Biocompatibility and surface structure of chemically modified immunoisolating alginate–PLL capsules

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Abstract: Grafting of encapsulated living cells has the potential to cure a wide variety of diseases. Large-scale application of the technique, however, is hampered by insufficient biocompatibility of the capsules. A major factor in the biocompatibility of capsules is inadequate covering of the inflammatory poly-L-lysine (PLL) on the capsules' surface. In the present study, we investigate whether tissue responses against alginate–PLL capsules can be reduced by crosslinking the surface of the capsules with heparin or polyacrylic acid. Our transplant study in rats shows a tissue response composed of fibroblasts and macrophages on alginate–PLL–alginate and alginate–PLL–heparin capsules that was completely absent on alginate–PLL–polyacrylic acid capsules. Atomic force microscopy analyses of the capsules demonstrates that the improved biocompatibility of alginate–PLL–capsules by polyacrylic acid coating should not only be explained by a more adequate binding of PLL but also by the induction of a smoother surface. This study shows for the first time that biologic responses against capsules can be successfully deleted by chemically crosslinking biocompatible molecules on the surface of alginate–PLL capsules. © 2003 Wiley Periodicals, Inc. J Biomed Mater Res 67A: 1219–1227, 2003

Key words: alginate microcapsules; crosslinking; polyacrylic acid; atomic force microscopy; biocompatibility

INTRODUCTION

Transplantation of cells for treatment of human disorders such as hormone deficiencies is only applied on limited scale as a consequence of the necessity to use lifelong immunosuppression for preventing rejection of the graft. To overcome the obstacle of application of immunosuppression, many investigators have focused on the design of techniques to encapsulate hormone-secreting cells in semipermeable membranes to protect donor cells against antibodies and cytotoxic cells of the host immune system. This immunoprotection by encapsulation not only allows successful transplantation of cells in the absence of immunosuppression[1,2] but also allows transplantation of cells from nonhuman origin, that is, xenografts, which overcomes the obstacle of insufficient availability of donor organs.

A commonly used procedure for immunoprotection is microencapsulation of tissues in alginate-poly-L-lysine (PLL)-based capsules as originally described by Lim and Sun.[2] During recent years, important advances have been made with this technology. The first allotransplantations in humans with encapsulated parathyroid cells[3] and islets[4] have been successfully performed.[4] Although this illustrated the principle applicability of the alginate–encapsulation technique, a fundamental barrier has to be overcome because graft survival varies considerably from several days to months.[5–10]

The variation in success rate is usually attributed to differences in the chemical composition[11–13] and, con-

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sequently, differences in biocompatibility of the applied capsules. A major factor causing bioincompatibility is insufficient covering of positively charged PLL at the capsules surface,[6,13,14] which has been shown to induce foreign body reactions by attracting macrophages and fibroblasts.[13,14]

In the present study we compared the biocompatibility of alginate–PLL capsules prepared with different crosslinking agents on their surface to cover positively charged PLL. We tested crosslinking of alginate–PLL with heparin and polyacrylic acid because these procedures have been reported to have beneficial, reducing effects on tissue reactions in other biomaterial applications.[15,16] Because it has been reported that modification of the surface of the capsules are not only associated with covering of positive charges but also with changes of the structure of the surface,[6,17,18] we applied transmission electron microscopy (TEM) and atomic force microscopy on the capsules. This approach allows us to correlate the tissue reactions against the different capsules with the structure of the capsule's surface, which, reportedly,[17,19,20] is also an important factor for attraction of cells involved in foreign body reactions.

MATERIALS AND METHODS

Study design

Alginate–PLL–alginate (i.e., conventional capsules), alginate–PLL–heparin (i.e., capsules with modified surface), and alginate–PLL–polyacrylic acid (i.e., capsules with modified surface) microcapsules were implanted in the peritoneal cavity, which is the usual transplantation site for an encapsulated islet graft, of Wistar rats. The capsules were inspected before and after implantation to confirm that the majority of the capsules was intact. The capsules were retrieved at 4 weeks after implantation to study the degree of overgrowth, that is, a measure for the capsules' biocompatibility.

In vitro, the smoothness of the surface of capsules with or without modifications was analyzed with TEM and atomic force microscopy.

Graft recipients and surgical procedures

Male Wistar/R rats served as recipients of microcapsules and were obtained from the Central Animal Laboratory of Rostock, Germany. Their body weights ranged from 300–350 g.

Capsules were injected into the peritoneal cavity with a 16-G cannula via a small incision (3 mm) in the linea alba. The abdomen was closed with a two-layer suture. The implanted volume was always 0.5 mL as assessed in a syringe with appropriate measure.

The microcapsules were retrieved 4 weeks after implantation by peritoneal lavage. Peritoneal lavage was performed by infusing 20 mL Krebs-Ringer-Hepes (KRH) through a 3-cm midline incision into the peritoneal cavity and subsequent flushing of the abdomen above a 50-mL centrifuge tube. The abdominal organs were flushed two to three times with KRH.

Microcapsules were either freely floating and nonadherent or adherent to the surface of abdominal organs. First, nonadherent microcapsules were retrieved by peritoneal lavage. Subsequently, the microcapsules adherent to the surface of abdominal organs were excised and processed for histology.

National Institutes for Health guidelines for the care and use of laboratory animals have been observed. All surgical procedures were performed under halothane anesthesia.

Purification of alginate

Only highly purified alginates were applied to exclude that contaminating components were the cause of an inflammatory response. Alginate (low-G alginate) was obtained from ISP Alginates (Tadworth, Surrey, UK). Crude sodium alginate was dissolved at 4°C in a 1-m*M* sodium EGTA solution to a 1% solution under constant stirring. Subsequently the solution was filtered over successively 5.0-, 1.2-, 0.8-, and 0.45-μm filters (Schleicher & Schüll, Germany). During this filtration step, all visible aggregates were removed.

Next, the pH of the solution was lowered to 3.5 by addition of 2*N* HCL + 20 m*M* NaCl. The solution was kept on ice to prevent hydrolysis of alginate. The next step was slow lowering of the pH from 3.5 to 1.5 and was associated with gradual precipitation of alginate as alginic acid. Routinely, the solution was brought at a pH of 2.0 and subsequently filtered over a Buchner funnel (pore size 1.5 mm) to wash out nonprecipitated contaminants. To extend the washout of nonprecipitated contaminants, the precipitate was brought in 0.01*N* HCl + 20 m*M* NaCl, vigorously shaken, and filtered again over the Buchner funnel. This washing procedure was performed three times.

Then, proteins were removed by extraction with chloroform/butanol. The alginic acid was suspended in 100 mL of 0.01*N* HCl + 20 m*M* NaCl and supplemented with 20 mL chloroform and 5 mL 1-butanol. The mixture was vigorously shaken for 30 min and filtered over the Buchner funnel. This chloroform/butanol extraction was performed three times. Next, the alginic acid was brought in water and slowly dissolved by gradually raising the pH to 7.0 by slow addition of 0.5*N* NaOH + 20 m*M* NaCl over a period of at least 1 h. The alginate solution obtained was subjected to a chloroform/butanol extraction to remove those proteins that can only be dissolved in chloroform/butanol at neutral pH. The solution was vigorously shaken in a mixture of chloroform (20 mL at each 100-mL alginate solution) and 1-butanol (5 mL at each 100-mL alginate solution) for 30 min. The mixture was centrifuged for 3–5 min at 3000 rpm, which induced the formation of a separate chloroform/butanol phase that was removed by aspiration. The extraction was repeated once.

The last step was precipitation of the alginate with ethanol.[21] To each 100 mL of alginate solution we added 200 mL absolute ethanol (ethylalcohol, absolute, NedAlco, Bergen op Zoom, The Netherlands). After an incubation period of 10 min all alginate had precipitated. It was filtered over the Buchner funnel and washed two times with absolute ethanol. Subsequently, the alginate was washed three times with ethylether and dialyzed to remove low-molecular-weight components in the alginate–solution. Finally, the alginate was freeze-dried overnight.

Endotoxin content of purified alginate samples was assessed by a commercial Limulus–lysate assay[22] following the protocol of the E-toxate kit recommended by Sigma. The alginate samples purified by this procedure was always close to zero and never higher than 0.01 U/mL.

Encapsulation and modification of the capsule's surface

Purified alginates were dissolved at 4°C in KRH with an appropriate osmolarity. Before application the solutions were sterilized by 0.2-μm filtration.

The 3% alginate solution was converted into droplets using an air-driven droplet generator.[23] Alginate–PLL–alginate encapsulation was performed as described elsewhere.[11] Briefly, the alginate droplets were transformed to alginate beads by gelling in a 100-m*M* $CaCl_2$ solution (containing 10 m*M* Hepes and 2 m*M* KCl) for at least 10 min. After washing with KRH containing 2.5 m*M* $CaCl_2$ for 1 min a PLL (PLL–HCl, 22 kDa, Sigma-Aldrich, Germany) membrane was formed by suspending the beads in a 0.1% PLL solution for 10 min. Subsequently, the coated beads were washed three times in Ca^{2+}-free KRH containing 135 m*M* NaCl for 3 min. Finally, positive-charged PLL molecules were covered with another alginate layer by incubation of the capsules in a 0.3% solution of alginate for another 5 min.

We tested two types of modifications of the surface of the capsules. The first is a substitution of the final incubation of capsules in alginate for a heparin treatment. This was done by suspending the alginate–PLL capsules for 5 min in 0.3% heparin (sodium salt, porcine mucosa, Fluka, Germany) in Ca^{2+}-free KRH containing 135 m*M* NaCl. This was followed by three washing steps with Ca^{2+}-free KRH containing 135 m*M* NaCl.

The second surface modification was performed by substituting the final incubation of capsules in alginate for a polyacrylic acid treatment. Therefore, capsules were incubated in 0.1% polyacrylic acid (250 kDa, Sigma-Aldrich, Germany) in Ca^{2+}-free KRH containing 135 m*M* NaCl. Before implantation, the capsules were washed with Ca^{2+}-free KRH containing 135 m*M* NaCl to remove the excess of nonbound polyacrylic acid.

After the procedure the capsules had a diameter of 650 ± 50 μm.

Microscopy

To assess overgrowth after implantation, samples of adherent capsules recovered by excision and nonadherent capsules were fixed in precooled Bouin solution, buffered with 0.05*M* phosphate in saline (pH 7.4), and processed for parrafin embedding. Sections were prepared at 2 μm and stained with hematoxylin and eosin (H&E) and applied for detecting capsules with and without overgrowth.

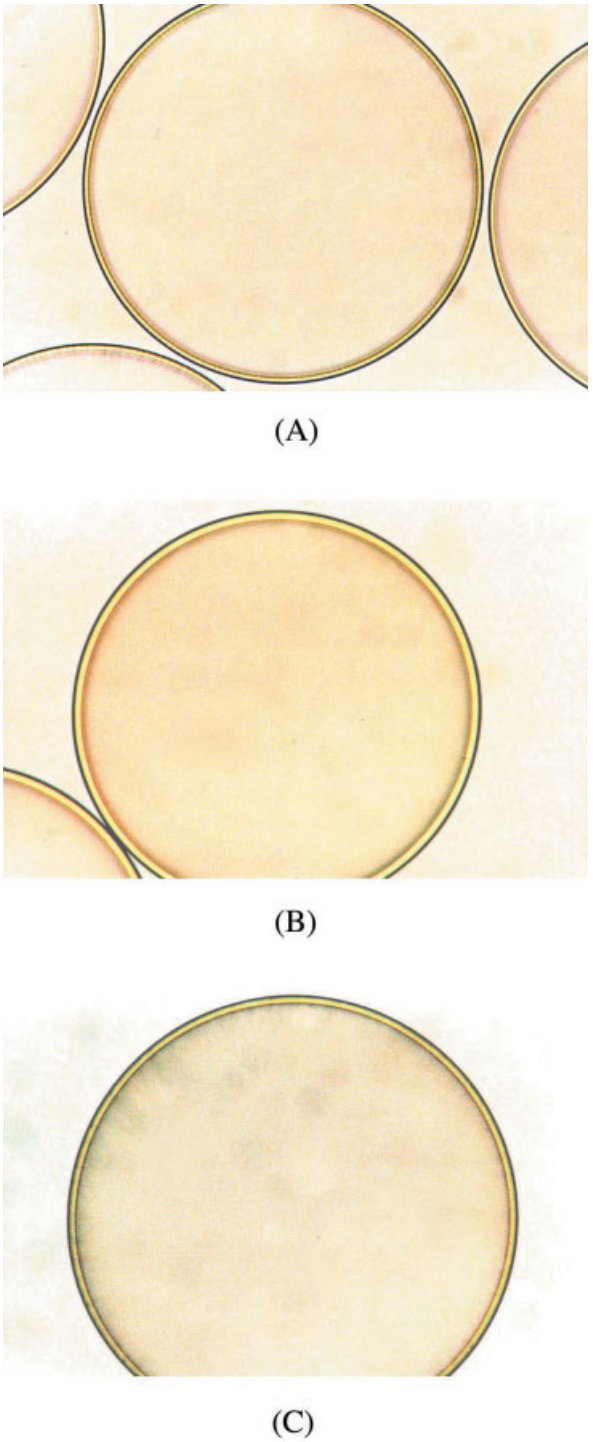


Figure 1. Conventional (A) alginate–PLL–alginate capsules, (B) alginate–PLL–heparin capsules, and (C) alginate–PLL–polyacrylic acid capsules before implantation. Note the absence of irregularities. (Light microscopy, original magnification 100×.) [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

To confirm the integrity of capsules before and after implantation, samples of capsules were meticulously inspected for the presence of irregularities or broken parts in the capsule membranes at both low magnification (by light microscopy, Leica, Hamburg, Germany) and high magnification (by TEM at 80 kV, EM902A, Carl Zeiss, Oberkochen, Germany).

For TEM, the capsules were processed as follows. Microcapsules were overnight fixed at 4°C in 4% glutaraldehyde (in PBS, pH 7.4). Next, they were rinsed three times in phosphate-buffered saline (PBS) for 15 min and fixed in 1% osmium tetroxide (pH 7.4) for 60min. This was followed by another rinsing in PBS and subsequent gradual dehydration in acetone series with increasing concentrations (30, 50, 75, 90, and 100%). Finally, the capsules were embedded in epon araldite. Ultrathin sections (Ultracut S, Leica, Wien, Austria) of 60–80 nm were stained with uranyl acetate and lead citrate for 2–3 min at room temperature.

Processing was not required for atomic force microscopy (Dimension 3100, Fa. Digital Instruments, Santa Barbara, CA) of the capsules. Topographic imaging of microcapsules was performed at room temperature using the tapping modus. The surface of the microcapsules was scanned by the tip of a siliziumnitrid centilever (Nanoprobes GmbH, Darmstadt, spring constant $k = 0.3N/m$), which is vertically oscillating (z-oscillating) near its resonant frequency with a tapping frequency (x-y raster scanning) less than 1 Hz. Intermittent contact of the tip with the sample causes attenuation of the root mean square (RMS) oscillation amplitude, which is used to monitor changes in sample height. RMS (R_q) is the standard deviation of the Z value within the given area and is calculated as

$$R_q = \sqrt{\frac{\sum (Z_i - Z_{ave})^2}{N}},$$

where Z_{ave} is the average of the Z value within the given area, Z_i is the current Z value, and N is the number of points within this area. A detailed description of the atomic force microscopy operation has been described elsewhere.[24,25] Surface roughness was evaluated by using the RMS parameter at a 10-μm scan. For fixation four to five microcapsules in KRH are fixed by a plastic ring in a Petri dish. For each type of microcapsule, three probes were examined to exclude variations between different sessions.

TABLE I
Biocompatibility of Conventional Alginate–PLL–Alginate, Alginate–PLL–Heparin, and Alginate–PLL–Polyacrylic Acid Capsules at 4 Weeks After Implantation in the Peritoneal Cavity of Rats

Capsule Type	n	Location Intraperitoneally	Types of Cells in Overgrowth
Alginate–PLL–alginate	5	Adherent	Fibroblasts
Alginate–PLL–heparin	5	Adherent	Fibroblasts, macrophages, endothelial cells
Alginate–PLL–polyacrylic acid	5	Free floating	—

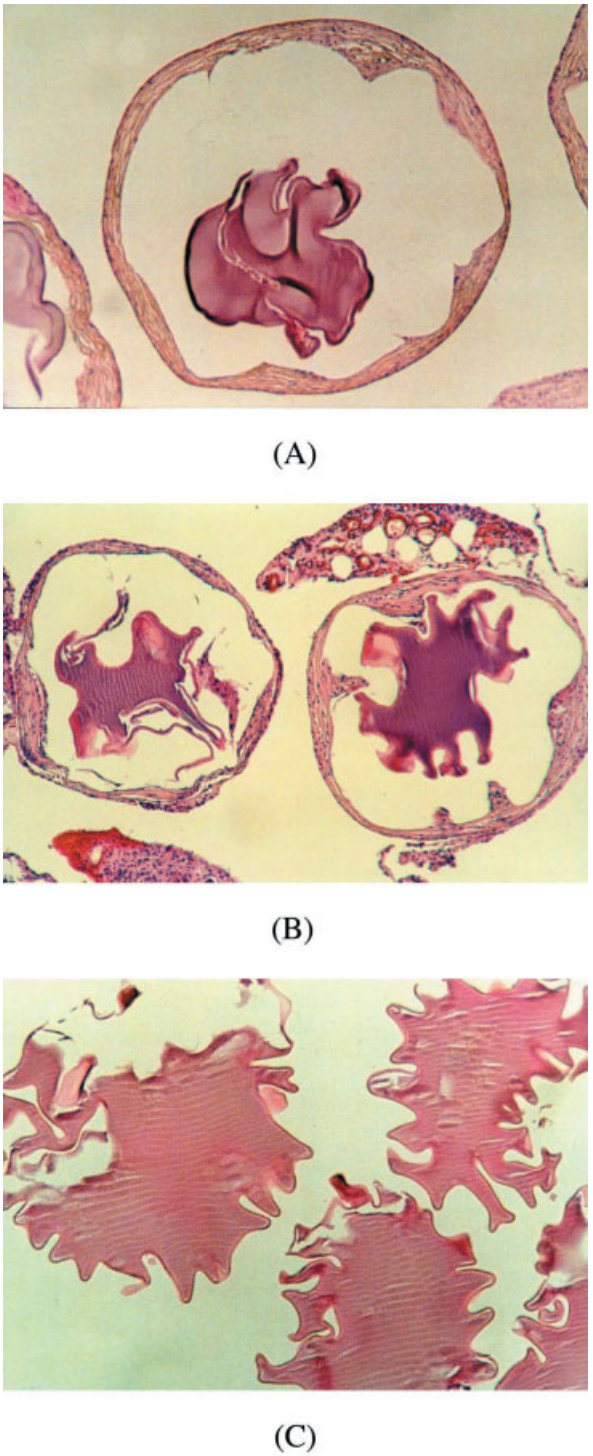


Figure 2. Tissue reactions against (A) conventional alginate–PLL–alginate capsules, (B) alginate–PLL–heparin capsules, and (C) alginate–PLL–polyacrylic acid capsules at 4 weeks after implantation in the peritoneal cavity of rats. Note the more severe reactions against (A) alginate–PLL–alginate capsules and (B) alginate–PLL–heparin capsules than against (C) alginate–PLL–polyacrylic acid capsules. (H&E staining, light microscopy, original magnification 100×.) [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

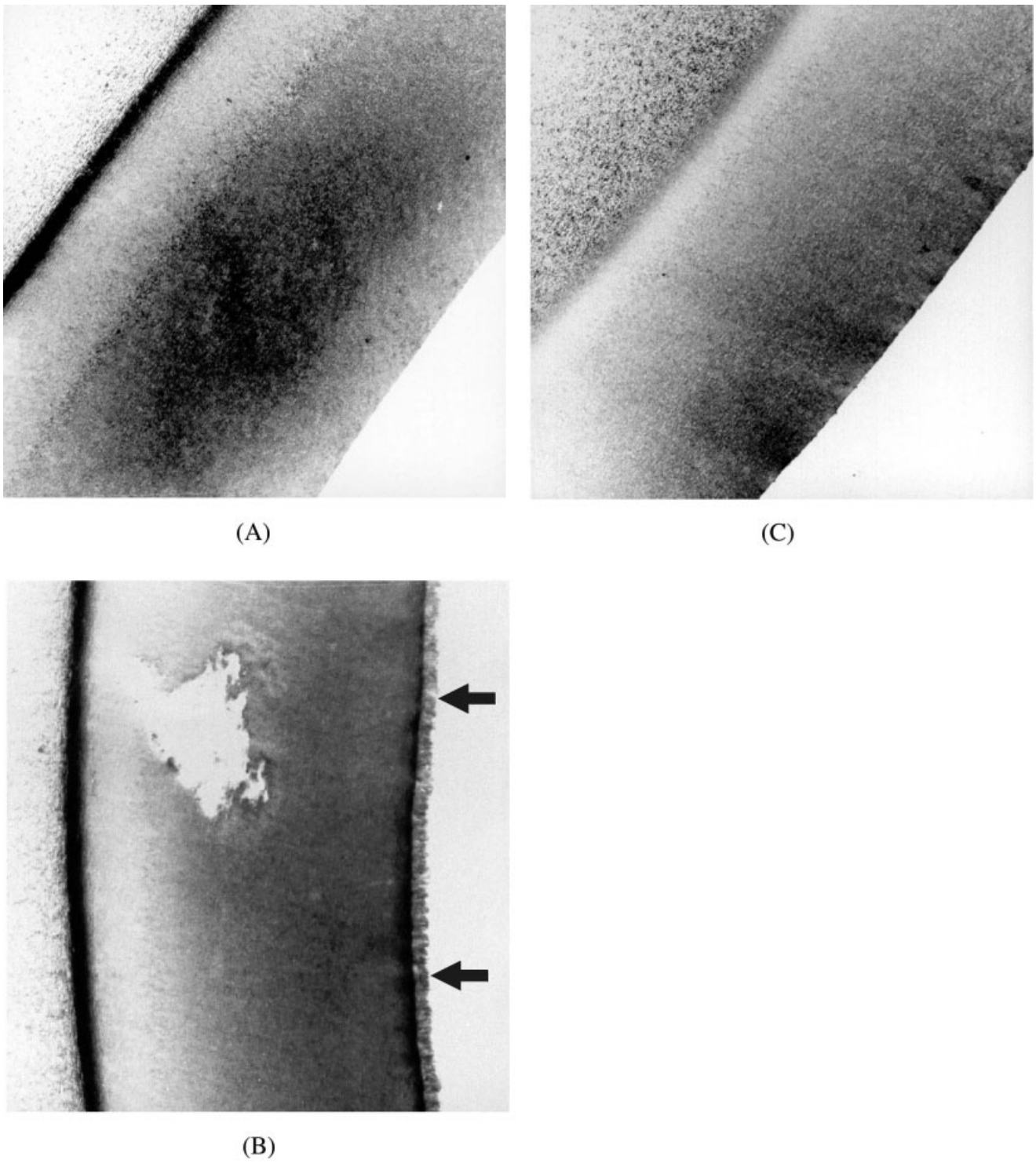


Figure 3. Transmission electron micrographs of (A) conventional alginate–PLL–alginate capsules, (B) alginate–PLL–heparin capsules, and (C) alginate–PLL–polyacrylic acid capsules. Alginate–PLL–heparin capsules showed a granular surface (see arrows) while polyacrylic acid crosslinked microcapsules showed a smooth surface. (TEM, original magnification 100×.)

RESULTS

In vivo responses against microcapsules

Prior and after implantation, we observed all batches of microcapsules to contain some imperfect capsules. The number of imperfect capsules was small and represented not more than approximately 5% in the batches of alginate–PLL–alginate capsules, alginate–PLL–heparin capsules, and alginate–PLL–polyacrylic acid capsules. Light microscopy showed all three types of capsules to have similar degrees of

integrity as illustrated by absence of strains and a surface without visible irregularities (Fig. 1).

Although there was no difference in the number of imperfect capsules, the responses were not similar but always less severe against alginate–PLL–polyacrylic acid capsules than against alginate–PLL–alginate and alginate–PLL–heparin capsules (Table I). At 4 weeks after implantation, the majority of alginate–PLL–alginate and alginate–PLL–heparin capsules were found to be adherent to the abdominal organs and virtually all were covered with cellular overgrowth [Fig.2(A,B)]. The overgrowth was composed of fibroblasts in the case of alginate–PLL–alginate capsules, but it was composed of cells with the morphological appearance of macrophages and some endothelial cells in immature blood vessels in addition to fibroblasts when alginate–PLL–heparin capsules were applied.

In contrast, the vast majority of alginate–PLL–polyacrylic acid capsules were not adherent to the abdominal organs but freely floating in the peritoneal cavity. The capsules could readily be flushed out of the peritoneal cavity and showed no signs of cellular overgrowth [Fig. 2(C)].

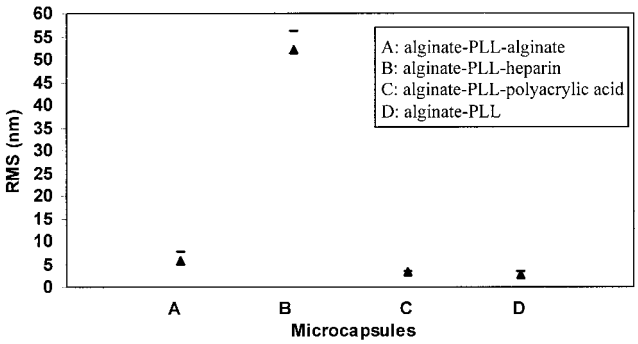


Figure 4. Surface smoothness as measured by atomic force microscopy (RMS parameter at a 10-μm scan) of (A) conventional alginate–PLL–alginate capsules (5.9–7.6 nm), (B) alginate–PLL–heparin capsules (52–56 nm), and (C) alginate–PLL–polyacrylic acid capsules (3.2–3.4 nm). Also, we analyzed (D) alginate–PLL capsules without an outer coating (2.8–3.3 nm). For each type of microcapsule three probes were examined to exclude variations between different sessions.

Structure of capsule's surface

Concomitantly, for the higher tissue responses TEM of capsules showed more irregularities on the surface of alginate–PLL–heparin capsules than on alginate–PLL–alginate and alginate–PLL–polyacrylic acid (Fig. 3). The irregularities were mainly granular depositions, which predictedly serve as a site for macrophages to adhere.[26] TEM showed no differences in structure of the surface of alginate–PLL–alginate and alginate–PLL–polyacrylic acid capsules.

The analyses of the capsule surface were also performed with atomic force miscroscopy in addition to TEM because atomic force miscroscopy allows for analyses in the nonmodified, normal liquid environment of the capsules[27] and is not associated with artifacts induced by processing for electron miscroscopy.

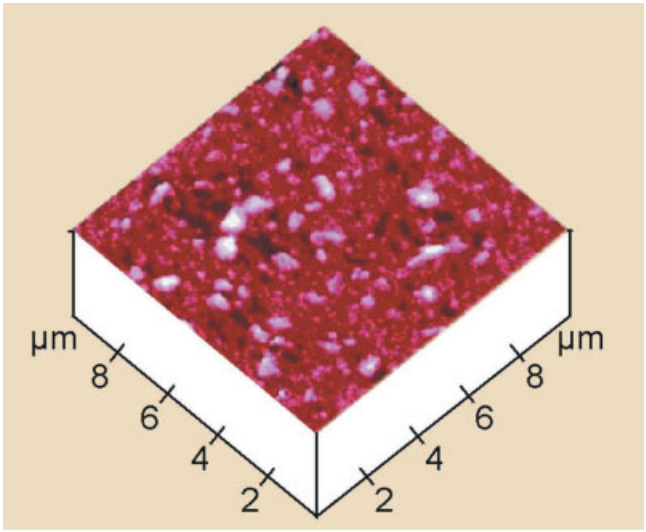
As shown in Figures 4 and 5, alginate–PLL–alginate, alginate–PLL–heparin, and alginate–PLL–polyacrylic acid capsules showed clearcut differences in their surface structure. The highest degree of roughness of the surface was observed with alginate–PLL–heparin [Fig. 4(B): 52–56 nm; Figure 5(B)] capsules, which also provoked the most severe tissue response while alginate–PLL–polyacrylic acid had the smoothest capsule surface [Fig. 4(C): 3.2–3.4 nm; Fig. 5(C)] and, concomitantly, the absence of a tissue response.

The conventional alginate–PLL–alginate capsules showed an elevated surface roughness value [Fig. 4(A): 5.9–7.6 nm; Fig. 5(A)] when compared to alginate–PLL–polyacrylic acid capsules. This elevation was induced in the last coating with alginate because the surface roughness value was only 2.8–3.3 nm [Fig. 4(D)] instead of 5.9–7.6 nm [Fig. 4(A)] with alginate–PLL capsules in the absence of any outer alginate. Obviously, conventional suspension in alginate–solutions to cover positively charged PLL induces irregularities on the capsule's surface.

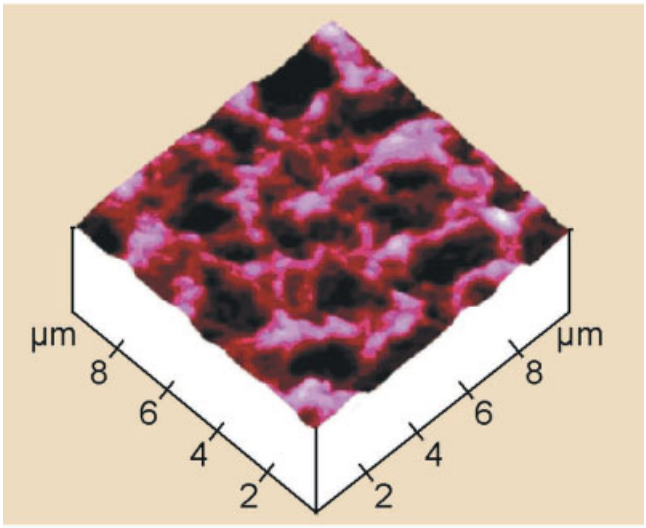
DISCUSSION

The present study shows that chemical modification of the surface of alginate–PLL capsules has a clear-cut effect on the biocompatibility of the capsules. When heparin was applied instead of alginate to cover positive charged PLL molecules we found a more severe reaction against the capsules than when polyacrylic acid was applied, which almost completely abrogated the responses against alginate–PLL capsules. The variations in reactions against the capsules *in vivo* were probably not only associated with the adequacy of covering the insufficient biocompatible PLL on the surface but also with differences in the smoothness of the capsule's surface as demonstrated in our TEM and atomic force microscopy study of the capsules.

The finding that heparin did not diminish but rather increased the tissue responses against alginate–PLL capsules does not corroborate the findings of Tatarkiewicz et al.,[28,29] who found a reduction of responses when heparin was applied in the construction of the capsules. Notably, however, those authors not only substituted the alginate but also the PLL and applied a protamine–heparin membrane instead. Although this combined substitution of PLL and alginate may



(A)



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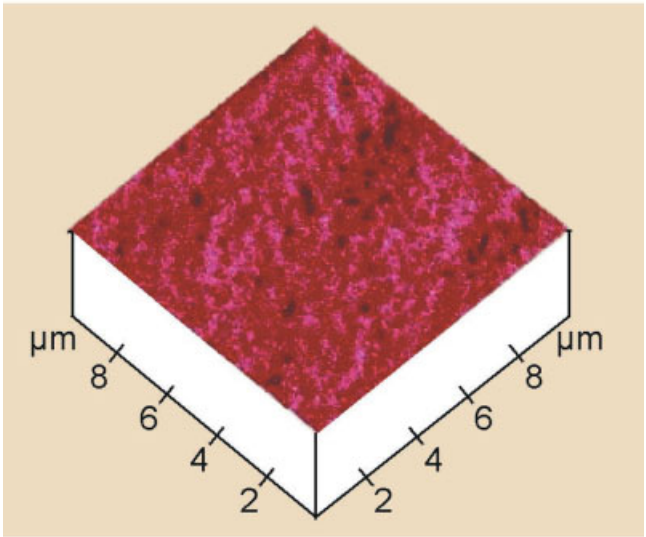

(C)

improve the biocompatibility of the capsules it will be impractical to apply for immunoisolation because the protamine–heparin coating does not allow for the gradual modulation of the permeability of the capsules, as can be accomplished with PLL.[30]

The heparin coating did not only induce more fibroses on the capsules but also provoked angiogenesis in the vicinity of the capsules as illustrated by the histological observation of blood vessels near the capsules. This can be explained by the fact that heparin on the capsule surface can serve as a ligand for angiogenic molecules such as vascular endothelial growth factor (VEGF) and fibroblast growth factors (FGF), which are members of the heparin-binding growth factor family.[31,32]

In contrast to others, we found a severe tissue response against conventional alginate–PLL–alginate capsules. Plausibly, this is caused by technical differences in the different studies because producing biocompatible alginate–PLL–alginate capsules is far from simple.[17] The chemical structure of alginate–PLL–alginate capsules, and thus its biocompatibility, is determined by many factors such as temperature, type and concentration of alginate (i.e., the guluronic acid content),[11] and type of polylysine.[13] Small differences in these factors induce variations in the capsules' chemical and mechanical properties[12,19,33] and biocompatibility.[11,12] This was the main rationale to change the procedure and seek a more practical and less laborious encapsulation technology. The present study suggests that a simple substitution of the outer alginate layer for polyacrylic acid can circumvent a number of biocompatibility problems associated with alginate–PLL capsules[6,17] and produce capsules in a reproducible fashion that do not provoke any harmful tissue response. Further, our results show that polyacrylic acid coating of alginate–PLL capsules reduces the inflammatory response and is not due to a reduction of adhesion of inflammatory cells to the capsule surface because the lavage from the peritoneal cavity was lacking any inflammatory cells in between the capsules.[34]

Another advantage of the polyacrylic acid coating is the durability, which is superior over the alginate–coating. Many investigators have shown that the ionic bonded membrane of the alginate–coating is not stable for longer periods of time.[11,35] The polyacrylic acid coating plausibly binds COOH and OH groups in alginate and NH_2 groups in polylysine in a covalent manner, which will provide a higher stability[36] and

Figure 5. Two-dimensional atomic force images (10 × 10 μm) of (A) conventional alginate–PLL–alginate capsules, (B) alginate–PLL–heparin capsules, and (C) alginate–PLL–polyacrylic acid capsules. The RMS roughness of the three films are (A) 7.3, (B) 54.6, and (C) 3.4 nm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

beneficial effects for the capsule's long-term biocompatibility.

Our atomic force microscopy study was performed with the tapping modus instead of the contact modus[27] because the tapping modus is associated with less shear forces on the sample,[24] which is essential when fragile, soft specimens such as microcapsules are analyzed. The atomic force microscopy analyses demonstrate that the crosslinking of chemical agents on alginate–PLL has a strong influence on the structure of the surface of microcapsules, which changes where not sufficiently manifested with light microscopy and TEM. This suggests that atomic force microscopy may serve as a valuable tool for predicting the biocompatibility of capsules before transplantation.

Our findings emphasize that optimal and flawless physical features of the capsule surface are required for an adequate biocompatible capsule and, consequently, a maximum chance for success of an encapsulated therapeutic agent producing transplant. In the present research efforts we focus on the effects of coating procedures on functional survival of cells enveloped in our modified capsules.

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References

1. De Vos P, van Straaten JF, Nieuwenhuizen AG, de Groot M, Ploeg RJ, De Haan BJ, Van Schilfgaarde R. Why do microencapsulated islet grafts fail in the absence of fibrotic overgrowth? Diabetes 1999;48:1381–1388.
2. Lim F, Sun AM. Microencapsulated islets as bioartificial endocrine pancreas. Science 1980;210:908–910.
3. Hasse C, Klöck G, Schlosser A, Zimmermann U, Rothmund M. Parathyroid allotransplantation without immunosuppression. Lancet 1997;350:1296–1297.
4. Soon Shiong P, Heintz RE, Merideth N, Yao QX, Yao Z, Zheng T, Murphy M, Moloney MK, Schmehl M, Harris M, et al. Insulin independence in a type 1 diabetic patient after encapsulated islet transplantation. Lancet 1994;343:950–951.
5. De Vos P, De Haan BJ, Wolters GHJ, Strubbe JH, Van Schilfgaarde R. Improved biocompatibility but limited graft survival after purification of alginate for microencapsulation of pancreatic islets. Diabetologia 1997;40:262–270.
6. De Vos P, Tatarkiewicz K. Considerations for successful transplantation of encapsulated pancreatic islets. Diabetologia 2002; 45:159–173.
7. Klöck G, Frank H, Houben R, Zekorn T, Horcher A, Siebers U, Wöhrle M, Federlin K, Zimmermann U. Production of purified alginates suitable for use in immunoisolated transplantation. Appl Microbiol Biotech 1994;40:638–643.
8. Sun YL, Ma XJ, Zhou DB, Vacek I, Sun AM. Normalization of diabetes in spontaneously diabetic cynomologus monkeys by xenografts of microencapsulated porcine islets without immunosuppression. J Clin Invest 1996;98:1417–1422.
9. Tatarkiewicz K, Garcia M, Omer A, Van Schilfgaarde R, Weir GC, De Vos P. C-peptide responses after meal challenge in mice transplanted with microencapsulated rat islets. Diabetologia 2001;44:646–653.
10. Zekorn T, Siebers U, Horcher A, Schnettler R, Klock G, Bretzel RG, Zimmermann U, Federlin K. Barium–alginate beads for immunoisolated transplantation of islets of Langerhans. Transplant Proc 1992;24:937–939.
11. De Vos P, De Haan B, Van Schilfgaarde R. Effect of the alginate composition on the biocompatibility of alginate–polylysine microcapsules. Biomaterials 1997;18:273–278.
12. De Vos P, Van Hoogmoed CG, Busscher HJ. Chemistry and biocompatibility of alginate–PLL capsules for immunoprotection of mammalian cells. J Biomed Mater Res 2002;60:252–259.
13. Vandenbossche GM, Bracke ME, Cuvelier CA, Bortier HE, Mareel MM, Remon JP. Host reaction against empty alginate–polylysine microcapsules. Influence of preparation procedure. J Pharm Pharmacol 1993;45:115–120.
14. Strand BK, Ryan L, In't Veld P, Kulseng B, Rokstad AM, Skjåk-Braek G, Espevik T. Poly-L-lysine induces fibrosis on alginate microcapsules via the induction of cytokines. Cell Transplant 2001;10:263–277.
15. van Wachem PB, Plantinga JA, Wissink MJ, Beernink R, Poot AA, Engbers GH, Beugeling T, van Aken WG, Feijen J, van Luyn MJ. In vivo biocompatibility of carbodiimide-crosslinked collagen matrices: Effects of crosslink density, heparin immobilization, and bFGF loading. J Biomed Mater Res 2001;55:368–378.
16. Falk K, Lindman B, Bengmark S, Larsson K, Holmdahl L. Sodium polyacrylate potentiates the anti-adhesion effect of a cellulose-derived polymer. Biomaterials 2001;22:2185–2190.
17. De Vos P, Van Schilfgaarde R: Biocompatibility issues. In: Kühtreiber WM, Lanza RP, Chick WL, editors. Cell encapsulation technology and therapeutics. Boston: Birkhäuser; 1999. p 63–79.
18. Lacík I, Brissová M, Anilkumar AV, Powers AC, Wang T. New capsule with tailored properties for the encapsulation of living cells. J Biomed Mater Res 1998;39:52–60.
19. De Vos P, De Haan BJ, Pater J, Van Schilfgaarde R. Association between capsule diameter, adequacy of encapsulation, and survival of microencapsulated rat islet allografts. Transplantation 1996;62:893–899.
20. De Vos P, De Haan BJ, Wolters GHJ, Van Schilfgaarde R. Factors influencing the adequacy of microencapsulation of rat pancreatic islets. Transplantation 1996;62:888–893.
21. Haug A, Myklestad S, Larsen B, Smidsrod O. Correlation between chemical structure and physical properties of alginates. Acta Chem Scand 1967;21:768–778.
22. R. Scheer. Pyrogene and limulustest. Pharm Unserer Z 1988;5: 137–146.
23. Wolters GHJ, Fritschy WM, Gerrits D, Van Schilfgaarde R. A versatile alginate droplet generator applicable for microencapsulation of pancreatic islets. J Appl Biomater 1992;3:281–286.
24. Siedlecki CA, Marchant RE. Atomic force microscopy for characterization of the biomaterial interface. Biomaterials 1998;19: 441–454.
25. Pang GKH, Baba-Kishi KZ, Patel A. Topographic and phase-contrast imaging in atomic force microscopy. Ultramicroscopy 2000;81:35–40.
26. Lin DT, Young TH, Fang Y. Studies on the effect of surface properties on the biocompatibility of polyurethane membranes. Biomaterials 2001;22:1521–1529.
27. Xu K, Hercules DM, Lacik I, Wang TG. Atomic force microscopy used for the surface characterization of microcapsule immunoisolation devices. J Biomed Mater Res 1998;41:461–467.
28. Tatarkiewicz K, Sitarek E, Fiedor P, Sabat M, Orlowski T. In vitro and in vivo evaluation of protamine–heparin membrane for microencapsulation of rat Langerhans islets. Artif Organs 1994;18:736–739.

29. Tatarkiewicz K, Sitarek E, Fiedor P, Sabat M, Orlowski T. Protamine–heparin membrane for cell microencapsulation. Transplant Proc 1994;26:3509.
30. King GA, Daugulis AJ, Faulkner P, Goosen MFA. Alginate–polylysine microcapsules of controlled membrane molecular weight cutoff for mammalian cell culture engineering. Biotech Progr 1987;3:231–240.
31. Amoroso A, Del Porto F, Di Monaco C, Manfredini P, Afeltra A. Vascular endothelial growth factor: a key mediator of neoangiogenesis. A review. Eur Rev Med Pharmacol Sci 1997;1:17–25.
32. Asahara T, Bauters C, Zheng LP, Takeshita S, Bunting S, Ferrara N, Symes JF, Isner JM. Synergistic effect of vascular endothelial growth factor and basic fibroblast growth factor on angiogenesis in vivo. Circulation 1995;92:II365–II371.
33. Vandenbossche GM, Bracke ME, Cuvelier CA, Bortier HE, Mareel MM, Remon JP. Host reaction against alginate–polylysine microcapsules containing living cells. J Pharm Pharmacol 1993;45:121–125.
34. De Vos P, Van Hoogmoed CG, De Haan BJ, Busscher HJ. Tissue responses against immunoisolating alginate–PLL capsules in the immediate posttransplant period. J Biomed Mater Res 2002;62:430–437.
35. Edwards-Lévy F, Lévy M-C. Serum–albumin–alginate coated beads: mechanical properties and stability. Biomaterials 1999; 20:2069–2084.
36. Torchilin VP, Maksimenko AV, Smirnov VM, et al. The principle of enzyme stabilization. Biochim Biophys Acta 1979;567: 1–15.