Polymers for microencapsulation in biomedicine

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INTRODUCTION

Grafting of therapeutic cells for treatment of human disorders such as hormone or protein deficiencies is not yet clinically applied on a large scale due to the necessity to use life-long immunosuppression for preventing rejection. The necessity to apply immunosuppression can be bypassed by immunoisolating hormone- or protein–secreting cells in semipermeable membranes to protect donor-cells against antibodies and cytotoxic cells of the host immune system. This immunoisolation by encapsulation not only allows for successful transplantation of cells in the absence of immunosuppression (Lim et al. 1980, Soon-Shiong 1992, De Vos et al. 1997) but also for transplantation of cells from nonhuman origin, *i.e.* xenografts, which could be a mean of overcoming the obstacle of limited supply of donor tissue (Omer et al. 2003, Zimmermann et al. 2005). The principle applicability of the technology has been shown for the treatment of a wide variety of endocrine diseases, including anemia (Koo et al. 1993), dwarfism (Chang et al. 1993), Hemophilia B (Liu et al. 1993), kidney (Cieslinski et al. 1994) and liver (Wong et al. 1986) failure, pituitary (Aebischer et al. 1986) and central nervous system insufficiencies (Aebischer et al. 1994), and diabetes mellitus (Lim et al. 1980).

Microencapsulation of cells or tissues in alginate-based capsules, as originally described by Lim and Sun (1980), is the most commonly applied procedure for immunoisolation. During recent years, important advances have been made with this technology. In spite of the simplicity of the concept of microencapsulation and the urgent need for alternatives to immunosuppressives in transplantation, the progress in the field during the past decades could not meet with the high expectations. A casual factor in this has been insufficient knowledge of the microcapsule structure and properties in relation to its biocompatibility. Therefore, a number of groups including ours have performed a step-wise examination of the microcapsules properties and its concomitant biocompatibility. This has included *in vivo*, *ex vivo*, and chemical analysis of the capsules and grafts. Quite often this has led to design and application of new concepts. As a consequence, during recent years, important advances have been made in the basic knowledge of immunoisolation and the factors determining success and failure.

A pertinent factor, possibly the most important factor in the medical field, is the biocompatibility of the applied capsules. Biocompatibility is usually defined as the ability of a biomaterial to perform with an appropriate host response in a 'specific application' (William 1987). With fully artificial organs such as artificial hips, knees or middle ears this definition is easy to interpret. It is, however, far from simple to interpret with bioartificial systems such as the immunoisolation technology. With immunoisolating devices there is not only an interaction between the biomaterial and the tissues of the exterior, host environment but also between the biomaterial and the encapsulated donor tissue. Although this aspect is not covered by the current definition of biocompatibility, it should be considered a true biocompatibility issue since long-term survival of the tissue is required for this 'specific application'. Both issues will be discussed in the presentation.
Both intravascular and extravascular immunoisolation devices have been studied for application in treatment of endocrine disorders such as Diabetes. In general, extravascular devices are beneficial because it requires not more than minor surgery with minimal risk for the patients.

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Microcapsules have been the most intensively studied extravascular device because of the spherical shape and small size that offers an optimal surface to volume ratio and an optimal diffusion capacity when compared to the larger macrocapsules. Other advantages are that microcapsules cannot be easily disrupted, are mechanically stable, and do not require complex or expensive manufacturing procedures. Microcapsules can be produced from different materials and are being applied as planar beads or as coated, multilayered systems.

PREVENTION OF CELL ADHESION

Prevention of cellular overgrowth of microcapsules is considered to be a crucial factor in biocompatibility of microcapsules. For some applications of biomaterials, such as implantation of artificial joints, growth of host cells and coverage of the implant with host-cells is considered as a benefit and a process that promotes the functional performance of the implant. For microcapsules, however, the growth of host cells on the capsule surface is considered to have negative effects because of reduced diffusion of oxygen and nutrients to the encapsulated graft resulting in necrosis of the enveloped cells. In addition, the cells on the capsule surface are found to be mainly inflammatory cells secreting cytokines and chemokines that may have a negative effect on graft function.

In the past decade many groups have studied the applicability of hydrogels for extravascular encapsulation. Hydrogels provide a number of features which are advantageous for the biocompatibility of the membranes. Firstly, as a consequence of the hydrophilic nature of the material, there is almost no interfacial tension with surrounding fluids and tissues which minimizes the protein adsorption and cell adhesion (Figure 1). Furthermore, the soft and pliable features of the gel reduce the mechanical or frictional irritations to surrounding tissue (De Vos et al. 1999, De Vos et al. 2002). The most commonly applied materials for microencapsulation are being presented in table 1.

Main component of the capsule	Source	Initially proposed by
Alginate	Alga	Lim and Sun
Chitosan	Alga	Zielinski
Agarose	Alga	Iwata
Poly(hydroxyethylmetacrylate-methylmethacrylate)(HEMA-MMA)	Synthetic	Dawson
Copolymers of acrylonitrile (AN69)	Synthetic	Kessler
Polyethyleneglycol (PEG)	Synthetic	Cruise

Table 1: the most commonly applied biomaterials for producing hydrogels

A number of issues are critical when considering a biomaterial for application in a hydrogel-capsule. First we have to apply specific compositions that are not provoking aspecific or specific immune responses. Also we have to prevent that inflammatory contaminations are present in the crude materials. This has been demonstrated to have hampered progress in the field of application of alginate. Crude alginate from seaweed contains polyphenols, proteins, and endotoxins (Skjak-Braek 1989). Polyphenols are known to be toxic to cells while endotoxins are potent stimulators of the immune system. Polyphenols are also responsible for ORD-catalyzed depolymerisation of alginates (Haug et al. 1963). Therefore, purification of alginate is required before application as an implantation material. The vast majority of groups nowadays apply pure alginates with low content of endotoxins and lacking immunogenic effects.

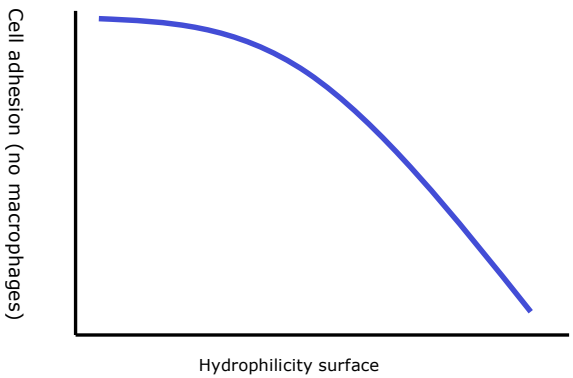

Figure 1: Decrease of cell adhesion with gradual increase of the hydrophilicity of the surface

INTERIOR BIOCOMPATIBILITY

The interior biocompatibility is defined as the (cyto)compatibility between the biomaterial and encapsulated tissue. It is an underestimated factor in the longevity of a graft. Many of the aforementioned hydrogels demonstrate excellent biocompatibility when the interaction with the host is considered but it is quite often not compatible with optimal functional survival of the cells. Eg it has been shown that encapsulation of islet-cells in capsules is associated with loss of 80% of the graft which is not acceptable when scare donor tissue is applied. Many do study the proliferation of cells before and after encapsulation and apply that as a measure for the interior compatibility. This however is not an adequate measure. It is essential to study also the functional performance of the cells in a specific application. We nowadays apply an assay to study the mitochondrial activity of the cells after encapsulation. This is done by quantifying the transformation of the tetrazolium salt WST-1 to a formazan-class dye by mitochondrial succinate-tetrazolium reductase. An advantage of this assay that it allows quantification of the mitochondrial activity of cells in intact capsules. This prevent artifacts or accelerated cell dysfunction associated with rupturing the capsules. By applying this assay in different applications we found that the compatibility of a specific material is very cell-source dependent. Also, it is dependent on the polymer concentration, the test-medium, the temperature, and the Ph. It is advisable to mimic the application as close as possible in order to perform adequate measures.

Other important considerations are to perform the measures after short but also after prolonged exposure to the capsule materials. Not rarely the capsule material is metabolized by the cell by which secondary, sometimes toxic, components are formed that are not observed in short term exposure studies. Also, we often observe disturbances in cell homeostasis and in the architecture of the cells. Figure 2 demonstrates giant cells with multiple nuclei that are formed in human CM (insulin producing) cells at two weeks after encapsulation. This phenomena is observed when the matrix in the capsule is having a high rigidity as the consequence of which the mitosis cycle cannot be full-filled. Cells that are in this multiple nuclei state do not function adequately and do not contribute to the functional performance the graft.

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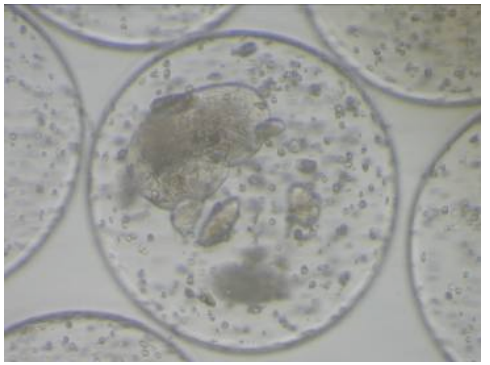

Figure 2: Multinucleated cells in a capsule at two weeks after encapsulation. The cells loose their functionality.

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