HAP/gelatin composite membranes for encapsulation and immunoisolation of islet cells

J.P. Chen and F.N.Chang
Chang Gung University, 259 Wen Hwa 1st Rd., Kwei-San,Taoyuan, Taiwan
(jpchen@mail.cgu.edu.tw)

Introduction

Xenogenic pancreatic islet transplantation represents a potential therapy for the treatment of insulin-dependent diabetes mellitus. However, xenotransplantation may also induce strong rejection and lead to dysfunction of transplanted islet. Encapsulation of insulin-secreting cells can prevent the passage of attacking cells from the humoral component of immune rejection, while the enclosing porous chamber can be permeated by glucose, nutrients, and insulin. Thus, encapsulated insulin-secreting cells might survive and maintain the effective insulin supply, which is synthesized as needed and available to the host on demand.
Clinically, bioceramics such as hydroxyapatite (HAP) has been tried for use as bone implants. The use of bioactive ceramics for defect filler was in powder and block forms. Although powder ceramics remain the form of choice for filling small irregular defects, the therapeutic effect of the filling implant was lost by migration of particles from the defect site. Furthermore, it was too difficult to handle and keep in place compactly for convenient fabrication and operation of block-type ceramics. Thus, it is necessary to mix a suitable binder with the granular material to overcome these problems. Recently, composites of ceramic powders with natural degradable polymers have attracted much interest. Fibrin, collagen and gelatin are generally considered as binders, as they have good adhesiveness and plasticity properties.
In this study, a new type of composite immunoisolation membrane combining cross-linked gelatin and HAP was designed for encapsulation of islet cells. In the composite, the glutaraldehyde cross-linked gelatin and HAP served as a continuous matrix (binding agent) and discontinuous particles, respectively. Gelatin crosslinked by glutaraldehyde has been used in clinical applications and has proven to be efficient for more than 20 years. For instance, bioprostheses made of bovine pericardium or porcine heart valve tissues were usually treated by glutaraldehyde (GA). The presence of gelatin in the composites serves two purposes: it immobilizes the HAP particles and it improves the mechanical properties. Gelatin is easily resorbable *in vivo*, the cross-linking agent being added can prolong its stay in the living tissue and improve the brittleness and control pore size of the composite.

Material and methods

Gelatin powder was extracted from porcine skin with an average molecular weight of about 50,000 to 100,000 Da. The gelatin powder was weighed and dissolved in the deionized distilled water until a homogeneous gelatin solution (5%, 10%, and 15 wt%) was attained. The dissolution process was done in a water bath at 60°C. HAP ceramic particles (4.2 g) were poured in and mixed with the gelatin solution (8 ml), and stirred continuously at the same temperature all the time, in order to achieve a better distribution of ceramic particles in the gelatin matrix. The composite solution was poured into a mold, waiting for solidification and fabrication into a membrane or a 1 cm length, 1 cm diameter cylindrical chamber. 1% glutaraldehyde (GA) was added to the HAP/gelatin mixture for cross-linking the gelatin at different times (6 to 72 h). The composites were then freezed dried at -80°C for 24 h. Ninhydrin method was used for measuring the free amino groups in gelatin and calculating the degree of crosslinking, which is defined as the percentage of free amino groups in gelatin that has been crosslinked by glutaraldehyde. The composite membrane was characterized by

XRD, FTIR, SEM, and BET. The cytotoxicity of the membrane was tested according to ASTM 10993-5 by using 3T3 fibroblast cells and MTT assays for cell viability. RIN-m5F rat insulinoma cells were used for encapsulation and test of insulin production. Cell viability and insulin secretion of the encapsulated cells were determined by MTS assays and ELISA. Glucose stimulation tests were carried out by challenging the cells alternatively with 2.7 and 16.7 mM glucose in RPMI-1640 medium. Permeability coefficients of glucose (MW = 180), Vitamin B12 (MW = 1,355), Myoglobin (MW = 17,600), and BSA (Mw = 68,000) in the composite membrane were measured in a side-by-side permeation cells at 37 ^{o}C.

Results and Discussion

The degree of cross-linking increases with time as the free amino group in gelatin decreases (Fig. 1). The efficiency of crosslinking also depends on gelatin concentration. For 5% and 10% gelatin (G5 and G10), crosslinking could be completed within 24 hr where the degree of crosslinking levels off at 90%. By choosing 48 h as the optimum crosslinking time, 20% gelatin (G20) was found to be less stable due to incomplete crosslinking and lost 25% of its original weight after shaken at 200 rpm and 37 $^{\circ}$C for 3 days where total recovery of initial weight were observed for G5 and G10. Increasing gelatin concentration will therefore leads to higher lost of material due to lower degree of crosslinking. The crystal structure of HAP powder remains unchanged regardless of the addition of gelatin or crosslinking from XRD analysis. The FTIR spectra in Fig. 2 confirms the imide bond formation between GA and gelatin from the new -C=N-C- peak at 1620~1640 cm^{-1}.

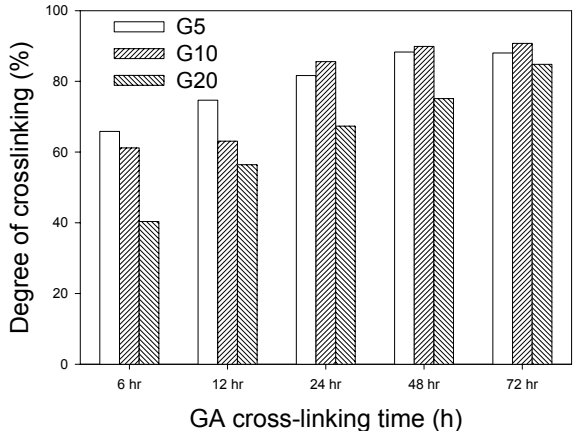

Fig. 1. Crosslinking of HAP/gelatin membranes by glutaraldehyde.▲: 20% gelatin (G20), ●: 10% gelatin (G10), ■: 5% gelatin (G5).

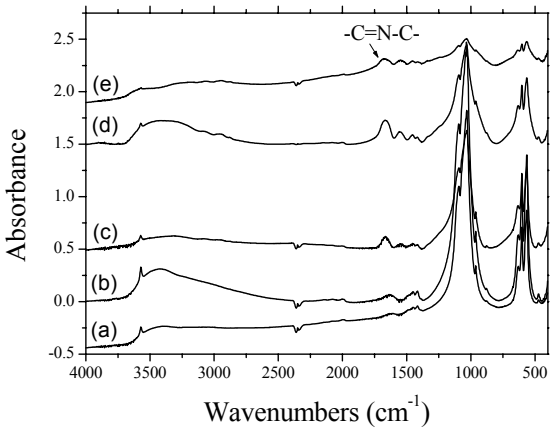

Fig. 2. FTIR spectra of HAP/gelatin composite membranes. (a) HAP, (b) HAP + gelatin, (c) G5, (d) G10, (e) G20.

From SEM micrographs in Figs. 3 to 5, with insufficient amount of gelatin added, only part of HAP powder was covered by gelatin for G5. In contrast, excellent cross-linking was observed for G10 and G20 samples, which will result in improved mechanical strength. The BET analysis in Table 1 indicates that pore size of the micropores on the surface of HAP/gelatin particles decrease with gelatin concentration. This can be confirmed from the SEM pictures as more gelatin cover the HAP powder leads to a smoother surface and larger particle size of the microparticles. However, the macropores, through which diffusion of molecules occur in the membrane, is larger at higher gelatin concentration. Binding coarse HAP/gelatin granules together (G20) is expected to lead to larger pores than binding together fine HAP/gelatin granules (G5) in the membrane. To verify the microstructure of the membrane, permeation studies of four molecules with different molecular weight was carried out to simulate the diffusion of glucose, insulin, and nutrients through the

membrane. As can be seen from Fig. 6, peameability coefficients of molecules increase with increasing gelatin concentration and are inversely correlated with molecular weight as expected.

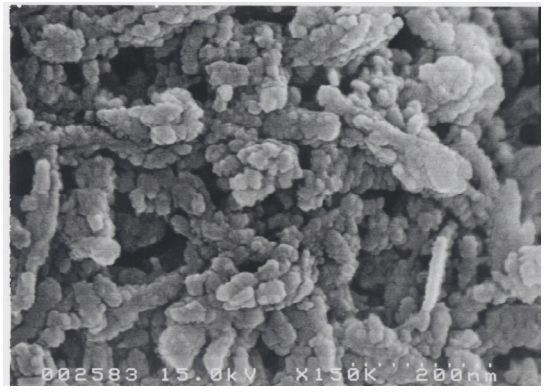

Fig. 3. SEM micrographs of HAP/5% gelatin (G5) membranes.

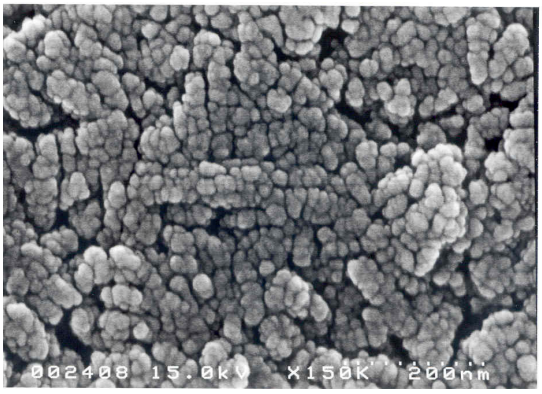

Fig. 4. SEM micrographs of HAP/10% gelatin (G10) membranes.

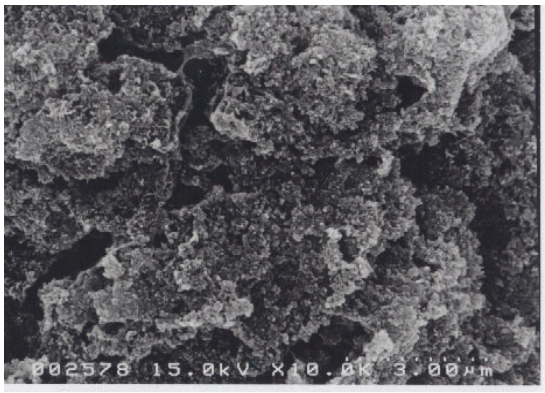

Fig. 5. SEM micrographs of HAP/20% gelatin (G20) membranes.

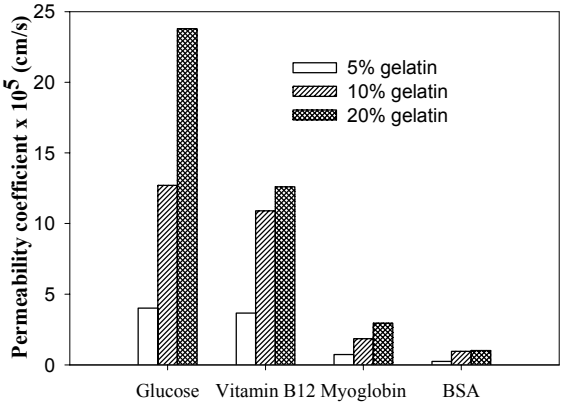

Fig. 6. Permeability coefficients of different molecules through HAP/gelatin membranes.

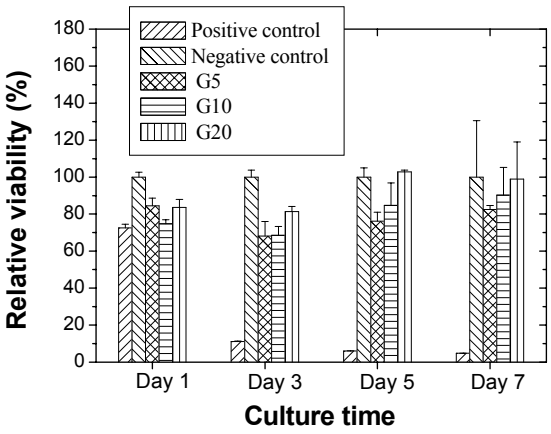

Fig. 7. Cytotoxicity of HAP/gelatin membranes. Positive control is latex, negative control is medium.

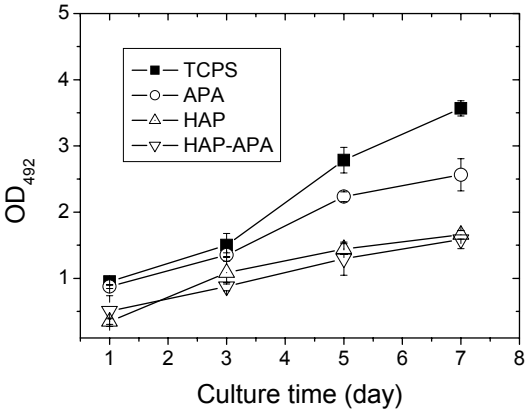

Fig. 8. Proliferation of viable cells in different systems by MTS assay. TCPS: culture dish, APA: APA microcapsules, HAP: HAP/gelatin chamber (G10), HAP-APA: APA microcapsules in HAP/gelatin chamber (G10).

Results from cell cytotoxicity studies of the composite membranes are shown in Fig. 7. Some cell toxicity was observed for the membrane when compared with negative control, which may come

from the residual GA. That G20 usually results in higher cell number than G5 and G10 may arise from beneficial effect of gelatin in the medium, which leaks out from the membrane during cell culture with incomplete crosslinking. The growth and insulin production of encapsulated cells were studied in different systems, including cells in tissue culture polystyrene (TCPS), alginate-polylysine-alginate microcapsules (APA), HAP/gelatin chambers (HAP), and APA microcapsules in HAP/gelatin chambers (HAP-APA). As can be seen from Figs. 8 and 9, all systems give similar increasing trend of cell growth and insulin secretion. However, the performance is generally the best for cells on TCPS, followed by APA, HAP, and HAP-APA systems. Mass transfer plays a role in the results observed as the performance can be correlated with the expected mass transfer resistance in each system. From results in Fig. 10, islet cell in HAP/gelatin chamber can modulate the rate of insulin secretion in response to glucose concentration as in APA microcapsule system.

Gelatin content (%)	Surface area (m^2/g)	Pore volume (cm^3/g)	Pore size (Å)
5	48.99 ± 3.39	0.146 ± 0.0082	119.28 ± 1.576
10	42.54 ± 2.608	0.1189 ± 0.026	105.75 ± 23.236
20	7.784 ± 3.754	0.0175 ± 0.0103	87.72 ± 11.881

Table 1. BET analysis of HAP/gelatin composite membranes

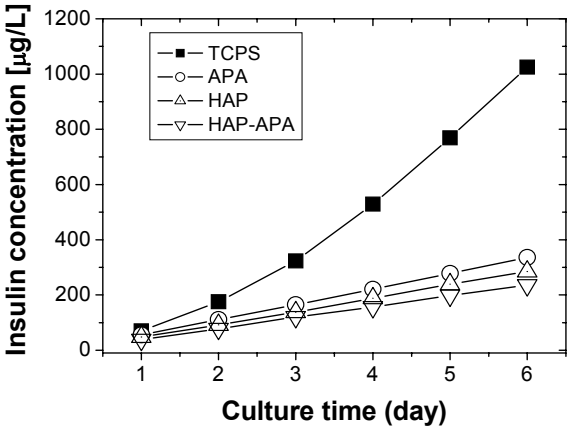

Fig. 9. Insulin secretion from cells in different systems. TCPS: culture dish, APA: APA microcapsules, HAP: HAP/gelatin chamber (G10), HAP-APA: APA microcapsules in HAP/gelatin chamber (G10).

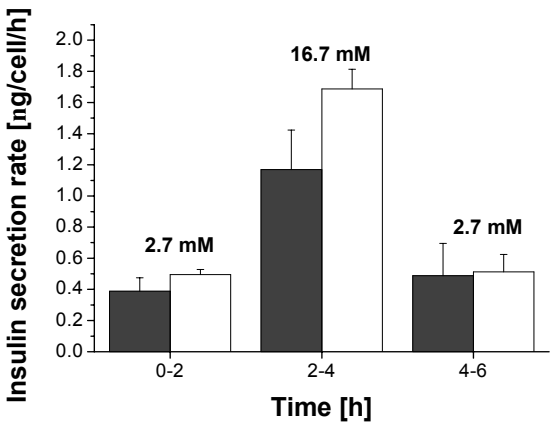

Fig. 10. Response of insulin secretion to glucose concentration. White bar: APA microcapsules, filled bar: HAP/gelatin chamber (G10).

Conclusions

HAP/gelatin composite materials intended for immunoisolation of islet cells were fabricated and characterized in detail in this study. With its good permeability to glucose, nutrients, and insulin; good mechanical property and biocompatibility, the newly developed HAP/gelatin chamber would be a good candidate for macroencapsulation of islets cells foe development of artificial pancreas.

References

Chang, M.C. *et al.* (2003) *Conformational change of hydroxyapatite/gelatin nanocomposite by glutaraldehyde*. Biomaterials, 24, 3087-3094.

Lin, F.H. *et al*. (1998) Biological effects and cytotoxicity of tricalcium phosphate and glutaraldehyde cross-linked gelatin. Biomaterials, 19, 905-912.