Development of a Micro-Encapsulation System for Controlled Cell Delivery

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Statement of Purpose: Injectable materials are a very attractive alternative as cell carriers for tissue engineering, thanks to the reduced surgical invasivity and the easier adaptation to anatomical sites. Nevertheless, in a 3D gel matrix, cells in the inner zone are likely to suffer or die due to limited diffusion and the consequent lack of metabolites.
To overcome this problem, the possibility of delivering cells encapsulated in microspheres prepared with gellable materials was explored.

Methods: A coaxial flow micro-encapsulation device, similar to the one described for pancreatic islands encapsulation [Wolters GH. J Appl Biomater. 1991; 3:281-286] was built. Briefly, to obtain small diameter capsules, a coaxial flow surrounding the needle of a syringe was used. The syringe was loaded with a suspension of cells in a solution of a gellable material (in phosphate buffer) and placed in a syringe pump. By contrasting the surface tension at the tip of the needle, the air flow allowed to reduce the diameter of the ejected drops. Below the syringe pump, a beaker containing a sterile solution was used to collect the microdrops.
Sodium alginate (Sigma Aldrich) was used as the main gel forming material and the sterile solution for droplet collection contained $CaCl_2$ to achieve alginate cross-linking. To adjust the degradation kinetic, a thermogelling bock copolymer (Lutrol® F127, BASF Chemical Company) was mixed to alginate.
The influence of airflow pressure (0,25 to 0,5 bar), diameter of the needle (16 to 22G) and suspension flow rate (10 to 50 ml/h) on the alginate microspheres dimensions were analyzed. Degradation and weight loss of the capsules prepared with different alginate (1 to 2% w/v) and/or $CaCl_2$ concentrations (0,05 to 0,25 M), as well as for Lutrol® containing alginate (20% w/v), were investigated by aging them in phosphate buffer (37 ± 1 °C) for up to 1 month.
Cell viability and release from the microspheres were preliminary assessed using the murine myoblast cell line C2C12. Alginate (1,5% w/v, $CaCl_2$ 0,05 M) and alginate /Lutrol® were dissolved in cell culture medium (DMEM + 10% Foetal Bovine Serum) and cells were suspended at a density of approximately 10^4 cell/ml. Cells containing microspheres were incubated (37 °C, 5% CO_2) in a standard plate containing the same medium and observed for up to two weeks to search for released cells.

Results/Discussion: When using the higher air flow pressure (0,5 bar), capsules with a diameter down to 100 µm were obtained, whereas diameter of the capsules prepared with the lower pressure were about 500 µm.
The needle diameter was also found to have some influence, but negligible if compared to the effect of the airflow. In PBS, all microspheres were characterized by an initial swelling, followed by a weight loss or a constant weight depending on the material.

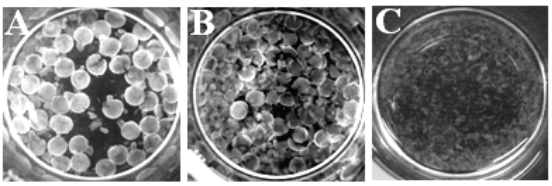

Figure 1. Appearance of 1% alginate capsules after 1 day (A), 1 week (B) and 4 weeks (C) in PBS at 37 ± 1 °C

By increasing sodium alginate and calcium chloride concentrations more stable samples were obtained. For low $CaCl_2$ and low alginate samples, complete break up of microspheres was observed after few days, whereas for the higher concentrations microspheres weight loss was negligible for up to 30 days. When Lutrol® was mixed to alginate microcapsules, degradation was also significantly faster.
When encapsulated cells were cultured in vitro, no cells were present on the bottom well for the first 2 days. At day 3, the cell release had begun for alginate/Lutrol® microspheres and groups of cells were visible at the bottom of the well. Conversely, after 7 days, only few released cells were found for alginate microspheres, even though fracture of the external layer of the microspheres was evident for a large number of capsules and elongated cells were visible inside.

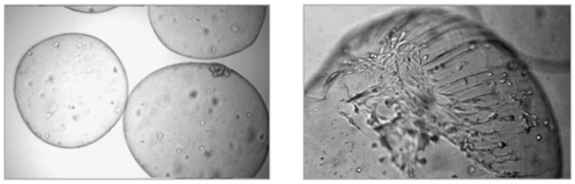
Figure 2. Appearance of microencapsulated C2C12 cells (1,5% alginate) after 1 day (left) and 1 week in culture

Confluent (for alginate/Lutrol microspheres) and semi confluent (for alginate alone) cell layers were found after two weeks.

Conclusions: Microencapsulation by coaxial flow is a simple method that allows to accurately control microsphere dimensions. When reducing microsphere diameter below 400-500 µm, enhanced diffusion is obtained and cell survival should be reasonably increased. By adjusting material composition, the degradation of microspheres can be programmed, in order to achieve a better control on cell release *in vivo*. Cell deliver by microspheres injections show therefore potential for tissue engineering, although *in vivo* tests are needed to confirm the good results obtained *in vitro*.

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