# Microcapsules for cell entrapment by template polymerization of a synthetic hydrogel coating around calcium alginate gel: preliminary development

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Stable, water-soluble copolymers of glycidyl methacrylate with n-vinyl pyrrolidinone (I) were prepared by free-radical polymerization. Water-soluble copolymers of 2-hydroxyethyl methacrylate with methacrylic acid (II) were examined as co-reactants with (I) to form hydrogel matrices. Upon mixing of (I) and (II) in solution, a covalently crosslinked hydrogel was formed, presumably by etherification of epoxy functionality on (I) with hydroxyls on (II). Synthetic hydrogel-coated gel beads were prepared from an aqueous mixture of sodium alginate and (II) by treatment with solution containing (I) and calcium ion. An elastic, defect free and indefinitely stable covalently crosslinked hydrogel coating was formed around the calcium alginate by surface reaction of (I) and (II). Encapsulated guinea-pig red blood cells suffered minimal lysis over a 4 day period due to isolation and protection from the reacting species by the interior alginate gel matrix.

## 1. Introduction

To bypass some of the limitations of conventional transplantation therapy for Type I diabetes, researchers have recognized that many of the problems associated with islet cell therapy may be overcome if the islets could be isolated by a semipermeable barrier, through which salts, glucose and other nutrients may be absorbed, and through which insulin and cell waste products may be excreted, while remaining impermeable to immunoglobulins and other components of the host's immune system.

In theory, therefore, the islets would respond to high levels of inwardly diffusing glucose by the secretion of insulin, which would diffuse across the membrane to restore normoglycaemia. In addition to protecting transplanted tissue, a plentiful supply of animal islets could be used in human therapy, thus circumventing ethical and supply problems associated with the use of foetal or donated tissue. If the islets are microencapsulated to maximize mass transfer, then transplantation would involve a simple periodic injection (frequency as yet unknown) of the entrapped cells into the intraperitoneal cavity, kidney subcapsule or portal vein.

The most advanced microencapsulation systems are based on the use of calcium alginate as a matrix gel former in which the cells of interest are embedded. Originally developed as an aid in high-density cell culture and as a means of immobilizing cells for use in large bioreactors [1, 2], alginate–polylysine technology was adapted by Sun and co-workers for islet cell entrapment purposes [3, 4]. Even so, concerns must be raised about the long-term biocompatibility and mechanical stability of this fragile polysaccharide–protein complex.

As a biocompatible and more robust alternative to the use of alginate, capsule-forming systems based on the use of uncharged and thermoplastic poly-(2-hydroxyethyl methacrylate)-containing polymers have been developed [5, 6]. Although most of the rather formidable technical problems associated with the required use of non-aqueous solvent systems have been solved [7] and encapsulated model cell lines have been shown to retain substantial function [8], questions remain regarding the incorporation of sufficient permeability into the capsule membrane to maintain islets *in vivo* and allow for the delivery of insulin in a timely manner.

The equilibrium water uptake and hence permeability of uncharged thermoplastic hydrogel polymers is limited by the requirement of complete water insolubility (for mechanical stability and elimination of leachables) to about 40 wt % of the swollen polymer. A higher water uptake, induced by, for example, the incorporation of some charged functionality, would result in a less stable membrane. Clearly,

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therefore, alternative approaches towards the development of a stable, highly permeable capsule membrane from synthetic polymer should be investigated.

An indefinitely stable synthetic hydrogel polymer with a higher equilibrium water uptake may be prepared if it is stabilized by a covalent bond network. However, covalent bond-forming reactions performed in solution invariably kill cells. In this paper we detail the preliminary development of a new concept for the entrapment of mammalian cells, based on the simultaneous formation of an alginate gel matrix and hydrogel coating. The alginate matrix isolates the cells from the capsule surface around which a synthetic, (hopefully) non-antigenic, covalently crosslinked hydrogel coating is being formed by co-reaction of water-soluble polymers.

## 2. Materials and methods

### 2.1. Polymer synthesis and characterization

Monomers were purified by vacuum distillation, stored at $-20\,^{\circ}C$ and used within 24 h of distillation. Abbreviations used in this paper are listed in Table I. Azo-bis-isobutyronitrile (AIBN) was recrystallized from warm ethanol. Solvents were of AR or HPLC grade. Polymers were prepared by solution polymerization in either ethanol or 1,4-dioxane, with a 10% (w/v) monomer loading, for 1–4 h at $70 \pm 0.05\,^{\circ}C$. Material was recovered by coagulation in petroleum ether and vacuum-dried to constant weight.

Polymer compositions were ascertained by proton nuclear magnetic resonance (PNMR) spectroscopy in either d4 methanol or d1 chloroform, using a Varian XL-300 spectrometer. Intrinsic viscosities were measured at $35 \pm 0.05\,^{\circ}C$ in Ubbelohde viscometers using dimethylformamide (DMF) solvent containing 0.2% (w/v) LiBr to suppress polyelectrolyte effects [9]. Copolymers containing the monomer residues A and B are designated as A-co-B or B-co-A throughout this paper, with no preference over the ordering of A and B.

### 2.2. Hydrogel formation

The hydrogel coating process was modelled by titrating an aliquot of aqueous epoxy-containing polymer with small amounts of dissolved hydroxylated polymer, recovering and weighing the precipitate after each titre.

### 2.3. Capsule preparation

Sodium alginate and the synthetic polyelectrolyte polymer were dissolved separately in appropriate medium and then mixed. No signs of precipitation or phase separation were observed visually. A mixture containing dissolved sodium alginate (Kelko LV: about 1 wt%) and copolymer of HEMA and MAA, usually around 3–5 wt%, was prepared (with or without cells) in physiologically buffered saline; pumped from an adjustable syringe pump and blown as droplets from a small-diameter needle tip with a coaxial air stream. Gel beads were prepared by a short (10–30 min) exposure to tris(hydroxymethyl)-aminomethane (Tris)-buffered 0.1 M $CaCl_2$ containing 3–5 wt% of the GMA–NVP copolymer, followed by a longer (1–4 h) exposure to the GMA-co-NVP copolymer in isotonic Tris-buffered saline in order to complete the hydrogel coating around the alginate. The treated beads were then transferred to physiological buffered saline for long-term storage. In some instances the interior alginate gel was liquefied by exposure to isotonic Tris-buffered citrate solution (100 mM sodium citrate + 200 mM NaCl).

### 2.4. Scanning electron microscopy and optical microscopy

Capsules or gel beads were lyophilized and examined, either intact or fractured, after gold-coating, in a Philips 315-M scanning electron microscope (SEM). Optical microscopy was performed using a Reichart Microstar IV microscope. Capsules were examined on medium hanging-drop slides (18 mm diameter $\times$ 0.5 mm depth). Black-and-white photographs were taken with an NPC black-and-white camera attachment on the microscope.

### 2.5. Infrared spectroscopy

A structural analysis of the hydrogel coating was

TABLE I Polymer compositions[a] and intrinsic viscosities

| Polymer | GMA (mol %) | NVP (mol %) | HEMA (mol %) | MAA (mol %) | MMA (mol %) | Intrinsic viscosity ($cm^3\,g^{-1}$) |
|---|---|---|---|---|---|---|
| PMAA | 0 | 0 | 0 | 100 | 0 | XX |
| PNVP | 0 | 100 | 0 | 0 | 0 | XX |
| 1 | 0 | 0 | 42 | 9 | 48 | 60.4 |
| 4 | 0 | 0 | 46 | 31 | 23 | 65.1 |
| 7 | 0 | 0 | 36 | 64 | 0 | 60.5 |
| 10a | 0 | 0 | 90 | 10 | 0 | 72.0 |
| 52 | 40 | 60 | 0 | 0 | 0 | XX |
| 53 | 9.6 | 90.4 | 0 | 0 | 0 | XX |

[a] Composition by NMR spectroscopy and acid–base titration where appropriate.
GMA, glycidyl methacrylate; NVP, n-vinyl pyrrolidinone; HEMA, 2-hydroxyethyl methacrylate; MAA, methacrylic acid; MMA, methyl methacrylate; PMAA, poly(MAA); PNVP, poly(NVP); XX, not measured.

made by infrared spectroscopy using the KBr disc method. Spectra were obtained, stored and subtracted using a Nicolet 5MX spectrometer. The spectra of other relevant materials (i.e. alginate, GMA-co-NVP, etc.) were also collected for comparison.

### 2.6. Drabkins test

Guinea-pig red blood cells (Brown Laboratories, Topeka, Kansas) were isolated by low-speed centrifugations. One portion (the control) was stored at 4 °C in physiological buffered saline. A second portion was mixed at a level of 10% haematocrit with sodium alginate and HEMA-co-MAA in isotonic Tris-buffered saline at pH 7.4, treated with $Ca^{2+}$ and dissolved GMA-co-NVP to form a hydrogel-coated alginate bead around the red blood cells.

Treated beads were stored at 4 °C for 4 days. The storage solution was changed daily and accumulated. Capsules were then teased open with a fine needle and the alginate was dissolved in isotonic Tris-containing sodium citrate (100 mM) and saline (200 mM). Lysed material, isolated from the cell pellet by centrifugation, was added to the storage solutions. Intact cells were then lysed in distilled water and the proportion of intact material was determined by investigation of both fractions by visible spectrometry at 540 nm using a Gilford Response spectrometer after fixing the haemoglobin in Drabkins reagent and adjusting the concentrations to keep absorbances below 1 a.u. [10, 11]. The control cells were treated in a similar manner and the percentage lysis of the encapsulated cells were reported against that recorded for the control.

## 3. Results and discussion

### 3.1. Polymer synthesis and characterization

The water-soluble polymers used to prepare the hydrogel coating around calcium alginate were all prepared by free-radical polymerization. Monomer residues are shown in Fig. 1. The coating was produced at and around the capsule surface by reaction of polymeric reagents as they diffuse into and out of the gel bead.

The reagents mixed with the alginate may be generalized as water-soluble copolymers of HEMA with MAA which were prepared by solution polymerization in ethanol. The synthesis of these copolymers was described in [12]. Structural information is summarized in Table I.

The reagents that diffuse into the capsule to react may be classified as water-soluble copolymers of GMA with NVP which are prepared by solution polymerization in 1,4-dioxane. We have previously determined the reactivity ratios for this system by the method of Kelen and Tudos as $r_{GMA} = 4.4$ and $r_{NVP} = 0.01$ [13, 14]. The polymers discussed here, therefore, contained considerable structural heterogeneity. Polymer molecular weights were kept low through the use of high initiator concentrations (1 mol % of the combined monomer charge). Small amounts of PMAA and PNVP homopolymers were prepared for reference purposes by solution polymerization in ethanol and in 1,4-dioxane, respectively.

Polymer 52 was prepared from a feed mixture containing 10 mol % GMA and 90 mol % NVP (1 h at 70 °C, yield 23.7%) and contained an "average" content of 40 mol % GMA as determined by PNMR spectroscopy using an established method [14]. Polymer 53 was prepared under the same conditions from a feed mixture containing 2.25 mol % GMA and 97.75 mol % NVP (yield 20.2%, GMA in polymer 9.6 mol %). Polymer 52 was used to prepare "free-standing" coatings for infrared and SEM analysis. Polymer 53 was used to coat cell-containing gel beads.

### 3.2. Hydrogel formation

Before performing the encapsulation experiments, hydroxylated polymers were reacted with polymer 52 and the rate, yield and product formation and its physical properties were used to guide selection of

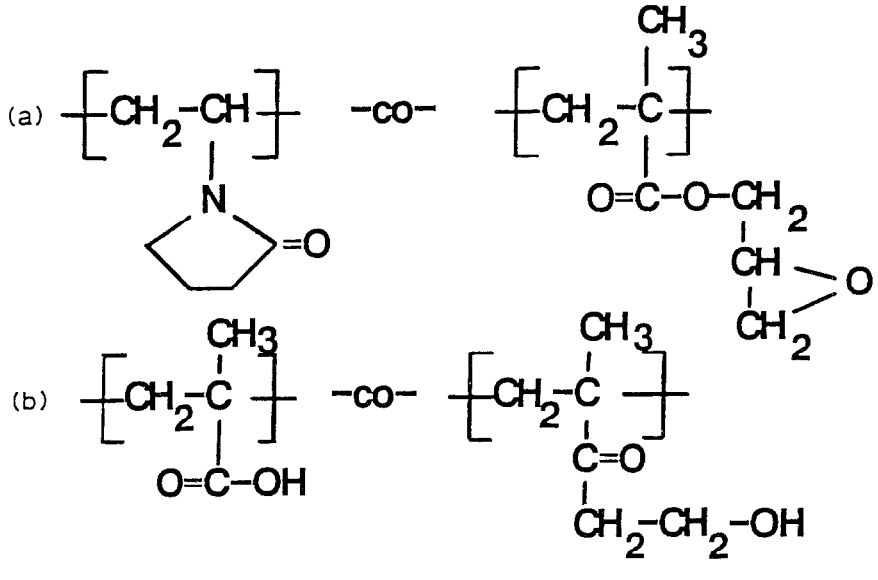


*Figure 1* Monomer residues in copolymers: (a) NVP-co-GMA and (b) MAA-co-HEMA.

materials for the coating process. The reaction in question is simple etherification of epoxy functionality on the GMA-co-NVP copolymer by hydroxyls on the HEMA-co-MAA copolymer as depicted in Fig. 2a. The opened oxirane ring then abstracts a proton from a third party (X–H) or from the attacking hydroxyl.

We chose a series of co- and ter-polymers of HEMA, MMA and MAA to test simultaneously the influence of hydroxyl content (from the HEMA), polymer hydrophilicity (lowered by incorporating MMA) and the feasibility of catalysis of the etherification reaction through the use of bound carboxylic acid groups (H–X from the MAA) as proton donors. Increments of the hydroxylated polymer were added to an excess of the epoxidized polymer to model (at least in the early stages of addition) the encapsulation experiment, in which the latter is present in excess over the former.

A 1% solution (0.1 g in 10 ml) of polymer 52 in buffered saline was titrated with a 2.5% solution of the polyhydric alcohol in 1 ml (0.025 g) increments, to a final titre of 0.1 g alcohol (in 2.0 ml). Precipitate or gel was recovered by centrifugation and freeze-drying. The cumulative yields of recovered coagulate, as shown in Fig. 2b, indicate that the product yield (especially after the all-important first titre, when the highest epoxy-containing and, therefore, most-reactive fractions of polymer 52 will react) increases

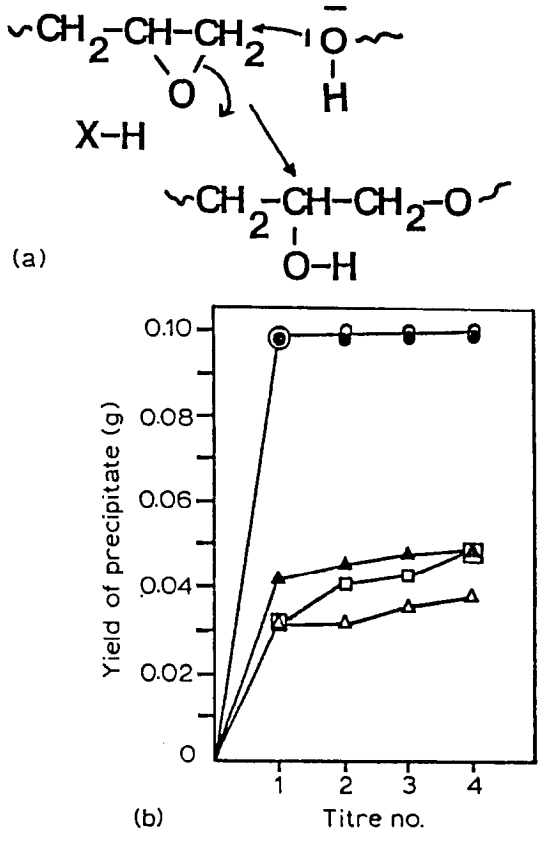


*Figure 2* (a) Etherification of epoxy group with hydroxyl functionality; ring-opened epoxy abstracts a proton either from the hydroxyl or from another proton donor (X–H). (b) Yield of precipitate formed by titrating a copolymer of NVP and GMA (polymer 52) with copolymers of MAA and HEMA with compositions as in Table I. (●) Polymer 10a, (○) polymer 1, (△) polymer 4, (▲) polymer 7 and (□) PMAA.

with the hydroxyl content in the polyhydric alcohol. These results are in agreement with those that would be predicted on the basis of the relative reactivities of hydroxyls and carboxylate groups with epoxy functionality [15]. Precipitate was observed through reaction of polymer 52 with PMAA through ester formation with the polyepoxide but, as expected, the yield from that reaction was less than from polymers containing a high proportion of alcohol functionality. Acid catalysis of the etherification reaction appeared not to occur, probably due to a nearly 100% ionization of the acid functionality in isotonic saline at pH 7.4 [11]. No precipitate was observed upon mixing polymer 52 with PNVP homopolymer, thus confirming that the GMA residue is the locus of reaction with the polymeric alcohol.

Coagulate from polymers 10a and 52 formed rapidly (within seconds) and exhibited an elastic response upon deformation with tweezers and puncture resistance upon probing with needles. Coagulate from polymer 52 and the more hydrophobic polymer 1 also formed rapidly but exhibited poorer mechanical properties (lowered elasticity and tear resistance, etc.), possibly because polymer 1 contains fewer hydroxyls and adopts a more compact configuration in aqueous solution than polymer 10a, both of which will result in less reaction with polymer 52 to form fewer crosslinks and, therefore, load-bearing chains. Coagulate from the other systems formed slowly and exhibited no load-bearing capability. In other experiments we found that soluble (89% hydrolysed) poly(vinyl alcohol) formed a semi-liquid coagulate with polymer 52. It would therefore seem that the development of mechanical properties is not only dependent on the hydroxyl content but is also a function of other structural elements of the water-swollen polyhydric alcohol.

### 3.3. Capsule formation

Surface-coated alginate beads were prepared by mixing the polyhydric alcohol (polymer 10a) with sodium alginate in aqueous solution, then gelling the alginate and forming the hydrogel coating by brief exposure to a solution containing $Ca^{2+}$ and dissolved epoxidized polymer (polymers 52 or 53). A more prolonged exposure to the epoxidized polymer in calcium-free buffer completed the coating process. The alginate core was then liquefied with citrate if desired.

Fresh GMA-co-NVP solutions were prepared for each experiment even though these polymers proved remarkably stable in physiological buffered saline. For example, 15 days storage of a 1 wt% solution of polymer 52 in Tris buffer at 37 °C changed the relative viscosity of the polymer solution from 1.133 to 1.149, and in Tris-buffered saline from 1.149 to 1.143.

Trial experiments proved that the coating was not formed in the absence of the polyhydric alcohol polymer (i.e. with pure alginate), from a mixture of alginate and PMAA or through the use of PNVP in place of the GMA-co-NVP copolymer. The alginate gel would liquefy in citrate and the capsule would disappear without a visible residue. A coating, but with

inferior properties, could be produced if the alginate were used in conjunction with polymers 1, 4 or 7. In contrast, a coating from the reaction of polymers 10a and 52 was found to be elastic and indefinitely stable even if the alginate core was liquefied by exposure to sodium citrate (over 1 year to date with no signs of visual deterioration; swelling, shrinkage, hole formation or loss of debris, etc.). Freeze-dried and reconstituted capsules from polymer 10a appeared to be undamaged by that procedure. Covalent bond formation was confirmed in freeze-dried capsules through a complete resistance to dissolution in organic solvent media. (A complete absence of crystallinity in these amorphous polymers would preclude that explanation for insolubility.)

It must be mentioned that the hydrogel coating need not be formed simultaneously with the interior alginate gel. For example, hydrogel-coated beads have been prepared by adding droplets of aqueous alginate and hydroxylated polymer to $Ca^{2+}$-containing solution free of epoxy polymer, then forming the hydrogel coating by transferring the beads to Tris-buffered saline containing epoxidized polymer. For example, capsules from a 1.3 wt % alginate + 2.5 wt % polymer 10a, gelled in isotonic Tris-buffered $CaCl_2$ for 30 min, then transferred to an isotonic Tris-buffered saline solution containing 5 wt % polymer 52, soaked for 4 h, then stored in isotonic Tris-buffered saline, have so far remained stable for more than 1 year. These capsules appear to possess a thinner and more transparent coating than others made by forming the gel and coating at the same time.

### 3.4. Scanning electron microscopy and optical microscopy

Coated alginate beads were examined by optical microscopy as shown in Fig. 3. Fig. 3a shows a typical microbead treated with polymers 52 and 10a (1.3 wt % alginate + 2.5 wt % polymer 10a, soaked for 30 min in a 0.1 M $CaCl_2$ solution containing 5 wt % polymer 52, then for 4 h in a Tris-buffered saline solution containing polymer 52, before long-term storage in isotonic Tris-buffered saline). The coated bead retained the shape of the original alginate gel and appeared opaque and otherwise featureless by optical microscopy. The differences between uncoated and coated alginate are illustrated in Fig. 3b. The optically transparent alginate (left) was transformed into an opaque structure which efficiently scattered light and resisted close penetration by visible light. We chose a capsule which had been prepared from an alginate bead possessing a "tail" to illustrate that the shape was retained during the coating process.

For a proper explanation of the hydrogel coating process and the morphology of treated alginate, we must first examine that of virgin calcium alginate. SEM micrographs of calcium alginate microbeads are reproduced in Fig. 4. A low-magnification micrograph (Fig. 4a) reveals that the alginate bead shrinks somewhat during lyophilization but otherwise displays a featureless surface, as emphasized at higher magnification in Fig. 4b. The dry alginate bead was difficult to handle because of the fragility of this surface layer which distorted and cracked upon application of small stresses (pictures not shown). Examination at low magnification of a fractured microbead (Fig. 4c) revealed a cellular, almost sponge-like, interior which is shown in more detail at higher magnification in Fig. 4d.

SEM micrographs of coated beads, prepared as for optical microscopy, are reproduced in Fig. 5. An intact capsule with tail is depicted at low magnification in Fig. 5a. The capsule surface appears to be covered with a meshwork of hydrogel. Close-ups of the capsule surface at successively higher magnifications reveal the macroscopically open (Fig. 5b) yet microscopically dense (Fig. 5c) nature of the coating. A capsule was treated with sodium citrate for 30 min then lyophilized and fractured (Fig. 5d) to show that the interior calcium alginate morphology was retained. The retention of the calcium alginate morphology and, by inference, the exclusion of citrate from the capsule interior over this short period, is strong circumstantial proof that the hydrogel coating is continuous and defect-free.

SEM micrographs of the surface of an intact hydrogel-coated gel bead after 1 h treatment with isotonic

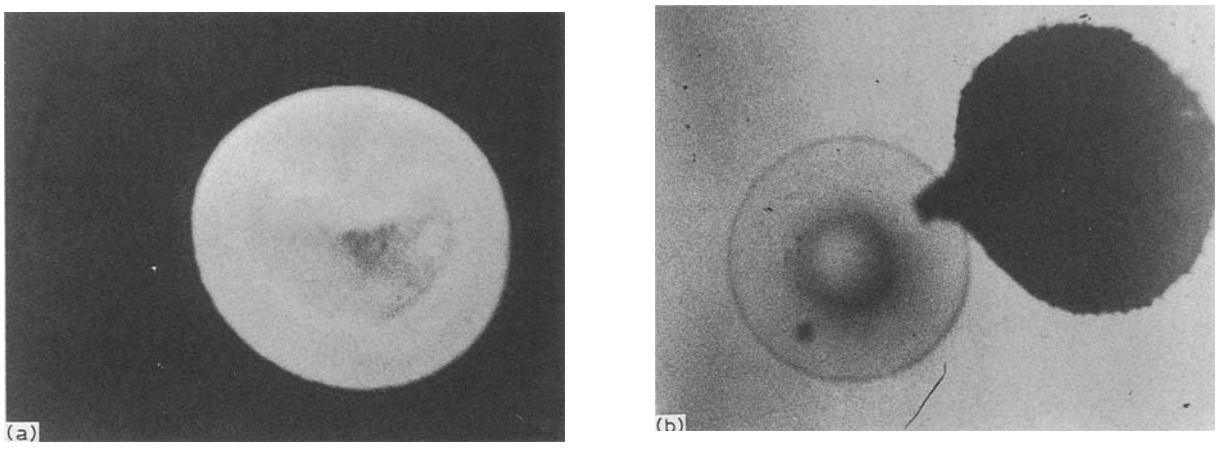


*Figure 3* Optical microscopy of hydrogel-coated alginate bead: (a) typical large capsule (long axis of micrograph 5.1 mm) and (b) comparison between coated (opaque) and uncoated (transparent) alginate bead at same magnification as in (a).

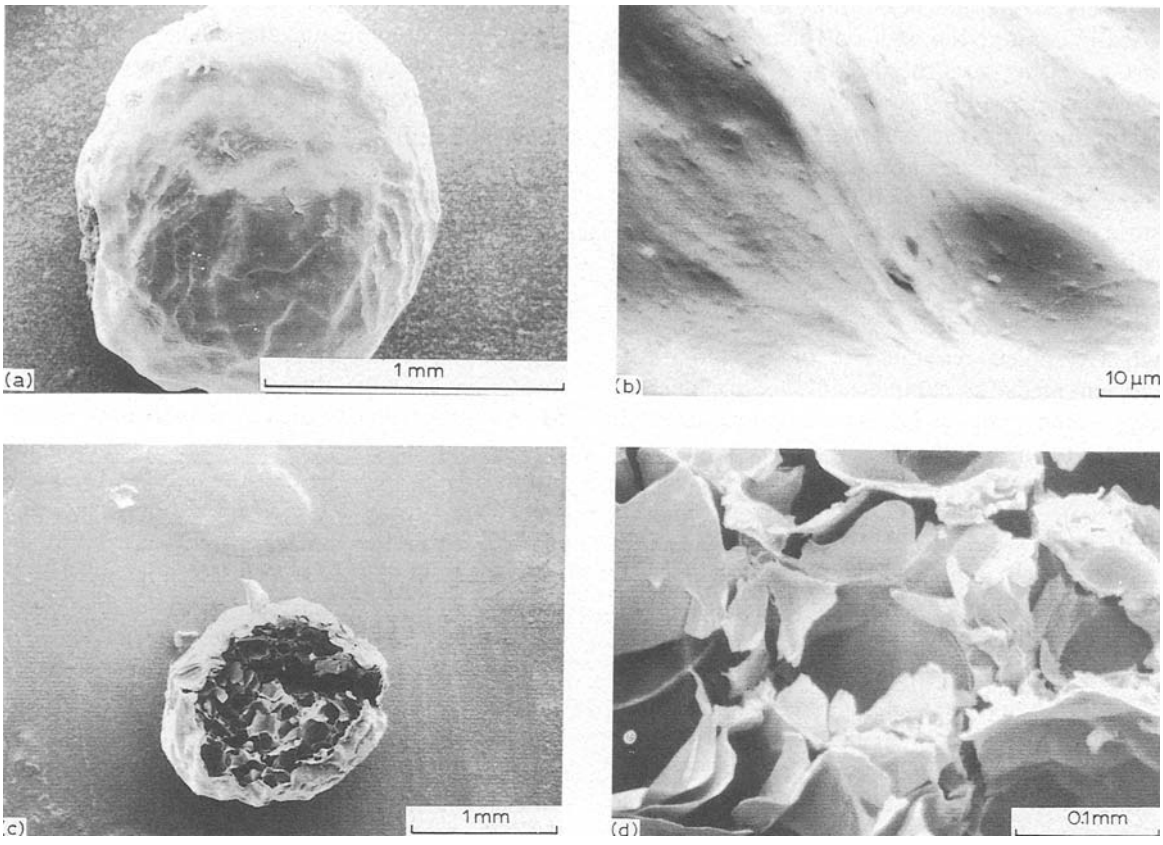


*Figure 4* SEM micrographs of calcium alginate beads: (a) intact bead at low magnification, (b) bead surface, featureless at higher magnification, (c) fractured alginate bead at low magnification, showing cellular interior and (d) close-up of fracture surface.

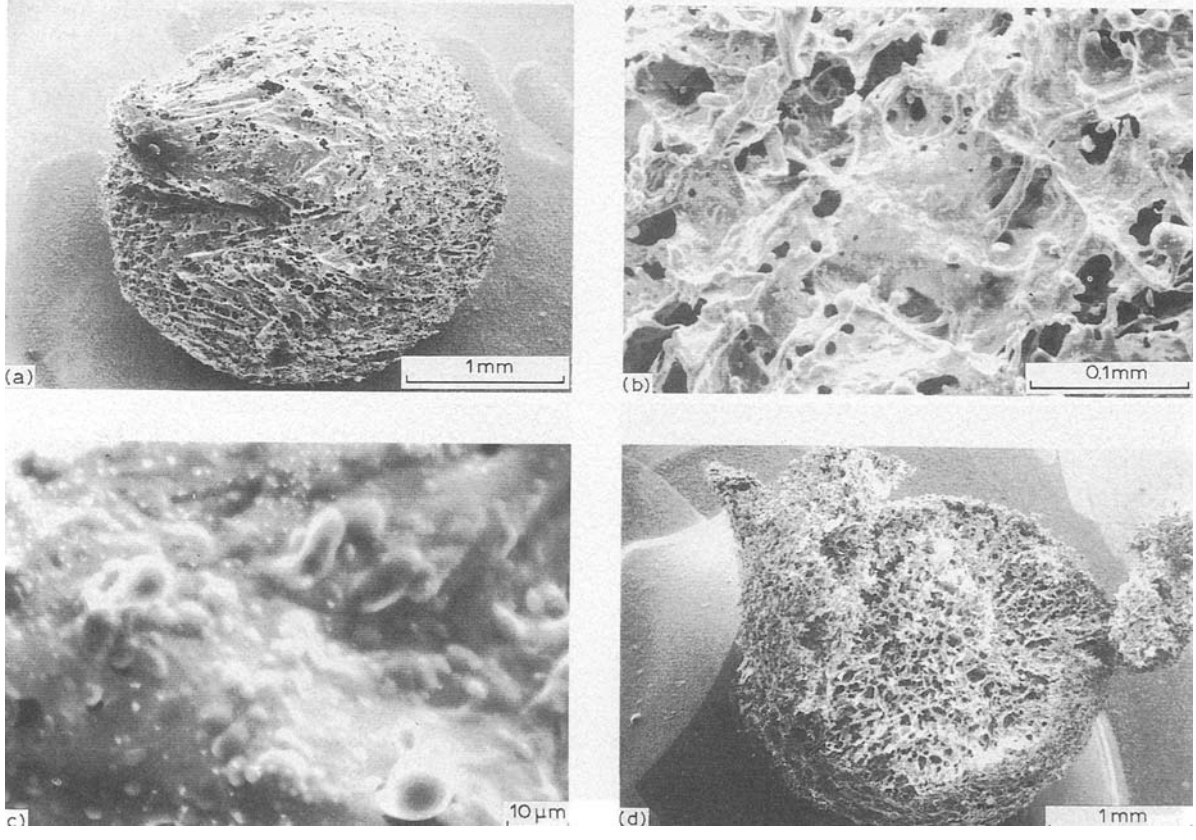


*Figure 5* SEM micrographs of hydrogel-coated alginate gel bead: (a) intact bead at low magnification, (b) and (c) bead surface at higher magnification and (d) fractured bead at low magnification.

citrate solution (Fig. 6a–c) clearly show that removal of the outermost layers of alginate is accompanied by a densification of the capsule surface. Fig. 6c most clearly shows that the hydrogel surface morphology is an "inverse" or negative of the parent alginate gel, hence the label "template polymerization" as applied to this process. The capsule depicted in Fig. 6d–f was treated overnight with citrate then cut open to wash out the liquefied alginate. The low-magnification view (Fig. 6d) indicates that a substantial thickness of hydrogel coating, approaching 0.1 mm in places, had been achieved. An oblique cross-section of the "capsule wall", as depicted in Fig. 6e, suggests that the surface morphology was replicated throughout the coating, an observation confirmed by examination of the interior surface at high magnification (Fig. 6f.)

A schematic of the coating process is illustrated in Fig. 7 and involves the simultaneous formation of a calcium alginate gel which is driven by the inwards diffusion of $Ca^{2+}$-ion; and a synthetic hydrogel coating as a product of reaction between the polyhydric alcohol and the epoxidized copolymer. A bead in the early stages of formation is illustrated in Fig. 7 (I). The more mobile $Ca^{2+}$-ions generate the calcium alginate matrix which traps the cells (filled circles) and protects them from contact with the hydrogel coating, which forms more slowly due to the hindered diffusion of the two polymers through the gel matrix. The two polymers meet and react within the cavities at the surface of the alginate bead (II) to produce a composite coating around and within the outer layers of the bead. The alginate provides mechanical support in the

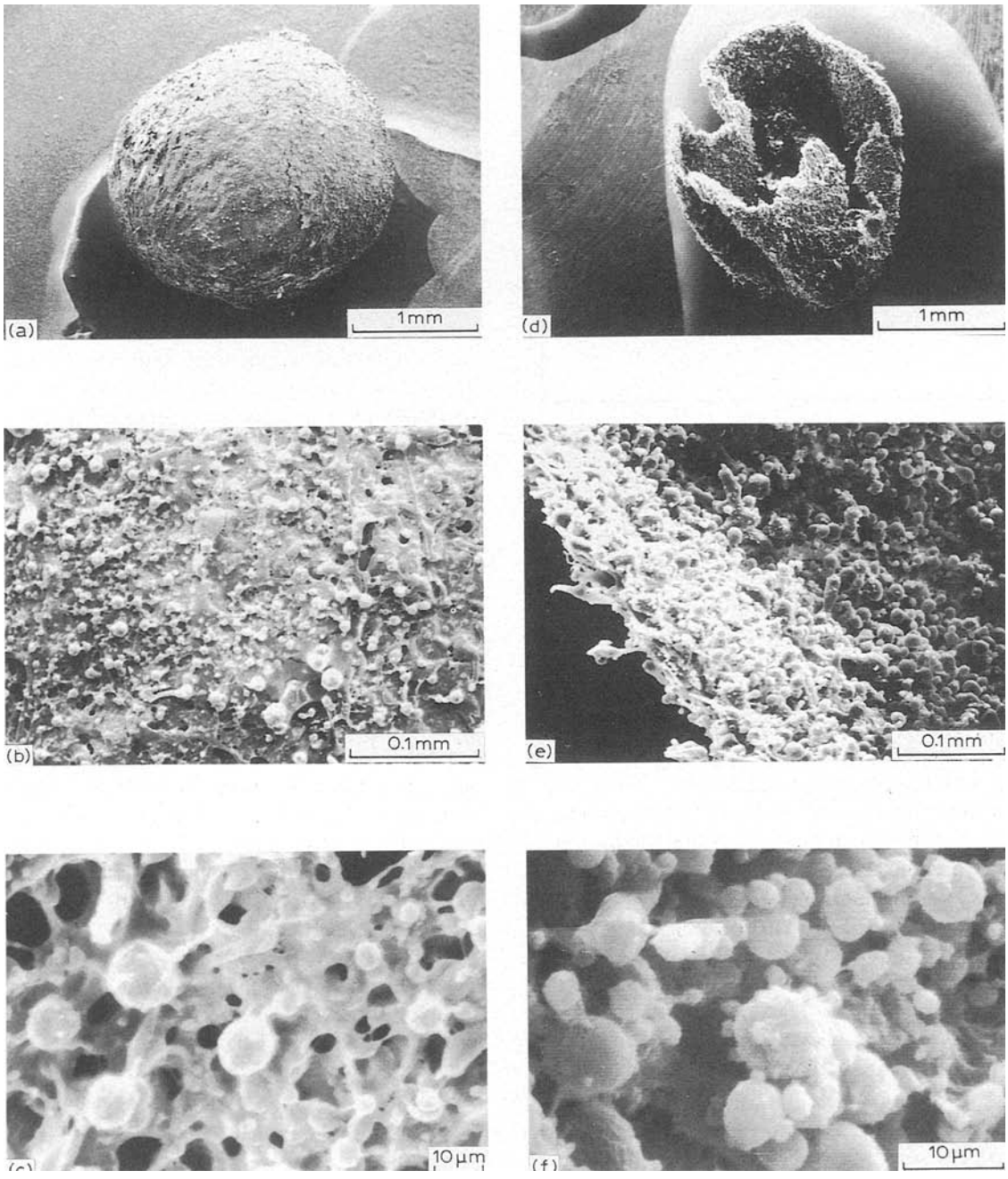


*Figure 6* SEM micrographs of hydrogel-coated alginate gel bead after treatment with sodium citrate, (a)–(c) for 30 min and (d)–(f) for 24 h: (a) intact bead at low magnification, (b) and (c) surface of intact bead at higher magnification, (d) fractured capsule at low magnification, and (e) and (f) cross-section and interior surface, respectively, of fractured capsule at higher magnification.

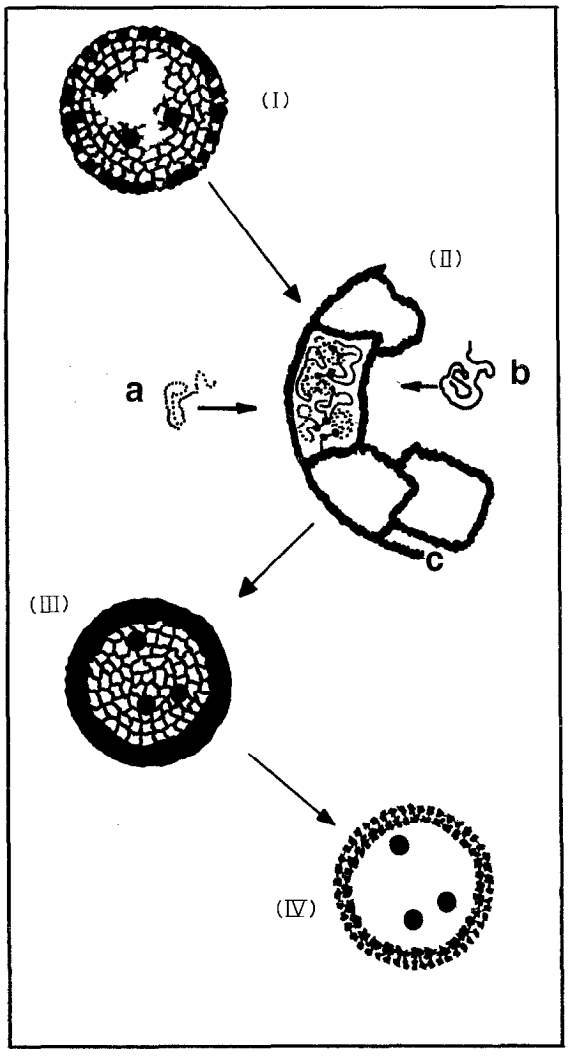


*Figure 7* Schematic representation of development of coating morphology. (I) Simultaneous ionotropic gelation of the alginate with synthetic hydrogel formation at the capsule surface; filled cavities at the bead surface denote hydrogel formation, filled circles denote cells. (II) Schematic of reaction of (a) epoxidized copolymer with (b) hydroxylated copolymer in alginate void space near surface of gel bead. (III) Hydrogel-coated alginate gel bead. (IV) Capsule after liquefaction of interior alginate and extraction of surface alginate in citrate solution.

early stages of the coating process and, of course, immobilizes entrapped cells so that only those originally near the capsule surface may be damaged by contact with the epoxy-bearing polymer. The process, and therefore the coating thickness, is self-limiting, as a product build-up hinders the interpenetration of reagents into the reaction zone. The coated alginate (III) may then be transferred to a citrate-containing solution to extract the alginate from the surface layers of the capsule and liquefy the capsule interior (IV). It must be emphasized that this is an optional step that may deteriorate the capsule contents. For example, it has been shown that liquefaction of the interior of alginate polylysine microcapsules results in a significant deterioration in the viability of encapsulated pancreatic islets [16].

## 3.5. Infrared spectroscopy

Supporting evidence for the formation of hydrogel coating through reaction of the external glycidyl

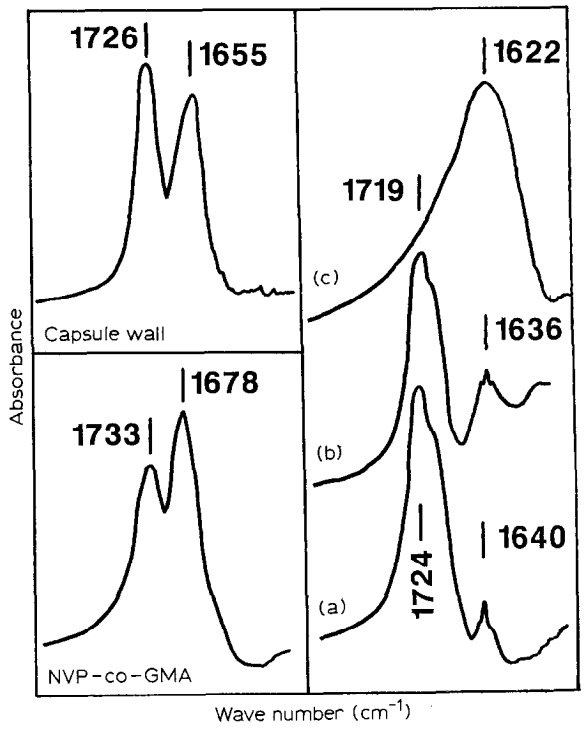


*Figure 8* Infrared carbonyl stretching frequencies: (left to right) 1700 to 1100 $cm^{-1}$. Shown are the spectra of NVP-co-GMA (polymer 52) and capsule wall from reaction of that copolymer with polymer 10a. Also shown are (a) copolymer containing 90 mol % HEMA and 10 mol % MAA (polymer 10a) as free acid, (b) as acid salt and (c) sodium alginate.

ether-containing copolymer with the internal hydroxylated copolymer was obtained by infrared spectroscopy. Capsules were prepared as for optical microscopy, teased open, soaked in isotonic citrate for 24 h, then in distilled water for 24 h, and freeze-dried. The coating residue was then examined by infrared spectroscopy by the KBr disc method. We also examined associated materials such as the parent polymers and alginate itself by this method.

Carbonyl stretching frequencies for the materials are illustrated in Fig. 8. The HEMA–MAA copolymer (Fig. 8a) displays an ester absorbance at 1724 $cm^{-1}$ from the HEMA residue and an acid absorbance at about 1640 $cm^{-1}$ from residual neutralized carboxylate anions in the MAA residue. If the same polymer is recovered from pH 7.4 buffer (Fig. 8b) the absorbance due to the carboxylate anions intensifies and shifts to about 1636 $cm^{-1}$ and the ester absorbance also downshifts to around 1719 $cm^{-1}$. Sodium alginate (Fig. 8c) produces a very broad absorbance maximizing at about 1622 $cm^{-1}$.

The GMA–NVP copolymer produces two absorbances, the first at 1733 $cm^{-1}$ attributable to the ester group in the GMA residue, and the second, an amide I absorbance of the NVP residue at 1678 $cm^{-1}$. The capsule coating also displays two resonances, downshifted to 1726 and 1655 $cm^{-1}$, respectively, and reversed in intensity with respect to the GMA–NVP copolymer.

The spectrum of the NVP–GMA copolymer from 2000 to 500 $cm^{-1}$ could be essentially recovered by a weighted subtraction of the spectrum in Fig. 8a from that of the capsule wall. Subtraction of the alginate

spectrum from that of the capsule wall could not reverse the about 1730 to 1660 $cm^{-1}$ absorbance ratio or produce meaningful results at other wavelengths. We must therefore conclude that the capsule coating is primarily composed of a product from the reaction of the GMA–NVP copolymer with the HEMA–MAA copolymer. Even so, we cannot assume the absence of small amounts of alginate as a co-reactant or entrained impurity in the hydrogel coating.

### 3.6. Drabkins test

Guinea-pig red blood cells were encapsulated in coated alginate microbeads using the protocol developed in Section 2.6. Capsules were prepared from a mixture of 1.3 wt % sodium alginate, 2.5 wt % polymer 10a and 10% haematocrit in isotonic Tris-buffered saline which was gelled in 0.1 M Tris-buffered $CaCl_2$ containing 5 wt % polymer 53 for 30 min, before exposure to a solution containing 5 wt % polymer 53 in isotonic Tris-buffered saline for 4 h to complete the hydrogel coating, followed by long-term storage in isotonic Tris-buffered saline.

The survival of encapsulated red blood cells after 4 days storage in the capsules (71.2%) compared favourably with the survival of the control sample (96%), indicating that the process of encapsulation followed by a short storage period does not unduly affect the cells. It must be emphasized that red blood cells are among the most robust of mammalian cell lines. Lysis or necrosis of more-fragile entities such as pancreatic islets may be higher.

## 4. Conclusions

We have demonstrated the feasibility of surface-modifying calcium alginate gel with a covalently cross-linked synthetic hydrogel coating. The alginate gel protects the trapped cells from potentially lethal contact with the water-soluble epoxidized polymer. The coating thickness is self-limiting through a balance of simultaneous diffusion–reaction of the polymeric reagents around and through the newly formed alginate gel matrix, which may be tailored through judicious choice of the polymer composition and molecular weight and the alginate concentration.

Although much about this process remains unclear (residual epoxy content and entrained alginate in the coating, host biocompatibility, permeability characteristics and relationships with capsule structure, etc.), desirable properties of the synthetic hydrogel coating such as (apparent) compatibility with entrapped cells, indefinite stability, a tough, elastic mechanical response and the capacity for substantial modification through an appropriate choice of reagents, suggest that this procedure merits further study as a means of stabilizing alginate gel towards the mechanical stresses and immunological responses experienced during and after transplantation.


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