Development of alginate-gelatin ‘artificial cell’ for cell encapsulation

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Abstract
Current study was aimed towards preparation of a prototype of ‘artificial cell’ for encapsulation of chicken hepatocytes using a biocompatible material and investigation on the influence of chicken bone marrow cells on viability of hepatocytes under in vitro culture conditions. A mixture of sodium alginate-gelatin (1:2 ratio) and isolated cells at definite seeding concentrations were added to 0.9% ice cold solution of calcium chloride for the preparation of very thin gelatin hydrogels with cells encapsulated in it. Viability of hepatocytes was evaluated at 48hrs of incubation under 3 different combinations of cultures, such as hepatocytes alone in monolayer culture, co-culturing of hepatocytes with encapsulated bone marrow cells and culture of co-encapsulated hepatocytes and bone marrow cells in hydrogels. Trypan blue cell viability tests confirmed reasonably good level of viability (70%) of encapsulated cells, confirming suitability of hepatocytes, bone marrow cells, hydrogel, ‘artificial cells’, tissue regeneration.

Keywords: Hepatocytes, bone marrow cells, hydrogel, ‘artificial cells’, tissue regeneration.

I. Introduction
Tissue damage due to diseases, age related degeneration or casualties are in the rise, creating ever increasing demand for organ transplantation. Development of new strategies plays an important role in the repair of diseased and damaged tissues1. These strategies include replacement of tissues with synthetic materials like ceramic, metallic, polymeric origin or tissue transplantation2. Synthetic materials pose the challenge of biocompatibility whereas tissue transplantation often gets hampered by immunorejection. Cell phenotype is an important factor in determining the success of repair and regeneration of damaged tissues, as the problem of immunorejection basically arises due to mismatch of cell phenotypes. Technique of using selectively permeable membranes for encapsulating mammalian cells was introduced in the early 1950’s as a strategy for immunosolation of live cells, to minimize immunorejection in tissue transplantation patients3. Selective permeability of the encapsulating membrane can avoid the flow of the components of immune system mediators4 reducing the challenges of immunorejection of xenogeneic or allogeneic cells when transplanted into a host.

Research work on immunosolation mode of delivery of live cells to target host have indicated that materials which are more similar to extracellular matrix (ECM) as successful delivery systems5. This information has lead to the concept of ‘artificial cells’. This concept involves preparation of artificial structures of cellular dimensions for possible replacement or to supplement deficient cell functions. Encapsulation of live cells in biocompatible membrane bound vesicles of varying sizes has evolved as a variation of the concept of ‘artificial cells’ and used in isolation of transplanted cells from the host tissue to varied extents depending on the type of material used for the process of encapsulation. ‘Artificial cells’ are used for encapsulation of different biological materials like enzymes, stem cells, proteins and islets5. Hydrogels have been identified as one of the suitable encapsulation materials for the preparation of ‘artificial cells’ due to their structural and morphological similarities with ECM.

Chronic and acute liver failure cases are at the increasing trend during recent times. Regeneration of liver tissue, though possible, requires long time and hence transplantation becomes imminent. Therefore, approaches that enhance the process of regeneration of hepatocytes would be of practical solution for avoiding risk of liver transplantation. Current study has attempted development of a process for the encapsulation of chicken hepatocytes and bone marrow cells in hydrogels and understanding the influence of bone marrow cells on viability of hepatocytes under in vitro conditions.

2. Materials and Methods
2.1 Materials
Sterile: Phosphate Buffer Saline (PBS) [0.1M, pH 7.2.], Dulbecco’s Modified Eagles Medium (DMEM) with 1.5% of Fetal Bovine Serum(FBS)), Trypsin [0.25%], antibiotic solution (1000units of penicillin and 10mg/ ml of streptomycin in normal saline), Forceps, Scalpels, Scissors, 100ml beaker with muslin cloth, 50ml beaker,100ml conical flask with magnetic bead, Centrifuge tubes [15 or 20 ml, or universal containers], Culture flasks, [25 cm²], or tissue-culture-grade Petri dishes [5–6 cm], pipette [1 ml], sodium alginate [1%], purified gelatin [2%], sodium chloride [0.9%], ice cold calcium chloride [10%], HEPES (2-[4-(2-hydroxyethyl)piperazin-1-yl] ethanesulfonic acid) [pH 7.2], stainless steel needle syringe.

Non sterile: Chicken liver tissue, thigh bone of an adult chicken, 70% ethanol, inverted phase contrast microscope.

2.2 Methods
2.2.1 Isolation of hepatocytes from chicken
Fresh liver tissue from chicken was washed two to three times with sterile PBS, transferred to a second dish and dissected off to remove unwanted parts such as fat or necrotic material. The trimmed tissue was transferred to a third dish chopped with crossed scalpels into

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small cubes of about 1-mm dimension. The tissue pieces were transferred to conical flask containing 10 ml of 0.25% of trypsin and stirred well using a magnetic stirrer for 10 min. The tissue suspension was filtered through muslin cloth and the filtrate was centrifuged for 10 min at 1000 rpm. After centrifugation the supernatant was removed and added to 5 ml of DMEM, mixed and the suspension was transferred carefully to a 50 ml culture flask. The flask was observed under inverted phase contrast microscope for viewing the cells and incubated at 37°C and 5% CO2 in CO2 incubator.

2.2.3. Monitoring viability of hepatocytes in culture
Cell viability was determined using the standard protocol of trypan blue exclusion test. The cell suspension was diluted and inoculated to three different culture flasks at a density of 0.5×10^6, 1×10^6 and 2×10^6 cells/ml respectively. Viability rate of the cells were recorded at 6, 12, 24 and 48 hours from the time of inoculation.

2.2.4. Isolation and culturing of chicken bone marrow cells
Thigh bone of fresh adult chicken was dissected out, washed and wiped with ethanol. One end of the bone was opened and bone marrow was transferred carefully to a petri dish using stainless steel needle syringe. 5 ml PBS was added to the bone marrow and the suspension was centrifuged for 10 min at 1000 rpm. The pellet was re suspended in 1x antibiotic solution and centrifuged for 10 min at 1000 rpm at 37°C. The pellet collected was suspended in DMEM, cell count was performed using a hemocytometer and seeded to culture flask containing DMEM medium at a concentration of 2×10^6 cells/ml. The flask was observed under inverted phase contrast microscope for viewing the cells and kept for incubation at 37°C and 5% CO2 in CO2 incubator.

2.2.5. Encapsulation of hepatocytes/bone marrow cells in 'artificial cell'
Alginate and gelatin were mixed in the ratio of 1:2 and transferred to three vials and bone marrow cells at three different densities (0.5×10^6, 1×10^6 and 2×10^6 cells/ml) were added separately to each vial. The cell suspensions were added drop wise in to 0.9% ice cold calcium chloride solution (pH 7.2) using stainless steel needle syringe. The droplets coming out of the tip of the syringe were sheared using a sterile needle tip. Upon contact with the calcium chloride, the droplets get wrapped by a thin membrane of calcium alginate resulting in small beads of 1-2 mm dimension. The tissue pieces were transferred to conical flask containing 10 ml of 0.25% of trypsin and stirred well using a magnetic stirrer for 10 min. The tissue suspension was filtered through muslin cloth and the filtrate was centrifuged for 10 min at 1000 rpm. After centrifugation the supernatant was removed and added to 5 ml of DMEM, mixed and the suspension was transferred carefully to a 50 ml culture flask. The flask was observed under inverted phase contrast microscope for viewing the cells and incubated at 37°C and 5% CO2 in CO2 incubator.

2.2.6. Co-culturing of hepatocytes and encapsulated bone marrow cells
The suspension of alginate - chitin beads containing trapped bone marrow cells inside was transferred carefully to culture flask containing monolayer culture of hepatocytes at densities of 0.5×10^6, 1×10^6 and 2×10^6 cells/ml and observed under inverted phase contrast microscope and kept for incubation as described above. Viability rate of hepatocytes was calculated at 6, 12, 24 and 48 hours of time intervals from the time of inoculation.

2.2.7. Co-encapsulation of hepatocytes with bone marrow cells
Using the method discussed in 2.2.5, hepatocytes were encapsulated inside the 'artificial cell’ along with bone marrow cells and the beads were maintained in DMEM medium in standard conditions. Viability rate of cells inside the beads were calculated at 6, 12, 24 and 48 hours of time intervals from the time of incubation.

2.2.8. Enumeration of viability of encapsulated cells
Culture medium was carefully removed from the culture flasks containing alginate-gelatin 'artificial cells” using 1 ml micropipette and the beads were suspended in PBS for 10 min. The suspension was filtered through muslin cloth and the beads were disrupted using scalpel. 10 ml of 1x antibiotic solution was added to the disrupted beads and centrifuged at 1000 rpm for 10 min. The pellets collected were resuspended in 5 ml DMEM and subjected to viability test according to the standard protocol.

3. Results
3.1. Morphology and changes in the viability rate of the cells.
Oval shaped hepatocytes adhered to the bottom surface of the culture flasks were observed under phase contrast microscope (Figure 1). The hepatocytes exhibited an average of 48 hrs for doubling and also for reaching confluency under in vitro conditions. The bone marrow cells appeared oval in shape and proliferated into monolayer in the culture flask (Figure 2). These cells reached confluency at 24 hrs and of incubation.

Figure 1: Microscopic view of hepatocytes in culture medium after 48 hours of isolation.

Figure 2: Microscopic view of bone marrow cells in culture medium.

3.2. Preparation of ‘artificial cell’.
The 'artificial cells” produced by combination of sodium alginate and gelatin appeared as spherical beads of 1-2 mm diameter (Fig. 3). The beads were translucent and the cells encapsulated within could be observed under phase contrast microscope (Fig. 4).

Figure 3: Alginate-gelatin hydrogels as ‘artificial cell’.

Figure 4: Cells encapsulated inside ‘artificial cell’.
3.3 Enumeration of viability of encapsulated cells

The average viability rate of cells at the end of 48 hrs of incubation under in vitro condition is presented in Fig. 5. Among the variants of in vitro culture trials notable difference was observed in the cell viability at the end of 48 hrs of incubation. Highest cell viability of 70% was recorded for the co-culturing of hepatocytes and encapsulated bone marrow cells. Viability of cells in co-encapsulated condition was found to be lesser than monolayer culture of hepatocytes alone and co-culturing of hepatocytes and encapsulated bone marrow cells.

![Figure 5: Percentage viability of cells in culture after 48 hours of incubation.](www.ssjournals.com)

H: Hepatocytes in monolayer; HBM: Hepatocytes and encapsulated bone marrow cells (in hydrogel capsules with alginate: gelatin at 1:2; BCE: co-encapsulated hepatocytes and bone marrow cells (in hydrogel capsules with alginate: gelatin at 1:2 concentration.

4. Discussion

Current study was intended to develop a prototype of the conceptual ‘artificial cells’ using chicken hepatocytes and bone marrow cells as model system. Combination of sodium alginate and gelatin at definite proportion has been tried for preparation of the encapsulating material in ‘artificial cells’. Chicken hepatocytes have been chosen for the study owing to their known requirements and behavior under in vitro cultures and intended application of the outcome in liver regeneration process. The study has attempted to investigate the influence of pleuripotent stem cells of chicken bone marrow cells in enhancing viability of hepatocytes.

Gelatin has been proposed as a prospective material for cell immobilization owing to its ability to form hydrogels. Earlier studies have reported up to 90% viability of entrapped hepatocytes in gelatin based hydrogels. During the current study, entrapment of the cells in gelatin hydrogels was not so successful and hence combination of sodium alginate and gelatin has been opted. Trials using different ratios of alginate-gelatin combination have yielded better result with 1:2 ratio [unpublished data and not include in this paper] and hence this ratio has been selected for enumeration of viability of encapsulated cells. In this study, the encapsulated bone marrow cells when cultured along with free hepatocytes showed high rate of viability, when compared with hepatocytes cultured alone in monolayer culture as well as hepatocytes co-encapsulated with bone marrow cells. The decrease in viability of cells observed inside ‘artificial cells’ may be because of the contact inhibition occurred inside the ‘artificial cells’ or leakage of desired growth components from the ‘artificial cells’ to outside medium due to porous nature of the membrane. Validation of the current result with multiple trials and necessary modifications in the protocol are required for fine tuning the technique. Overall this technique will have a promising future in the area of tissue engineering and regenerative medicine.

5. Conclusion

Current study has developed a prototype of alginate-gelatin hydrogel based ‘artificial cell’ for encapsulation of chicken cells in general and hepatocytes and bone marrow cells in particular. Presence of bone marrow cells was found to enhance viability of hepatocytes under co-culturing. Co-encapsulation of the two cell types yielded lesser viability than co-culturing with encapsulated bone marrow cells. Further validation of the current result and standardization of ‘artificial cell’ composition and in vitro conditions are needed for making use of the concept at practical level.

Reference