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PREPARATION AND EVALUTION OF MICROENCAPULATED MONONUCLEAR CELLS IN NATURAL POLYMER

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Abstract

Microencapsulation of cells represents an alternative to cell transplant, abrogating the requirement for immunosuppressive drugs to avoid graft rejection. Encapsulation allows the use of cells of other animals, and also the use of stem cells, aiming to overcome the limited access to organs of cadaveric donors. The present study involves encapsulating the Mononuclear cells (MNC) and various parameters like swelling, the mechanical stability of the beads, their degradation and viability of encapsulated monocytes will be studied. These beads would provide the required immune isolation for the MNC to avoid incompatibility with an immune system. A chemotherapeutic drug, Vincristine used as a standard drug for this study which has immune potentiating activity apart from the anti-cancer activity. The needle having maximum accommodation (18 G) has been selected, and lower concentration of sodium alginate (0.5 M) has been used for the microencapsulation of monocytes and vincristine sulfate. The criterion for this selection is degradation and swelling of the beads. It may conclude that the degradation and swelling of alginate beads containing vincristine sulfate are less which is followed by beads containing monocytes and then by hollow alginate beads. The viability test for monocytes released from the beads was performed, and the monocytes were found viable for three days. At high concentration of sodium alginate, the degradation and swelling are less.

Key Words: Microencapsulation, Cell therapy, Mononuclear cells, Sodium alginate.

INTRODUCTION

Oral delivery is the preferred route of drug administration due to high patient comfort and compliance, low administration costs and low risk of contamination/infection. However, orally administered active agents face the dual challenge of surviving in active form in the harsh degradative conditions of the gastrointestinal environment and crossing the intestinal epithelium in amounts sufficient to provide a therapeutic effect.A drug that is released from a dosage in a controlled manner in the stomach will have the whole surface of the small intestine available for absorption. These considerations have led to the development of oral controlled gastro-retentive dosage forms possessing gastric retention capabilities (DurgaJaiswal et al., 2009). Microencapsulation is described as a process of enclosing micron-sized particles of solids or droplets of liquid or gasses in an inert shell, which in turn isolates and protects them from the external environment. Microcapsules can be divided into two parts viz: the core and the shell.The “core” (the intrinsic part) contain the active ingredients e.g. a hardener or a biocide while the "shell" (the extrinsic part) protects permanently or temporarily from the external atmosphere.

Microencapsulation of cells represents an alternative to cell transplant, abrogating the requirement for immunosuppressive drugs to avoid graft rejection. In cell encapsulation, the transplanted cells are protected from immune rejection by an artificial semi-permeable membrane, allowing transplantation without the need for immunosuppression. Microencapsulation of mononuclear cells could enable transplantation in the absence of immunosuppression. Alginate is often used for microencapsulation of the cell.

The present study involves encapsulating the mononuclear cells (MNC) which are used in the diseases such as cancer. Various parameters like swelling, mechanical stability of the beads,degradation, and variability of encapsulated monocytes will be studied. Vincristine sulfate, an anticancer agent which has immunopotentiation activity, will also be encapsulated for comparison.

MATERIALS AND METHODS

Materials:

All chemicals used in this investigation were biological grade and purchased from SRL chemicals and Himedia.

Isolation of monocytes:

Monocyte cells were separated according to the procedure Franklin Lakes (2003): 2ml of human blood collected into anticoagulant containing tube. Diluted with 2ml of phosphate buffer and layered on ficollhypeque and centrifuged at 3000rpmfor 30minutes at 180-200oc.the lymphocyte layer was transferred into a clean centrifuge tube.

Preparation of beads:
The beads were prepared by inotropic gelation technique and procedure is as follows: sodium alginate (0.5M) dispersion was extruded dropwise into calcium chloride solution and stirred at 100rpm for 15minutes.the beads were then separated by filtration washed with distilled water and dried.The beads of different sizes are prepared using different size needles (18G, 20G, 21G, 22G, 23G, 24G, 26G), and their diameters were compared. In the same way, beads are prepared using another concentration (1M) of sodium alginate using different sizes of needles. Different size of beads of monocyte cells and vincristine sulfate were also prepared in the same way. For monocyte cells-containing beads: 1ml of lymphocytes fraction was added to sodium alginate solution. For vincristine sulphate-containing bead; 100mg of vincristine sulfate added to sodium alginate solution.

Measurement of size of beads:
Empty alginate beads, beads of monocyte cell, beads of vincristine sulfate were spread over a flat surface using a spatula. The diameter was then measured using a calibrated scale.

Swelling test of beads:
A petri dish was taken and filled with phosphate buffer (pH7.4) then the weight was measured. Ten beads were taken and their weight measured. The beads were then placed in the petri dish containing buffer and the swelling rate determined by measuring the weight periodically for 100minutes.Different bead sizes of cells of monocyte cells and vincristine sulfate were also studied in the same manner, and their swelling rate was compared.

Degradation study of beads:
A petri dish filled with phosphate buffer (pH7.4) was taken, and its weight was measured.10 beads were taken, and their weight was also measured. The beads were then placed in a petri dish containing phosphate buffer (7.4), and the beads were periodically weighed for 14 days. Different bead sizes of monocyte cells and vincristine sulfate were also studied in the same manner and their degradation was compared.

Mechanical Stability test of beads:
300 beads were taken in a petri dish, and they were subjected to the shearing force at 133rpm for 1hour. The numbers of bead that are damaged were counted after 1 hour. The percentage stability of beads was calculated by using following formula:

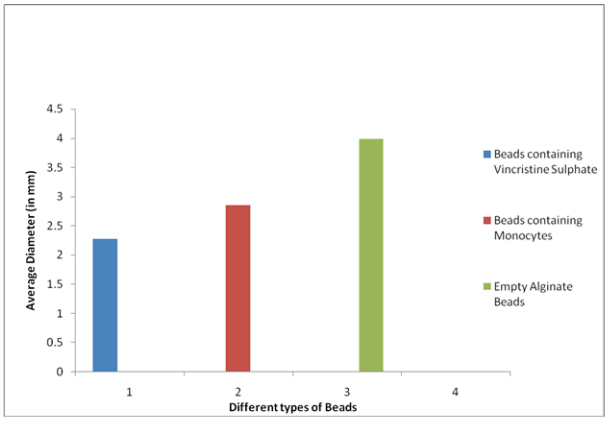
$$\% \text{ stability} = (100 - \%\text{damaged})$$

$$\%\text{damaged} = \frac{\text{Number of beads damaged}}{\text{Total number of beads taken}}$$

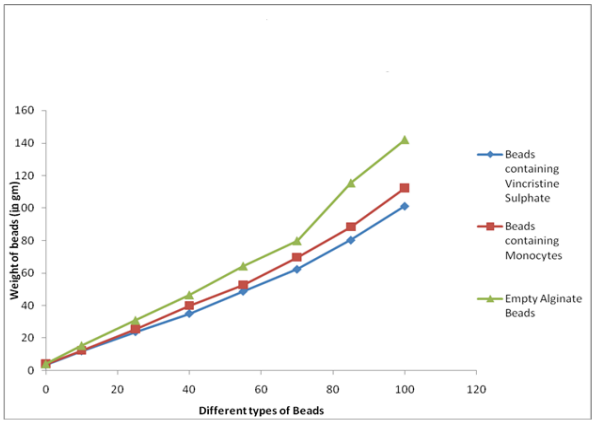
Different bead sizes of monocyte cells and vincristine sulfate were also studied in the same manner and their mechanical stabilities were compared.

Results and Discussion

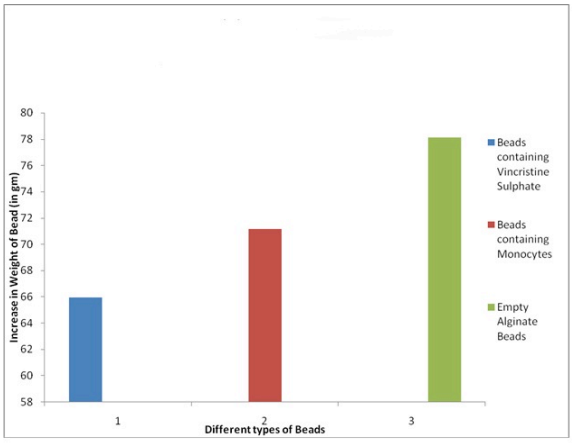
Microencapsulation involves enclosing micron-sized particles of solid or droplets of liquids or gasses in an inert shell which in turn isolates and protects them from the external environment. This technique is an alternative to cell transplantation because it prevents the usage of immunosuppressive drugs to avoid the newly implanted grafts. Immunity can be boosted by the right choice of products that increase the effectiveness of white blood cells. Mononuclear cells (MNC) or monocytes are such cells which are derived from bone marrow promonocytes. Monocytes serve two primary functions: firstly, they ingest and destroy particulate matters; secondly, it is involved in the initial recognition, processing and presentation of antigen to T-cell to elicit the specific immune response. A chemotherapeutic drug, vincristine has immunopotentiation activity apart from anticancer activity, so monocytes and vincristine sulfate were selected for the preparation of microcapsules.



Graph1: Comparison of size of beads containing Vincristine sulphate, Monocytes and empty alginate beads.



Graph2: Swelling of beads containing Vincristine sulphate, Monocytes and empty alginate beads.



Graph3: Degradation of beads containing Vincristine sulphate, Monocytes and empty alginate beads.

In the present work, microcapsules were prepared by using inotropic gelation technique by using sodium alginate and calcium chlorides.Different sizes of needles (26, 24, 23, 22, 21, 20, 18G) and two concentrations of sodium alginate (0.5M and 1M) were used for the preparation of different sizes of empty beads.Lower concentration of sodium alginate(0.5M) and a needle having maximum accommodation(18G) has been used for the microencapsulation of monocytes and vincristine sulfate. The hollow alginate beads, beads of monocyte cells and vincristine sulfate were compared in their sizes, swelling, degradation and mechanical stability;

Size: Empty alginate beads have the greater diameter when compared to that of beads containing monocytes and vincristine sulfate. Vincristine sulfate- containing beads are the smallest.

Degradation and swelling: Less in beads containing vincristine sulfate which is followed by beads containing monocytes and then by hollow alginate beads.

Viability test: Viability test for monocytes released from the beads was performed, and the monocytes were found viable for 3days. The concentration of the drug released from the beads was determined using UV-Spectrophotometer and also HPLC.

CONCLUSION:

The obtained results were encouraging more the concentration of sodium alginate more is the stability of the bead. The degradation and swelling are less. This result can be used further for the development of in vivo procedures for determining the Immuno-enhancer activity of monocytes as well as vincristine sulfate. In future, monocytes can be encapsulated by Nanoencapsulation technique. These may lead to the development of a novel method to enhance the immunity with the use of cell therapy. Also, Nanocapsule may be an alternative approach to reducing the side effects of immunosuppressant drugs.

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