Engineered Hydrogels for Cell Microencapsulation and Subsequent Transplantation

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INTRODUCTION

Cell microencapsulation is being widely investigated for cell therapies. However, the lack of optimal material is considered as a major drawback for promising therapy development. In this study, polycation-free hydrogel microspheres are produced under physiological conditions using a one-step encapsulation process. The hydrogel consists of calcium alginate (Ca-alg) which is reinforced by covalently cross-linked poly(ethylene glycol) (PEG). The physical properties of these microspheres (Ca-alg-PEG) are adjustable over a wide range by the selection of the components and the preparation conditions. The biocompatibility was demonstrated *in vitro* and *in vivo*, and the suitability of Ca-alg-PEG for cell immobilization and subsequent transplantation was confirmed. One application study targeted the treatment of liver fibrosis by transplantation of bone marrow-derived mesenchymal stem cells (MSCs).

MATERIALS AND METHODS

The key step combines ionotropic gelation of sodium alginate (Na-alg) in the presence of calcium ions and the Michael-type conjugate addition of vinyl sulfone terminated poly(ethylene glycol) (PEG) in the presence of thiolated molecules (Figure 1).

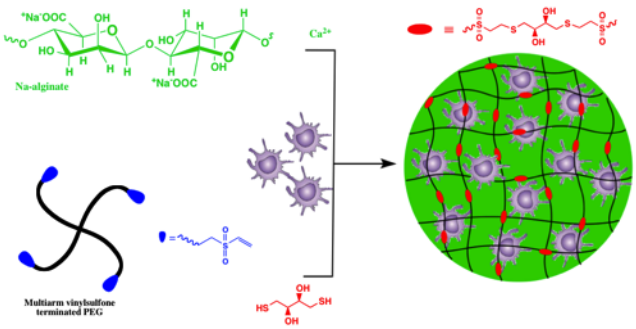

Figure 1. Formation of microspheres by combining electrostatic and covalent cross-linking
(Mahou 2010, 2012)

Process optimization
The cells were suspended in Na-alg/PEG solution and encapsulated using a BUCHI Encapsulator B-395 Pro (BÜCHI Labortechnik AG, Switzerland). The bead-producing unit of the Encapsulator was specially modified and employed as an air-flow (coaxial) technique to encapsulate bigger cells or cell clusters. The goal of the modification was to achieve the reproducible production of microspheres with diameters of 400 to 600 µm under very low shear stress.

Physical characterization of microspheres
The mechanical resistance, durability, deformability, and permeability of Ca-alg-PEG were assessed.

Microencapsulation and transplantation of MSCs
MSCs were encapsulated in microspheres of 550 µm diameter and subsequently transplanted into a mice model of liver fibrosis.

RESULTS AND DISCUSSION

Process optimization
It was shown by several research groups that coaxial airflow around a centred nozzle (inner diameter approximately 400 µm) could be used to encapsulate cell clusters in microspheres. In our studies, the preparation parameters (air-flow, extrusion rate, nozzle shape and dimension) were independently studied and correlated to the viscosity of the precursor solution (Na-alg or Na-alg/PEG). **Figure 2** shows photomicrographs of two batches of Ca-alg beads prepared with 1.5 wt% Na-alg (viscosity 55 mPa.s). The effect of changing the airflow can be seen.

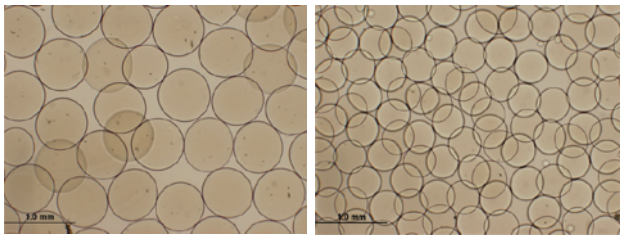

Figure 2: Ca-alg beads produced with an air-flow of 1.5 L/min (left) and 2.0 L/min (right). The photomicrographs have the same magnification.

The use of specially designed nozzles with 400 µm inner diameters and a length less than 1 cm (**Figure 3**) enables the production of a high number of droplets without generating a counter pressure, and consequently exposes the cells to very low shear stress. This technology prevents the unwanted reduction in cell viability during the encapsulation process.

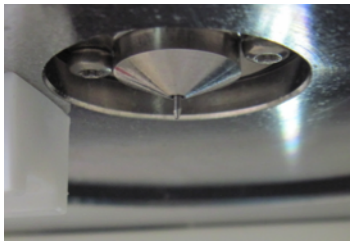

Figure 3: Centred nozzles with inner diameters of 400 µm were used to produce the cell containing droplets on a BUCHI Encapsulator B-395 Pro.

Physical characterization of microspheres

It was demonstrated that the mechanical resistance of the microspheres could be tailored by adequate choice of the PEG concentration (**Figure 4**). At least 5 wt% PEG is needed to reinforce the microspheres and improve their durability (**Figure 5**) (Mahou 2013).

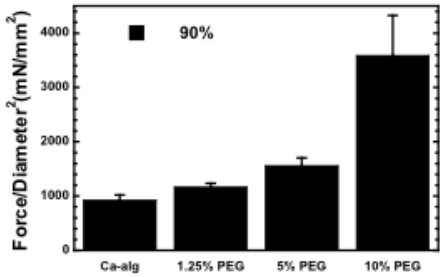

Figure 4: The mechanical resistance of Ca-alg-PEG as a function of the PEG concentration

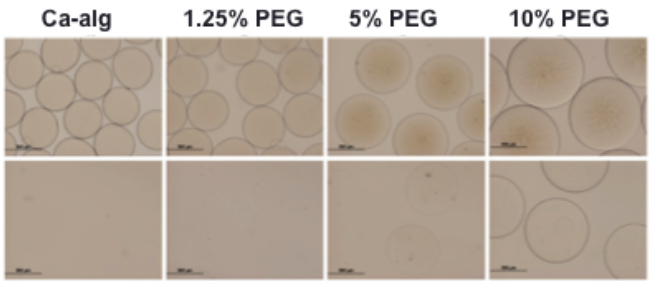

Figure 5: Photomicrographs of Ca-alg-PEG beads initially (top) and after incubation in sodium citrate (bottom)

Microencapsulation and transplantation of MSCs

(Meier 2013)

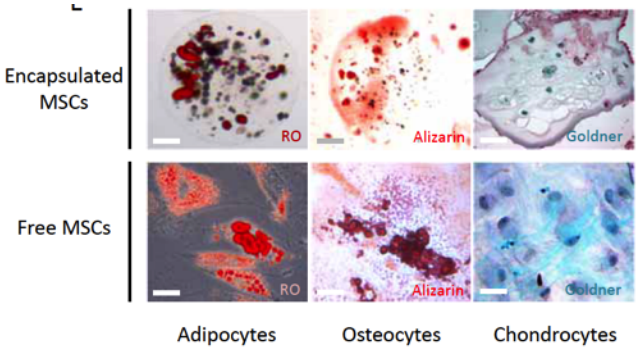

Figure 6: Photomicrographs of free and encapsulated MSCs and their differentiation into adipocytes, osteoblasts, and chondrocytes

Human encapsulated MSCs were viable and continued proliferation up to 6 months after encapsulation. The MSCs maintained their differentiation capacity into adipocytes, osteocytes, and chondrocytes (**Figure 6**). When transplanted into mice with bile duct ligation, encapsulated MSCs delayed the development of liver fibrosis, if compared to empty microspheres and encapsulated fibroblasts (EDX cells) (**Figure 7**).

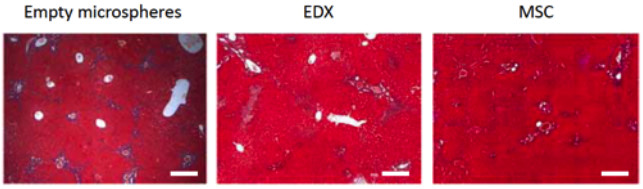

Figure 7: The quantification of fibrosis was performed on multiple liver sections.

CONCLUSIONS

The reinforcement of physical hydrogels with covalent bonds and the use of a new coaxial extrusion technology offer a versatile tool for engineering microspheres with well-defined and controllable properties. We demonstrated that microspheres with optimal dimensions are obtained when using a coaxial air-flow technique with the BUCHI Encapsulator. The physical properties of the microspheres can be tuned by the selection of the components and the preparation conditions, and are obtainable in a range envisioned for biotechnological, biomedical, and pharmaceutical applications. The durability of Ca-alg-PEG beads was demonstrated *in vitro* and *in vivo* studies.

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