make the perfect capsules' is still a question with many answers. During recent years it has become clear that different types of tissues, different types of diseases, and even different types of hosts species require specific adaptations of the capsules that are being used for immunoisolation. For example some cell types cannot survive is some commonly applied alginate types and require specific adaptations of the matrix. In some applications such as in the brain where rejection cannot occur it is not allowed that cells escape from capsules. As outlined in this newsletter, this requires new means to kill cells that may grow out of capsules. Another pertinent issue is the struggle going from different experimental animals towards human studies. Not only do we need to scale up we also need to adapt the microenvironment in the capsules as different species have different desires for optimal function in a capsule, and than we did not even discuss the many different immune response that might be encountered during bringing an encapsulation technology from experimental animals to humans.

Although many research questions remain to be answered, the future for encapsulation in the medical area looks very bright. As outlined in the present newsletter new tailored made polymers are being developed that allow for reproducible production of capsules with documented properties. New approaches to encapsulate islets are being explored in large animal models on their way to humans and hepatocytes in capsules seem to survive in an adequate fashion to bridge the period of liver failure till the time that a donor liver is available. Also new ways to overcome some obstacles in the application of therapeutic cells to treat brain tumors open doors for novel therapies for this deadly disease. After reading this news letter we hope you understand that 'How to make the perfect capsules' will for always remain a question with many answers.

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ARTICLE

SHEET ENCAPSULATION FOR CELL THERAPY

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INTRODUCTION

Medical uses of encapsulation include delivery of drugs and biologics, as well as the subject of this report, delivery of living cells where the activity of the living cells is essential to the therapeutic effect. Treatment of insulin-requiring diabetes is the most investigated application of encapsulated cell technology, where insulin-producing islets of Langerhans are encapsulated in a natural hydrogel, alginate. Here we share some of what we have learned during the twenty year development of the Islet Sheet .

Encapsulation of therapeutic cells has unusual and stringent requirements related to the needs of living cells. The process for making the capsules must not harm the viability or functionality of the cells. The surface of the capsule must not provoke a deleterious response and must simultaneously permit rapid exchange of nutrients with adjacent tissues while preventing direct contact with the host's tissues. Oxygen, which in most cases is delivered by passive diffusion, is typically the limiting nutrient. The dimensions of the device must be small enough that sufficient oxygen is available to every encapsulated cell. Capsule strength and integrity must endure months or years in the host. The overriding requirement is that the living cells be able to perform their biological function for an extended period.

One approach to encapsulating cells is to individually coat each cell. This is most often accomplished by some mechanism of sputtering from a small orifice, which results in stochastic positioning of the cell within the capsule. The Islet Sheet takes a different approach, and different engineering principles, to effectively mold the islets into the interior of a flat sheet of alginate.

The sheet configuration poses its own challenges, when compared with spheroidal microcapsules. There are many benefits of the sheet configuration. These include complete retrievability of the device, an important safety consideration; the ability to characterize the completeness of coverage prior to implantation; and the maintenance of a defined distribution of many cells such that they don't unnecessarily compete with each other for vital nutrients such as oxygen.

ISLET SHEET MEDICAL

Finally, our chosen target is islets of Langerhans to treat insulin insufficiency. Islets have unusually high metabolic rates and unusually rapid secretory responses. The response to changing glucose levels must occur within minutes, especially as glucose levels drop. A capsule to treat diabetes must accommodate these extraordinary features of islets.

CELL ENVIRONMENT DURING ENCAPSULATION

The sheet capsule is made by cationic crosslinking of uronic acid block copolymers called alginate, which are natural products derived from kelp. Islets of Langerhans (or a different cell for other uses) are suspended in liquid sodium alginate solution and then cross-linked to make a hydrogel. Not only does alginate permit formation of a useful capsule surface, impermeable to invasive immune cells from the host, but the phase change of the suspension of cells from liquid alginate to calcium-gelled alginate has no measurable effect on the viability and functionality of the encapsulated cells. Moreover, many researchers have reported that islets encapsulated in alginate spheroids have improved viability and insulin secretion compared with control islets in ordinary culture. Islets in alginate sheets show similar improved viability and insulin secretion.

We believe that the mild ionic crosslinking of alginate as well as the benign sodium alginate solutions used for suspending islets before gelation explain the fortunate fact that islets and cells are undamaged and conti-

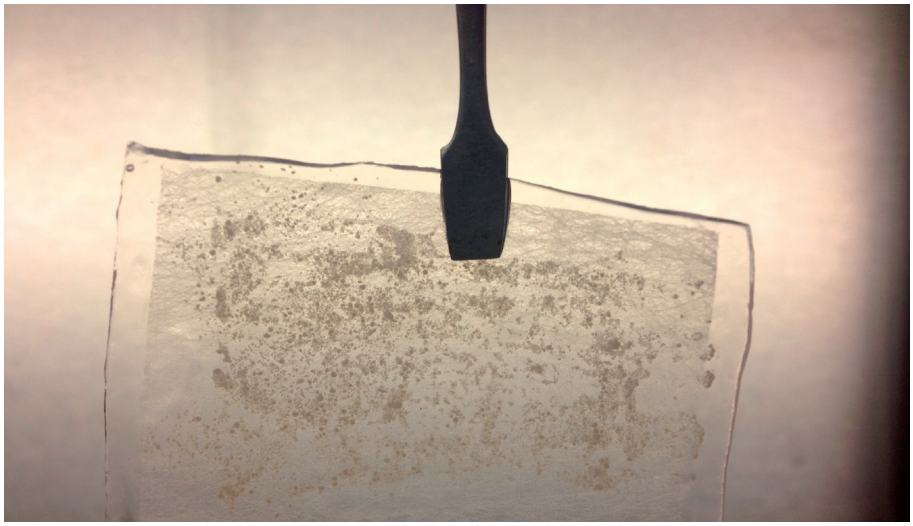

Fig. 1 Islet Sheet made from piglet islets, 3% alginate and polyester scrim

nue function after gelation. Other encapsulation methods that use non-ionic formation of gels (e.g., thermal) or covalent bonds (e.g., free-radical polymerization) have the potential to damage cells.

CAPSULE SURFACE AND TISSUE RESPONSES

Our bodies have evolved complex and robust mechanisms for identifying and eliminating foreign bodies. To be successful a cellular encapsulation device must evade these mechanisms of elimination. Medical encapsulation researchers disagree on whether the primary cause of the inflammatory and foreign body response is cell debris diffusing from the capsule or the physical and chemical properties of the surface. Our data support the latter view.

The subject of most cell encapsulation research is microcapsules. The nature of their fabrication complicates investigations. Such capsules are made by a nebulization step followed by suspension in a solution (usually of calcium chloride) that gels the alginate. This initial step produces a grooved and pitted surface and islets often protrude. Usually additional steps add further layers of polymer such a polylysine or more alginate. There are many variables and nearly as many capsules described in the literature, making comparisons of results difficult. Outstanding work on this problem been published by the European Science Foundation COST bioencapsulation consortium which has concluded :

In spite of the tremendous growth of the industrial and clinical application of encapsulation in the past decade, it is still difficult if not impossible to define the requirement capsules have to meet in order to provide long-term functionality of the enveloped cells or bioactive components. For a further development of the technology an and exchange of technologies it is mandatory to standardize and define technologies that measure specific characteristics.

By contrast few studies have addressed the chemical and microtopological surface of sheets. Dufrane reports no alginate degradation using their sheet design that incorporates collagen as a bed for islets . We have observed minimal biological responses to test sheets (both empty and containing islets of various species) in mice, rats, pigs and dogs. We find the best (i.e., least) response to sheets that are formed with a very smooth surface and no participation by polycations.

DIMENSIONS AND OXYGEN DIFFUSION

It is intuitive that the thinnest capsules provide the best oxygen availability. Conforming coatings (as opposed to capsules) are under investigation because of their minimal thinness. The same concern applies to the sheet, namely that islets should be as close to the surface of the sheet as possible without breaking through to the surface and exposing cells to the host immune system.

The simplest way to achieve this is to make a thin sheet, so thin that an islet in the center experiences reasonable ambient oxygen diffusing in from both sides. Computer modeling and selective measurement show this thickness to be approximately 350 micrometers, depending on islet density. Thicker sheets may have better stability and durability, and can be successful by the careful use of oxygen diffusion enhancers such as fluorocarbons and hemoglobin.

Our collaborators at the University of California – Irvine have observed in real time the development of vasculature adjacent to the sheet through a dorsal window .

CAPSULE STRENGTH

Properly purified and gelled, alginate can be indefinitely stable in biological fluids. Spheroidal capsules have a single breaking strength that can be used to estimate in vivo stability. Sheets are flat objects that conform to a nearby surface and generally are secured by sutures. Sheets can fold, crease, delaminate, pull out from sutures or lose physical integrity in a variety of ways. This cannot be simulated with a single laboratory test. Properly fabricated and sutured in an appropriate site, alginate sheets have been demonstrated in our studies to be stable for over a year in animal models.

We have observed sheets in large animals for periods up to three months. The most important factor leading to failure is the location of the implant. Clean sheets with little overgrowth are observed when implanted subcutaneously, sutured to the abdominal wall, or sutured to the pancreas; sheets have folded or detached when sutured to more flexible tissues such as the omentum or mesentery. Suturing sheets to the liver is initially successful but soon the liver 'ejects' the sutures and thus the sheets detach and crumple.

The sheet configuration has at least one unique advantage compared with spheroidal capsules. A sheet can be retrieved intact with all its encapsulated cells. We have done so from mice, rats, dogs and pigs. In one instance we removed an Islet Sheet from a diabetic rat and immediately implanted into another diabetic rat. The blood glucose of the first rat quickly rose and the blood glucose of the second declined. Given the importance of safety in medical product licensure this feature might be very useful to secure regulatory approvals.

PERMEABILITY

Many researchers believe that soluble factors diffusing out of capsules play an important role initiating tissue responses. We do not agree. In our sheet system preventing exposure of all cells at the surface (that is, complete coverage of islets) largely prevents tissue reaction due to cells. We believe the technical challenge of achieving complete coverage with spheroidal capsules and the tendency of spheroids to roll so that the heavy islets are at the lowest point (thus hiding protruding cells) has masked this result in microencapsulation systems.

We have implanted islet sheets incorporating rat, dog, pig and human islets into rats, both normal and diabetic. We can discern no difference between allogeneic and xenogeneic islet sheet tissue reactions. We believe this supports our hypothesis that the surface of the sheet governs tissue reaction, which can only then be amplified by soluble antigens from perfectly encapsulated islets.

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It follows that permeability control of capsules is less important than generally thought, assuming perfect coverage of cells by the capsule.

ISLETS OF LANGERHANS

Rapid exchange of nutrients and insulin between the islets and the vasculature is essential for control of diabetes in a large mammal. In particular, cessation of insulin secretion should occur within 15 minutes of normalization of blood sugar to prevent hypoglycemia. Encapsulating the islets in hydrogels imposes a diffusion barrier and slows the kinetics of this response. This is another argument for keeping the coatings as thin as possible. We have demonstrated that our thin sheets produce such favorable kinetics.

CONCLUSION

Sheet capsules can have fundamental advantages compared with more traditional spheroidal capsules including smoother and more biocompatible surfaces as well as retrievability. In a series of animal studies we have demonstrated that alginate sheets are biocompatible, durable, and maintain viability of encapsulated islets of Langerhans for months. Ongoing studies in diabetic large mammals are intended to demonstrate that clinical success with implantation of Islet Sheets into diabetic humans is possible.

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President and CEO of Islet Sheet Medical and one of its founders, Scott received a BS in chemistry from the University of Chicago in 1977 and an MA in biochemistry from Harvard University in 1978. He was an investment analyst specializing in biological and medical technology at F. Eberstadt & Co. in New York and at Montgomery Securities in San Francisco from 1979 to 1986. He wrote Wall Street's first investment report on the diabetes industry. He was diagnosed with type 1 diabetes in 1978, and has sought a cure ever since. He has more than 35 years of experience in diabetes research as well as previous ventures in the islet transplantation field, including Transtech Medical and Metabolex. He is also president of PlasmaSeal LLC. He composes music in his spare time.

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ARTICLE

TAILORED ALGINATES FOR ENCAPSULATION

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INTRODUCTION

Alginates are commonly used as a core component in microcapsule systems for living mammalian cells as it allows entrapment of cells at physiological conditions ensuring conservation of cell viability and function post encapsulation. It is a low-immunogenic and non-toxic polysaccharide naturally occurring in brown algae and some bacteria, that cross-links into a gel with divalent ions (Ca2+, Ba2+, Sr2+). The gelling enables the encapsulation of cells and tissue in a porous 3D network allowing rapid diffusion of nutrients and oxygen (Figure 1). Immune protection is based on the prevention of cell-cell contact between the encapsulated cells and the host immune cells, but also larger immune molecules (e.g. antibodies) can be prevented from entering the capsules [1,2].

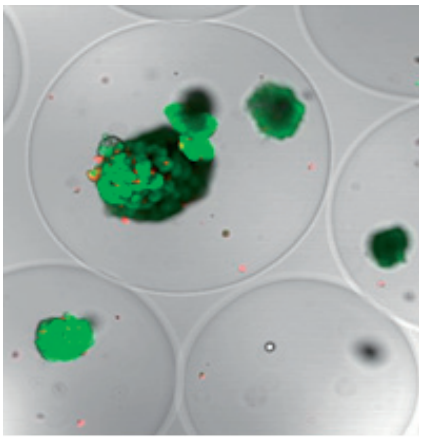

Figure 1. Human pancreatic islets in alginate gel beads stained by calcein/EthD to visualize living (green) and dead (red) cells. The photomicrograph is a cross section obtained using confocal microscopy. Bead diameter is ca. 500 µm [3]

Alginate properties are highly dependent on the structure of the polysaccharide. Hence, this paper will review some of the details of alginate composition and subsequent properties of alginate ionic gels that form the basic for the use as encapsulation material. Further, a review on novel tailored alginate materials will be presented, mainly focusing on our own research. A recent review on alginates as biomaterials in tissue engineering is given in RSC advances [3].

ALGINATE COMPOSITION

The composition of the alginate, together with the molecular weight, determines the properties of the alginate gel and hence properties of alginate as encapsulation material. The building blocks of the linear polysaccharide are Ð-D-mannuronic acid (M) and Ð-L-guluronic acid (G) that form blocks of M-, G- and MG- of various lengths (Figure 2A). The most probable ring conformations are 4C1 for the M residues and 1C4 for the G-residues [4,5,6]. Hence, by connecting the monomers with 1-→4 glycosidic linkages, the resulting block structures have different affinity for the divalent ions and different ability to form crosslinks with other alginate polymers.

Alginates are macromolecules of heterogeneous structure both regarding composition and molecular weight. Alginates do not have any regular repeating unit and the sequen¬tial structure is not determined by the monomer composition (¬monad frequencies) alone, but by measurements of diad and triad frequencies. The four diad (nearest neighbour) frequencies (FGG, FGM, FMG and FMM) and the eight possible triad frequencies (FGGG,¬ FGGM, FMGG, FMGM, FMMM, FMMG, FGMM and FGMG) can be measured by NMR techniques [7,8,9]. From the frequencies the average length of blocks of consecutive G units,

$$N_{G>1} = FG - FMGM/FMGG,$$

and M units,

$$N_{M>1} = FM - FGMG/FMMG$$

can be calculated.

Both the composition and block-struct¬ure varies in diff¬erent types of algina¬tes. In algal alginates, FG varies typically in the range of 0.3 to 0.7 [10]. Alginates with more variations can be isolated from the bacterium A. vinelandii, which in contrast to Pseudomonas species produces polymers containing G-blocks [11]. Recent development of specific alginate lyases and thorough analyses of degradation products have revealed the existence of considerably amounts of extremely long G-blocks and M-blocks (DP > 90-100) in seaweed alginates [12]. The function of these sequences in the gel is still elusive.

Alginates are macromolecules with a distribution of molecular weights. The molecular size of the native alginate is not known as the polymers are degraded during processing. Commercial alginates are delivered in molecular ranges from

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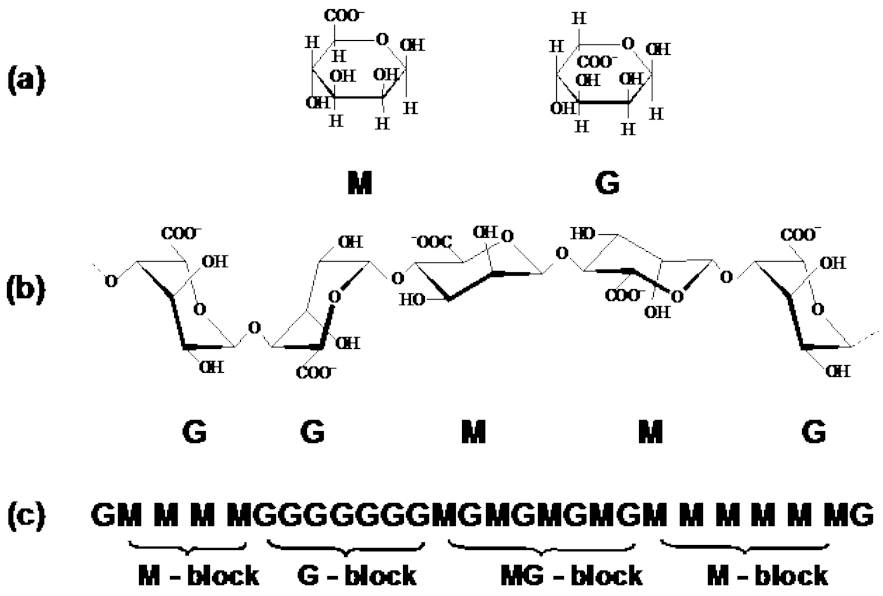

Figure 2. Structure of alginate. ß-D-mannuronic acid (M) and Ð-L-guluronic acid (G). Haworth formulas (a), ring conformation in the alginate chain (M: 4C1and G: 1C4) [6](b), and the alginate chain sequence (c).

4 - 500kDa. The molecular weight of the alginate is essential in the means of the viscosity and gelling properties. Alginates are polydisperse with respect to molecular weight, which implies that the molecular weight is an average over the whole distribution of molecular weights.

ALGINATE GELS – CROSSLINKING WITH DIVALENT IONS

Alginate has high affinity for divalent cations. The affinity series for various divalent cations are

Pb > Cu > Cd > Ba > Sr > Ca > Co, Ni, Zn > Mn

[13,14]. Calcium, strontium and barium ions bind prefer¬ent¬ially to the G-blocks in a highly co-operative manner. The size of the co-opera¬tive units for Ca-binding is reported to be between approximately 8 and 20 mono¬mers [15,16]. Recently, by the production of pure fractions of M-, G- and MG-blocks it has been found that calcium ions also cross-link MG-blocks with MG- and G-blocks [2,17,18]. Also, barium ions have been shown to bind to both G- and M-blocks, and strontium ions to G-blocks solely [2]. Figure 3 gives an overview over alginate crosslinked with calcium ions.

The crosslinks form junction zones in the polymer network that are interspaced with non-gelling zones responsible for the expanded hydrogel. The number and strength of crosslinks determines the stiffness (Youngs modulus) of the gel as well as the stability. Hence alginates with many long G-blocks (and MG-blocks) produce strong and stable gels with calcium. For high concentration of calcium, more than two G-blocks can be crosslinked forming condensed crosslinking zones [20].

Although crosslinking of alginate with calcium ions are the mostly used encapsulation system for mammalian cells, also barium and strontium ions are popular candidates [21], binding even more strongly in the G-blocks resulting in gels that are stronger (mechanically more stiff) and more resistant to destabilization due to exchange of cross-linking ions with sodium that is in excess in culture medium and in vivo [2,22]. In fact, only tiny amounts of barium can be used to stabilize a calcium gel of alginate containing many G-blocks as barium is accumulating in the alginate gel due to the high affinity of alginate towards barium [2,23]. As barium and strontium do not bind to MG-blocks, and only barium weakly binds to M-blocks, exchange of calcium with these ions in gels of alginate containing mainly M- and MG-blocks do not have any effect on physical properties [2].

Barium ions are toxic and the leakage of barium ions from alginate capsules in vivo may be a concern. By using tiny amounts of barium in the gelling solution combined with calcium, the leakage of barium ions from alginate gels with many G-blocks are reduced about four times compared to a gel crosslinked with barium solely, being of relevance compared to tolerable intake values for barium [23].

EPIMERISATION – ENZYMATIC MODIFICATION

Alginates are synthesized in both seaweed and bacteria as mannuronan (poly-M). In bacteria, the alginates are O-acetylated on C-2 and/or C-3 on mannuronic acid residues [24]. M-residues are then converted to G in the polymer chain by enzymes known as mannuronan C-5 epimerases [25] (Figure 4). The presence of mannuronan C-5 epimerase activity was first demonstrated in A. vinelandii [26,27] and was later reported in P. aeruginosa [28] as well as in a wide range of brown algae [29].

Screening of an A. vinelandii

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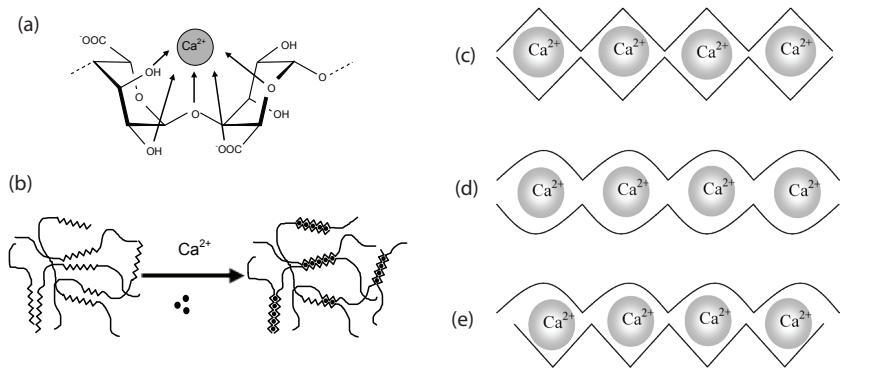

Figure 3. Crosslinking alginate polymers by calcium ions. Probably Ca-binding site in a GG-sequence (a) and ionic cross-linking of two homopolymeric blocks of G-residues by the egg-box model (b) [19]. (c) GG/GG junctions, (d) MG/MG junctions (e) GG/MG junctions [17].

gene library has proved the existence of a family of seven mannuronan C-5 epimerase genes, called the algE genes (algE1-algE7), six of which are clustered in the genome [30,31,32]. These genes have all been sequenced and expressed in Escherichia coli [31,32]. The protein products of the algE genes (the AlgE epimerases) vary both in size and activity [11,32,33].

Based on the mannuronan C-5 epimerases studied so far, it is clear that each enzyme catalyses the production of alginates with distinct monomer distribution patterns. The AlgE4 epimerase catalyses the formation of MG-blocks, while AlgE2 and AlgE6 predominantly make G-blocks. The epimerase activity of AlgE7, on the other hand, leads to formation of alginates with both single G residues and G-blocks [11,32,34] (Table 1). Thus, it is possible to increase the amount of MG- and/or G-blocks in any alginate by using one of the mannuronan C-5 epimerases or a mixture of them.

Table 1. The seven AlgE epimerases from A. vinelandii: Molecular weights [kDa], and product specificity [11,31,32,33].

Initial studies using the AlgE4 epimerase to introduce single G`s in M-blocks of natural alginates in vitro revealed the formation of more compact calcium gels (higher syneresis), higher mechanical stiffness of the gels [18], higher stability towards swelling in saline [35] and higher resistance to rupture upon compression [36]. Later studies have used mannuronan produced in an epimerase-negative mutant (AlgG-) of Pseudomonas fluorescence [37] as a starting material to produce poly-MG by AlgE4 and then introduce G-blocks by using AlgE1 and AlgE6 in vitro [38,39]. This latter strategy results in total depletion of M-blocks in the alginate and results in calcium-gels that are highly syneretic, very stable against osmotic destabilization and have a higher stiffness and rupture strength than natural alginates [36,39]. The high degree of syneresis and resistance towards swelling is probably the main reason for these calcium gels also to be less permeable to IgG than natural alginates of comparable content of G [39,40,41].

Alginate substitution and other modifications

Enzymatic tailoring can be combined with chemical modification. By chemically modifying the mannuronan followed by epimerisation, alginates can be made that are specifically modified on the M-units (since modified M residues are not epimerised). Hence, chemically modified alginates can be made that have intact G-blocks for ionic crosslinking. By using this method we have produced alginates substituted with galactose for hepatocyte immobilization [40] and methacrylate for photocrosslinking to produce covalently crosslinked alginate gels [41].

Alginates substituted with peptides are popular candidates for cell immobilization and tissue engineering [42,43]. Alginate gels are not known to interact with cells, being highly hydrophilic with no anchorage towards cell receptors such as integrins. However, the covalent attachment of RGD-peptides to the alginate has shown cell attachment and morphological effects on 2D culture of myoblasts [43].

Ionically crosslinked alginate gels can be degraded by the exchange of gelling with non-gelling ions. As no alginate degrading enzymes are known in humans, the degradation of the alginate polymer in vivo is due to hydrolysis or depolimerisation by free radicals. Hence, the degradation is slow and favours stable encapsulation systems. For purposes where degradation of the alginate could be favorable (e.g. for resorption of material by the body in some tissue engineering application), the degradation rate of alginate can be increased by using periodate oxidised alginates that is easily degraded by hydrolysis [44].

CONCLUSION

In conclusion, ionic hydro-

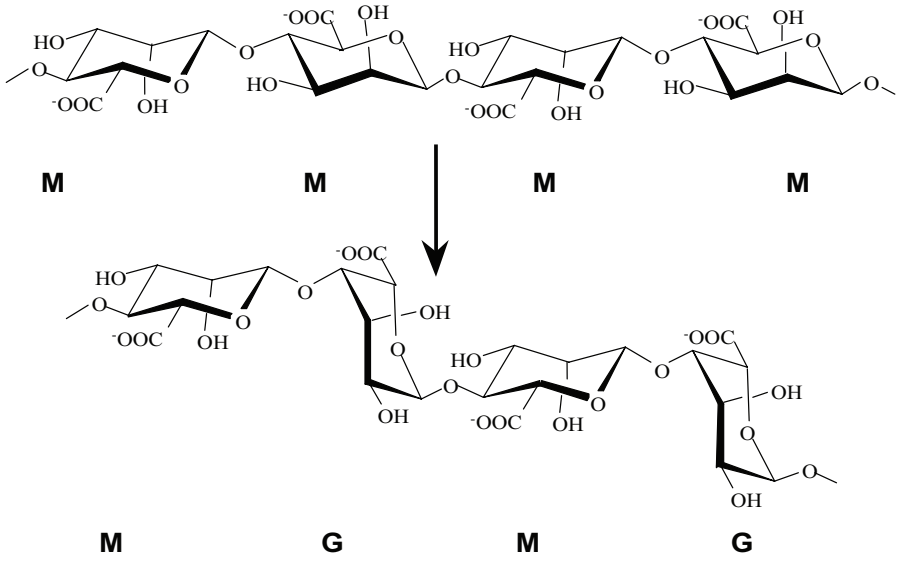

Figure 4. The mannuronan C-5 epimerases converts M-residues to G-residues in the alginate polymer chain.

gels of alginates are favorable systems for cell encapsulation allowing entrapment of cells under physiological conditions conserving cell viability and function. The strong structure-function relationships in alginates are provided by the different sequences ability to bind ions. The alginate structure can be tailored by the use of specific mannuronan C-5 epimerases that converts M to G in the polymer chain. Chemical modification may disrupt the ability of alginate to crosslink with ions and form a gel. However, introducing G by epimerization after chemical modification allows the formation of gelling sequences not being disturbed by the chemical modification. Most modifications of alginate take place before the encapsulation of cells as many modification protocols involve the use of substances that are toxic to cells.

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Berit Løkensgard Strand completed her PhD at NTNU in 2002 entitled "Alginate microcapsules for cell therapy – A study of capsules made of native and enzymatically tailored alginates". She continued her post doc at NTNU and University of Alberta, Canada, and is now a senior research scientist at NTNU with work covering biopolymer/biomaterials at Department of Biotechnology and cell culture/immunology at Department of Cancer Research and Molecular Medicine.

The current research focus is alginate based materials for tissue engineering. Particular interest has been the use of alginate capsules for immune protection of transplanted cells and tissue. This is relevant for type 1 diabetes and transplantation of pancreatic islets without the need of immune suppression. Alginate structure – function relationships is essential in the work. Making new alginate materials by enzymatic modification enables the tailoring of alginate to specific properties.

Combining enzymatic modification and chemical modification allows further tailoring of the alginate for specific purposes. Important biomaterial properties include stability, biocompatibility and permeability in addition to the function as growth and differentiation matrix for encapsulated cells. Strand has extensive collaborations with universities in Europe and the US.

PHD THESIS SUMMARY

MICROENCAPSULATION OF HEPATIC CELLS FOR VIROLOGY STUDIES

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University of Technology of Compiegne, France
Supervisor : Cécile Legallais (cecile.legallais@utc.fr)

Abstract: Three dimensional (3D) hepatocyte cell culture and tissue engineering nowadays finds new applications in bioengineering, including virology, particularly in virology about hepatitis C. Indeed, the establishment of new antiviral strategies requires culture model for hepatitis C virus (HCV) that mimics the physiology of natural infection. New culture models permissive to HCV would thus appear as a promising innovation for HCV studies and mass production of virus. This PhD thesis is part of this perspective and focuses on the development of a 3D culture model of human hepatic cells. It is based on the microencapsulation of a hepatic cell line (Huh-7), permissive to HCV in calcium alginate porous beads.Our experimental approach is based on the physical and biological characterization of this model, after changes in the composition of the material (viscosity and concentration of sodium alginate, or polyethylene glycol (PEG) grafting). The results of these studies permitted to identify a condition for encapsulation which combines matrix porosity compatible with viral infection with an adequate cell behavior due to a 3D cell organization and the expression of HCV membrane receptors.This culture model was submitted to various infection tests with HCV and others hepatotropic viruses. In contrast with the hypothesis leading to optimal encapsulation conditions, we showed that hepatic cells were not infected or did not produce viral particles, regardless of the virus and infection conditions used. These unexpected results open up innovative prospects in the context of cell transplantation.

Desired career: I have been fascinated with science throughout my studies and also PhD experiences. What interests me most is the multidisciplinary but also communication. Collaborations and exchanges between teams and laboratories during my PhD confirm my interest not only in the technical aspect but also in terms of communication. My goal after my thesis is to work in a multidisciplinary environment with multitasking activities yet still remaining in connection with science. Therefore, doing research in the private sector is my desired career.

DAIRY PROTEINS AS FOOD GRADE IMMOBILISATION MATRICES FOR BIOACTIVE INGREDIENTS

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University College Dublin, Ireland, March 2012

Abstract : A range of dairy protein matrices were prepared by food grade processes to immobilise and protect bioactive ingredients in food products.

Cross-linked spray-dried sodium caseinate microparticles (<10μm) achieved excellent (>90%) retention of small pharmaceutical bioactives when dispersed in aqueous solutions representative in pH and ionic strength of a fermented milk drink.

A novel emulsification/ internal gelation method was optimised for the production of cold-set whey protein (WP) microgels (<100μm), with efficient immobilisation (>95%) of model lipid bioactives. Under in-vitro gastro-intestinal conditions, the presence of immobilised lipids provided a composite matrix resulting in a sustained release of water-soluble bioactive vitamins.

WP microgels were also applied as pH-dependent sorbents of ionisable bioactive peptides, and a computer model was developed in order to predict the pH at which maximum interaction between the matrices and a variety of peptides occurred.

Overall, dairy protein matrices proved excellent food grade and effective encapsulants of small bioactive molecules not only to protect the bioactives in food products, but also to target release them due to ionic, pH or digestive enzyme changes in the gut.

Research interests: The physico-chemical characterisation and development of innovative functional foods including novel processing and design of encapsulation systems for bioactives.

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ARTICLE

HEPATIC CELL MICROENCAPSULATION FOR LIVER SUPPORT

Stéphanie Capone*, Nhu Mai Tran* and Cécile Legallais

University of Technology of Compiegne, France

LIVER FAILURE AND PROMISES OF TISSUE ENGINEERING

Both acute and acute-on-chronic liver failures are associated with high morbidity and mortality, because the organ's metabolic functions (detoxification, biotransformation, excretion and synthesis) are severely impaired. Up to now, liver transplantation is the only efficient treatment for patients suffering from acute or fulminant failure. However, its cost, the shortage of organs and the surgical and post-surgical complications strongly limit the number of patients that may benefit from this procedure. For the past 20 years, the expanding gap between the number of patients on waiting lists and the number of transplants has highlighted the requirement for a temporary liver support. Such a temporary liver support can be an artificial organ that can be employed either as a bridge to transplantation or to support regeneration.

There are two types of liver assist devices: artificial and bioartificial types (Carpentier et al. 2009). The purely artificial devices are helpful in some cases but cannot replace all liver functions. Bioartificial approaches are more versatile. By applying tissue engineering, bioartificial livers (BAL) can be developed to fulfill all the liver functions. Such systems can be either extracorporeal (using a cell-housing bioreactor inserted in a perfusion circuit) or implanted. Both of these approaches rely on biohybrid constructs, combining biomaterials and liver cells from several origins (human/mammalian, primary/cell line, mature/stem cells). The materials hosting the cells should offer biocompatibility, adequate surface properties, desired porosity to allow the transfer of nutrients, oxygen, metabolites and low molecular weight proteins such as albumin, but also immune-protection, and mechanical stability. They can be native or benefit from chemical and biological modifications to better mimic the cells' niche and create microenvironments to control their response.

MICROENCAPSULATION IN LIVER TISSUE ENGINEERING

A spherical shape of beads or capsules provides some interesting characteristics that can be considered advantageous to other geometries such as a plate or cylindrical shape. A spherical shape promotes mass transfer as a sphere is associated with an optimal surface-to-volume ratio leading to fast solutes bidirectional diffusion. A prerequisite however is that the sieving properties of the material are tuned. The size of the beads or capsules should also be within certain limits to prevent diffusion delays. Usually, the diameter should not exceed 1 mm, to avoid cell necrosis in the center of cell clusters in the beads. Another reason to keep the capsules below 1 mm is that these beads or capsules of this size can be easily frozen without disturbing morphological features of cells or capsules. Microencapsulation decreases cell damage during cryopreservation and allows an easy storage before use, leading to readily available encapsulated cells when needed.

Cell viability and biocompatibility are essential criteria for the selection of the material and the method of encapsulation.

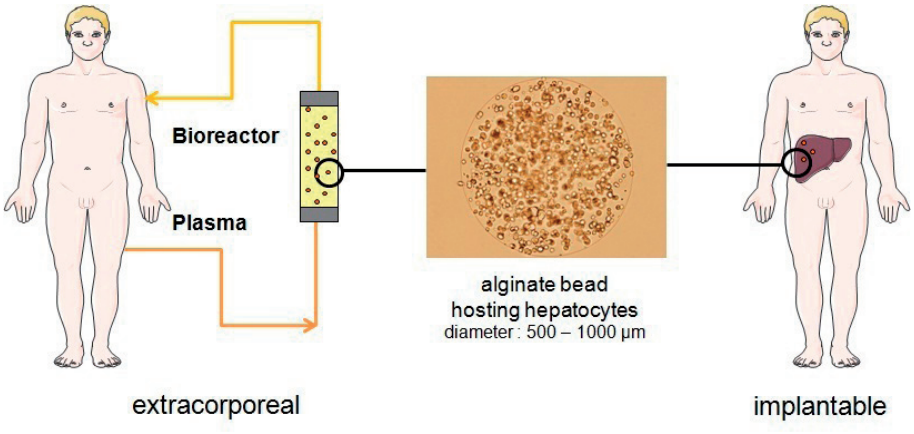

Fig. 1 : Concept of extracorporeal (EBAL) or implantable (IBAL) biortificial liver using hepatocytes encapsulated in alginate beads.

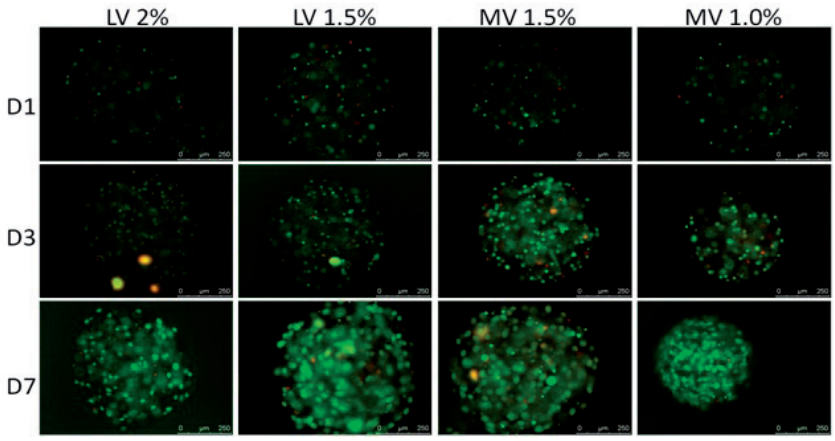

Fig. 2 : Huh7 cell proliferation in alginate beads with different alginate composition : from day 1 to day 7.

ALGINATE AS BASIC MATERIAL

Alginate is an appealing material for the construction of "biohybrid organs" because its hydrated 3D network allows cells to spread, migrate and interact with others. Alginate bead-entrapped hepatocytes can thus be proposed as the biological component in an extracorporeal or implantable bioartificial liver (Fig.1) and have been studied by several teams worldwide (Dixit et al., 1992, Selden et al., 2000, Park et al., 2008, Yang et al., 2010).

Alginate beads hosting hepatocytes are easy to produce by extrusion techniques such as co-axial air flow, electrostatic force or a vibrating nozzle to adjust the bead diameter (David et al., 2004, Kinasiewiez et al., 2008, Coward et al., 2009). The encapsulation can be performed at room or physiological temperatures, at physiological pH, and in isotonic solutions. The alginate bead or capsule might be coated in some approaches with a poly-L-lysine layer (PLL) to improve mechanical stability and reduce external porosity. Covalently crosslinked components, such as pegylated alginate, have become attractive due to their mechanical properties and low degradation degree (Mahou et al. 2012).

However, there is still no clear consensus on the requirements for the biomaterial and the cell culture conditions leading to an efficient system that can be applied for future clinical applications.

WHAT ARE THE BEST CONDITIONS FOR CELL ENCAPSULATION ?

In our laboratory, we have been working for about ten years on hepatic cell microencapsulation, with two human hepatoma-derived cell lines, HepG2/C3A and Huh-7.5.1. The first one was already employed in EBAL development, and the second one is up to now used in studies dealing with hepatits C because of its permissiveness for this virus. The cells were encapsulated in different compositions of alginate (low viscosity (LV), medium viscosity (MV), with concentrations from 1 to 2% in the presence or absence of collagen type I or a poly-L-Lysine layer. Our objective was to determine the encapsulation conditions that meets some or all of the following requirements: optimal cell function and proliferation, optimal porosity for efficient mass transfer, and an adequate mechanical stability.

Hepatic cells were encapsulated in alginate beads of 500µm diameter using an air-extrusion method adapted in our laboratory (Gautier et al., 2011). The alginate matrix porosity was determined by applying both a cryo-microscopic approach, and by diffusion tests with known molecules such as vitamin B12 or more recently with polystyrene nanoparticles (Tran et al., 2012). Hepatic cell viability within alginate beads was qualitatively assessed by fluorescence staining with propidium iodide and acridine orange and quantified using the lactate dehydrogenase (LDH) assay. The growth kinetics of hepatic cells was followed by an Alamar Blue test and by DNA quantification. Albumin concentrations in the supernatant was determined by a sandwich enzyme-linked immunosorbent assay (ELISA) to study the cell functionality in a 3D culture.

We demonstrated that extrusion with a co-axial air flow provided safe encapsulation for both types of cells, without too much adverse effects on cell-viability for densities varying from 0.5 to 10 millions of cells/ mL of alginate solution. Cell viability varied from 60 % to 90 % post encapsulation. After 2 days, cells started to proliferate in most of the structures, the rates being limited with dense alginate networks. Under some conditions, cells can reorganize themselves in 3D multi-cellular structures that can be either spheroids or channel-like (Fig.2). In the last case, this rearrangement resembles the organization in native liver lobules. Cell cluster size could be controlled according to the duration of the culture.

We confirmed the functionality of the encapsulated cell by assessing the production of albumin. Albumin secretion is commonly assessed in the evaluation of liver in situ models as a measure for functionality. We showed the ability of Huh-7.5.1 and HepG2/C3Acells cultured under different conditions of alginate beads to secrete albumin even though variability in the amount of albumin released was observed. The different amount of albumin released when comparing LV and MV alginate beads can contribute to improvement of hepatic support, due to the presence of multicellular channel-like structures in MV alginate, in contrast with LV alginate. The porosity of alginate beads could also influence the diffusion of secreted albumin from the beads to the supernatant as we found that the presence of collagen in alginate, with or without a PLL layer, resulted in decreased mass transfer coefficients while improving the bead mechanical properties.

As far as mass transfer was concer-

ned, we also demonstrated the advantage of using perfusion systems, i.e. fluidized bed bioreactors, to improve nutrients, oxygen and metabolites transport in the supernatant.

NEED FOR COMPROMISE DEPENDING ON THE APPLICATION

3-D cultured hepatic cells represent a promising physiologically relevant system for different applications in tissue engineering. The fluid dynamics of the 3-D culture system promote in vivo-like exchange and efficient cell-to-cell interactions. The alginate beads mixed with collagen meet the specifications linked to in vivo application, but do not present results significantly different from those obtained with "pure" alginate. PLL layer does not provide specific advantages for both implantation and extracorporeal circulation. The alginate bead density is the key parameter: it should be as low as possible to favor mass transfer, while maintaining bead integrity.

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Since 2011, she is the Secretary General of the European Society for Artificial Organs, for which she organised the 36th Congress in 2009 in Compiègne. She is also Section Editor of the International Journal of Artificial Organs.

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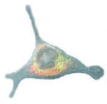

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ARTICLE

TAILORED PEG AS ALTERNATIVE TO POLYCATIONS IN ALGINATE-BASED MICROCAPSULES

Redouan Mahou and Christine Wandrey

Ecole Polytechnique Fédérale de Lausanne, Switzerland

INTRODUCTION

For medical applications, currently cell microencapsulation is under study, for example, for three-dimensional (3D) cell culturing, for external organ systems in bioreactors and for cell allo- and/or xenotransplantation. While all of these applications include as a first step the entrapment of cells in a hydrogel microsphere, the requirements concerning the encapsulation material and technology can differ substantially and are governed by the cell type and final application. The main different requirements are illustrated in Figure 1.

Cell culturing in 3D environments instead of in 2D provides some advantages. The first is that 3D cell culture systems mimic in vivo conditions. Especially the spherical shape and micro-size achieved upon cell microencapsulation promote growth to high densities. Moreover, the surface to volume ratio is favorable for cell nutrition, gas, and metabolic product exchange. Compatibility with the enveloped cell is a primary material requirement, though physical properties of the gel such as polymer network density, stiffness or hydrophilicity can have an impact on cell proliferation, differentiation, and/or function.

For applications in bioreactors not only cell compatibility but also mechanical stability is important in particular if technologies such as fluidized bed reactors are used. The microsphere material has to resist friction and frequent shear forces over extended periods of time.

The most demanding applications are those for which transplantation to human bodies is intended. Here, in addition to cell compatibility and optimal physical properties, host compatibility and minimal immune response after transplantation are crucial.

Despite these different requirements, all applications will benefit from a simple production process, preferably a one-step production technology, in terms of cell friendliness and process economy.

CURRENT SITUATION

Overall, hydrogels of any shape can provide conditions to cells, which are similar to those of tissue. A variety of hydrogels prepared from different macromolecular origin, synthetic, natural, and modified natural, are under study. In particular, cell immobilization materials prepared from sodium alginate (Na-alg) or poly(ethylene glycol) (PEG), as well as derivatives of these, have been reported as promising.

PEG has many advantages as it is synthetic, can reproducibly produced, and is biocompatible. The formation of PEG hydrogel microspheres however is a challenge. This is due to the fact that covalent cross-linking, which is necessary to form a PEG hydrogel, occurs slowly, in the timeframe of minutes or even hours for total completion. This is different with alginate-based microspheres where this process occurs within seconds.

In spite of the nontoxicity, nonimmunogenicity, and advantageous gelling behavior of Na-alg, the application of microspheres obtained by gelation with divalent cations is limited because they are suffering, for example, from low mechanical stability, limited durability, and permeability drawbacks. While the physical properties of the initially formed alginate hydrogels can be tuned and improved with one or more coatings of polyelectrolyte complexes using polycations such as poly(L-lysine) (Figure 2) such coatings seem to induce biocompatibility problems. Moreover, any additional coating step complicates the technology, worsens the economics of application and can compromise cell survival.

Considering the principal advantage of both alginate- and PEG-based hydrogels but also their shortcomings, a combination of both chemistries would combine the advantages and hopefully delete the shortcoming. Two strate-

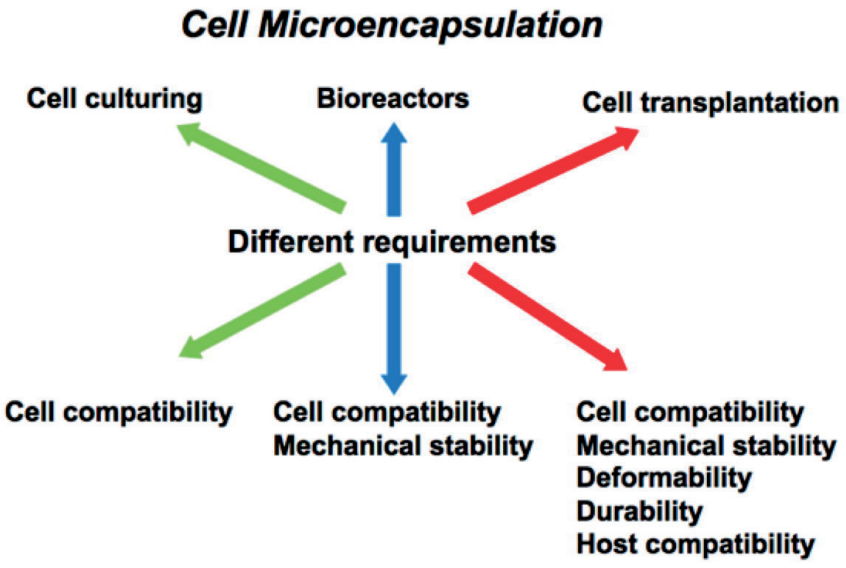

Fig. 1 : Different requirements for cell microencapsulation for three selected medical applications

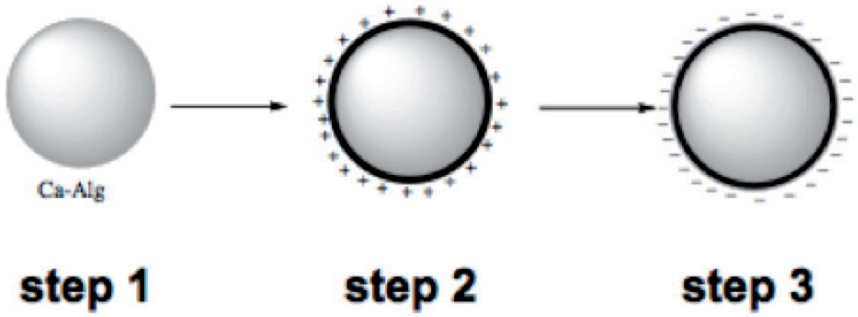

Figure 2: Multi-step technology, consisting of gel formatting, coating with polycation and polyanion, as well as washing steps, to adapt the physical properties for cell transplantation

gies to avoid polycations and multi-step processes for the production of microspheres for cell microencapsulation combining alginate and PEG are presented here (Figure 3).

ALGINATE-PEG HYDROGELS FOR CELL MICROENCAPSULATION

Strategy 1

The first strategy combines Na-alg and end group functionalized branched PEG. The fast gelling Ca-alg provides the spherical matrix for the slow covalent cross-linking of PEG yielding a two-component interpenetrating hydrogel designated as alg/PEG-M (1). (Figure 3a)

Strategy 2

For the second strategy, Na-alg was modified by partial grafting with heterobifunctional linear PEG. The remaining carboxyl groups pre-form the spherical matrix upon gelation with calcium ions. Simultaneously, the slower cross-linker-free covalent cross-linking of the PEG end groups occurs yielding a combined ionic-covalent hydrogel designated as Ca-alg-PEG (2) (Figure 3b).

Alg/PEG-M: Properties and cell microencapsulation

Alg/PEG-M are obtained by mixing the solutions of Na-alg and multi-arm vinyl sulfone terminated PEG before extrusion into the gelation bath containing calcium ions and the cross-linker. A coaxial air-flow droplet generator provides homogeneous drops. The size can be adjusted by the needle diameter and air-flow. Cells are mixed with the solution before extrusion. The one-step process works in cell culture media such as MOPS or DMEM. Upon liquefaction of the Ca-alg gel with sodium citrate, pure PEG hydrogels are principally obtainable.

It was demonstrated that the PEG gel almost exclusively determines the mechanical properties and permeability of the hydrogel. The presence or absence of Ca-alg has no significant influence on the mechanical resistance and permeability. These properties can be tuned by varying the PEG concentration and/or its molar mass/arm length. A minimum of 5wt% of PEG was required to yield stable microspheres after liquefaction of Ca-alg. With more than 20wt% PEG, the hydrogel can become heterogeneous. For stable and homogeneous microspheres, the MWCO is tunable in the range of 20 to 150 kg/mol.

Microencapsulation of several cell types such as mesenchymal stromal cells (MSC), human foreskin fibroblasts and islets of Langerhans confirmed the feasibility of cell microencapsulation with alg/PEG-M.

MSC adapted well to the alg/PEG-M hydrogel and recovered their natural shape one day after encapsulation at the latest. They proliferated and differentiated into adipocytes. In vitro survival was observed for more than 6 months. In vivo, encapsulated MSC were viable and functioning at 26 days after intraperitoneal transplantation in mice. Moreover, the microspheres were free floating and clean (Figure 4). Transplantation of encapsulated MSC represents a promising strategy for local and systemic delivery of anti-inflammatory and immunomodulatory molecules secreted by MSC (3).

Human foreskin fibroblasts were also successfully encapsulated within alg/PEG-M. The cells recovered their normal shape. Already after 24h, the proliferation rate after 24h was similar for free and encapsulated fibroblasts.

From encapsulation studies with islets of Langerhans became obvious that the gel density has an influence on the cell response to glucose stimulation. From these studies can be hypothesized that less stiff hydrogels will be advantageous.

Ca-alg-PEG: Properties and cell microencapsulation

Grafting 5% to 10% of the carboxylic groups of Na-alg with α-amine-ω-thiol PEG with molar masses in the range of 300 to 2000 g/mol allows for tailoring the hydrogel properties. To ensure both ionic gelation and covalent cross-linking of the thiol groups, more than 5% but less than 12% grafting seem to

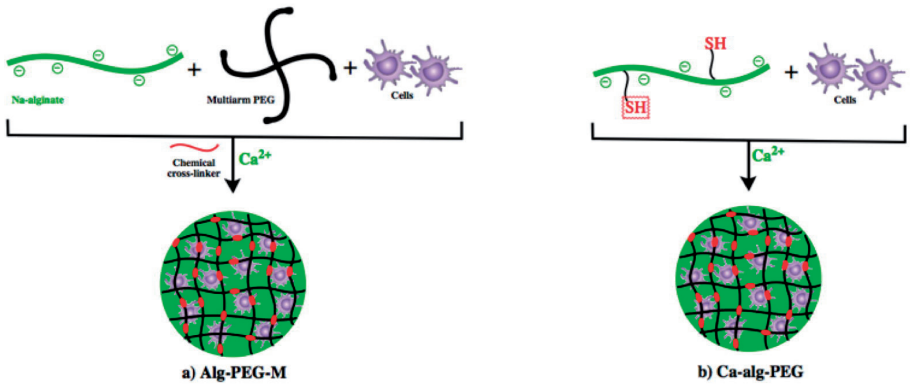

Figure 3: Two strategies to produce hydrogel microspheres by a one-step technology

be necessary. In case of less than 5% grafted backbone units, there are not sufficient covalent bonds formed and the microspheres completely dissolve upon liquefaction with sodium citrate. Contrarily, in case of more than 12% grafted backbone units, an insufficient number of carboxylic groups remained for fast ionic cross-linking and no microspheres are formed upon extrusion in the gelation bath, which contains calcium ions. If the alg-PEG is exposed at sufficient concentration to oxygen/air, self-cross-linking takes place within hours. Adding reducing agents such as TCEP, makes the gel formation reversible. Due to the slow covalent cross-linking reaction, microspheres cannot be formed by covalent cross-linking alone, using common extrusion technologies.

Microencapsulation of hepatocellular carcinoma cells (Huh-7) confirmed the feasibility of cell microencapsulation within Ca-alg-PEG. The one-step extrusion process under physiological conditions yielded microspheres with a narrow size distribution (<5%). The microsphere size was tunable by modifying the air stream, needle diameter, or extrusion rate. Cell viability, proliferation and albumin secretion of Huh-7 were maintained during a study of two weeks (4). (Figure 5)

CONCLUSIONS

Alg/PEG-M and Ca-alg-PEG seem to be promising as alternatives to existing cell microencapsulation materials. These hydrogel microspheres extend the materials basis for cell microencapsulation. A particular advantage is the fabrication of both materials by a one-step technology, which allows for modifying the hydrogel properties by varying controllable process parameters. Further studies will identify and prove for which applications alg/PEG-M and Ca-alg-PEG are particularly suitable.

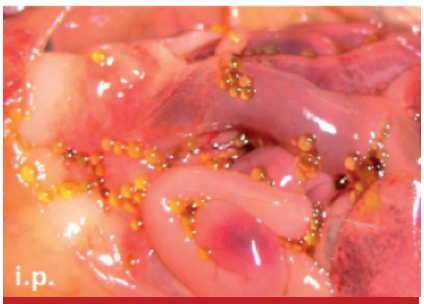

Figure 4: Alg/PEG-M 10 days after intraperitoneal transplantation in mice

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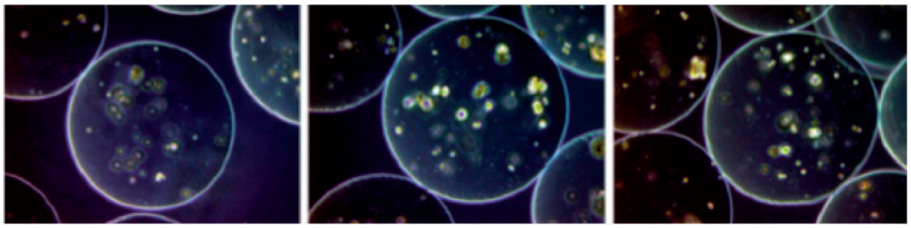

Figure 5: Huh-7 encapsulated in Ca-alg-PEG at days 1, 2 and 3 (from left to right).

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ARTICLE

IS THERE A FUTURE FOR BIOENCAPSULATION IN THE TREATMENT OF BRAIN TUMORS?

Swapnil Vilas Bhujbal 1,2, Simone P. Niclou 1, Paul de Vos 2

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INTRODUCTION

There are many cancer types where chemotherapy is poorly effective. Brain tumors are such a family of cancers. Brain tumors are protected by the blood brain barrier, which prevents effective transport of chemotherapeutic agents to the tumor. Novel approaches are urgently required for the treatment of malignant brain tumors, which have a very poor prognosis and ultimately lead to the death of the patients.

CELL ENCAPSULATION AS THERAPEUTIC OPTION

Implantation of encapsulated cells has been proposed as a promising treatment option. Encapsulated cells that produce anti-tumor agents are implanted in close vicinity of the tumor. The encapsulated cells could be introduced during the neurosurgical resection of the tumor, normally applied to remove the largest part of the tumor. The encapsulated cells can subsequently function for months to target remnant tumor cells that could not be removed by the surgery, such as highly invasive tumor cells. This strategy holds a number of important advances over the conventional therapy of surgery combined with chemotherapy. The release of the therapeutic agent is local avoiding undesired systemic side-effects of high drug concentrations in the blood. Further, the therapeutic molecules are produced continuously and over prolonged periods of time, which guarantee the targeting of remaining malignancies that may have survived surgery. Before encapsulation can be proposed as a realistic treatment option a number of issues have to be solved.

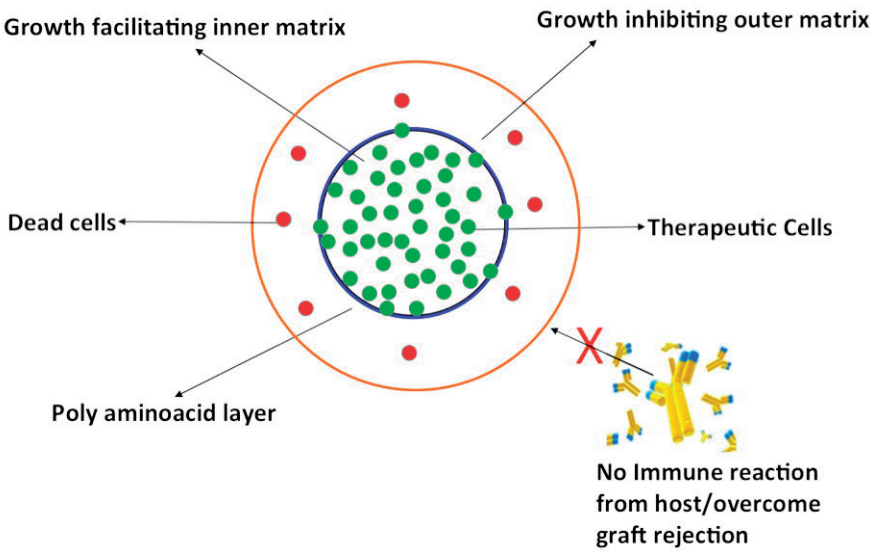

Figure 2 – Concept of Perfect Trap. The inner capsule of Perfect Trap system contains a matrix that facilitates growth of cells while the outer shell does prevent outgrowth of the cells into the host.

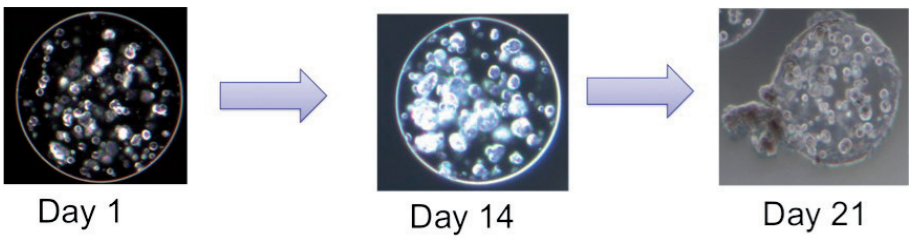

Figure 1- Protrusion of cells is a common problem in application of encapsulated cells as vehicle for therapeutic cells

IMMUNOCOMPROMISED PATIENT GROUP

Because of the immunesuppressing factors released by tumor cells, cancer patients have a weak immune system. Additionally the brain is considered an immune privileged site. Any cell therapy proposed for anti-cancer treatment should not be associated with the possibility of introducing tumor growth in itself. Therefore the encapsulation approach should prevent leakage of the therapeutic cell from the capsule. Most conventional

encapsulation systems do not meet this prerequisite as protrusion of cells is more the rule than an exception (figure 1). We therefore developed a new system that is not associated with protrusion and even will kill cells that grow out of the capsules

THE APPROACH

A prerequisite for application of cell encapsulation in the treatment of brain tumors is thus to guarantee that the therapeutic cells cannot leak out of the capsules. To this end we have designed a new approach in which we apply a novel capsule type that overcomes outgrowth of cells in two ways. First we apply an additional layer around the capsules that prevents leakage of the cells. Second we have used in the engineering of this additional layer molecules that are not compatible with the survival of the outgrowing cells. Thus every cell that protrudes and grows out of the inner capsule will die in the outer layer. We name this system the "Perfect Trap".

THE PERFECT TRAP AT WORK

The perfect trap capsule system is composed of fully biocompatible materials. The concept is based on application of materials with different physiochemical properties. Every cell type has its own threshold for withstanding shear forces. In the inner capsule we apply molecules that facilitate survival of cells and that has a rigidity that allows for extension of the population of cells by replication. The matrix is very flexible and allows for placing daughter cells. This is different in the outer layer 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 cells death (figure 2).

Time scale microscopic images of mammalian cells (Figure 3) show the principle applicability of the system. Producer cells were encapsulated in the matrix of the outer layer. As shown in figure 3 most of the cells died within 21 days after encapsulation in matrix of outer layer. This was not observed in the agent applied for the inner capsules. The cells grew effectively and formed clusters after day 7. When applied in the Perfect Trap system we observed the same phenomenon. In the first 14 days after encapsulation the cells were growing mainly in the core i.e. the growth facilitating matrix. As of day 14 we observed the first outgrowth of cells towards the outer layer. These cells however did not have a chance to leak out of the Perfect Trap system as they died in the outer rigid layer. The live/dead staining of the cells with calcein AM and ethidium homodimer-1 illustrates the proof of principle of the "Perfect Trap" system. At present the first animal trials are being performed to study the efficacy of the system in killing malignant human tumors.

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Currently he is working on joint PhD program between CRP Santé, and University of Groningen under supervision of Simone Niclou and Paul de Vos respectively on the topic -Application of cell encapsulation to treat brain tumor. His research focuses on designing a novel cell encapsulation system to overcome the existing challenges in encapsulation. He possess skills in both wet lab techniques and bioinformatics.

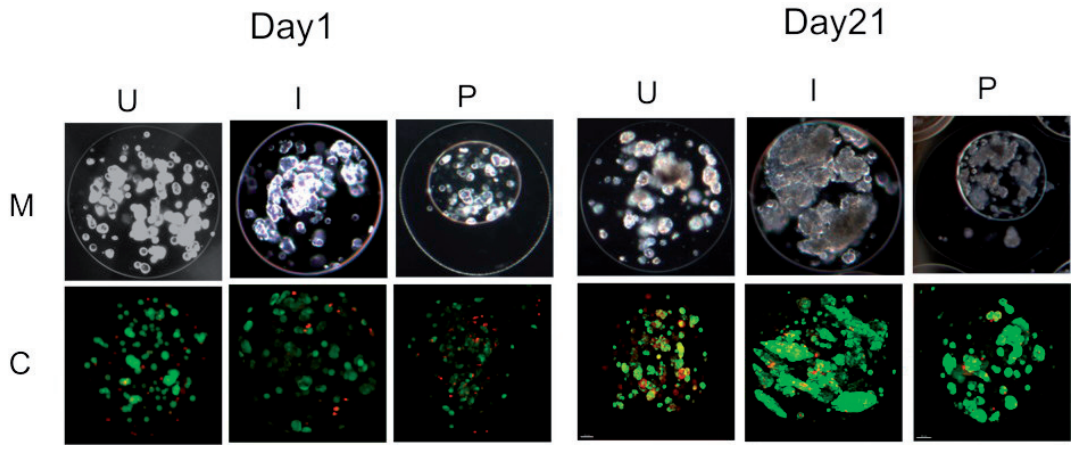

Figure 3- Microscope(M) and confocal(C) microscope images of live (in green) and dead (in red) cells in the unfavorable outer matrix (U), the growth facilitating inner matrix (I) and the perfect trap system (P) on day 1 and day 21. Cells in green are live cells (stained with calcein AM), cells in red are dead cells (stained with ethidium homodimer-1). Note the pronounced differences in cell survival between U, I and P.

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INTRODUCTION

The General Assembly held at the annual conference in Orillia, Ontario Canada on September 22. All participants at the conference were invited to attend the General Assembly.

ACTIVITY REPORT

Four issues in the BRG newsletter were published during 2011 and sent to 7000 people. Two issues where printed and distributed at different conferences and events.

Three events were organised

- A training school in Valdivia, Chile on April 25-27, 2011
- The 14th Industrial Symposium and 6th trade fair on Microencapsulation co-organized by South West Research Intitute in San Antonio, Texas, USA on March 7-9, 2011
- The 19th International Conference on Bioencapsulation, held in Amboise, France on October 5-8, 2011

The three events where a success both in terms of attendance and the quality of the contributions (see Table 1).

FINANCIAL REPORT

The accounting of the Valdivia meeting was handled directly by the Austral University. Table 2 and 3 provides a summary of the incomes and expenses for the San Antonio and Amboise meetings. The following remarks can be made:

- For San Antonio, part of the registration took place in 2010
- Taking this into account, the balance for this event is 19 633 euros
- For Amboise, the grants amount to 23450 euros as free registration and 11 500 euros for travel expenses.
- The profit of the San Antonio Symposium and LVMH sponsorship was dedicated to the conference grants

Table 1	Participants					Contributions		
2011 events	Industrials	Academics	Students	Exhibitors	Total	Orals	Posters	1to1*
Valdivia	7	47	59		113	14	22	
San Antonio	112	13		14	139	10		678
Amboise	41	97	59	8	205	39	92	

* 1to1: one to one meeting between participants

Table 4 provides the accounting for the association restricted to operations carried out in 2011 (Amboise and San Antonio being considered as single operations). The net negative balance (3065 euros) may be compensate by 5374 euros collected in 2010 for the San Antonio symposium, resulting mainly in a financial balance. The global volume of exchanges is 204 000 euros.

STEERING COMMITTEE

General Assembly has unanimously appointed the following people until next General Assembly to be held at the end of August 2013:

- Denis Poncelet (President)
- Thierry Van Damme (Secretary)
- Ron Neufeld (Teasurer)

The steering committee will be completed by a group of people taking responsibility for the organisation of the activities in 2012 and 2013 (see last page).

The General Assembly entrusts the Steering Committe to revise the BRG status.

2012-2013 ACTIVITIES

Three events were organised in 2012 :

- 15th Industrial Symposium and 6th trade fair on Microencapsulation held in Archamps, France on March 20-22
- South American Symposium on Microencapsulation held in Limeira, Brasil on April 30 - May 2, organised by Prof. Silvia Prata Soares from Unicamp.
- 20th International Conference on Bioencapsulation, held in Orilllia, Ontario, Canada on September 21-24, organised by Prof. Neufeld from Queen's University.

Three events are scheduled for 2013:

- A training school on Microencapsulation, to be held in Nantes, France on April 9-12, 2013
- 16th Industrial Symposium and 7th trade fair on Microencapsulation held in Madison, WI, USA on June 25-27, 20123, co-organized by Don Josefchuck from Encapsys
- 21th International Conference on Bioencapsulation to be held in Berlin, Germany on August 28-30, co-organized by Stefan Drush from TU Berlin and Thorsten Brandau from Brace Gmbh.

Three issues in the BRG newsletters are already scheduled:

- In October, one issue will be dedicated to biomedical applications of microcapsules, edited by Paul De Vos from Groningen Medical University, the Netherlands
- In December, a special issue on the last annual conference, based on the 10 best student contributions
- In February, Yves Freres from CNRS (France) and Bojana Boh from Lubljana University will edit an issue on chemical methods of encapsulation.

Other issues are in preparation.

CLOSING.

As no questions were raised by the participants, the General Assembly was closed by the president.

Table 2 : 2011 Amboise Conference	
Registration	134 671 €
Sponsoring	13 000 €
Reception & acommodation	-83670 €
Proceedings & printing	-11 333 €
Organisation & management	-13 814 €
Divers	-6 981 €
Grants	-34 950 €
Balance	-3077 €

Table 3 : 2011 San Antonio Symposium	
Registration in 2010	5 374 €
Registration in 2011	77 312 €
Reception & acommodation	-24375 €
Proceedings & printing	-4610 €
Organisation & management	-25 539 €
Missions & Speakers	-7 620 €
Divers	-2609 €
Balance (12 559 € in 2011)	19 633 €

Table 4 : 2011 BRG accounting	
2011 San Antonio Symposium	12 559 €
2011 Amboise Conference	-3 077 €
2011 subscription + divers	-3 565€
2010 meeting residual invoices	-5 370 €
2012 Archamps invoices (renting)	-4 830 €
2012 Archamps registration	2 000 €
2011 Divers (web site, newletters)	-5 912 €
Balance	-3 065€

Bioencapsulation Research Group

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Bioencapsulation Research Group is a non-profit association promoting networking and research in the encapsulation technology of bioactives. It organises academic conferences and industrial symposiums, publishes newsletters and manages a website.

More information : http://bioencapsulation.net

KEEP CONTACT BY REGISTERING ...

Registration is based on a voluntary annual fee. If you wish to simply receive the newsletter and be advised about future events, register online at: http://bioencapsulation.net

Be an active member pay the registration fee and get more services

- Reduced registration fees to BRG events
- Full access to conference proceedings (> 1700)
- Access to the forum and internal mailling
- Possibility to contribute to the newsletter
- Reduction for the conference registration
- Priority for awarding of conference grants

Class	Annual fees
Industry members	100 €
Researchers [1]	60 €
Students [2]	30 €
Honorary member and corporate registration [3]	1000 €

[1] public and non-profit organizations, contact us for group registration
[2] registered for a master or PhD program, less than 30 years old.
[3] Open access to 1 full page in 1 issues (1/2 page in 2 issues ...) in the newsletter
Registration fees may be paid by credit card (preferably), bank transfer or cheque.

For more information or an invoice, see the registration page on http://bioencapsulation.net

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REGISTRATION DATA

- Title: .....
- First name: ..... Last name: .....
- Affiliation: ..... Department: .....
- Address: ..... Address (cont.): .....
- Zipcode: ..... City: .....
- State: ..... Country: .....
- Phone: ..... Fax: .....
- Email: ..... Website: .....
- Password: ..... Repeat password: .....
- Registration class: ..... Registration fees: ..... €

Send your registration to : Bioencapsulation Research Group 5 rue de la maison blanche44240 Sucé sur Erdre France