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Research Article

Bio Artificial Pancreas - Encapsulation of Human Pancreatic Islet Cells Using Calcium Alginate Micro Beads for the Use in the Clinical Therapy

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Abstract

Objective: Transplantation of pancreatic Islet cells is one of the promising therapies in treating Type 1 diabetes. Shortage of human pancreas and the usage of immuno suppressive drugs are the two main limitations to prevent the transplantation. Microencapsulation an emerging technology in transplantation of islet cells in which islet cells were encapsulated by a bio compatible membrane composed of the molecular sequence of calcium alginate.

Methods: Islet cell encapsulated were prepared by using Sodium alginate and Calcium carbonate followed by characterization of UV-VIS spectrometer, Turbidity, Viscosity and Scanning Electron Microscopy.

Results: Spherical islet cells were formed and observed under microscope. The viscosity was found to be 310 m Pa S and turbidity was found to be 15 NTU units. From UV-VIS spectrophotometer a max emission peak was observed at 429 nm which shows that cells are entrapped in calcium alginate beads which was further confirmed by Scanning Electron Microscopy images.

Conclusion: From the results it was concluded that Islet cells were entrapped in calcium alginate beads which will be a good therapeutic vehicle of controlled drug delivery system.

Keywords: Islet Pancreatic Cells; Transplantation; Scanning Electron Microscope; Calcium Alginate Beads

Background

Transplantation of Pancreatic islet cells is known to be a promising approach to treat Type 1 Diabetes. Diabetes mellitus, the most prevalent and destructive disease affects more than 451 million people and supposed to increase to 693 million by 2045 million according to Cho [1]. Diabetes Type 1 is an autoimmune disease, mainly seen in adolescent and young adults exhibiting dysfunction of diastolic which is an early marker of heart failure [2]. The inflammatory process and immune response are the key factors in the development of diabetes such as auto reactive T lymphocyte cells and islet cells reactive B lymphocyte cells play an important role in the destruction of insulin cells [3,4]. This restricted the treatment of diabetes with life exogenous insulin supplementation only, though it is actually indispensable for granting type 1 diabetes, this is not a cure for this metabolic disorder and it might attenuate but never eliminate the risk for developing secondary complications of the disease such as cardiovascular , renal, neuropathy and retinopathy with often severe disabling squeal [5].

Cell therapy is one of the most promising therapies for the treatment of type 1 diabetes previously in which intrahepatic grafts of healthy pancreatic islets retrieved from cadaveric donor

organs into totally immuno suppressed type 1 diabetes patients [6]. The main limitation and or problem of substituting destroyed cells with healthy and functional insulin producing cells and also this procedure has been associated with limited clinical success Prolonged graft survival is achieved by using continuous immuno suppressive drugs but the toxicity associated with the usage of immunosuppressive exhibits adverse effects Continuous usage of immuno suppressive drugs leads to damaging the survival and functionality of the transplanted cells [7].

Microencapsulation is a possible alternative to cell therapy graft of islets or insulin producing cells. Microencapsulation has gained much more importance in the controlled drug delivery process [8].

Microencapsulation is a defined process which is a having a core surrounded by a polymeric coated wall with in second material with a continuous coating of polymeric material yielding microscopic material. Alginate is linear anionic polysaccharide and calcium is one of the most commonly used cation for ionotrophic gelation of alginate which can be easily produced by extrusion methods by dripping the alginate solution into a calcium ion solution. The main advantages of these microencapsulation technologies are material structuration, protection of the enclosed product and delivering at right time at target tissues [9].

The present research paper focuses on the encapsulation of islet pancreatic cells using sodium alginate. Authors previously standardized and optimized the sample preparation for Scanning Electron microscopy of microencapsulation beads without islet cells using sodium alginate and calcium carbonate [10].

Materials and Methods

Materials

Sodium Alginate and Poly L Lysine were purchased from Sigma Aldrich and Calcium chloride was purchased from SRL chemicals, India.

Methodology

Preparation of Islet cells

Microencapsulated Pancreatic islet cells were prepared according to Opara [11]. Briefly place 100 µl of solution containing islet cells, centrifuge with 1400rpm for 5 minutes followed by excess removal of media with pipetting. Sodium alginate of 1.2% w/v was dropped using a syringe with nozzle size about 500 micrometer solutions was added. 100 mM calcium chloride solution in 10mM HEPES solution was prepared and islet cells in alginate solutions were added drop by drop into this solution. Following two washes with normal saline, the micro beads are perm-selectively coated with variable concentrations of 0.1% PLL f which helps as an immuno protective barrier. Excess of PLL encapsulated beads were washed with saline.

Shape and size

To characterize shape and morphology of the alginate beads and encapsulated cells were first observed under microscope and then Scanning Electron microscope. Size of the particles was analyzed by Particle size analyzer.

UV-VIS spectrophotometer

UV-VIS spectroscopy studies were carried out room temperature using UV-VIS spectrophotometer to determine the spectral changes after encapsulating the islet pancreatic cells in the calcium alginate beads.

Viscosity and turbidity measurement

Viscosity measurement was done using a rotational viscometer using Haake Viscotester 6 plus with a speed range of 5-200rpm. Turbidity measurement was carried out by using a portable Turbidometer.

Scanning electron microscopy (SEM) analysis

Calcium alginate beads were fixed in 2.5% glutaraldehyde treatment for 2 hours at 4ºC followed by washing with PBS (Phosphate-Buffered Saline) and dehydrated again in a graded alcohol series. The samples were then incubated in HMDS for 10 minutes, air-dried in desiccators and subjected to SEM analysis.

Results

Shape and size

Uniform spherical shaped spheres were formed which were observed under microscope (Figure 1). Sizes of the beads were determined by particle size analyzer which is in the range of 100µm.

UV-VIS spectrometer

Wavelength scan of calcium alginate islet solution exhibited λ max at 429 nm. This showed that islet cells which are called as

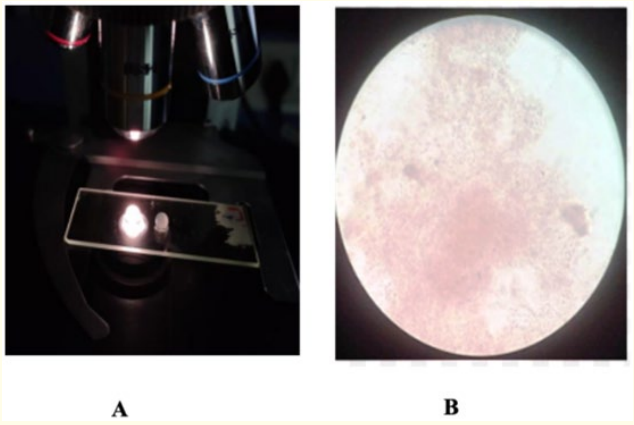

Figure 1: (A): Microencapsulated Islet Cells (B) Microencapsulated cell under microscope.

drug carrier were inside the alginate beads (Figure 2). Viscometer measurement test was conducted for 1 min and the values were observed at 310m Pa.S. A value of 15 NTU units was observed by Turbidometer when Turbidity of encapsulated cells measured.

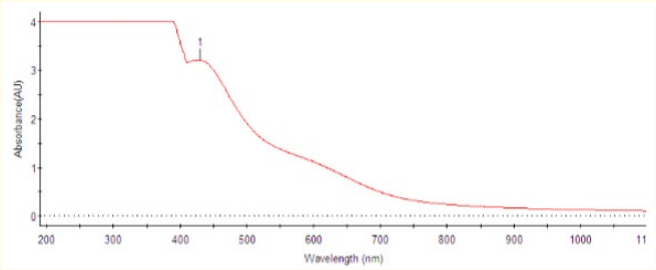

Figure 2: UV-Vis spectroscopy analysis of Islet cells encapsulates in Calcium alginate beads.

Viscometer and turbidity

Measurements were performed using rotational viscometer Haake Viscometer 6 Plus with speed in the range of 5–200 rpm. Tests were conducted for 1 min, and then viscosity values was observed to be at 310 mPa.s Turbidity measurement was carried out by using a Laboratory portable Turbidometer with a turbidity of 15 NTU units.

Scanning electron microscopy (SEM) analysis

Cells encapsulated in Calcium alginate beads were identified using glutaraldehyde (2.5%) Spherically shaped encapsulated islet cells were analysed for SEM analysis represented in Figure 3.

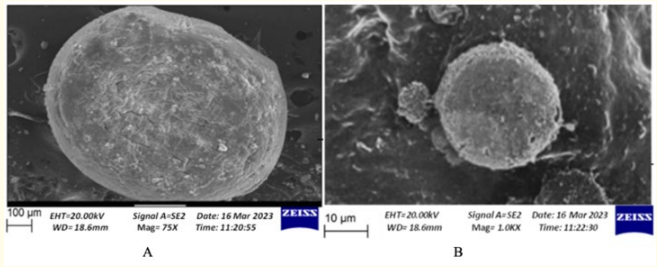

Figure 3: (A) Pancreatic Islet cells entrapped in Calcium alginate Beads (B) Islet cells at higher magnification.

Discussion

Diabetes is a chronic disease characterised by hyperglycemia which occurs in two forms Type 1 and 2. Diabetes has an increased risk of developing a number of serious life threatening pathologies such as retinopathy and cardiovascular disorders. Of these two forms Type 1 is an autoimmune disease in which patients depends on exogenous insulin supply in which long term continuous usage of exogenous insulin shown the failure of biological actions of endogenous insulin [12]. To overcome this, Transplantation of Islet cells provide a good alternative to insulin treatment for type 1 diabetes. These can improve the blood glucose homeostasis efficiently on a regular basis. The limitation of this transplantation is the shortage of donor material. Islet allo or xenotransplantation limited by inflammatory and immune reactions resulting in low survival. Many efforts have been devoted to find alternative sources of insulin producing cells such as stem cells and xenogeny sources [13].

Controlled drug delivery system using microencapsulation technology has greatly enhanced pharmaceutics and cell therapy. Encapsulation of Islet pancreatic cells compromises of polymer science and emulsion technologies for controlled studies; this technique has significant advantages such as protective against enzyme degradation, time control and aims at targeted organ tissue [14]. Usually for this microencapsulation, Sodium alginate and calcium carbonate are the main sources of forming encapsulation

beads. These are normally spherical in shape with size varying from 05nm to 1mm containing a core substance. Sodium alginate is a natural biodegradable, biocompatible, non toxic when taken orally and hydrophilic polymer suitable for entrapment.

Shape and size plays a vital role in the encapsulation technology. Microencapsule beads are spherical in shape when observed microscope. Size of the microencapsulate islet cells found to be in the range of 100µm. There is a decrease in the weight of beads during drying process before SEM analysis. SEM analysis UV-VIS spectrophotometer is one of the characterization parameter to determine the stability of encapsulated beads. In the present study we have observed a maximum peak at 429 nm which represents the islet cells are entrapped in the beads. The viscosity was found to be at 310 m Pas which is a moderate viscosity [15]. Rasyid., et al. [16] categorized the viscosity into three forms (i) low viscosity <240m Pas (ii) moderate viscosity 240-3500 m Pa s (iii) high viscosity which relates to fluidity of a liquid. SEM analysis revealed the presence of Islet pancreatic cells in the calcium alginate beads.

Conclusion

The bioengineering approach for designing a bioartificial pancreas has generally involved the development of either microcapsules or microcapsules, or other devices such as biocompatible sheet of encapsulated islets. When implanted, these constructs would substitute for the defective native endocrine pancreas.

Many studies are performed in several different small and large animal models have demonstrated the promise of encapsulated islet xenotransplantation to treat diabetes. While currently therapy doesn't provide permanent independence from exogenous insulin, the next steps are clear. Effective methods of oxygen delivery, the use of anti-inflammatory factors, optimized biomaterial and encapsulation methods, as well as greater clinical experience with transplantation will eventually result in a solution that eliminates the need for immunosuppression in transplantation and the unreliable glycemic control achieved by exogenous insulin administration. Furthermore, gains made in islet encapsulation will lend to other encapsulation applications, resulting in exciting novel therapies for disease.

Conflict of Interest

We hereby declare that we have no conflict of interest of any form pertaining to our research study titled, 'Bio Artificial Pancreas - Encapsulation of Human Pancreatic Islet cells using Calcium Alginate Micro Beads for the use in the clinical therapy.

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