EFFECT OF INTEGRATING POLYETHYLENE GLYCOL TO ALGINATE-POLY-L-LYSINE AND ALGINATE CHITOSAN MICROCAPSULES FOR ORAL DELIVERY OF LIVE CELLS AND CELL TRANSPLANT FOR THERAPY

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ABSTRACT

Microencapsulation is an emerging technology that has a wide range of applications ranging from drug delivery to tissue engineering. The primary advantages of microencapsulation are that the technology can be used to orally administer drugs directly to the gastrointestinal system and it may eliminate the requirement of immunosuppressant drugs when used in cell therapy procedures. For the technology to be implemented, it is necessary to obtain an appropriate membrane material. Presently, the most commonly used membrane for cell encapsulation is alginate coated with poly-l-lysine, however, there still remains limitations associated with this membrane. The current study investigates the advantages of adding polyethylene glycol (PEG) to the conventionally studied alginate-poly-l-lysine-alginate (APA) and alginate-chitosan (AC) membrane microcapsules. Stability tests using osmotic pressure reveal the addition of PEG to improve mechanical stability of both APA and AC capsules by over 50% which was determined by a decrease in the number of broken capsules when subjected to osmotic pressure. Morphological studies performed in subjecting the capsules to simulated gastrointestinal fluids show that the addition of polyethylene glycol (PEG) prolongs the stability of APA capsules significantly. APA microcapsules disintegrate within 2 hours compared to 24 hours for capsules integrating PEG. Cytotoxicity tests using human HepG2 cells indicate positive MTT showing the membranes containing PEG can support cellular growth. This study implies that incorporating PEG to alginate microcapsules may lead to improvements in the membrane properties for use in oral delivery or transplantation.

INTRODUCTION

Microencapsulation of live cells is a novel approach for the target delivery and controlled release of therapeutic products and has been studied widely by several researchers. The application of microencapsulation has been investigated for the treatment of diabetes by encapsulation of islet cells, treatment of hepatic failure by direct transplantation of encapsulated liver cells and the treatment of IBD via oral delivery.[2,5,12,10] The primary requirements for a functional biomaterial for cell encapsulation include that the membrane must be impermeable to high-molecular weight species such as antibodies to avoid immune rejection, be permeable to small molecules to allow transport of metabolites, and the microcapsule material must be able to support cell growth without interfering with their differentiated function.[12] Microcapsules, due to their small size (0.3-1.5mm), have a high surface to volume ratio which is advantageous for mass transfer. The permeability of microcapsules can also be controlled by varying membrane composition. This permits the diffusion of anabolic compounds such as oxygen and glucose as well as cellular products such as hormones and proteins while excluding immunoglobulins.[12,8]

The most commonly studied biopolymer membrane includes the alginate-poly-l-lysine-alginate (APA) membrane and the chitosan membrane. Previous research has shown that APA membranes can induce necrosis of encapsulated cells when implanted due to the cationic attraction of inflammatory cells from the poly-l-lysine (PLL).[8, 9,14] Similarly, the structural stability of APA membranes needs to be increased for long-term cell encapsulation. As a result, studies continue to be conducted to attain an appropriate membrane material for use in microencapsulation technology.[1,12,8,9] Chitosan is a biocompatible and biodegradable polymer which has been investigated for drug and protein encapsulation and may be an alternative to poly-l-lysine.[3,16] Polyethylene glycol (PEG) is a water soluble, non-ionic polymer that is protein resistant and has both low toxicity and immunogenicity. PEG is a widely studied product that may have the potential to improve biocompatibility and mechanical strength of microcapsule membranes.[1,8,9,11]

The current study compares the effect of adding PEG to APA and chitosan membranes to verify whether it can lead to an improved membrane material for use in cell encapsulation either in the area of

bioartificial organ development or for the oral delivery of therapeutic products.

MATERIALS AND METHODS

Sodium Alginate of low viscosity (2%), PEG (MW 10000), MTT (Thiazolyl blue), Poly-l-lysine hydrobromide (MW 27,400), were all purchased from Sigma Chemical. Chitosan 10 was obtained from Wako. Cell line HepG2 was purchased from ATCC and routinely subcultured in MEM (minimum essential eagle media) supplemented with 10% FBS and 1% antibiotics. All experiments were performed in triplicate and results are expressed as an average with standard deviations.

Microcapsule Preparation

Alginate microcapsules were prepared using a 1.5% sodium alginate solution extruded through an INOTECH microencapsulator. The gelation process took place in a 0.1M $CaCl_2$ solution for 10 minutes. APA microcapsules were formed by immersing the alginate capsules in a 0.05% poly-l-lysine solution for 10 minutes and then coating with a layer of 0.1% alginate for 5 minutes. To prepare alginate-PLL-PEG-alginate (APPA) capsules, the capsules were exposed to a solution of 0.5% PEG for 10 minutes prior to being recoated with dilute alginate. Alginate-chitosan (AC) microcapsules were prepared by immersing the alginate capsules for 30 minutes in a solution of 0.5% chitosan in 1% acetic acid to allow cross linking. For PEG integration (ACP), the chitosan capsules were exposed to a 0.5% solution of PEG for 10 minutes. All microcapsules were stored in physiological solution (0.85% NaCl) prior to testing.

Cell Encapsulation

HepG2 cells were encapsulated in alginate microcapsules using previously established procedures by Chang[5]. The capsules were then coated to form APA, APPA, AC and ACP beads. The ability of the membrane to sustain living cells was determined using an MTT calorimetric assay which was used to detect metabolic activity of cells within the microcapsules. The procedure was followed with slight modifications to Uludag [12]. Approximately 20 capsules were incubated with 100µL of media and 25µL of MTT solution for 4 hours. The microcapsules were then washed with physiological solution and the formazan crystals formed by MTT was dissolved in 100µL of DMSO. Absorbance was measured using a multiwell spectrophotometer at a wavelength of 570nm.

Stability Test

The mechanical stability of the microcapsules was determined by observing the integrity of the capsules when exposed to a hypertonic solution. Approximately 100 cell-free microcapsules of each type (APA, APPA, AC and ACP) was removed from the 0.85% saline solution and immersed in distilled water. The number of broken capsules was analyzed using a light microscope.

Exposure to simulated GI fluid

The ability of the microcapsules to sustain the harsh GI environment was analyzed microscopically by exposing approximately 100 cell-free capsules to SGF for approximately 2 hours and then transferred to SIF and analyzed at various time intervals. Simulated GI fluids were prepared in accordance with USP XXII. The SGF was composed of a solution containing 2 g sodium chloride and 3.2 g pepsin in 7 ml hydrochloric acid. A pH of 1.2 to simulate the stomach environment was attained by adding H_2O. The SIF was prepared by dissolving 6.8 g of monobasic potassium phosphate in 250 ml of H_2O. 190 ml of 0.2N sodium hydroxide, 400 ml of H_2O and 10 g of pancreatin were added. The pH was then adjusted with 0.2 N sodium hydroxide to 7.5 ± 0.1 and a total volume of 1L was obtained by adding H_2O. All chemicals were purchased from Sigma.

RESULTS AND DISCUSSION

Microcapsule morphology

Microcapsules having a diameter of 400 ± 30 µm were prepared. After coating with PLL, PEG and chitosan, all membranes retained uniform, spherical shape with a smooth surface as revealed by microscopic analysis. Figure 1 shows a picture of APPA microcapsules.

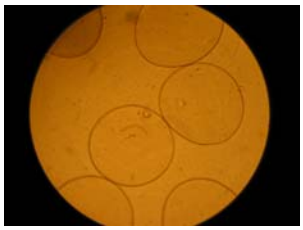

Figure 1: APPA microcapsules. Magnification 10X

Osmosis test

Although chitosan, due its charge distribution, can form stronger bonds with alginate than PLL, results indicate the membrane is weak at sustaining osmotic pressure. Results are shown in figure 2. It was observed that over 32 ± 0.3% of chitosan coated microcapsules were broken within the 60 minute analysis. APA microcapsules displayed greater

stability with a total of 26 ± 1% of broken capsules. In both cases, the addition of PEG greatly enhanced mechanical stability. PEG incorporated in the chitosan membrane resulted in all the capsules to remain intact. Within 60 minutes, a total of 11 ± 0.7% of APPA microcapsules were ruptured which is a reduction of over 50% of the number of broken capsules observed for the poly-l-lysine membrane without PEG. From the graph, osmotic pressure appears to affect APA microcapsules more rapidly than the remaining three membranes tested in this study. As shown in the figure, a total of 6.6 ± 0.7% of the capsules had broken within the first 5 minutes.

These results imply that the addition of PEG may enhance the mechanical stability of microcapsules. The exact mechanism of how PEG interacts with chitosan and PLL remains to be studied. It is hypothesized that the PEG polymer may form an interpenetrating network near the microcapsule membrane surface which may be a reason for the significant improvement in the stability of the chitosan coated microcapsules containing PEG. Other studies reveal that PEG incorporates itself directly into the alginate matrix.[1, 11,16]

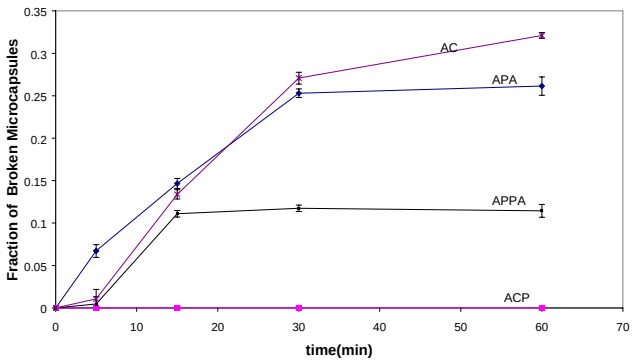

Figure 2: Mechanical stability comparison of AC, APA, APPA and ACP microcapsules. Fraction of broken beads verses time (min) after subjecting to osmotic pressure

Stability in Simulated GI fluid

The possibility of using PEG integrated microcapsules for the use in oral delivery was analyzed morphologically by observing the effect of gastrointestinal fluids on the membranes. All capsules remained intact with no differences observed in membrane size or shape in the SGF at a pH of 1.2. AC and ACP capsules expressed similar characteristics when transferred to SIF. The two microcapsules began to rupture within 2 hours and APA microcapsules completely disintegrated. After 24 hours, all of the AC and ACP microcapsules, although still visible, were deformed and broken. ACP microcapsules did not rupture, however, they showed extensive swelling similar to APA microcapsules and were no longer visible after 24 hours. These results imply that the addition of PEG does not affect the mechanical behaviour of the conventionally studied AC microcapsules. Both types of microcapsules would most likely release therapeutic products in "bursts" occurring when the capsules rupture. APA and APPA microcapsules behave significantly differently. Since they do not visually show signs of membrane rupture, they may potentially provide a controlled release of therapeutic products through the GI tract. The results indicate that the incorporation of PEG to APA microcapsules prolong the time the microcapsule can exist in the GI tract. The APA microcapsules disappeared within 2 hours, while the APPA microcapsule lasted up to 24 hours. This suggests that APPA membranes may be more appropriate for prolonged controlled release and since it retains its membrane longer in the GI tract than APA, it may be more suitable for oral administration of encapsulated cells. In addition to these morphological studies, in order to test the benefit of PEG for oral delivery of therapeutic products, live cells will need to be encapsulated and analyzed.

Cell encapsulation

HepG2 cells were encapsulated in pure alginate and then coated with PLL, chitosan and PEG. Figure 3a shows a picture of encapsulated hepatocytes in an ACP membrane. Figure 3b shows a comparison between the absorbance of the hepatocytes encapsulated in alginate and the absorbance after being coated with the various membranes and being incubated for 24 hours. Results indicate positive metabolic activity of cells within the membranes. Therefore, the membrane materials do not exert toxicity to the cells. The corresponding absorbances were found to be: 0.84± 0.04 for alginate microcapsules, 0.81±0.06 for APPA, 0.67±0.03 for AC, 0.80±0.01 for ACP and 0.89±0.1 for APA. No significant changes were observed in the viability of the cells after the coating procedure. Although results reveal chitosan is the least favourable for cell attachment, the addition of PEG to the chitosan membrane clearly improves biocompatibility.

Previous studies have revealed that PEG can lead to increased biocompatibility. It has been observed that PEG can eliminate the immunogenicity of proteins while preserving their biological properties. The addition of PEG to polymers reduces the adsorption and adhesion of cells and proteins to the membrane surface.[11,15] The results from this study show that

membranes containing PEG can support cellular growth.

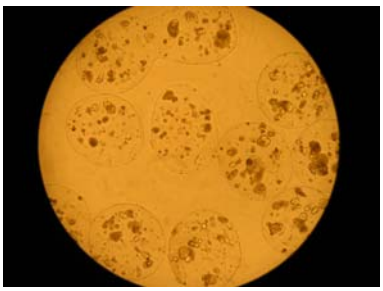

Figure 3a: Hepatocytes encapsulated in ACP membrane. Magnification 10X

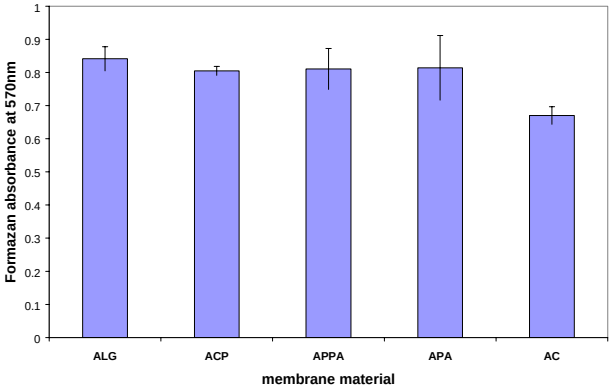

Figure 3b: Cytotoxicity test for membranes. MTT absorbance at 570nm of encapsulated hepatocytes after 24 hour incubation.

CONCLUSION

The current study has shown that the incorporation of PEG to microcapsule membranes may enhance the mechanical stability of microcapsules and lead to improvements to AC and APA capsules. It has been shown that PEG integrated microcapsules can support cell proliferation and they may have the potential to be used for both oral delivery and cell transplantation. Further research remains to be conducted on the possibility of long term cell encapsulation.

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