Immobilization of cells in biocompatible films to cell therapy

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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.

The pneumonectomy (pulmonary resection) is a surgical procedure to remove a lung and it is used to excise tumourous tissue arising from lung cancer, and rarely for treatment of tuberculosis. Despite careful selection in the pre-operative period, high rates of early mortality (9.1 %) and morbidity remain after phneumonectomy (Jimenez, 2006). One of the most serious complications after the surgical procedure is the post pneumonectomy bronchopleural fistula (BPF). BPF is defined as a communication between the bronchial tree and the pleural space through the bronchial stump that usually results after pulmonary resection (Cerfolio, 2001).

One successful approach to the treatment of this surgical complication is the tissue repairing by polymeric systems containing different cells lines, such us monocytes, mononuclear and fibroblasts (Perets, 2003). Coverage of the bronchial stump with autologous tissue of fibroblast (Goto, 1999; Kobayashi, 2007), vascular endothelial growth factor (VEGF) (Yancopoulos, 2000) and basic fibroblast growth factor (bFGF) (Yancopoulos, 2000) reduces the risk of postoperative death due to BPF after pneumonectomy.

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.

In this work it is demonstrated a method for non-autologous cellular transplantation that mitigates immune rejection without immunosuppressants. To do that, cells were 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).

During the last years, the major efforts have been dedicated to develop spherical microcapsules to cell therapy (Herrero, 2007), but recently other type of cell immobilization polymeric devices is taking importance, the immobilisation within polymeric films (Jain, 1995). Cell immobilisation within films presents some advantages in comparison with microcapsules in some kinds of treatments; they are easy to retrieval in case of complications (Kizilel, 2005) and are useful in tissue repairing (Alperin, 2005) and wounds treatment. In addition their mechanical strength must be adequate to the applications required. Films will be fixed using sutures by surgery, and in cases as

myocardial repair will be exposed to considerable tautness that barium alginate compounds should resist. Mechanical resistance tests have been made to ensure that barium alginate films present an appropriate elastic modulus.

There are two habitual techniques proposed to produce films using alginates: first process consists in dehydrate an alginate layer before crosslinking (Hermes, 2002), this technique is not adequate because desiccation process will damage cells; second process consists in extend a thin alginate layer over a Petri dish and then crosslinking (Wan Chan, 2006), this process could be applied to our case, but currently it is difficult to control film thickness and uniformity.

In this paper a relevant and new cellular immobilization system based on biocompatible alginate barium-films is described. This system allows a better control over film thickness and makes better film uniformity, avoiding roughness over film surface that can produce fibrosis and rejection after transplantation.

Based on this new technology of producing biocompatible films with a high mechanical stability and a specific thickness, the main aim of this work is to achieve the cell immobilization of different cell lines within the immobilization system. Cell viability was investigated using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay.

MATERIAL AND METHODS

The production of the films was optimized, to control and reduce the thickness (300 microns) maintaining the mechanical strength. The device to generate the biocompatible films was composed of two porous plates, fixated in a metallic support, in contact with a gripper. In each part of the device was placed a filter drenched with a 3 % wt. barium chloride. On the plate that was set in the support, was spilled 0.7 mL of a 4 % wt. alginate solution with cells, in the center of the filter. The other plate was then vertically placed over the first one, and the device was fixed with the gripper. Through the entrance on the top of the device was introduced 4 mL of barium chloride solution so that it could cross the porous plate and contacting with the alginate thus enabling the formation of the gel which constitutes the membrane.

RESULTS AND DISCUSSION

The main aim in the present work is to achieve the viability of cells immobilized in the alternative supports o films produced by gelation processes. Nobody has achieved the cell immobilization in these conditions before.

To test whether the cells could survive within the micrometric alginate-barium films, the cell viability was monitored using an MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay.

According to Figure 1, in the first day after immobilization of monocytes cell line, cell population increases 50% with respect to the initial cells quantity. Cells continue proliferating and reach repeatedly local maximums. Generally analyzing the variation of cell viability in the barium alginate membranes produced, related to the initial number, it's possible to see a general trend of cell proliferation.

In the case of the mononuclear cell line (Figure 2), analyzing the data in the fifteen days of the analysis it can be referred that the used system Ba-ALG allows cell viability but doesn't permit cell proliferation. There isn't any trend of cell growth, but the number of viable cells has remained at or above the initial value.

With fibroblast cell line (Figure 3), generally analyzing the variation of cell viability in the barium alginate membranes produced, related to the initial number, it's possible to see that cell proliferation occurs since it is detected a quantity of cells always higher than the initial amount. On the other hand there are two reading points discordant of the trend of cell proliferation that can be explain by not significant sample, which contained a smaller quantity of cells then expected.

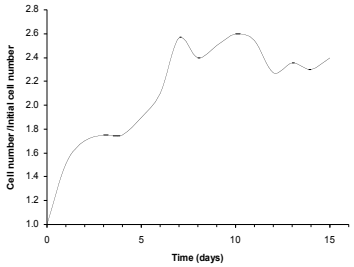

Figure 1 : Cell viability for monocytes

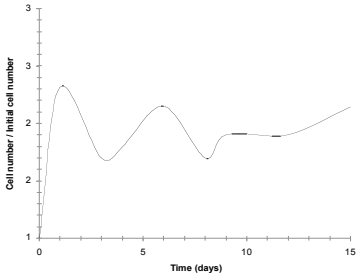

Figure 2: Cell viability for monoclonal cells

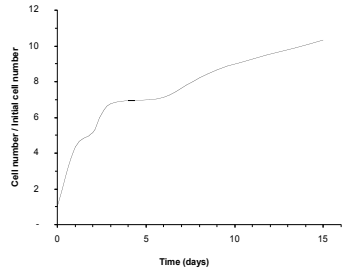

Figure 3 : Cell viability for fibroblast

CONCLUSIONS

In this study it was successfully achieved the immobilization of monocyte, mononuclear and fibroblast cell lines, in micrometric (≈300 microns of thickness) barium alginate membranes with supplemented growth medium, based on gelification processes.

The system used proved to be a good alternative to encapsulate the types cells mentioned, revealing itself an suitable system to maintain cell viability of all the three types of cells and enable cell proliferation of monocyte and fibroblast cell lines for at least fifteen days.

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