Development of a new technology of microencapsulation to cell therapy based on air-blast atomizers

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INTRODUCTION

Cell therapy is one of the most exciting fields in translational medicine and may develop into a new therapeutic platform to treat a vast array of clinical disorders. The aim of cell therapy is to replace, repair or enhance the function of damaged tissues or organs.

Multiple Sclerosis is a degeneration of the myelin sheath surrounding nerves in the brain and spinal cord. Without the protective coating, nerve cells have difficulty doing their job and sending signals. The devastating effects of this disease are due to the death of neurons that cannot be replaced and the inability of the surviving neurons to regenerate their axons. Several different strategies are being investigated for treatment of this disease, one of the most promising is being the application of neurotrophic factors which have been shown to prevent death of neurons and stimulate the regeneration of injured axons. An actual approach to this treatment involves the transplantation of cells that produce a neurotrophic factor at the injury site (Pluchino, 2003).

Several factors complicate the development of cellular therapies. Of primary importance is protection of the implanted cells from the host's immune system to prevent the freshly grafted cells from attack by native killer cells. A highly undesirable solution to immuno-rejection is the regular administration of a cocktail of immunosuppressants that can result in serious side effects.

The solution to these problems would be the inclusion of the therapeutically active cells in microcapsules with semi-permeable membranes that mitigates immune rejection without immunosuppressants. In these systems cells are immobilized in supports with semi-permeable and biocompatible membranes that provides mechanical protection to block entry of immune mediators, allowing outward diffusion of the growth factors produced by the cells to allow treatment of the disease. In addition, this membrane permits the entry of nutrients and oxygen, and the exit of waste (Angelova, 1999; Benoit, 1996; De Vos, 2002; Orive, 1999; Orive, 2004).

Most methods of microencapsulation involve one of two harsh conditions which would be a problem, especially for biomaterials handling (Herrero, 2007). The previous work done on microencapsulation without harsh conditions produce capsules with diameters ranged between 300-1000 microns (Herrero, 2007). In order to achieve the cell therapy is needed the administration of the microcapsules via injection Hamilton that requires particles diameter between 1 and 50 microns.

Until now there is not a described cell immobilization technology that allows to obtain micrometric biocompatible microcapsules (1-50 μm) with the enough mechanical stability, of controlled size, uniforms, based on the reaction of ionic gelification barium-alginate to achieve the administration of the microcapsules via injection and aerosol to cell therapy. Based on atomization processes, a microencapsulation technology of polyelectrolyte complex beads (alginate-barium) which produced very small particles with control size and narrow distribution (1-50 microns) has been developed.

Based on this new technology of producing very small microcapsules with a high mechanical stability, the main aim of this work is to achieve the cell immobilization of two different cell lines, mesenchymal stem cells and monocytes using this technology. Cell viability will be investigated using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay.

MATERIAL AND METHODS

The microencapsulation procedure, optimized to produce microcapsules in the size range 1-50 μm, can be described as follow. Sodium alginate (0.7 % w) was fed from a beaker (Figure 1-1) to an atomization nozzle (Figure 1-3) of 1.8 mm by air intake. After that, sodium alginate is sprayed into a crystallizer (Figure 1-4) containing 1000 ml of hardening solution, 2 % w calcium chloride solution, which induces the gelation. The device to the atomization works with purified pressurized air (Figure 1-6) that mixes with the liquid, forcing liquid droplets out through the orifice of the nozzle. Both the liquid and the air flow rates are measured by means of rotameters (Figure 1-2,5). The divalent barium ions crosslink the droplets of sodium alginate on contact to form the microcapsules. The microbeads were kept 5 minutes under crosslinked conditions to form a semi-permeable membrane. The resultant microbeads were collected by filtration and washed with 30 ml of 0.9 % of barium chloride and kept in distilled water. The scheme of the microencapsulation device is shown in Figure 2.

It was prepared two productions of each cell line encapsulation, one was dispersed by ultrasonication, and the other was not dispersed to find out the ultrasonication effect on the cell viability.

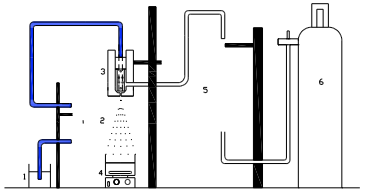

Figure 1 : scheme of the encapsulation device

RESULTS AND DISCUSSION

Microcapsules were studied before cell immobilization to its characterization. Based on the previous studies, it has been chosen the best conditions to produce the microcapsules, with a good mechanical strength, in the range between 1-50 microns to can be administered via injection Hamilton. Also, the size of the pores of the capsules should be in range between 16-42 KDa to allow the release of the growth factors that secrete the mesenchymal stem cells and the monocytes. It was chosen a low alginate concentration (0.7 % wt.), a barium concentration of 2.0 % wt, and a high air flow rate (95.0 L/min). It was used a medium molecular weight sodium alginate and a low alginate flow rate (0.006 L/min) that as well as producing smaller microcapsules, it help to save the high added value products used in this work (mesenchymal stem cells and monocytes). With this

conditions it is achieved microcapsules with a Sauter Mean Diameter of 53.46 microns as is shown in the Figure 2. Microcapsules displayed a consistent appearance of spheres.

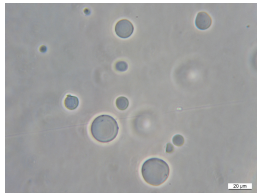

Figure 2 : Alg.-Barium capsules

To test whether the cells could survive within the micrometric alginate-barium microcapsules, the cell viability was monitored every day for a period of 13-15 days using an MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay. The Figure 3 represents the mesenchymal stem cells viability for the microcapsules contained in 100 µl of culture medium. The figure 4 represents the monocytes viability for the microcapsules contained in 100 µl of culture medium. Both figures show, as well, the effect of the dispersion of the microcapsules by ultrasonication. Figures 3 and 4 show a similar behaviour, but with some differences.

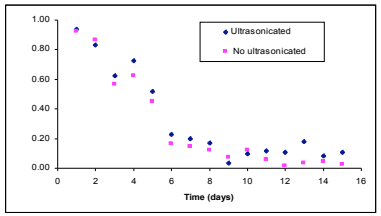

Figure 3 : cell viability for mesenchymal cells

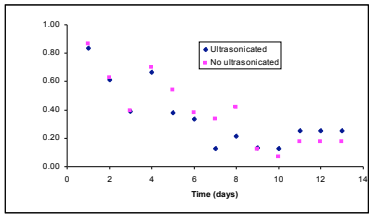

Figure 4 : cell viability for monocytes

Mesenchymal stem cells line behaviour is shown in Figure 3. It was tested also the influence of the dispersion by ultrasonication in the cell viability (Figure 3- b). The cell viability was monitored daily for 15 days. In the ultrasonicated microcapsules was observed, within the first 6 days after encapsulating, a gradual decrease in cell viability. After the sixth day the cell viability remained almost constant. At this point approximately 15-20% of the cells within the beads were alive. With regard to the microcapsules without dispersion the behaviour was slightly different. Within the first 6 days after encapsulating was observed a gradual decrease in cell viability, but in this case, the cell viability continued to decrease until the ninth day, where it remained constant in a 5-10%.

As regards the monocytes line, its behaviour is shown in Figure 4. It was also tested the influence of the dispersion by ultrasonication in the cell viability (Figure 4- b). The cell viability was monitored daily for 13 days. In the ultrasonicated microcapsules was observed, within the first 3 days after encapsulating, a gradual decrease in cell viability. After the third day the cell viability remained almost constant. At this point approximately 25-40% of the cells within the beads were alive. With regard to the microcapsules without dispersion the behaviour was slightly different. Within the first 9 days after encapsulating was observed a gradual decrease in cell viability. After the ninth day the

cell viability remained almost constant. At this point approximately 10-20% of the cells within the beads were alive.

Immediately after encapsulation most of the stem cells and monocytes (93 and 85%) were viable in both microcapsules productions (with and without dispersion).
The reduction of the cell viability within the first days is due to the cells need a period of accommodation to the new conditions within the microcapsules. After this period of accommodation the cell viability remained almost constant because of cells need a minimal number to proliferate. Within the microcapsules there are only few cells because of the small size of the microcapsules, and this cause the absence of proliferation.

The dispersion improves the cell viability. Figures 3-b and 4-b show a reduction in cell viability in the final period (cell viability constant) in comparison with the Figure 3-a and 4-a (microcapsules ultrasonicated). It may be speculated that this reduction may be due to the microcapsules require space to membrane permits the entry of nutrients and oxygen, and the exit of waste. The microcapsules dispersion by ultrasonication gives them the required space to allow the bidirectional exchange. Therefore, ultrasonication not only permits the cell viability, but increases it.

CONCLUSIONS

It was developed a microencapsulation technology of polyelectrolyte complex beads which produce very small particles (1-50 microns) with particle size control. The polymers used is sodium alginate as polyanion and barium chloride as polycation. To do that, it was used an airblast atomizer. The microcapsules that have been formed in this work are spherical. It was obtained a narrow particle size distribution.

It has been achieved successfully the immobilization of mesenchymal stem cells and monocytes, in micrometric (1-50 microns) alginate-barium microcapsules based on atomization processes. This size is necessary to the administration of microcapsules via injection Hamilton.

REFERENCES

Angelova N et. al. (1999) *Rationalizing the design of polymeric biomaterials*. Trends Biotechnol 17 409-421.
Benoit et. al. (1996) *Microencapsulation methods and industrial applications*, Marcel Dekker: New York.
De Vos P. et. al. (2002) *Considerations for successful transplantation of encapsulated pancreatic islets*. Diabetología 45 159-173.
Herrero EP. et. al. (2007) *Immobilization of Mesenchymal stem cells and monocytes in biocompatible microcapsules to cell therapy*. Biotechnol Prog 23 940-945.
Orive G et. al. (2003) *Cell encapsulation: promise and prog*ress. Nat Med 9 104-107.
Orive G et. al. (2004) *History, challenges and promises microencapsulation*. Trends Biotechnol 22.
Pluchino S. et. al. (2003) *Injection of adult neurospheres induces recovery in a chronic model of multiple sclerosis*. Nature 422 684-688.