O7-2	**Two novel types of hybrid hydrogel microspheres for cell microencapsulation**

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INTRODUCTION AND OBJECTIVES

Hydrogel microspheres with sodium alginate (Na-alg) as major component are among the most studied materials for cell immobilization. Ionotropic gelation of Na-alg in presence of divalent cations yields such hydrogels. However, they suffer from mechanical stability deficiency, limited durability, and permeability drawbacks. Frequently used reinforcement with polycations requires multi-step processes and can have a negative impact on the biocompatibility (Rokstad 2011). Our approaches follow two strategies, which combine ionotropic gelation of Na-alg and covalent cross-linking of poly(ethylene glycol) (PEG) derivatives in one step and yield Na-alginate-PEG hybrid microspheres (alg-PEG-M and alg-PEG HH), partially convertible into PEG microbeads (PEG-M). The first strategy uses the fast gelling Ca-alg as a spherical matrix for simultaneous covalent cross-linking of PEG derivatives. Recently, we reported the physicochemical properties of alg-PEG-M, which can be designed in a range suitable for cell and tissue immobilization (Mahou 2010). For the second strategy, Na-alg was grafted with heterobifunctional PEG. Extruding this graft into the gelation bath, fast Ca-alg formation occurs accompanied by cross-linker free covalent cross-linking of the PEG side chains. Here we report and discuss the microsphere preparation under physiological conditions and the results of *in vitro* and *in vivo* studies and we demonstrate the potential suitability of the two novel types of hybrid microspheres as materials for cell microencapsulation applications.

MATERIALS AND METHODS

Na-alg (PRONOVA UP LVM) was obtained from FMC BioPolymer (Novamatrix, Norway). Multi-arm PEG of different molar masses was purchased from Shearwater Polymers (Huntsville, USA). Vinyl sulfone terminated PEG (PEG-VS) was synthesized by reacting PEG with an excess of divinyl sulfone (Mahou 2010). Na-alg-PEG was synthesized by conjugation of α-amine-ω-thiol PEG to Na-alg. Components used for the formation of the hybrid microspheres were dissolved in DMEM (special formulation without NaCl and KCl, Cell Culture Technologies LLC, Switzerland). All microspheres were prepared at 37 °C employing a coaxial airflow droplet generator. Mechanical properties were characterized using a Texture analyzer TA-XT Plus (Stable Microsystems, UK). Osmolality was adjusted by a Micro-Sample Osmometer (Fiske®, USA). Permeability was monitored by inverse size exclusion chromatography (ISEC).

The following assays were used to evaluate the cytocompatibility, cell survival and function as well as immune response: MTT (Schmitt 2010), Griess microassay (Schmitt 2010), BD Mouse Inflammatory Cytokine Beads Array, static glucose stimulation test, LDH assays, Alamar blue assay, ELISA tests.

RESULTS AND DISCUSSION

Figure 1 shows the formation for both microsphere types.

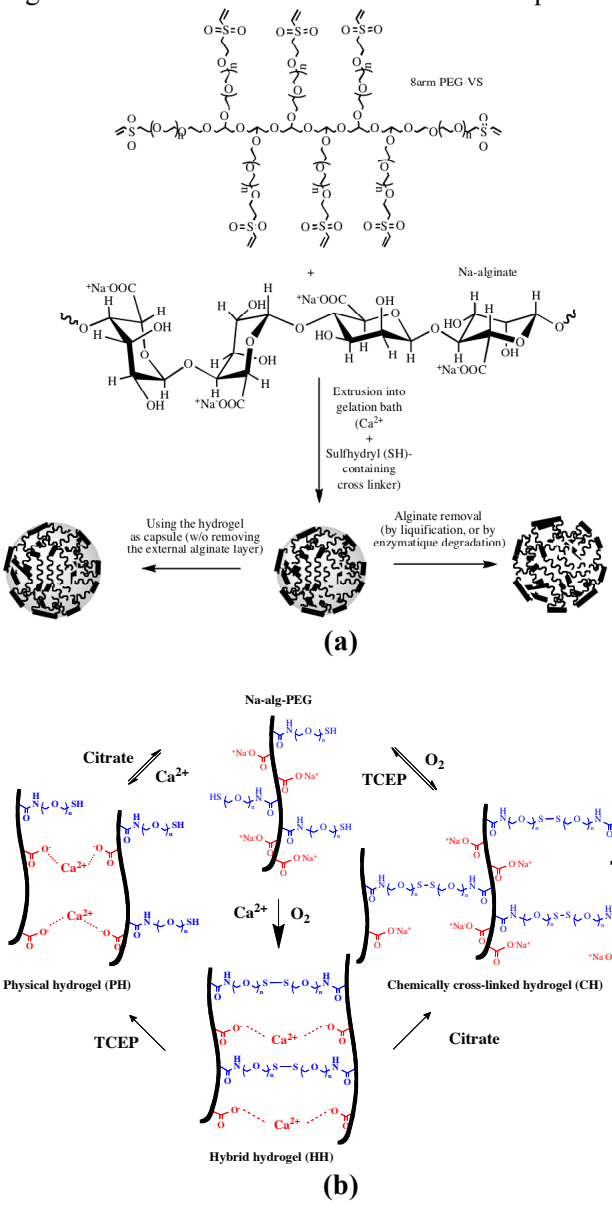

Figure 1: Formation process to obtain spherical and uniform alg-PEG-M and PEG-M (a). Preparation of different hydrogel types from grafted Na-alg-PEG (b).

Alg-PEG-M were prepared in one step under physiologi-

cal conditions (pH=7.4, T= 37°C). The diameter could be tuned in the range of 300 μm to 1200 μm by changing process conditions such as airflow, extrusion rate, and/or syringe diameter. Spherical microspheres have been obtained with less than 5% relative standard deviation of the diameter. Figure 2 shows an example of alg-PEG-M prepared from PEG-8-40 (8-arms, 40 kg/mol) in DMEM. The efficiency of PEG cross-coupling was confirmed after liquefaction of the Ca-alg hydrogel by sodium citrate yielding stable PEG hydrogel beads.

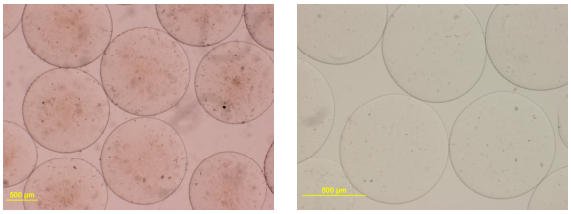

Figure 2: Alg-PEG-M in DMEM (left) and PEG-M in water after liquefaction of Ca-alg (right).

In addition to good elasticity, the microspheres exhibited high mechanical resistance to compression approaching 1.5 to 2 N at 95 % compression. The ISEC analysis quantitatively revealed that the MWCO correlates with both the molar mass and the concentration of the PEG-VS microsphere component. Permeability can be tuned by these parameters in the range of 20 to 150 kg/mol.

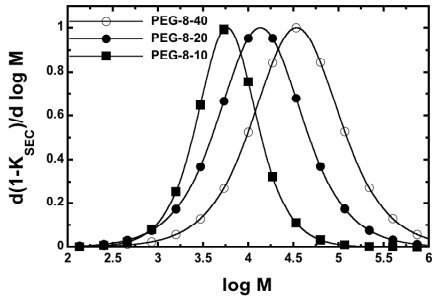

Figure 3: Differential pore size distribution obtained by ISEC showing the effect of the molar mass of PEG-VS on the permeability of alg-PEG-M prepared with a constant PEG-VS concentration of 10% (w/v).

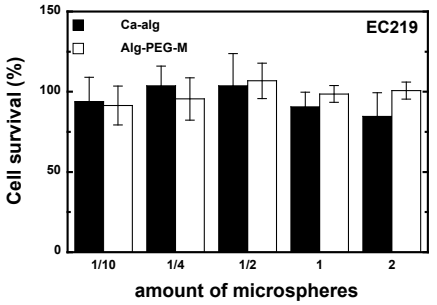

Figure 4: Cell survival of EC219 rat endothelial cells after 24 h of incubation with alg-PEG-M or Ca-alg. The survival of exposed cells was compared to unexposed control cells, defined as 100% survival.

Cell survival after 24 h incubation with alg-PEG-M remained almost unchanged for three cell lines (EC219 rat endothelial cells, ECp23 murine endothelial cells, and RAW 264.7 murine macrophages) compared to the control. It was independent of the microsphere concentration up to an equivalent of two microsphere layers covering the cells and almost identical for alg-PEG-M and Ca-alg. Figure 4 shows the results for EC219 cells.

The immune response, cytokine production, upon intraperitoneal implantation into mice was comparable to the control (Hank's solution) and pure Ca-alg microbeads. No significant increase was observed for IL-6, IL-10, IL-12p70, MCP-1, TNF-α and γ-IFN up to 6 weeks.

The suitability of the materials and processes for cell microencapsulation was evaluated for several cell types. Human islets responded to stimulation after microencapsulation in alg-PEG-M. The viability and proliferation of microencapsulated human mesenchymal stromal cells (MSC) was similar as for free cells. The MSC adapted well to the hydrogel of alg-PEG-M (Figure 5).

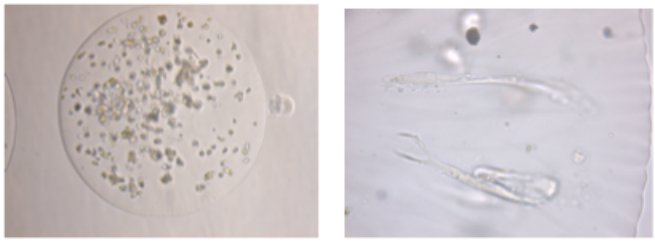

Figure 5: MSC in alg-PEG-M, 0.5 mm (left), adaptation of the MSC shape to the hydrogel on day 2 upon encapsulation (right).

Upon microencapsulation of hepatic cells in the hybrid hydrogel alg-PEG HH (Figure 1b), the viability was similar as in Ca-alg while the metabolic activity was slightly higher than in Ca-alg during two weeks of culturing.

CONCLUSIOS

Two novel types of hybrid hydrogel microspheres have been evaluated as potential materials for biomedical applications. The tunable physical properties, good cell compatibility, no significant *in vitro* immune response as well as good cell matrix properties identify them as promising materials cell microencapsulation. Moreover, the chemistry and technology allow for future specific adaptation to respective types of cells.

REFERENCES

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