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# Contribution of polymeric materials to progress in xenotransplantation of microencapsulated cells - A review

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# Contribution of polymeric materials to progress in xenotransplantation of microencapsulated cells - A review

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**Abstract**: Cell microencapsulation and subsequent transplantation of the microencapsulated cells require multidisciplinary approaches. Physical, chemical, biological, engineering, and medical expertise has to be combined. Several natural and synthetic polymeric materials and different technologies have been reported for the preparation of hydrogels, which are suitable to protect cells by microencapsulation. However, owing to the frequent lack of adequate characterization of the hydrogels and their components as well as incomplete description of the technology, many results of *in vitro* and *in vivo* studies appear contradictory or cannot reliably be reproduced. This review addresses the state of the art in cell microencapsulation with special focus on microencapsulated cells intended for xenotransplantation cell therapies. The choice of materials, the design and fabrication of the microspheres, as well as the conditions to be met during the cell microencapsulation process, are summarized and discussed prior to presenting research results of *in vitro* and *in vivo* studies. Overall, this review will serve to sensitize medically educated specialists for materials and technological aspects of cell microencapsulation.

**Keywords**: Cell microencapsulation, hydrogels, mechanical resistance, permeability, biocompatibility, xenotransplantation.

**Abbreviations**: Alg-CS, alginate-cellulose sulfate; APA, alginate-poly(L-lysine)-alginate capsules; Ca-alg, calcium alginate; cryo-SEM, cryo-scanning electron microscopy; DDA, degree of deacetylation; ECM, extra-cellular matrix; FGF-1, heparin-binding growth factor 1; G, α-L-guluronic acid; HPC, hydroxypropyl cellulose; IL, interleukin; ISEC, inverse size-exclusion



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chromatography; LCST, lower critical solution temperature; M, D-mannuronic acid; MWCO, molecular weight cut-off; Na-alg, sodium alginate; NICCs, neonatal pig islet-like cell clusters; PEG, poly(ethylene glycol); PLL, poly(L-lysine); PLO, poly(L-ornithine); PMCG, poly(methylene-co-guanidine); PNIPAA, poly(N-isopropylacrylamide); PNVIBA, poly(N-vinylisobutyramide); RGD, arginylglycylaspartic acid; TNF, tumor necrosis factor; UCST, upper critical solution temperature; VEGF, vascular endothelial growth factor;

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## Introduction

Cell-based therapy is an attractive approach to treat several end-stage diseases. The Food and Drug Administration (FDA) defines cell-based therapy as “the prevention, treatment, cure or mitigation of diseases or injuries in humans by the administration of autologous, allogeneic or xenogeneic cells that have been manipulated or altered ex vivo" [1]. While the whole-organ transplantation is limited by the shortage of donors and the need of major surgery, cell-based therapy could overcome both obstacles. Indeed, xenotransplantation will offer an inexhaustible source of cells, and these cells could be delivered near the target site using non-invasive procedures.

In spite of the enormous potential of such approach, progress in the field of cell-based therapy has been hampered for several reasons, in particular due to issues of maintaining cell viability and of the identification of "non-self" cells by the immune system causing transplant rejection. Although better patient and transplant survival rates are achievable by the administration of immunosuppressive treatment, major challenges such as adverse effects associated with these drugs and the risks of long-term immunosuppression are still to be overcome.

The immobilization of cells within a hydrogel material has been identified as efficient strategy to provide mechanical and immune protection to the cells, and to maintain their viability and metabolic functionality for subsequent therapeutic applications [2-3]. Successful applications

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undoubtedly require a multidisciplinary input from materials scientists, chemists, biologists, engineers, and surgeons.

The focus of the present review will be on cell microencapsulation, even though the immobilization and protection of cells is achievable also by other techniques such as immobilization in films, extravascular chambers or in hollow fibers. This review provides an overview of suitable materials under study for cell microencapsulation and discusses the special features of the technologies applied so far. The paper is mainly addressed to medically educated readers working on developing therapies that rely on hydrogels. The selection of suitable material, the design and preparation of spherical hydrogels, as well as the main requirements to be fulfilled during the cell immobilization process are summarized and discussed. Recent trials towards transplantation of xenogeneic microencapsulated cells in order to treat congenital or acquired hormone/enzyme deficiencies as well as degenerative/inflammatory diseases complete this review.

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## Cell microencapsulation

***Microencapsulation*** denotes the physical entrapment of a gas, liquid, or solid within a surrounding material with dimensions in the micrometer range. It has gained interest in domains such as agriculture, food, cosmetics, construction, and analytics. Microencapsulation also includes the entrapment of biologically active substances such as cells, tissue, enzymes, bacteria,

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or DNA. For such applications, the term ***bioencapsulation*** is frequently used. An ambitious challenge in bioencapsulation is ***cell microencapsulation***, which denotes the entrapment of cells while maintaining their viability and metabolic functionality (Figure 1).

Cell immobilization in polymer-based hydrogels was first proposed in 1933 by Bisceglie [4], who demonstrated that insulin-producing cells remained viable and metabolically active after immobilization. Three decades later, Chang proposed the use of semi-permeable membranes as immune-isolating devices, and introduced the term "artificial cells" to define the concept of cell microencapsulation [5]. As shown in Fig. 1, cell microencapsulation offers protection against mechanical stress or deteriorating environmental effects. The surrounding hydrogel allows for bidirectional diffusion of molecules essential for cell metabolism such as oxygen and nutrients, and the release of metabolic products. Simultaneously, the passage of immune cells and antibodies is restricted, giving rise to an immunoprotection for the encapsulated cells. Therapies relying on microencapsulated cells could therefore result in the reduction or even avoidance of the administration of immunosuppressive drugs on the one hand, and on the other hand permit the transplantation of nonhuman cells, which is a promising alternative considering the limited availability of donor organs [6-7]. The therapeutic potential of the transplantation of microencapsulated cells has been reported for the treatment of a variety of diseases, including liver failure [8-10], renal failure [11-13], cancer [14], and diabetes mellitus [15-18].

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**Hydrogels for cell microencapsulation**

Since the pioneering work of Wichterle on cross-linked poly(hydroxyethyl methacrylate) [19], hydrogels have been of great interest to biomaterial scientists [20-24]. Hydrogels consist of a three-dimensional network of natural [25] or synthetic [26] polymer chains. Due to their high water content, hydrogels have been recognized to meet the requirements for bioencapsulation [27-30]. The cross-linking mechanism determines their classification as physical or chemical hydrogels. Hydrogels are called “physical” or “reversible” if the networks are held together by physical forces only. Chemical hydrogels, also referred to as “permanent”, are obtained when polymers having reactive groups link together via covalent bonds. Hydrogels used for cell microencapsulation are hereinafter designated as ***microspheres***, which is a generic name that refers to the size and shape of the materials. According to other classifications of microspheres, which take into account the physical structure of the hydrogel, microspheres are called ***microbeads*** if the hydrogel is radially homogeneous. Contrary, the term ***microcapsule*** is used if the microsphere is radially heterogeneous, for example, if a microbead was additionally coated with other polymers or if hydrogel surrounds a liquid core.

Physical hydrogels

Physically cross-linked hydrogels possess physical junction domains associated with chain entanglements, ionic or hydrogen bonding, and hydrophobic interactions [31-36]. The interest in



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these physical hydrogels is obvious since they are easily obtainable in a one-step process while avoiding the use of cross-linking agents. Only physical hydrogels obtained via ionic bonding or induced by temperature change are discussed herein.

***Physical hydrogels by ionic bonding***. The principle of preparing physical hydrogels via ionic bonding is schematically represented in Fig. 2. First, when a polyelectrolyte (polymer bearing many positive or negative charges) interacts with multivalent ions of the opposite charge, it may form a physical hydrogel known as "ionotropic" hydrogel. Second, when polyelectrolytes of opposite charges are mixed, they may gel depending on their polymer backbone constitution, concentrations, the ionic strength, as well as the pH of the solution. The products of such polyanion/polycation interactions are known as complex coacervates, polyelectrolyte complexes, or simplexes. A polyanion was selected in Fig. 2 as example to demonstrate the principle. The complexation of polycations is achievable similarly.

A description of polymers that have been explored in terms of their ability to form physical hydrogels via ionic bonding is presented below.

*Sodium alginate.* The designation sodium alginate (Na-alg) does not refer to a unique polymer structure but to a variety of polymers, which are composed of the same two monomeric units, β-D-mannuronic acid (M) and α-L-guluronic acid (G), but arranged in different linear sequences



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[37, 38]. As shown in Fig. 3, the monomeric units can be arranged as blocks of different lengths or randomly alternated in the polymer chains. Due to the advantageous gelling properties in contact with divalent cations, the Na-alg family is the most frequently used polymeric material for cell microencapsulation. However, the properties of alg-based hydrogels are very sensitive to the nature of the Na-alg and the preparation conditions. Consequently, the knowledge of the molecular and macromolecular characteristics of Na-alg is crucial for the production of defined hydrogels. Fig. 4 summarizes characteristics of Na-alg that influence the properties of alg-based hydrogels.

The chemical composition of Na-alg has an impact on the stability and permeability of the alg-based hydrogels [38-40], attributed to ionic bonding between G units and divalent cations, referred to as the egg-box model [41]. The importance of the G units is highlighted by the fact that the strength of alg-based hydrogels is directly related to the total content of G units and the average length of the G blocks in Na-alg [42]. Furthermore, it has been demonstrated that calcium alginate beads (Ca-alg) prepared from Na-alg with high G content are more permeable and exhibit less water uptake compared to Ca-alg prepared from Na-alg with high M content [43]. The composition-biocompatibility relationship, however, is still a matter of controversy. Some studies reported that Na-alg with a high content of M evoke an inflammatory response by stimulating monocytes to produce cytokines such as interleukin IL-1, IL-6 and TNF [44].

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Moreover, antibodies were found when high-M alg-based hydrogels were transplanted, but not in the case of high-G alg materials [45]. In contrast, other studies claim high-G alg-based hydrogels to be associated with more severe cell overgrowth [46]. In addition to the composition, the purity of the Na-alg cannot be neglected [47]. There is a consensus that *in vitro* and *in vivo* studies have to be conducted using highly purified Na-alg, free of endotoxin, proteins, and polyphenols.

The molar mass of the Na-alg and the concentration of its solution are further important parameters. Na-alg is available with molar mass in the range of 50 to 3000 kg/mol [48, 49]. The viscosity of a Na-alg solution increases with both the molar mass and the concentration of Na-alg. Therefore, a compromise between the molar mass of Na-alg and its concentration is needed to adapt the viscosity of the solution to a specific application in terms of cell type and technology. A selection of applications of Na-alg-based physical hydrogels is listed in Table 1.

In spite of favorable properties, alg-based physical hydrogels suffer from drawbacks such as limited mechanical stability, insufficient durability, and too high permeability. They are dissolved when chelators such as phosphate, lactate, citrate and non-gelling cations are present above a certain concentration [62]. To overcome such problems, subsequent coating of the initially formed hydrogels with polycations to form a polyanion-polycation complex has been proposed. Poly(L-lysine) (PLL), poly(L-ornithine) (PLO), and chitosan are the main polycations that have been suggested for the coating of alg-based hydrogels. A final layer of Na-alg is needed to neutralize the excess of positive charges, to turn the surface charge into negative, and thus to



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avoid biocompatibility problems caused primarily by attachment of proteins to a surface with positive charges.

*Poly(L-lysine) and poly(L-ornithine).* Since the first transplantation of islets of Langerhans microencapsulated in alg-based hydrogel coated with PLL was published [63], the application of PLL as coating material for alg-based hydrogels has been widely reported. Typically, the alginate-poly(L-lysine)-alginate (APA) microcapsule comprises three main components: a core of Ca-alg surrounded by an alginate-PLL complex and an outer coating of Na-alg. The coating reinforces the mechanical resistance of Ca-alg and allows for controlling the permeability. The surrounding polyanion-polycation complex reduces the osmotic swelling and thus stabilizes the microcapsule size. Numerous studies using APA to encapsulate cells have been reported [64-70]. Despite improvement of the physical properties of alg hydrogels upon coating with PLL, the immunological response upon transplantation of APA remains a major challenge. A multitude of studies have demonstrated that Ca-alg is better tolerated *in vivo* than polycation-coated microcapsules [71-73]. The proposed mechanism involves the adhesion of proteins to the surface of the hydrogel when this latter is exposed to blood, plasma or peritoneal fluid. This adhesion initiates the reactive protein cascades and serves as cellular anchor [74-78]. To overcome these biocompatibility issues, different methods have been proposed to reduce the protein adsorption to the surface of APA. For instance, grafting biocompatible poly(ethylene glycol) (PEG) pendent



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chains [79-81], optimizing the APA composition [82] and size [83], or using epimerized Na-alg [84] were reported.

Other efforts were directed towards exploring the suitability of other polycations as coating materials. It has been suggested that using PLO has advantages compared to the use of PLL [85-87]. PLO is more hydrophilic than PLL due to the difference in the chemical structure, as shown in Fig. 5. PLO binds more efficiently to the surface of Ca-alg, which in turn has a positive impact on the physical properties of the microspheres. Microspheres coated with PLO exhibited higher hydrophilicity, improved mechanical properties, and superior resistance to swelling and damage under osmotic stress [88, 89]. In terms of biocompatibility, however, the suitability of using PLO instead of PLL is still controversial. Likewise PLL, the immunological response to PLO containing microcapsules was reduced by PEGylation of the outer layer [90]. While some studies claim that promising results are obtained when replacing PLL by PLO [91-93], others suggest that PLL remains the best option [94, 95].

*Chitosan.* Chitosan is a linear polysaccharide consisting of randomly distributed $\beta(1\rightarrow4)$ linked D-glucosamine and N-acetyl-D-glucosamine units, as illustrated in Fig. 6. The cationic properties of chitosan depend on the degree of deacetylation (DDA), which is the fraction of glucosamine units. The molar mass depends on the source and isolation procedure, and it can reach values up



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to 500 kg/mol. Lower molar masses and oligomers are obtained by chain degradation [96]. Chitosan is a non-permanently charged cationic polyelectrolyte. Its charge density and solubility strongly depend on the DDA and pH. With a $pK_a$ value of approximately 6.5, chitosan is positively charged and soluble in acidic to neutral media, only if the DDA exceeds 60%. Exceptions are oligomers.

Similarly to PLL and PLO, chitosan has been applied to coat Ca-alg or Ba-alg [97]. However, microspheres with a Ca-alg core covered by an alginate-chitosan complex membrane can also be made in a one-stage process by dropping Na-alg into an aqueous solution containing chitosan and calcium ions [98]. The opposite process, dropping chitosan and $Ca^{2+}$ solutions into Na-alg yields microspheres with a chitosan core and a chitosan-alginate membrane [99]. The stability, permeability, and biocompatibility of alg-chitosan microcapsules were intensely studied [100-106]. Several cell types were successfully encapsulated in alg-chitosan microspheres including islets of Langerhans [107, 108], hepatocytes [109-114], and mesenchymal stem cells [115, 116].

*Sodium cellulose sulfate and poly(methylene-co-guanidine).* Alginate-cellulose sulfate-poly(methylene-co-guanidine) microcapsules (alg-CS/PMCG) are prepared by a two steps process. First, a polyanion blend of Na-alg and CS is gelled in the presence of calcium ions. Second, a membrane is formed via the addition of PMCG. It was demonstrated that both mechanical resistance and permeability of alg-CS/PMCG are tunable by changing the ratio of Na-



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alg/CS in the polyanion blend and the chemical composition [117-119]. A subsequent coating with lower molar mass Na-alg allowed adjusting the permeability over a wide range, suitable for cell microencapsulation and immunoprotection, without compromising the durability of the microspheres. A number of studies using alg-CS/PMCG microcapsules were published [120-125]. However, the *in vivo* biocompatibility remains a major issue. Indeed, when a human whole blood model was used to assess the inflammatory properties of alg-CS/PMCG microspheres, they have triggered complement and leukocyte activation over time, although they were still less activating than PLL-containing microcapsules [71].

***Physical hydrogel by temperature-response***. The preparation of temperature-responding hydrogels is emerging as a promising tool for various biomedical applications [126], including cell microencapsulation. Such hydrogels are obtained from polymers that respond to temperature change and undergo a sol-gel transition [127, 128]. Derivatives of methylcellulose [129], chitosan [130], hydroxypropyl cellulose (HPC) [131], poly(N-vinylisobutyramide) (PNVIBA) [132], and poly(N-isopropylacrylamide) (PNIPAAm) [133-136] have been reported to exhibit gelation upon temperature change. The latter is a very attractive temperature-responsive polymer since it exhibits a sharp sol-gel transition in water at 34.3°C. Thus injecting a polymer solution prepared at room temperature (rt) can lead *in situ* to the formation of hydrogels at 37°C.

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Moreover, the formation of hydrogels from PNIPAAm is tunable by changing the preparation conditions [137-139].

Chemical hydrogels

Chemical hydrogels are mostly applied in cell microencapsulation when high mechanical resistance and long-term durability are required. For this purpose, several combinations of polymers and preparation conditions have been tested in regards to their suitability to encapsulate cells within chemical hydrogels. However, only a limited number of combinations have been identified for this purpose. Table 2 lists some examples that have been used to immobilize cells. Numerous criteria must be considered when designing a chemical hydrogel for cell microencapsulation. The process of hydrogel formation must not negatively influence cell integrity and viability and should not involve harsh conditions, toxic solvents and reactants [158]. Because cells are suspended in a liquid precursor solution prior to the encapsulation process, the choice of precursors is limited to water-soluble components. The aqueous solution must be buffered with appropriate osmolality to prevent cell lysis. The rheological properties of the precursor solution are crucial to maintain cell viability and cell-cell adhesion during the encapsulation process. Mixing cells with highly viscous solutions can lead to a significant decrease in cell viability [159].



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Hybrid hydrogels

Novel hydrogel types that combine physical and chemical cross-linking, are being emerging as adequate candidates for cell microencapsulation. The physical interactions allow for fast gelation and spherical shape formation, while biocompatible covalent cross-linking ensures the reinforcement of the hydrogel networks, along with tunable permeability and gel stiffness.

For the preparation of APA microspheres with covalent cross-linking between adjacent layers, PLL was equipped with phenyl azide residues that create covalent bonds with Ca-alg when irradiated with UVA [160]. The photo-initiated cross-linking was shown to be cell compatible, and yielded stable microspheres up to 3 years in alkaline buffer (pH 12), whereas standard APA disappeared within 1 min. Another strategy reported the replacement of the final alginate layer of APA capsules by poly(methyl vinyl ether-alt-maleic anhydride) (PMM) and poly(vinyl dimethyl azlactone-co-methacrylic acid) (PMV) to form stable covalent amide bonds with PLL, neutralizing the polycation layer [161].

Further, the involvement of methacrylate polymers into physical microspheres, and subsequent covalent cross-linking was reported [162, 163]. It was demonstrated that either cross-linked shells or cross-linked cores are obtainable by adjusting the molar mass of the cross-linker. Approaches based on chemical cross-linking through complementary reactive groups attached to two oppositely charged polyelectrolytes were also investigated [164, 165]. Similarly, microbeads were prepared by ionotropic gelation of a combination of Ca-alg and sericin as inner core



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followed by coating with chitosan and further cross-linking with genipin [166, 167]. This combination effectively reduced swelling and physical disintegration of the microspheres induced by non-gelling ions and calcium chelating agents. Higher resistance to mechanical shear force and improved durability against enzymatic degradation were achieved. The entrapment of vinyl sulfone terminated PEG in Ca-alg and subsequent Michael-type cross-linking has been reported. The mechanical properties of such hybrid microspheres were adjustable and suitable for cell microencapsulation [168-170].

Rather than using hydrogels obtained from oppositely charged polyelectrolytes bearing complementary reactive groups for chemical cross-linking, one-component hybrid hydrogels are also being developed and tested for cell microencapsulation. A one-component system denotes hybrid hydrogels prepared from polymers able to form simultaneously both physical and chemical links. Such polymers are very often a modified macromolecule or biopolymer. For instance, the preparation of hybrid alg-based microspheres was achieved by equipping Na-alg with azide-terminated PEG pendent chains [171]. The azide end group forms chemical cross-links via the Staudinger reaction by incubation in a gelation bath containing phosphine-functionalized agents. Human pancreatic islets were encapsulated using such systems [172-174]. Similarly, Na-alg with thiol end groups was prepared [175, 176]. The modified Na-alg maintained the gelling capacity in the presence of calcium ions, while the thiol end groups ensured the preparation of a chemically cross-linked network via disulfide bond formation. Being



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biocompatible, spontaneous, and catalyst free, the formation of disulfide bonds yielded hybrid microspheres in a one-step extrusion process under physiological temperature, pH, and osmolality. Good survival rate and improved proliferation were obtained upon microencapsulation of liver-derived cells within hybrid microspheres [177].

Nonetheless, the strategies described above mainly focused on the robustness of the biomaterials but other parameters must also be underlined. The lifetime and biocompatibility of encapsulated cells should be enhanced to overcome the problem of graft failure [178]. Actually, the survival of engrafted cells depends on the cells adhesion to the hydrogel matrix, on the vascularization improving the delivery of nutrients, oxygen and metabolites but also on the immune response of the host body. For this purpose, hybrid hydrogels containing peptides or proteins were developed. Mimicking the ECM, and the covalent incorporation of adhesives ligands such as galactose [179], RGD [180], and other types of laminin-derived recognition sequences and collagen type I sequence [181] have been reported. Besides these molecules, proteins such as fibrin [182] and collagen [183] but also glycoproteins [184] were also encapsulated to enhance cell viability. Moreover, the co-encapsulation of vascularization promoting factors such as VEGF [185] and FGF-1 [186] promoted the neovascularization by improving the viability of engrafted encapsulated islets. Last but not least, co-encapsulation of anti-inflammatory agents such as complement receptor 1 sCR1 [187], the chemokine CXCL12 [188] or the drug dexamethasone [189] reduced the inflammatory response and thus prolonged the graft survival. Also, anti-



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cytokine agents [190, 191] were covalently attached on the hydrogel matrix sequestrating the pro-inflammatory cytokines highly expressed in wounded environment.

Properties of hydrogel microspheres and their assessment

In addition to the size and size stability as well as chemical stability, the most important characteristics of microspheres, which determine the applicability for subsequent transplantation, are their mechanical resistance, permeability, and biocompatibility. The selection of suitable methods to assess these parameters is not always a straightforward decision.

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***Mechanical resistance***. The mechanical resistance of hydrogel microspheres is not an absolute parameter. It can be evaluated by several methods, each of them providing different information. One method to assess the mechanical resistance is the osmotic pressure test. The microspheres are simply exposed to water [192]. The sudden influx of water causes the microspheres to swell and break. A method to study the resistance to mechanical stress is the microsphere shearing test. A suspension of microspheres is subjected to a controlled fluid shear [193]. The number of broken microspheres as a function of the applied mechanical stress gives an indication of their mechanical resistance. Such essays have the advantage of being very simple. However, they have limitations:

1) only qualitative information, restricted quantification; 2) the osmotic pressure test is reliably applicable only to physical hydrogels.

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Contrary, the evaluation of the resistance and deformability of the microspheres under compression is considered a quantitative method [194]. A mobile probe compresses the microspheres at a defined speed. Microspheres having the same size can be characterized and compared by measuring the force corresponding to the rupture of the microsphere, and by comparing the mechanical resistance at a given degree of compression [195, 196]. Dependent on the equipment sensitivity and the hydrogel stiffness, two methods are commonly used:

- Each microsphere is individually compressed. The mechanical resistance of 20 to 30 microspheres needs to be analyzed to obtain statistically meaningful data.
- A layer of microspheres is compressed. This method is suitable for capsules that burst at low force. A narrow size distribution is the prerequisite.

Although several methods have been proposed to study the mechanical properties of hydrogels, only few have focused on measuring the mechanical properties of cell-embedding hydrogels. Ahearne et al. reported a non-destructive, online and real-time method that allows measuring the mechanical properties of hydrogels with incorporated cells [197].

***Permeability***. The ability to deliver metabolic products and therapeutic proteins, but to block the diffusion of immune cells and antibodies, is the basis of the use of cell microencapsulation in biomedical applications. A defined and controllable permeability is therefore an essential prerequisite of hydrogel microspheres intended for transplantation.



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The permeability of microspheres can be described by the mass transfer or diffusion coefficient [198, 199]. It defines the rate of diffusion of a given solute into the hydrogel microsphere. Another quantification is the minimum size of a solute, which is completely excluded from the hydrogel pores. This is usually referred to as the exclusion limit or molecular weight cut-off (MWCO). The determination of solute permeation can be either by diffusion into the microspheres (ingress) or from the microspheres (egress).

Several experimental techniques to assess the permeability of microspheres have been described relying on different solute types and monitoring methods for quantifying the chosen solute. These include, for example, dextrans and pullulans as solutes and spectroscopy (fluorescence, UV/vis), measurement of radioactivity, size exclusion chromatography with concentration-sensitive detectors, and protein assay kits as monitoring methods [200, 201].

An efficient technique that is being used for measuring the permeability is the inverse size-exclusion chromatography (ISEC) [202, 203]. It has the advantage that the MWCO and its distribution can be determined simultaneously. The drawback is that a minimum of 10 mL of microspheres is so far needed for each measurement. The optimization of this method to reduce the volume of hydrogel microspheres would be a great achievement.

The hydrogel mesh size, which is the space available between the macromolecular chains, has been also used to describe the permeability of hydrogel microspheres. Direct determination of the mesh size is achieved by cryo-scanning electron microscopy (cryo-SEM), a technique that images



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the internal structure of hydrogel microspheres on a nanometer scale [204]. However, the manipulation of the hydrogel during freezing is critical.

***Biocompatibility***. The assessment of the biocompatibility of hydrogel microspheres is a complex task due to the multitude of interactions between host body and foreign material and because biocompatibility is not simply a property of a material but a property of a biomaterial-host system [205]. Biocompatibility issues of microspheres are often connected with their ability to perform with an "appropriate host response" in a "specific application" [206]. A biocompatible hydrogel is considered to be a system that elicits no or not more than a minimal foreign body reaction. The success of cell microencapsulation intended for transplantation strongly depends on minimizing this immune response. Upon implantation of microencapsulated cells, the immune response is activated by the adsorption of proteins onto the microspheres surface, which will subsequently stimulate the recruitment of immune cells [207], and the rejection of the transplant through one of the many well-documented pathways [208, 209]. The immune response also elicits fibrosis around the hydrogels, which subsequently starves the encapsulated cells by limiting the diffusion of nutrients, and limits the efflux of bioactive molecules secreted from the cells [210]. Thus, most approaches developed to minimize the immune response to hydrogels are focused on preventing protein adsorption and cellular adhesion to the surface of hydrogels through the encapsulation of cells into biologically inert hydrogels, or modification of the hydrogel surface with biocompatible



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materials. Very recently, beads size and shape have shown to influence the immune response of the foreign body [211]. It was demonstrated that larger beads (1.5 mm) reduced immune reactions and fibrosis in comparison to smaller beads (0.5 mm) when using different materials, including alginate in rats and non-human primate models [212].

In addition to the host reaction, the cell compatibility of the hydrogel is a general biocompatibility prerequisite for a successful therapy using microencapsulated cells. Different cell types could require different hydrogel properties in terms of stiffness or hydrophobicity. In this context, also a minimum of microsphere volume was reported as being advantageous to ensure sufficient oxygen supply and to avoid necrosis [92]. This is in contradiction with results reported in [212].

**Cell microencapsulation techniques**

Extrusion techniques

Numerous techniques have been developed for the production of hydrogel microspheres [213, 214]. Most of them are based on forcing the passage of the cell-containing solution through a needle or a nozzle, and its extrusion into a gelation bath. The simplest method comprises dripping with only gravitational force as the driving force. This mode is restricted to low-viscous solutions and yields hydrogel microspheres with diameters in the range of 1.5 to 3 mm. However, the challenge in cell microencapsulation is to produce preferably microspheres with diameters in the



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range of 100 to 400 μm. Indeed, hydrogel microspheres with such dimensions have the advantage of a higher surface-to-volume ratio, exhibiting good transport of nutrients [215]. Subsequently, different processes of droplet formation, which is the first step of cell microencapsulation, are discussed and schematically shown in Fig. 8. A comprehensive database of different encapsulation technologies is available on http://www.genialab.de/WG3/.

***Droplet formation by coaxial air-flow***. The principle of the coaxial air-flow droplet generator [216] is the use of air-flow to separate droplets from a needle or nozzle tip before they fall due to gravity into a gelling bath. Surface tension gives the droplets their near-spherical shape. Microspheres in the range of 200-1000 μm with good uniformity are obtained. However, the size distribution significantly increases for diameters below 400 μm [217]. Reasonable production rates are attainable by optimization [218].

***Jet breakage by a vibrating nozzle***. Small (<1000 μm) and uniform (< 3 % size deviation) microspheres can be obtained by employing the jet-break vibrating technique [219]. The technology principle is that a laminar liquid jet breaks up into equally sized droplets by superimposed vibration of the nozzle. However, given that the droplet diameter obtained by this technique is 2 to 3 times larger than the nozzle diameter, the production of capsules with diameters < 400 μm needs extrusion nozzles of 150 μm. Therefore, this technique is not optimal



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for the microencapsulation of large cells or cell clusters. The high mechanical stress to which the cells are exposed might compromise their viability.

***Droplet formation by an electrostatic potential***. To obtain small uniform droplets, a high electrostatic potential between the needle and the receiving bath can be used [220, 221]. The diameter of the droplets can be tailored by controlling the electrostatic pulses [222-224].

***Jet breakage by rotating elements***. This technology is especially suitable for the production of large batches of microspheres. Droplets are formed by rotating nozzles, a rotating disk or a cutting wire [225].

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Emulsion techniques

Emulsion techniques allow the formation of microspheres with relatively small diameters. The aqueous phase (cell-containing solution) is mixed and dispersed in an organic phase. When the dispersion reaches equilibrium, gel formation is initiated by cooling or by the addition of a gelling agent. Although the emulsion process is advantageous for large-scale production [222-226], its use for cell microencapsulation is limited due to the broad size distribution generally obtained. Further, the use of an organic phase and the significant shear stress during emulsion may compromise cell survival [227].

Microfluidic-based cell encapsulation

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The recent advances in microfluidics and microlithography provided effective tools to control the formation of hydrogel microspheres with desirable shape, size, and size distribution [228-231]. Microfluidics allow for the preparation of hydrogels with defined morphology such as Janus particles, multi-compartment microspheres and non-spherical microgels [232-239]. Droplets are produced when mixing an aqueous precursor solution with an oil phase such as mineral oil, silicone oil, corn oil, hexadecane or fluorinated oil [240]. The droplets are further internally crosslinked by ionic gelation or by photo-polymerization to obtain cells entrapped in hydrogel microspheres [241]. Obviously, the immiscibility of the two phases is a prerequisite to ensure the formation of spherical droplets [242, 243]. However, the viscosity of the oil has been identified as an important factor controlling the formation of the droplets. The results have shown that highly viscous liquids are emulsified into larger droplets with lower polydispersity [244]. As a proof of concept, several cell types have been encapsulated within hydrogel microspheres using microfluidic droplet formation devices [245-251]. Moreover this technology exhibits ideal solution for single cell encapsulation [252, 253]. The production of a sufficient numbers of droplets in a short-time period remains a major drawback to be overcome. There is therefore a need for scale up studies to adapt this technology for cell microencapsulation [254].

Conformal polymer coating



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If the average diameter of a cell aggregate is considered as 150 µm, the total volume of a 450 µm diameter microcapsule is 27 times larger. This "dead" volume is an often-overlooked issue that can be related to a delayed metabolic response and a slowed diffusion of oxygen and nutrients causing cell necrosis, especially at the center of the microsphere. This issue may also represent a limiting factor if the transplantation of microencapsulated cells is aimed, as only limited space is available at the transplant site. To reduce the microsphere size, depositing a polymer coating directly on the surface of a cell was proposed [255]. As shown in Fig. 9, the total volume equals the size of the cell aggregate plus the thickness of the coating. The advantages from a mass transfer perspective are achieved because of the high ratio of surface to volume. The direct deposit of polymer coatings on the surface of cells has been achieved following different processes such as emulsification [212], discontinuous gradient density centrifugation [256], selective withdrawal [257], or interfacial polymerization [258]. Because of the relatively large cell or cell cluster diameter, conformal polymer coating was preferably applied to islets of Langerhans, using alginate [259], agarose [260], and PEG [261-263] and different chemical strategies were explored to modify covalently the surface of the cells [264]. Polymers such as PLL have been applied for conformal coating after conjugation with biocompatible molecules to attenuate their toxicity [265-267].

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**Cell microencapsulation for xenotransplantation**

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Sites of transplantation

Different surgical sites have been so far used for the transplantation of encapsulated cells [268, 269]. Choosing the surgical site is critical to provide capsules proper intimacy with the outside environment and graft vascularization. In addition to biological functionality, the ideal site for transplantation should be easily accessible for both placement and microcapsule retrieval.

To accomplish these needs, several studies have investigated the peritoneal site. Indeed, the peritoneum is currently considered by several authors a feasible and effective site to implant both encapsulated islets and hepatocytes [270-275]. However, it was also shown that the peritoneal cavity has less chance to provide sufficient oxygen to microencapsulated cells compared to the kidney capsule and muscle [270]. Nonetheless, the peritoneum has shown good results, when used as implantation site for encapsulated cells, despite impaired insulin secretion to glucose stimuli and progressive loss of function in islet transplantation has been reported. Furthermore it has been shown that the capsules are likely to float into the peritoneal cavity with lack of engraftment, clot formation and subsequent poor nutrition. On the other hand, the peritoneum allows the transplantation of large quantity of tissue and further infusions in case of loss of graft function with respect to other sites [276]. In addition, the biocompatibility can be significantly improved by using barium-alginate microcapsules [277]. To overcome low oxygen tension, some authors proposed the construction of an omental pouch providing a more efficient blood supply by capillary neoangiogenesis [278, 279]. Indeed, it was shown that encapsulated islets,



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transplanted into the omental pouch, are able to restore euglycemia in NOD mice without immunocytes infiltration around the capsules [279, 280].

The portal vein is traditionally used for human islet transplantation and it is now considered the standard site to make islets exert their function. Moreover closer contact to the vessels should provide efficient nutrition and blood supply. On the other hand the risk of portal vein occlusion and the difficult retrieval of the capsules (harboring both islets and hepatocytes) are important issues to be solved to optimize this site of implantation [281]. Nevertheless, portal puncture during acute liver failure for HT should be balanced as a highly hazardous maneuver due both to coagulopathy and the enhanced risk of occlusion in pathologic liver parenchyma.

Skin and subcutaneous tissue represent the most accessible site for microsphere transplantation regarding surgical implantation and retrieval [282]. Lack of blood supply could be improved by prevascularization of the site [283]. However, para-physiological conditions for the cells to work properly is difficult to obtain in these sites, thus the skin should be used for host-biocompatibility and immunoprotection tests. Vascularization could be improved by using the intramuscular site to maintain good surgical accessibility and graft monitoring.

The kidney capsule has been widely used for murine islet transplantation [284, 285]. It provides good surgical accessibility and retrievability. However, the volume of encapsulated cells required to achieve insulin independence makes this site unconvincing for clinical translation.

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Sources of cells for xenotransplantation

The use of porcine cells for xenotransplantation has been intensively studied, in particular for islet transplantation [286, 287]. Shin et al. demonstrated that adult porcine islets from pathogen-free miniature pigs transplanted into immunosuppressed diabetic monkeys induced fast glucose levels normalization. In addition, normoglycemia was maintained for more than six months in four cases, with no serious advert effects resulting from the transplantation [288]. Several reports highlighted the advantages to use neonatal pig islet-like cell clusters (NICCs) instead of adult pig cells. In particular, NICCs are easily digested and purified, show low level of T-cell response and high resistance to ischemia and inflammation [289]. Optimal functionality was observed after 12 days of culture [290] and tolerance to xenotransplantation of NICCs can be improved by treatment with expanded regulatory T cells or molecules targeting innate immunity [291]. The ideal age at which these cell clusters should be isolated from porcine donors was established to be during the first month of life, with slight advantages for pigs within the first week of life [292]. Pig-to-monkey islet xenotransplantation was also attempted from adult genetically engineered pigs to overcome the metabolic and immunological barriers between species. However, the multi-transgenic islet grafts did not show consistent long-term functionality for several months [293]. The use of pig cells was also investigated in the context of xenotransplantation for the treatment of acute liver-failure [294]. Alginate encapsulated re-aggregated neonatal pig liver cells demonstrated promising efficacy for the treatment of mice with acute liver failure. In addition,



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coating of the capsules with chitosan resulted in a reduction of the attachment of macrophages [295]. Recently, No et al reported on the development of a method for the tri-culture of three types of cells from rats to produce uniformly sized and shaped micro liver tissues. Encapsulated in collagen-alginate composites, these cells induced a long-term survival rate of 80% in mice with 90% hepatectomy [296]. Other sources of transgenic cells have been investigated. In particular, wide-type and transgenic tilapia donors were considered for the harvesting of islet cells [297, 298]. Transplantation of alginate encapsulated tilapia islets into diabetic mice resulted in long-term (up to 210 days) metabolic control but required immunosuppressive treatment [299]. The significant difference between tilapia insulin and human insulin is nevertheless a strong limitation to clinical application of tilapia islets in cell therapy protocols.

Translation of cell microencapsulation into a xenotransplantation cell therapy

Since the first reports, in the 1980s, of hydrogel encapsulation of islet-β-cells for the treatment of type I diabetes mellitus, many studies on animal models addressed the different parameters which can improve biocompatibility, stability and functionality of encapsulated cells. Table 4 lists some of the animal experiments and pre-clinical studies that evaluated the xenotransplantation of encapsulated islets and hepatocytes.

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The encapsulation of different islet types in Ca- and Ba-alg materials allowed restoring normoglycemia in xenogenic settings, without immunosuppression. Similarly, encapsulated xenogenic hepatocytes were able to revert acute liver failure in rodent models, in absence of immunosuppressive treatment. Despite these encouraging results, the demonstration of effective protocols in patient protocols is still very scarce. In 2011, Calafiore et al reported a phase I clinical trial testing alginate encapsulated islets, transplanted into the portal vein, in 4 type I diabetic patients obtaining transient insulin independence only in one of them, but showing safety, feasibility and biocompatibility of alginate capsules in humans [304]. More recently, Matsumoto et al published the results from a phase I/IIa xenotransplantation study of APA encapsulated neonatal porcine islets in 14 patients with unstable type 1 diabetes [305]. Without immunosuppressive treatment, the transplantation was safe and resulted in the reduction of unaware hypoglycemia events, but did not result in insulin independence.

**Concluding remarks, future directions and open questions**

The Holy Grail for transplant investigators is to avoid the use of immunosuppression. Encapsulation of cells and cell clusters could finally lead to this goal by setting up the conditions for long-term function cell therapy without the adverse effect of immunosuppressive agents. Nonetheless, adequate protection of cells from the immune system could expand the field of xenogeneic transplantation. However, despite the recent breakthrough-findings, crucial issues



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still remain unsolved. Purity and stability of encapsulation materials should be improved to gain effective biocompatibility [306]. Highly purified alginate-based materials have brought promising results, but capsular overgrowth is still an issue leading to inadequate blood and nutrients supply. Conformal coating of cells and cell clusters seems to be one possibility to overcome problems of cell malnutrition by reducing the distance between cells and capillaries and has shown better quality in terms of immunogenicity. Despite the progress achieved in biotechnology, the balance between immunoprotection and nutrition delivery requires further attention. Interestingly, necrosis of the central region of the encapsulated cells was identified in several studies as the common characteristic for graft failure and thus it was hypothesized that poor nutrition and lack of blood supply are the main causes of loss of function other than failure in immunoprotection [279].

There is no consensus about the ideal site of transplantation. Many reports gave evidence for the advantages of intraperitoneal placement of both encapsulated islets and hepatocytes. Peritoneum is well surgically accessible but the shortage of nutrition impairs the engraftment of encapsulated cells [307, 308]. Moreover the lack of contact with vessels causes a reduced insulin response to glucose stimuli for the islets. On the other hand, the omental pouch has shown sufficient angiogenesis potential and could be an ideal site for islet transplantation by virtue of direct portal delivery. Intraportal infusion has the advantage of providing more accessible blood supply to the encapsulated cells but it is affected by the risk of occlusion. Hence, optimization of the



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microcapsule size is advised in order to avoid unnecessary hydrogel dead-volume. Islet encapsulated therapy has been demonstrated to be effective in the treatment of diabetes in rodent models and large animals. Feasibility and biocompatibility studies in human beings have recently highlighted the potential of this fascinating technology in translational medicine, but effective results in patients are still lacking [309]. However, enough evidence has been gathered to justify clinical trials, in particular for porcine islet xenotransplantation [310]. The other therapeutic area, which might largely benefit from xenotransplantation, is the reversal of acute liver failure. In murine models, the engraftment of encapsulated xenogenic hepatocytes brought new advances in cell therapy [270, 271]. Xenogeneic sources of hepatocytes should be considered in translational protocols given the shortage of liver donors. Further studies in large animal models are required to prove the effectiveness of this strategy. However, encapsulated hepatocytes have shown, by metabolites de-tossification rate analysis, loss of function within 30 days after transplantation. This short-term cell functionality could be acceptable for therapies intending to provide a bridge for the native liver to regenerate upon acute failure, but is objectionable for a long-term function protocol in order to treat chronic liver diseases. In the near future, cryopreserved microencapsulated cells could be stored in a cell therapy bank with the aim to secure large quantities for patients. Also for this purpose, alginate-based hydrogels were successfully tested. Cryopreservation and re-warming did not negatively affect the function of microencapsulated



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hepatocytes [311, 312]. In conclusion, while the potential of cell microencapsulation for the development of cell therapies has been demonstrated in animal models, long-term studies are still scarcely reported. Despite promising results, translating in particular xenotransplantation of microencapsulated cells into a therapy will require further multidisciplinary efforts. The final success will not least depend on the development of suitable encapsulation materials and technologies.

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*Table 1*. Selected alg-based physical hydrogels applied for cell microencapsulation

| Divalent ions | Cell type | Target | Reference |
|---|---|---|---|
| $Ca^{2+}$ | BMSC[1] | Treatment of stress urinary incontinence | [50] |
| | Hepatocytes | Development of bio-artificial liver | [51] |
| | Pig islets | Impact of implantation sites on the biocompatibility | [52] |
| | ADSC[2] | Study of angiogenic and osteogenic potential of ADSC | [53, 54] |
| | CSP[3] | Therapeutic approach for cartilage regeneration | [55] |
| $Ba^{2+}$ | Rat islets | Study of islets function *in vitro* and *in vivo* | [56] |
| | Neuroblastoma | Cryopreservation of neurospheres by encapsulation | [57] |
| | WJMSC[4] | Optimized microencapsulation of MSC by vibrational nozzle | [58] |
| $Ba^{2+}/Ca^{2+}$ | Human islets | Viability and function after transplantation into diabetic mice. | [59, 60] |
| | ARPE-19[5] | *In vitro* study of encapsulated human retinal pigment epithelial cells | [61] |

[1]Bone marrow mesenchymal stem cells; [2]Adipose-derived stem cells; [3]Human mesenchymal progenitor cells from the subchondral bone marrow; [4]Wharton's jelly mesenchymal stem cells; [5]Human retinal pigment epithelial cells

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*Table 2*. Examples of chemical hydrogels applied for cell immobilization

| Material | Preparation | Cell | Reference |
|---|---|---|---|
| **Alginate** | Click reaction with tetrazine-norbornene modified hydrogel (no ionic cross-linkings) | EGFP-expressing 3T3 fibroblast | [140] |
| **PEG** | Thiol-ene reaction of PEG diacrylate with thiolated gelatin | Fibroblasts; keratinocytes | [141, 142] |
| | Maleimide, acrylate and vinyl sulfone-modified PEG cross-linked with peptides | C2C12 myoblast | [143] |
| | Photo-polymerization of fibrinogen-g-PEGacryloyl and PEG diacrylate | BMSC | [144] |
| | Photo-polymerization of PEG diacrylate | Huh-7.5 | [145] |
| **Chitosan** | Photo-polymerization of chitosan grafted with lactic acid and methacrylate | Chondrocytes | [146] |
| | Chemically cross-linked chitosan hydrogel loaded with gelatin | | [147] |
| | N-succinyl-chitosan gelation with aldehyde hyaluronic acid | | [148] |
| **Dextran** | Photo-polymerization of dextran with benzophenone | Osteoblast-endothelial cell | [149] |
| | Gelation of methacrylate and lysine functionalized dextran | Smooth muscle cells | [150] |
| | Photopolymerization of dextran-acrylate | Embryonic stem cells | [151] |
| **HA** | HA cross-linked via disulfide bond formation reaction | Fibroblasts, stem cells | [152] |
| | Methacrylated HA cross-linked by UV exposure | MSC | [153] |
| | Peroxidase catalyzed oxidation of tyramine-substituted HA | Chondrocytes | [154] |
| | Conjugate addition of thiol-modified HA onto PEG diacrylate | Adipocyte-stem cells | [155] |
| **PVA** | UV photopolymerization | L929 fibroblast | [156] |
| | Click hydrogels formed by hydrazone bonds | | [157] |

PEG: Poly(ethylene glycol); HA: Hyaluronic acid; PVA: Poly(vinyl alcohol); MSC: Mesenchymal stem cell

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*Table 3*. Xenotransplantation of encapsulated cells in animal models

| Transplanted model | Cell type | Transplantation site | Materials for encapsulation | Reference |
|---|---|---|---|---|
| Mice | Neonatal porcine islets | Peritoneal cavity | Ca-alg and Ba-alg | [273] [274] |
| | Rat islets | Subcutaneous tissue | Agarose / poly (styrene sulfonic acid) | [283] |
| | | Intraperitoneal space | Alg-PLL-PEG | [80] |
| | | Peritoneal cavity | Agarose | [187] |
| | Human islets | Omental pouch | Agarose | [279] |
| | | Peritoneal cavity | Ba-alg | [300] |
| | Neonatal pig hepatocytes | Abdominal cavity | Ba-alg | [295] |
| | Rat hepatocytes<br>Human hepatocytes | Peritoneal cavity | Alg-PLL-alg | [270, 271] |
| | Fish islets | Abdominal cavity | Ba-alg | [299] |
| Rat | Pig islets | Subcutaneous tissue | Ca-alg | [282] |
| | | Abdominal cavity | Ba-alg | [301] |
| | Guinea pig hepatocytes | Peritoneal cavity | acrylonitrile-sodium methallyl-sulfonate copolymer | [302] |
| Non human primates | Pig islets | Intraportal injection | No material | [288] [293] |
| | | Intraperitoneal space | Ca-alg-PLL | [303] |

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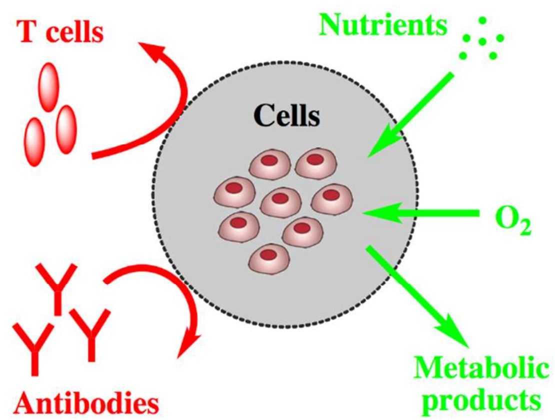


*Fig. 1*. Schematic representation of cell microencapsulation.

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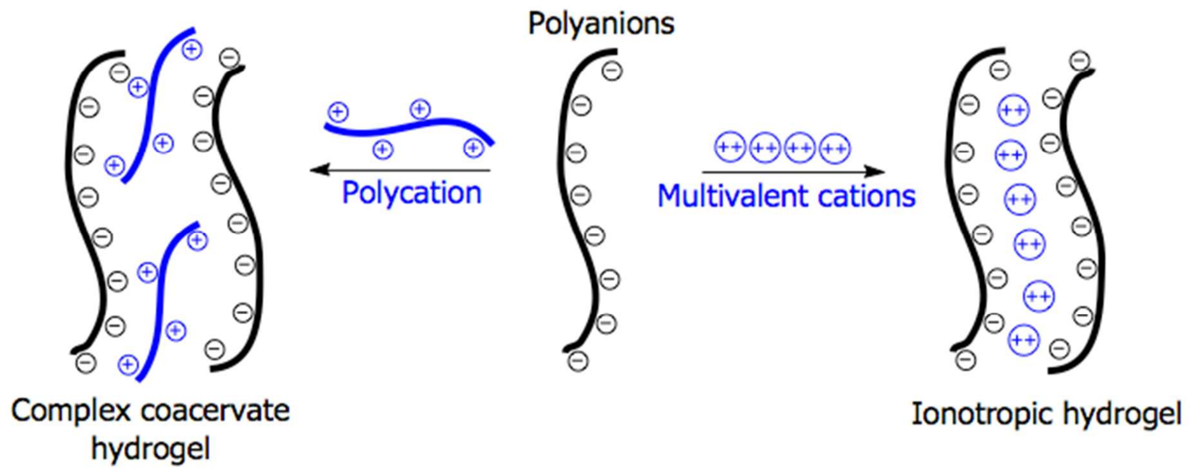


*Fig. 2.* Principles to form physical hydrogels by ionic bonding.

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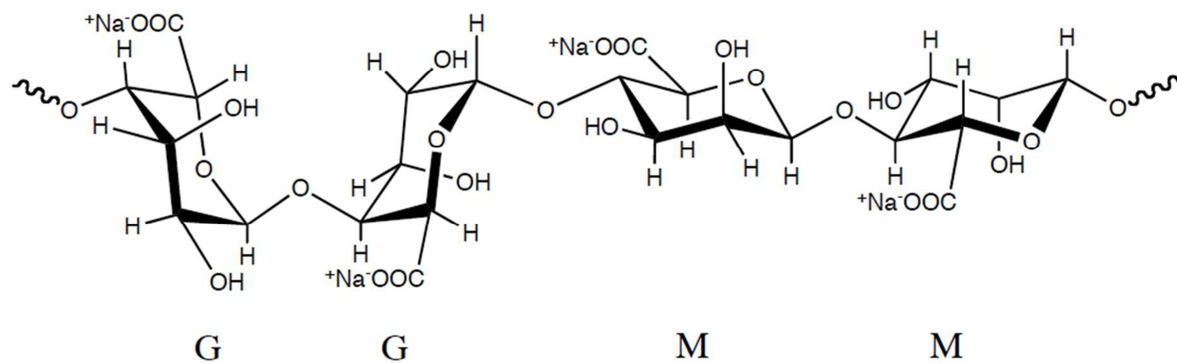


*Fig. 3.* Na-alg consists of α-L-guluronic and β-D-manuronic acid residues.

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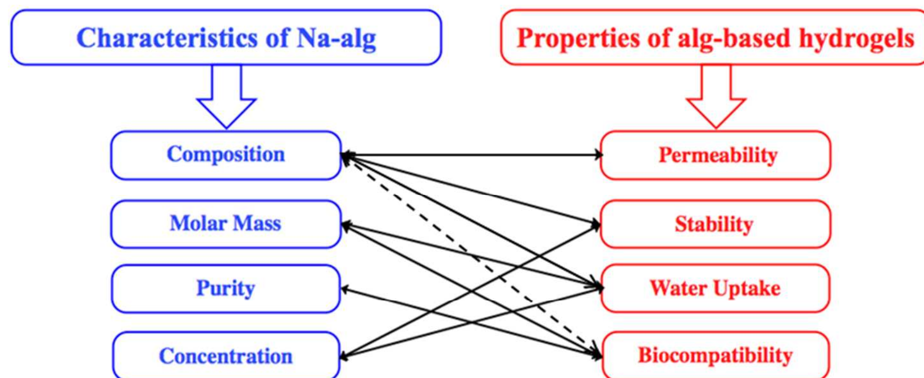


*Fig. 4.* The properties of alg-based hydrogels depend on the nature of the applied Na-alg, (solid line) confirmed influence, (dotted line) controversially discussed.

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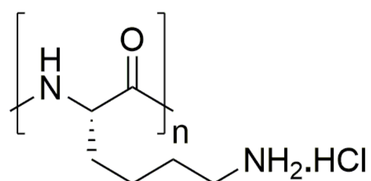


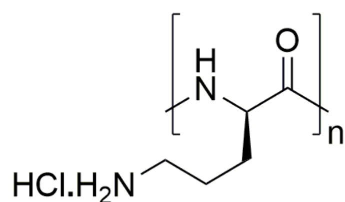


*Fig. 5.* Chemical structure of poly(L-lysine) hydrochloride and poly(L-ornithine) hydrochloride.

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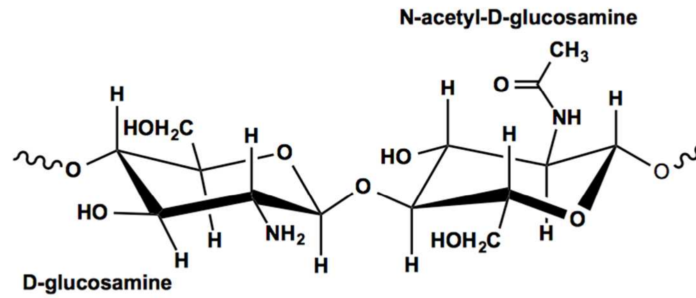


*Fig. 6.* The chemical structure of chitosan.

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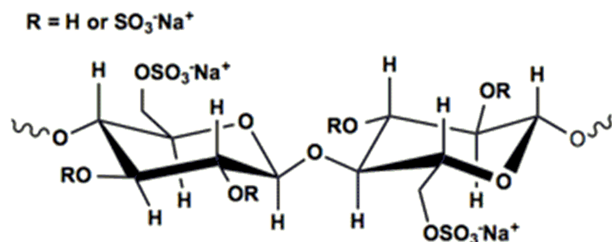


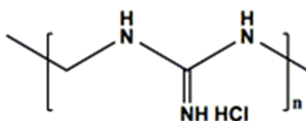


*Fig. 7.* The chemical structures of sodium cellulose sulfate (CS) and poly(methylene-co-guanidine) hydrochloride (PMCG).

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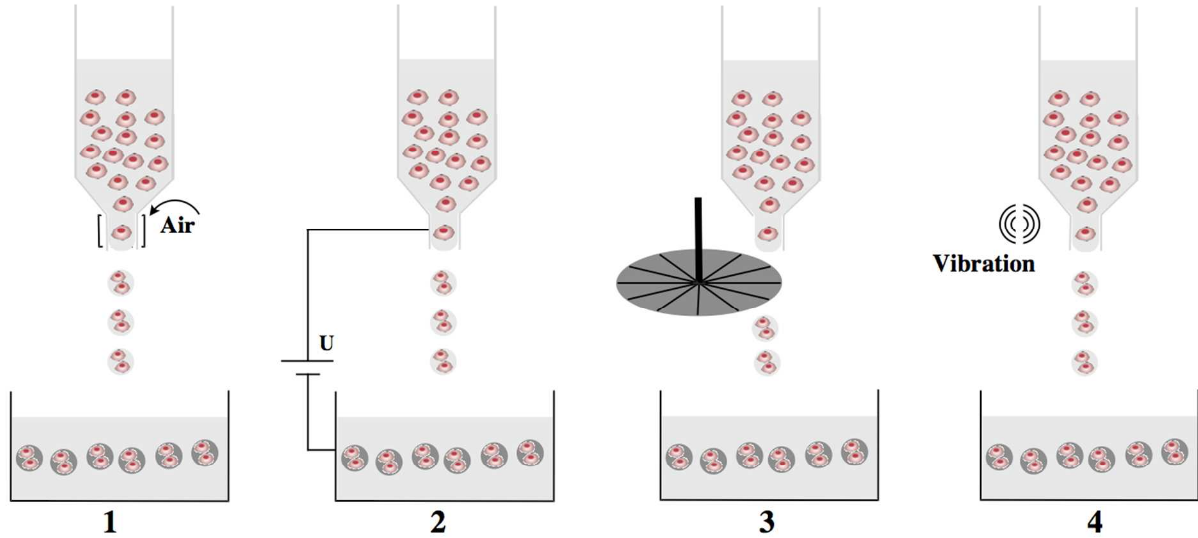


*Fig. 8.* Formation of droplets by: coaxial air-flow (1), electrostatic potential (2), rotating disk (jet cutter) (3), and vibrating nozzle (4). Cells are finally entrapped in hydrogel microspheres after falling down in the gelation bath.

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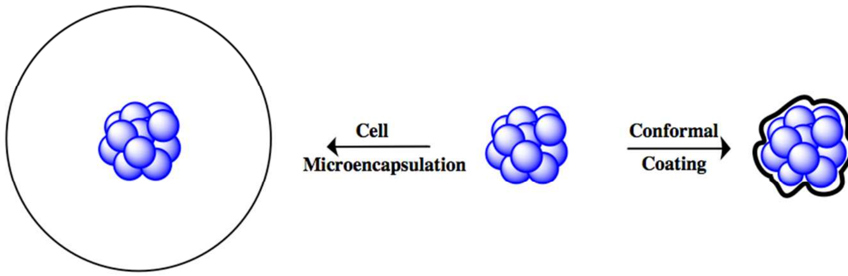


*Fig. 9.* Comparison of conformal coating and cell microencapsulation.

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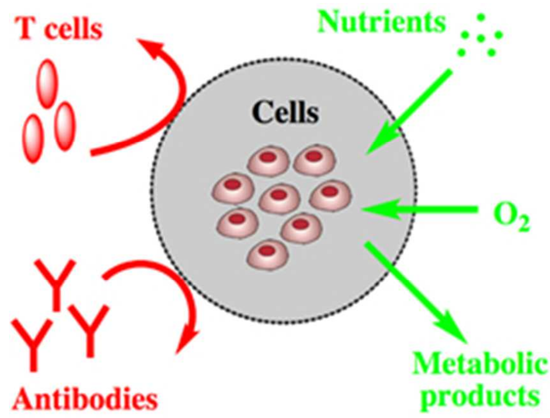


Figure 1. Schematic representation of cell microencapsulation
95x73mm (72 x 72 DPI)

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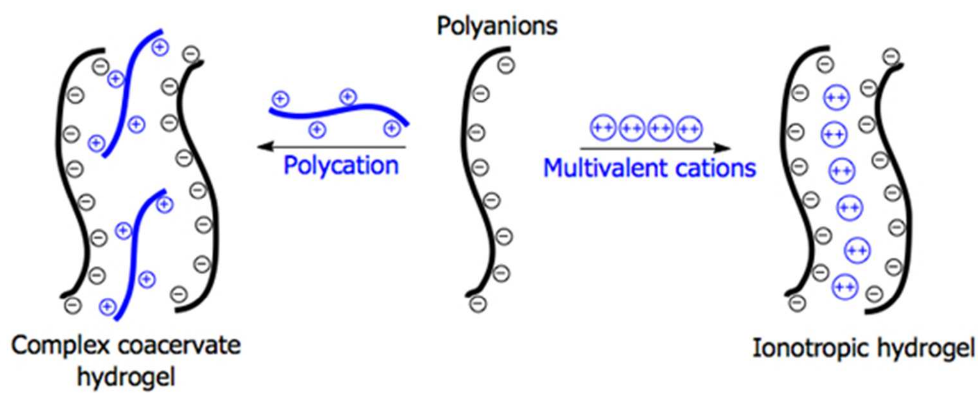


Figure 2. Principles to form physical hydrogels by ionic bonding
214x85mm (72 x 72 DPI)

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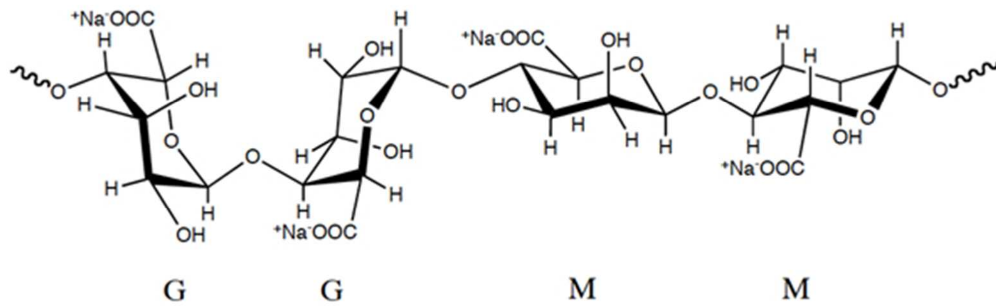


Figure 3. Na-alg consists of α-L-guluronic and β-D-manuronic acid residues
209x67mm (72 x 72 DPI)

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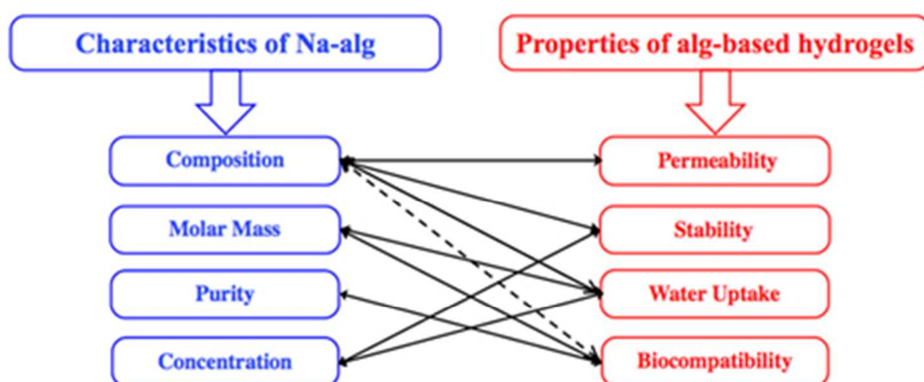


Figure 4. The properties of alg-based hydrogels depend on the nature of the applied Na-alg, (solid line) confirmed influence, (dotted line) controversially discussed
168x74mm (72 x 72 DPI)

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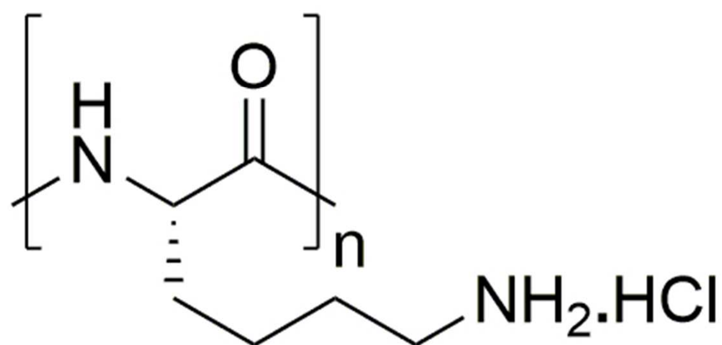
**Poly(L-lysine) hydrochloride**




**Poly(L-ornithine) hydrochloride**

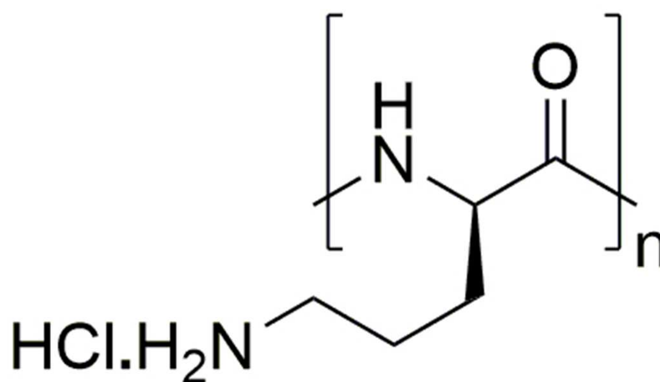



Figure 5. Chemical structure of poly(L-lysine) hydrochloride and poly(L-ornithine) hydrochloride
40x65mm (300 x 300 DPI)

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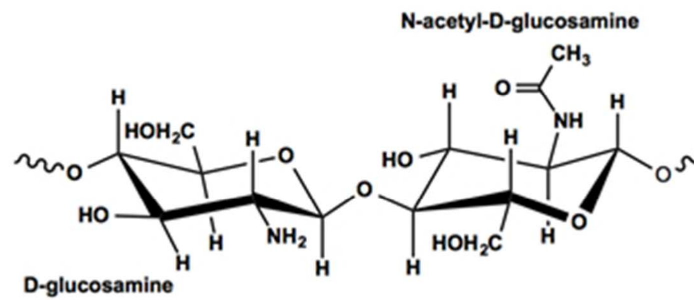


Figure 6. The chemical structure of chitosan
124x55mm (72 x 72 DPI)

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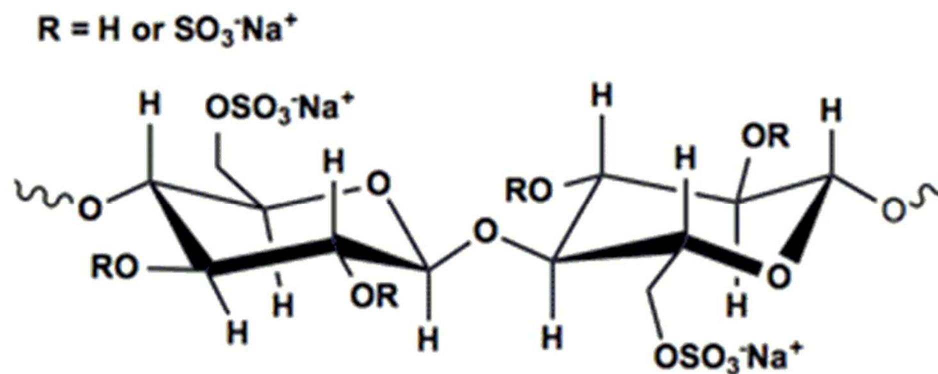


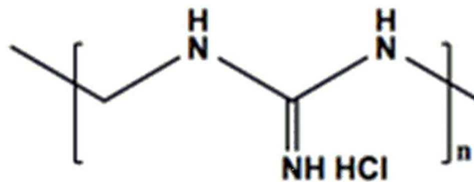


Figure 7. The chemical structures of sodium cellulose sulfate (CS) and poly(methylene-co-guanidine) hydrochloride (PMCG)
81x68mm (300 x 300 DPI)

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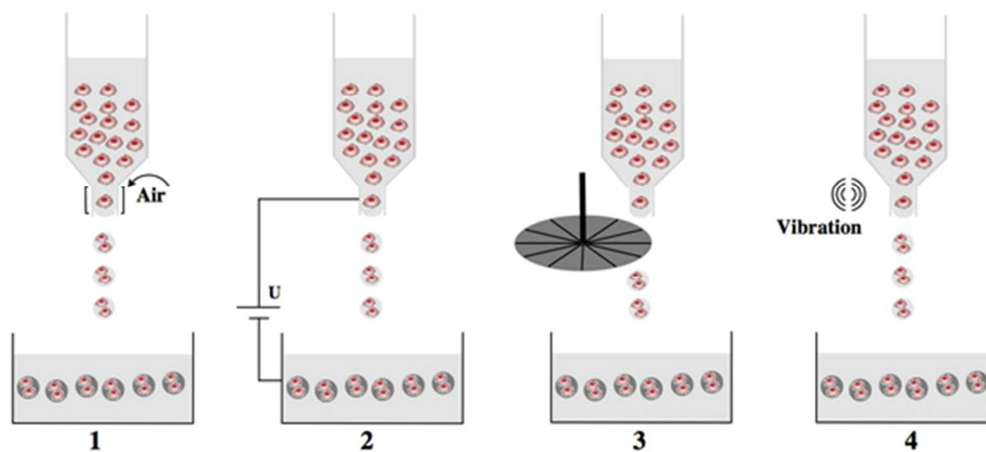


Figure 8. Formation of droplets by: coaxial air-flow (1), electrostatic potential (2), rotating disk (jet cutter) (3), and vibrating nozzle (4). Cells are finally entrapped in hydrogel microspheres after falling down in the gelation bath

213x96mm (72 x 72 DPI)

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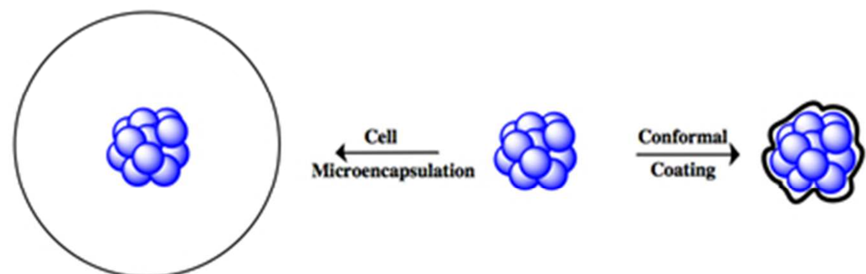


Figure 9. Comparison of conformal coating and cell microencapsulation
154x50mm (72 x 72 DPI)

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*Table 1*. Selected alg-based physical hydrogels applied for cell microencapsulation

| **Divalent ions** | **Cell type** | **Target** | **Reference** |
|---|---|---|---|
| **$Ca^{2+}$** | BMSC[1] | Treatment of stress urinary incontinence | [50] |
| | Hepatocytes | Development of bio-artificial liver | [51] |
| | Pig islets | Impact of implantation sites on the biocompatibility | [52] |
| | ADSC[2] | Study of angiogenic and osteogenic potential of ADSC | [53, 54] |
| | CSP[3] | Therapeutic approach for cartilage regeneration | [55] |
| **$Ba^{2+}$** | Rat islets | Study of islets function *in vitro* and *in vivo* | [56] |
| | Neuroblastoma | Cryopreservation of neurospheres by encapsulation | [57] |
| | WJMSC[4] | Optimized microencapsulation of MSC by vibrational nozzle | [58] |
| **$Ba^{2+}/Ca^{2+}$** | Human islets | Viability and function after transplantation into diabetic mice. | [59, 60] |
| | ARPE-19[5] | *In vitro* study of encapsulated human retinal pigment epithelial cells | [61] |

[1]Bone marrow mesenchymal stem cells; [2]Adipose-derived stem cells; [3]Human mesenchymal progenitor cells from the subchondral bone marrow; [4]Wharton's jelly mesenchymal stem cells; [5]Human retinal pigment epithelial cells

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*Table 2*. Examples of chemical hydrogels applied for cell immobilization

| Material | Preparation | Cell | Reference |
|---|---|---|---|
| **Alginate** | Click reaction with tetrazine-norbornene modified hydrogel (no ionic cross-linkings) | EGFP-expressing 3T3 fibroblast | [140] |
| **PEG** | Thiol-ene reaction of PEG diacrylate with thiolated gelatin | Fibroblasts; keratinocytes | [141, 142] |
| | Maleimide, acrylate and vinyl sulfone-modified PEG cross-linked with peptides | C2C12 myoblast | [143] |
| | Photo-polymerization of fibrinogen-g-PEGacryloyl and PEG diacrylate | BMSC | [144] |
| | Photo-polymerization of PEG diacrylate | Huh-7.5 | [145] |
| **Chitosan** | Photo-polymerization of chitosan grafted with lactic acid and methacrylate | Chondrocytes | [146] |
| | Chemically cross-linked chitosan hydrogel loaded with gelatin | | [147] |
| | N-succinyl-chitosan gelation with aldehyde hyaluronic acid | | [148] |
| **Dextran** | Photo-polymerization of dextran with benzophenone | Osteoblast-endothelial cell | [149] |
| | Gelation of methacrylate and lysine functionalized dextran | Smooth muscle cells | [150] |
| | Photopolymerization of dextran-acrylate | Embryonic stem cells | [151] |
| **HA** | HA cross-linked via disulfide bond formation reaction | Fibroblasts, stem cells | [152] |
| | Methacrylated HA cross-linked by UV exposure | MSC | [153] |
| | Peroxidase catalyzed oxidation of tyramine-substituted HA | Chondrocytes | [154] |
| | Conjugate addition of thiol-modified HA onto PEG diacrylate | Adipocyte-stem cells | [155] |
| **PVA** | UV photopolymerization | L929 fibroblast | [156] |
| | Click hydrogels formed by hydrazone bonds | | [157] |

PEG: Poly(ethylene glycol); HA: Hyaluronic acid; PVA: Poly(vinyl alcohol); MSC: Mesenchymal stem cell

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*Table 3*. Xenotransplantation of encapsulated cells in animal models

| Transplanted model | Cell type | Transplantation site | Materials for encapsulation | Reference |
|---|---|---|---|---|
| Mice | Neonatal porcine islets | Peritoneal cavity | Ca-alg and Ba-alg | [273] [274] |
| | Rat islets | Subcutaneous tissue | Agarose / poly (styrene sulfonic acid) | [283] |
| | | Intraperitoneal space | Alg-PLL-PEG | [80] |
| | | Peritoneal cavity | Agarose | [187] |
| | Human islets | Omental pouch | Agarose | [279] |
| | | Peritoneal cavity | Ba-alg | [300] |
| | Neonatal pig hepatocytes | Abdominal cavity | Ba-alg | [295] |
| | Rat hepatocytes<br>Human hepatocytes | Peritoneal cavity | Alg-PLL-alg | [270, 271] |
| | Fish islets | Abdominal cavity | Ba-alg | [299] |
| Rat | Pig islets | Subcutaneous tissue | Ca-alg | [282] |
| | | Abdominal cavity | Ba-alg | [301] |
| | Guinea pig hepatocytes | Peritoneal cavity | acrylonitrile-sodium methallyl-sulfonate copolymer | [302] |
| Non human primates | Pig islets | Intraportal injection | No material | [288] [293] |
| | | Intraperitoneal space | Ca-alg-PLL | [303] |

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