PEG Incorporated Polymeric Microcapsules for Intramyocardial Delivery of Stem Cells Genetically Modified by Baculovirus

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ABSTRACT

The ability of mesenchymal stem cells to self-renew and differentiate into specialized cell lineages makes them promising tools for regenerative medicine. Local injection and use of scaffolds had been employed earlier to deliver these cells; yet, an optimal delivery system remains to be identified. Through the present work we developed a new microcapsule delivery system using polymeric alginate-chitosan microcapsule coated with PEG (AC-PEG) to deliver human bone marrow derived stem cells (hBMSCs), genetically modified by recombinant baculoviruses (Bac-MGFP) carrying GFP as a reporter gene. The data indicates that the cells encapsulated in AC-PEG microcapsules can grow within the capsules. The capsules can also protect the cells against immune rejection. ACPEG microcapsules also had significantly higher mechanical and osmotic stability than commonly used AC microcapsules. The entrapped genetically modified cells showed transgene expression for at least two weeks. The encapsulated cells also retained their inherent potential to differentiate into multiple lineages. Thus, microencapsulated stem cells carrying therapeutic genes have immense potential to facilitate functional improvement in cellular heart failure therapy, although preclinical studies need to be done to establish their functional benefits on myocardial implantation.

Keywords: polymeric microcapsules, stem cells, regenerative therapy, virus

1 INTRODUCTION

Stem cell therapy has been suggested as a novel approach to address myocardial cell loss, particularly using hBMSC or other progenitor cells [1]. These pluripotent cells have the potential for self-renewal or regeneration of myocardium, bone, and cartilage. It has been shown that hBMSCs get mobilized in response to ischemia and exhibit tropism in an ischemic heart. In order to enhance the therapeutic efficacy, hBMSC can be genetically engineered using a suitable gene delivery system prior to cell transplantation in order to allow the engrafted cells to proliferate and integrate with the host cells. This genetic modification can promote angiogenesis, tissue regeneration of the infarcted portion and even support differentiation of the stromal cells *in vivo* [2]. But the success of such experimental cell-based gene therapies is mainly based on the cell delivery system, its biosafety profile, efficiency of gene transfer and the subsequent expression profile of the transplanted cells without affecting the host cells' natural biological capabilities. This is particularly important in direct intramyocardial transplantation where, apart from rapidly losing their viability due to the harsh environment, the beating condition of the heart makes the retention of the transplanted cell at the target site very difficult [3]. **Figure 1** illustrates the method of preparation used here for generation of recombinant baculovirus, stem cell trasduction and microencapsulation for myocardial regenration therapy.

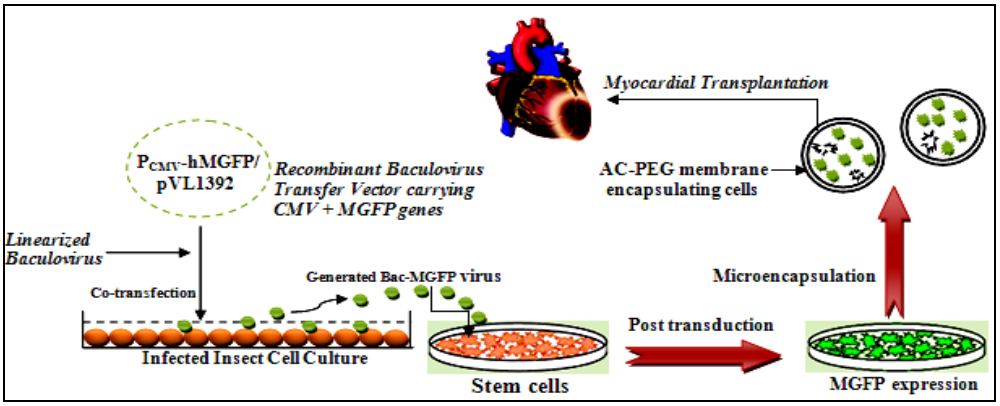


Figure1: Schematic representation for generation of recombinant baculovirus and microencapsulation of transduced hBMSCs for myocardial transplantation. The P_{CMV}-hMGFP/pVL1392 transfer vector was co-transfected with linearized baculovirus into the insect cells. The generated Bac-MGFP virus was used to transduce the hBMSCs, which were then microencapsulated in AC-PEG membrane for transplantation.

In this work, recombinant baculoviruses carrying MGFP transgene under the control of mammalian CMV promoter were generated Bac-MGFP to transduce the cells before encapsulation. Baculovirus is a powerful tool for different gene therapy applications as it combines the advantages of viral transient expression, ease of generation, and a wide cell tropism.. To deliver the transduced cells, we developed PEG incorporated AC microcapsules and investigated their properties and potential as a novel stem cell based gene delivery system for cardiac transplantation.

2 METHODS

The recombinant baculovirus carrying MGFP reporter gene under the cntrol of CMV promoter was generated using molecular cloning techniques as mentioned elsewhere [4]. This was followed by optimisation of transduction conditions to achieve the maximum transduction efficiency. The transduced cells were then encapsulated in polymeric microcapsules.

Briefly, right after viral incubation, the cells were trypsinized and suspended in 1.5% sodium alginate (Sigma Chemicals) at a cell concentration of $2x10^6$ cells/ mL. The suspension was extruded through an encapsulator (Inotech) fitted with 300µM nozzle at a voltage of 0.577kV and frequency of 710Hz. The gelation process took place in a 0.1M $CaCl_2$ solution for 15 min. AC microcapsules were prepared by immersing the calcium alginate capsules in 0.5% chitosan solution dissolved in dilute acetic acid at a pH of 5.2 for 30 min. The capsules were washed for 5 min after every coating with physiological solution. To prepare AC-PEG capsules, the AC microcapsules were treated with 0.5% solution of PEG (MW 10000; Sigma Chemicals) dissolved in 0.45% NaCl for 10 min. The capsules were then washed twice with physiological solution. The washed capsules were then transferred to stationary cell culture flasks, and resuspended in medium with antibiotics (Sigma) for further culture in 37^oC incubator with 5% CO_2. These microcapsules were replenished with fresh media every alternate day. This was followd by monitoring the viability of encapsulated cells from timt to time. In order to investigate the potential of the membranes to provide immunoprotection to the encapsulated cells, encapsulated hBMSCs were co-cultured in static condition with lymphocytes for 2 weeks. This was followed by checking the stability of the microcapsules under mechanical and osmotic stress. The encapsulated cells were then studied to evaluate whether the encased stem cells can retain their differentiation potential.

3 RESULTS

The recombinant baculoviruses were generated as depicted in Fig. 1 using the BacMam expression system. The titre of the amplified virus stock was $2.4x10^9$pfu/mL, as determined by an immunological assay. The ability of the AC-PEG capsules to provide immunogenic protection to the encapsulated human stem cells were studied by co-culturing microencapsulated Bac-null transduced cells with a significantly high population of lymphocytes derived from mouse origin. It was observed that growth of the encapsulated cells in presence of lymphocytes started to decrease significantly from day 3 of encapsulation, when no PEG was used; while in AC-PEG capsules there was slow ceasing of growth from a much later time, from day. Even the AC-PEG proved to be mechanically much stronger than AC as illustrated in **Figure 2.**

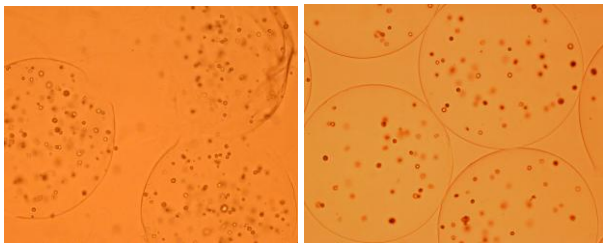
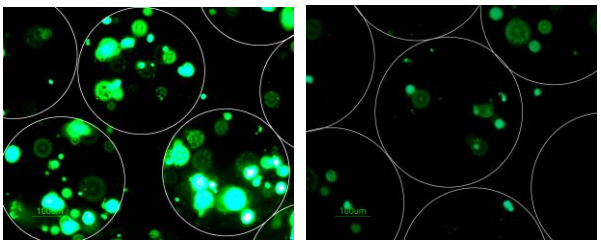


Figure 2: Effect of PEG incorporation on mechanical stability of the microcapsules. (A) represents AC microcapsules which were mostly broken under mechanical and osmotic stress compared to intact AC-PEG microcapsules (B).

To confirm the retention of differentiation potential of the baculovirus transduced encapsulated hBMSCs, the cells were isolated from the capsules by depolymerisation after 15 days of culture [5]. They were then seeded at high confluency and induced towards adipogenic, chondrogenic and osteogenic lineages with appropriate differentiation media. Data showed that both the encapsulated and non-encapsulated control cells have differentiated into adipocytes, chondrocytes and osteocytes with high efficiency. From the above obtained results, it can be concluded that neither encapsulation nor viral transduction altered the differentiation potential of the stem cells. This is an important finding which demonstrates that microcapsules can be a potential delivery system for stem cells which can not only protect the stem cells from external hostile environment but also can retain their therapeutic differential potential.

To check whether viral incubation time plays an important role in transduction of the cells the hBMSCs were incubated with viral solution for 4h and 8h. This was followed by microencapsulation of the cells and culturing for 24h. Fluorensce images of the microencapsulated cells confirm the efficient GFP expression of the transduced cells under encapsulation condition; but cells which had an incubation time of 8h resulted in much higher transgene expression than 4h incubation, as confirmed by **Figure 3**.



(A) **(B)**

Figure 3: AC-PEG microencapsulated hBMSCs transduced with Bac-MGFP under optimal transduction condition with varied incubation times. Representative photographs of corresponding transduced encapsulated cells were taken using fluorescence microscope on 24h post encapsulation with 8h (A) and 4h (B) viral incubation. The white circles show the peripheral surface of the microcapsules. Higher expression was noticed with 8h viral incubation than 4h incubation.

4 DISCUSSIONS

Microencapsulation is the procedure by which materials such as enzymes, genes or even cells are enclosed within microscopic, semipermeable containers. These synthetic semipermeable microcapsules, sometimes referred to as artificial cells, were designed in our lab to retain the cells inside while allowing permeable molecules to cross the membrane. These encapsulated cells are supported by external oxygen and nutrients. Their secreted products are diffused out of the microcapsules in order to carry out their functions. Furthermore, microencapsulated cells are protected from immune rejection because leukocytes and antibodies cannot penetrate the capsule. Thus, it becomes an ideal tool for allogeneic and xenogeneic transplantation[3,6]. The concept of microencapsulation may therefore eliminate the requirement for immune suppressants when used in transplantations. The positively charged chitosan binds with negatively charged alginate through electrostatic interaction, which results in interfacial adsorption of chitosan by the alginate core. An improper coating can make the capsular surface charged. Hydrophilic, non-ionic and inert nature of PEG makes it an ideal candidate to neutralize the surface charge and hence, increase the immunoprotective potential of the capsules by alleviating the chance of having surface protein adhesions [7]. The current study revealed that AC-PEG microcapsules can be used as an efficient delivery system for stem cell transplantation as well as for cell based gene therapies using baculoviruses for therapeutic protein production. The incorporation of PEG gave the capsules a better mechanical stability as well immunogenic property without interfering with their cell viability, proliferation and differentiation ability. AC-PEG microcapsules may thus become an innovative and integral part of stem cell based regenerative medicine that can be useful in the growing fields of myocardial tissue engineering. In order to attain a better understanding of its future potential and concerns for biomedical applications, much more comprehensive *in vitro* and *in vivo* studies on the biocompatibility and biodegradability of the membrane need to be done.

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