Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 251 (2003) 175–181
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

The preparation and characterisation of drug-loaded alginate and chitosan sponges

Hui Ling Lai, Asad Abu'Khalil, Duncan Q.M. Craig *

The School of Pharmacy, The Queen's University of Belfast, 97 Lisburn Road, Belfast BT9 7BL, UK

Received 28 August 2002; received in revised form 17 October 2002; accepted 29 October 2002

Abstract

Sponges composed of sodium alginate and chitosan were prepared via a freeze drying process in order to assess the utility of mixed sponges as potential wound dressings or matrices for tissue engineering. Sponge preparation involved dissolving both polymers (either individually or mixed) in 1% acetic acid and freeze-drying the corresponding solutions. The mechanical properties of the sponges were assessed using texture analysis and the microstructure examined using scanning electron microscopy. The dissolution of a model drug (paracetamol) from the sponges was assessed as a function of polysaccharide composition. It was noted that the sponges had a flexible yet strong texture, as assessed macroscopically. Measurement of the resistance to compression ('hardness') indicated that the chitosan sponges were the 'hardest' while the alginate sponges showed the least resistance to compression, with all sponges showing a high degree of recovery. In contrast, the breaking force (tensile force) of the sponges were greatest for the single component systems, while the elongation prior to breaking was similar for each material. SEM studies indicated that the mixed systems had a less well-defined microstructure than the single component sponges. This was ascribed to the two polysaccharides interacting in aqueous solution via coulombic forces, leading to a more randomly ordered network being formed on freezing. Dissolution studies indicated that systems containing chitosan alone showed the slowest release profile, with the mixed systems showing a relatively rapid dissolution profile. The use of chitosan and alginates together, therefore, appears to allow the formulator to manipulate both the mechanical properties and the drug release properties of the sponges.

Keywords: Alginate; Chitosan; Paracetamol; Sponge; Texture analysis

1. Introduction

Sponges may be defined as dispersions of gas (usually air) in a solid matrix. There has been considerable recent interest in the use of sponges within the pharmaceutical and biomedical arena, particularly as matrices for controlled drug delivery, as wound dressings and as matrices for cell growth within the tissue engineering field. In particular, sponges based on polysaccharides such as alginate and chitosan have been studied due to the low toxicity, favourable mechanical

* Corresponding author. Tel.: +44-28-9027-2129; fax: +44-28-9024-7794
E-mail address: duncan.craig@qub.ac.uk (D.Q.M. Craig).

0378-5173/02/$ - see front matter
PII: S0378-5173(02)00590-2

properties and capacity for bioresorption of the constituent materials (Shapiro and Cohen, 1997; Kofuji et al., 2001; Kataoka et al., 2001; Singla and Chawla, 2001). Alginates are anionic block copolymers of α(1-4)-L-guluronic and β(1-4)-D-mannuronic acid and are usually presented as the sodium salt, while chitosan is a cationic copolymer of β(1-4)-2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-glucopyranose, obtained via deacetylation of the naturally occurring chitin. Alginate sponges have attracted particular interest as matrices for tissue engineering, with examples including nerve (Kataoka et al., 2001; Sufan et al., 2001) and cartilage (Miralles et al., 2001) regeneration. Similarly, chitosan sponges have been studied for bone regeneration (Park et al., 2000; Lee et al., 2000) and as a medium for culturing pancreatic islets (Cui et al., 2001), as well as for wound dressings in which antibiotics may be included (Mi et al., 2001, 2002).

In order to develop effective sponges for biomedical applications it is essential to consider their properties in the context of their application. Tissue engineering matrices must not only sustain growth of the cells, be suitably robust so as to withstand the mechanical trauma associated with in vivo application and be capable of incorporating and releasing growth factors at an appropriate rate (Whitaker et al., 2001). Similarly, wound dressings must be both robust and flexible so as to allow adherence to the tissue in question for a sustained period, while also maximising patient comfort and convenience. To this effect, many workers have examined the use of cross-linking agents as a means of improving mechanical strength (Kataoka et al., 2001; Park et al., 2000), while others have examined the use of mixed polymeric systems either to promote mechanical strength or to modify the functionality of the resulting sponge (Choi et al., 1999; Leffler and Muller, 2000). On this basis it is perhaps surprising that comparatively little work has been performed on mixed alginate–chitosan sponges, given the possibility of forming a robust polyelectrolyte complex (Kumar, 2000; Coppi et al., 2002) and the frequency with which these materials have been used in conjunction in other applications such as microspheres (Coppi et al., 2002; Vandenberg et al., 2001) and membranes (Wang et al., 2001). Two studies that have used this approach for sponge manufacture include that of Kim et al. (1999), whereby, silver sulfadizine was incorporated into mixed chitosan and sodium alginate sponges as a wound dressing, while Yang et al. (2001) prepared hepatocyte-loaded alginate/galactosylated chitosan sponges for the growth of liver tissue. A further, more general issue that has received comparatively little attention in the context of biomaterials is the assessment of the mechanical properties of the sponges. These properties vary with gas loading (Jeronomidis, 1988; Gibson and Ashby, 1988; Lillford, 1989), whereby, for systems containing low air contents (nominally $<20\%$) the elastic modulus of the sponge (E_s) is given by:

$$E_S = E_M\left(\frac{\rho_S}{\rho_G}\right) \tag{1}$$

where, E_M is the elastic modulus of the matrix, ρ_S is the density of the sponge and ρ_G is the density of the inclusion material. These high density sponges tend to be brittle and show negligible dependence on inclusion phase geometry; examination of Eq. (1) indicates that the elastic modulus is simply proportional to the density of the sponge system for a given matrix material. The second class of sponge, in which most pharmaceutical materials will be included, includes a higher disperse phase volume, leading to a stronger influence of geometry on deformation due to bending and bucking of the cell walls. The elastic modulus is then given by:

$$E_S = \mathrm{K}E_M\left(\frac{\rho_S}{\rho_G}\right)^2 \tag{2}$$

where, K is a constant, hence, $E_S \propto \rho_S^2$. The objectives of this investigation were, therefore, 2-fold. In the first instance, we have examined a range of alginate/chitosan sponges prepared via a lyophilization protocol, specifically in terms of the mechanical, structural and drug release properties in relation to composition. Secondly, we describe the use of texture analysis as a novel means of assessing the mechanical properties of the sponges.

2. Materials and methods

Chitosan (150 kDa) and sodium alginate (48 k–186 kDa) were obtained from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). Stock solutions of both 1% w/v chitosan and 1% w/v sodium alginate were prepared in 1% w/v acetic acid and mixed systems containing varying weight ratios of alginate to chitosan (single polysaccharide, 3:1, 1:1 and 1:3) were prepared by mixing the two stock solutions as appropriate, all systems containing a total polysaccharide content of 1% w/v and all formed clear solutions at room temperature. For the dissolution studies, paracetamol (Sigma) was added to each solution to give a drug concentration of 0.1% w/v. The solution was then poured into four plastic weighing boats ($\sim$25 ml each) with dimensions $60 \times 60 \times 8$ mm, frozen overnight at -18 °C and freeze dried overnight in an Edwards freeze dryer. Foaming agents were not used in this study to prevent any possible effect they may incur on the morphological structure or mechanical strength of the sponges.

The mechanical strength of the sponges was assessed using a texture analyser (TA-XT2, Stable Micro Systems, Leatherhead, UK). This instrument involves the application of a mechanical stress to a sample in one of a number of modes and subsequent measurement of the force profile associated with that stress. In this investigation, three modes were used which were selected so as to indicate the strength, flexibility and ability of the sponges to recover from compression. In the first instance the ability of the sponges to withstand compression was assessed (termed 'hardness' in the parlance associated with the instrument). This consisted of placing the sponge under a 36 mm diameter probe and compressing a distance of 4 mm (i.e. approximately half the thickness of the sponge) at a test speed of 0.5 mm s^{-1}, followed by removal of the probe ($n = 10$). The hardness is designated by the maximum peak force during the compression cycle. The recovery was assessed by compressing the sample twice and measuring the ratio between the maximum compression force.

Secondly the breaking force (tensile force) was measured for each of the sponges by clamping the sample between two sets of grips with a separation distance of 15 mm. The instrument was then set to extend the sample at a speed of 0.5 mm s^{-1} ($n = 4$). The instrument measured both the rupture force and the distance to which the sponge extended prior to rupture. This latter parameter is expressed as the extensibility and is calculated by expressing the increased in length prior to rupture as a percentage of the original length.

Dissolution tests were carried out at 37 °C using dissolution baskets covered with nylon gauze (125 mm pore size); crushed (500–1000 μm) 500 mg commercial uncoated paracetamol tablets (Hillcross) were used for comparison. PU 8700 Series UV/VIS Scanning Spectrophotometer was used to determine the release of paracetamol at wavelength 257 nm. Small pieces of sponges (10×10 mm) were examined using Scanning Electron Microscopy (SEM, Jeol 6400). Samples were fixed on an SEM sample holder using carbon double adhesive and coated with a thin layer of gold ($\sim$ 30 nm) using Agar Sputter Coater.

3. Results and discussion

Initial inspection of the sponges indicated that all had flexible textures, as shown in Fig. 1, with considerable bending possible prior to fracture. Given the necessity of a combination of mechan-

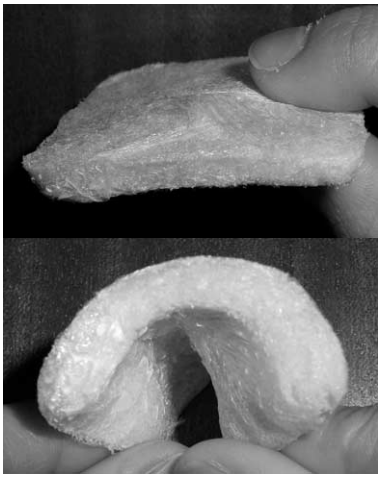

Fig. 1. Photograph of 3:1 (alginate:chitosan) sponge showing the pliability of the freeze dried material.

ical robustness and flexibility that will inevitably be required for in vivo applications, these initial indications were considered to be promising.

The mechanical properties were assessed quantitatively using texture analysis, as discussed above. Fig. 2a shows the maximum force ('hardness') for the sponges under compression as a function of composition. It is interesting to note that the chitosan sponge was clearly considerably more resistant to compression than the alginate or mixed systems ($P < 0.05$). Similarly the alginate alone gave the weakest sponge, while the mixed systems showed intermediate 'hardness' values. Clearly, therefore, the expected coulombic interaction between the two materials has not resulted in a more robust structure using the preparation conditions described here. It is also interesting to note that the compression profiles were almost identical between the first and second cycles, indicating that in all cases recovery was effectively complete. This is significant given that the sponge was compressed to 50% of its thickness this could be reasonably considered to be a practically realistic degree of mechanical trauma.

The tensile force profile of the sponges (Fig. 2b) showed a different rank order with respect to sponge composition in that the chitosan and alginate alone showed greater breaking strengths than the mixed systems ($P < 0.05$). The elongation values (Fig. 2b) were similar in all cases (no statistically significant differences), with only very limited extension taking place prior to breakage. The lack of correlation between the hardness and tensile force is of interest as it indicates that while the chitosan sponges show favourable rigidity (hardness) and resistance to breakage (tensile force), the alginate sponges appear to be more pliable while still having a relatively high strength. Clearly the use of either of the polysaccharides alone or the mixed systems affords the operator a range of options with regard to mechanical characteristics.

In order to further elucidate the relationship between the composition and the mechanical properties, the sponges were examined using SEM, with specimen data shown in Fig. 3. Two interesting observations arose from these studies. In the first instance, there were clear differences in the appearance of the fibrillar structure of the sponges depending on the composition. The pure alginate and chitosan showed a reasonably regular network, as shown in Fig. 3a and b for the chitosan and alginate alone, with approximate pore sizes of approximately 20 and 10 μm, respectively. The mixed systems, however, showed a much more irregular morphology, as indicated in Fig. 3c. This may at least partially explain the relatively low values for the tensile force seen for the mixed sponges, as the less well defined and regular mesh network may be less resistant to rupture, probably due to the presence of weak areas caused by particularly thin fibrillar architecture which may allow propagation of a tear through the system. In terms of the molecular mechanism responsible for this effect, we suggest that the mixed systems may have interacted prior to freezing and, therefore, would have already formed a random fibrillar network. The single

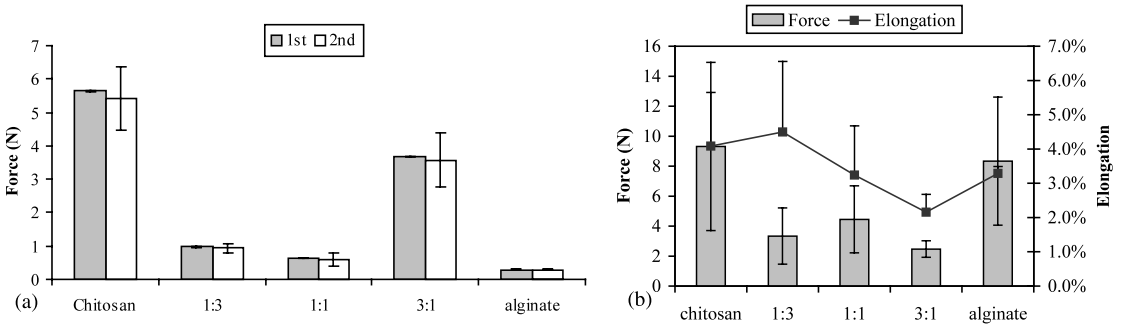

Fig. 2. Texture analysis data showing (a) the compression force on first and second compression (b) the tensile force and elongation for mixed and single component alginate and chitosan sponges (3:1, 1:1 and 1:3 alginate:chitosan).

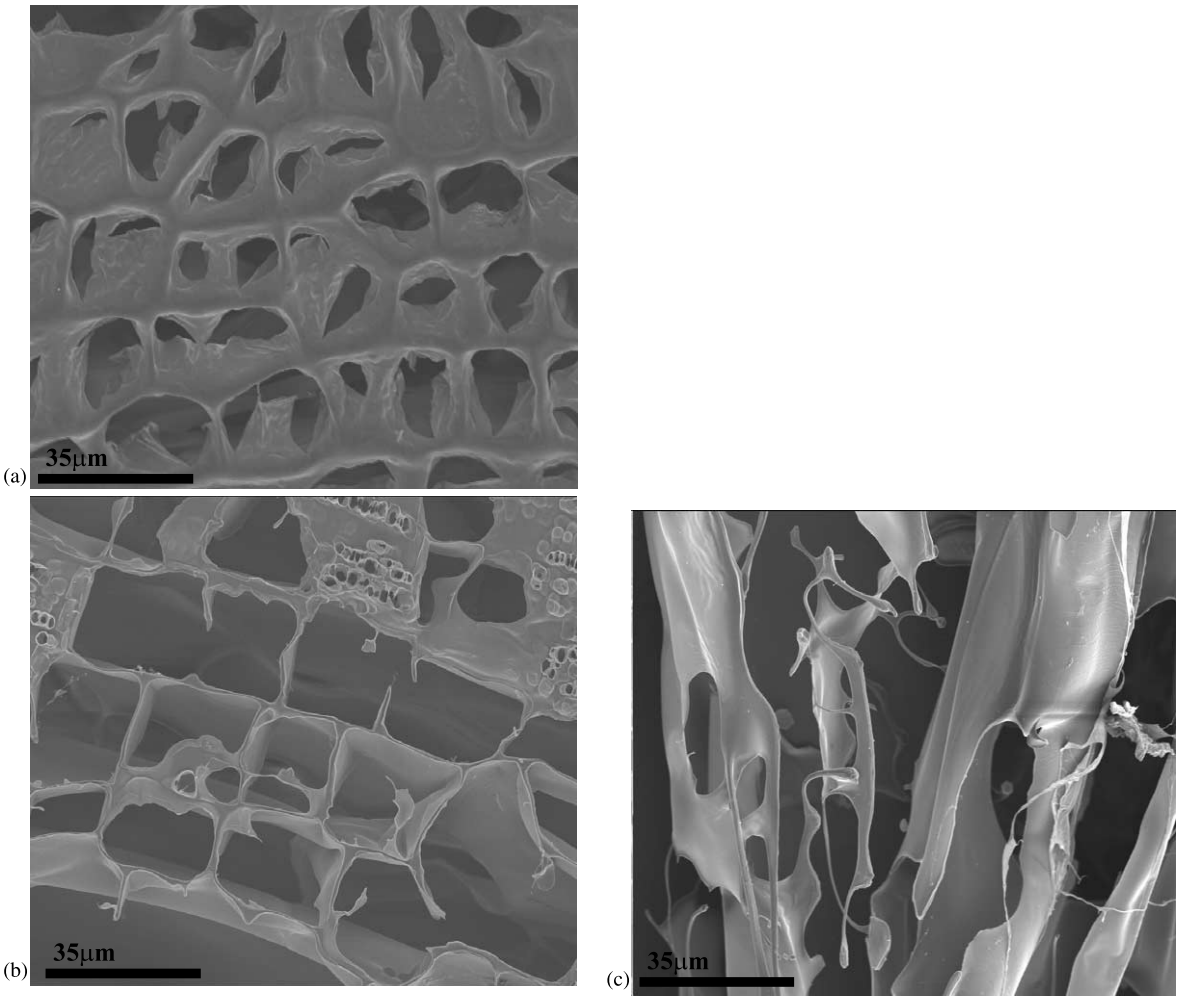

Fig. 3. SEM images for sponges containing (a) alginate alone (b) chitosan alone and (c) 3:1 alginate:chitosan.

component systems, however, would have remained in solution and, hence, the pore size determined by ice crystal formation during the freezing process. This may be reasonably expected to be homogeneous throughout the sample, leading to a more regular pore size and fibrillar structure. Inspection of Eq. (2) indicates that the strength of the sponges will be dependent on the robustness of the solid matrix and the density of the sponge system, with the latter being the predominant factor due to the corresponding power law dependence. In fact from the SEM images the chitosan systems appeared to be less dense than the alginate materials, hence, it may be assumed that the high degree of robustness is a function of the strength of the polysaccharide itself. Similarly, it is also surprising that the mixed systems produced comparatively weak gels, given the well-known interaction between alginates and cationic systems that generally lead to increases in moduli. This may be a function of any increase in matrix strength being counteracted by the irregularity of the sponge network, although more work is required in order to confirm this.

The dissolution profiles of the paracetamol from the sponges (Fig. 4) indicated that slow release was

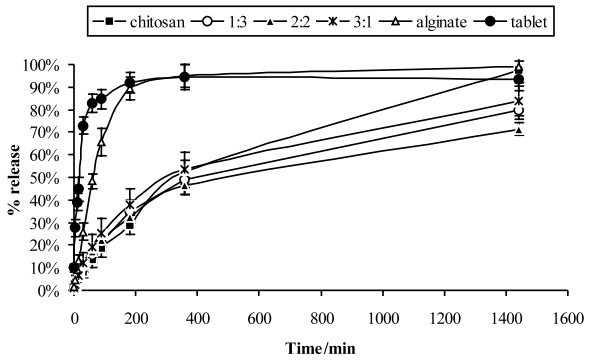

Fig. 4. Percentage release of paracetamol from alginate:chitosan sponges.

apparent for the mixed systems and the chitosan, using the paracetamol tablet as a comparator. However, perhaps surprisingly, the alginate alone did not produce a significant delay in drug release compared with the crushed tablet. It is also interesting to note that only the pure chitosan and alginate sponges showed complete drug release after 24 h.

Overall, therefore, it appears to be possible to manipulate the mechanical characteristics of the sponges via judicious choice of composition so as to tailor the properties of the sponge to the biological need. Similarly the release profiles may also be manipulated, although surprisingly there does appear to be similarity between all the chitosan-containing systems. Evidence is presented for the properties of the system to be a function of both the architecture of the sponge and the composition of supporting material. It should, however, be appreciated that both chitosan and alginate are available in a range of molecular conformations, while the freeze drying protocol may also be altered to control, for example, pore size via the initial freezing rate. Consequently while the present study has examined a limited range of formulation variables it should be appreciated that the use of these polysaccharide sponges, either alone or as mixed systems, does appear to afford the operator a potentially very wide range of performance characteristics depending on composition and processing.

4. Conclusions

The study has indicated that the mechanical, release and morphological properties of alginate/chitosan sponges are highly dependent on composition. The resistance to compression was greatest for the chitosan alone and was markedly lower for the mixed systems and alginate alone, while the resistance to breakage was greater for the single component systems. SEM studies indicated a more regular structure for the single component systems, possibly due to a pre-freezing interaction between the two polysaccharides. Dissolution studies showed reasonable slow release behaviour for all systems except the alginate alone. Clearly, therefore, it is possible to manipulate both the mechanical and drug release properties of the sponges by altering the polysaccharide composition.

References

Choi, Y.S., Hong, S.R., Lee, Y.M., Song, K.M., Park, M.H., Nam, Y.S., 1999. Study on gelatin-containing artificial skin: 1. Preparation and characteristics of novel gelatin-alginate sponge. Biomaterials 20, 409–417.

Coppi, G., Iannuccelli, V., Leo, B., Bernabei, M.T., Cameroni, R., 2002. Protein immobilization in crosslinked alginate microparticles. J. Microencapsulation 19, 37–44.

Cui, W.X., Kim, D.H., Imamura, M., Hyon, S.H., Inoue, K., 2001. Tissue-engineered pancreatic islets: culturing rat islets in the chitosan sponge. Cell Transplant. 10, 499–502.

Gibson, L.J., Ashby, M.F., 1988. Cellular Solids: Structure and Properties. Permagon Press.

Jeronomidis, G., 1988. Structure and properties of liquid and soft foams. In: Blanshard, J.M.V., Mitchell, J.R. (Eds.), Food Structure. Butterworth.

Kataoka, K., Suzuki, Y., Ohnishi, K., Suzuki, K., Tanihara, M., Ide, C., Endo, K., Nishimura, Y., 2001. Alginate, a bioresorbable material derived from brown seaweed, enhanced elongation of amputated axons of spinal cord in infant rats. J. Biomed. Mat. Res. 54, 373–384.

Kim, J.J., Lee, H.C., Oh, J.S., Shin, B.A., Oh, C.S., Park, R.D., Yang, K.S., Cho, C.S., 1999. Polyelectrolyte complexes composed of chitosan and sodium alginate for wound dressing application. J. Biomater. Sci. Polym. Edn. 10, 543–556.

Kofuji, K., Ito, T., Murata, Y., Kawashima, S., 2001. Biodegradation and drug release of chitosan gel beads in subcutaneous air pouches of mice. Biol. Pharm. Bull. 24, 205–208.

Kumar, R.M.N.V., 2000. A review of chitin and chitosan applications. Reactive Functional Polym. 46, 1–27.

Lee, Y.M., Park, Y.L., lee, S.J., Ku, Y., Han, S.B., Choi, S.M., Klokkevold, P.R., Chung, C.P., 2000. Tissue engineered bone formation using chitosan/tricalcium phosphate sponges. J. Peridontology 71, 410–417.

Leffler, C.C., Muller, B.W., 2000. Influence of the acid type on the physical and drug liberation properties of chitosan–gelatin sponges. Int. J. Pharm. 194, 229–237.

Lillford, P.J., 1989. Structure and properties of solid food foams. In: Wilson, A.J. (Ed.), Foams: Physics, Chemistry and Structure. Springer.

Mi, F.L., Shyu, S.S., Wu, Y.B., Shyong, J.Y., Huang, R.N., 2001. Fabrication and characterization of a sponge-like asymmetric chitosan membrane as a wound dressing. Biomaterials 22, 163–173.

Mi, F.L., Wu, Y.B., Shyu, S.S., Schoung, J.Y., Huang, Y.B., Tsai, Y.H., Hao, J.Y., 2002. Control of wound infections using a bilayer chitosan wound dressing with sustainable antibiotic delivery. J. Biomed. Mater. Res. 59, 438–449.

Miralles, G., Baudoin, R., Dumas, D., Baptiste, D., Hubert, P., Stolz, J.F., Dellacherie, E., Mainard, D., Netter, P., Payan, E., 2001. Sodium alginate sponges with or without sodium hyaluronate: in vitro engineering of cartilage. J. Biomed. Mat. Res. 57, 268–278.

Park, Y.L., Lee, Y.M., Park, S.N., Sheen, S.Y., Chung, C.P., Lee, S.J., 2000. Platelet derived growth factor releasing chitosan sponges for periodontal bone regeneration. Biomaterials 21, 153–159.

Shapiro, L., Cohen, S., 1997. Novel alginate sponges for cell culture and transplantation. Biomaterials 18, 583–590.

Singla, A.K., Chawla, M., 2001. Chitosan: some pharmaceutical and biological aspects-an update. J. Pharm. Pharmacol. 53, 1047–1067.

Sufan, W., Suzuki, Y., Tanihara, M., Ohnishi, K., Suzuki, K., Endo, K., Nishimura, Y., 2001. Sciatic nerve regeneration through alginate with tubulation or nontubulation repair in cat. J. Neurotrauma 18, 329–338.

Vandenberg, G.W., Drolet, C., Scott, S.L., de la Noue, J., 2001. Factors affecting protein release from alginate-chitosan coacervate microcapsules during production and gastric/intestinal simulation. J. Control. Release 77, 297–307.

Wang, L.H., Khor, E., Lim, L.Y., 2001. Chitosan-alginate-$CaCl_2$ system for membrane coat application. J. Pharm. Sci. 90, 1134–1142.

Whitaker, M.J., Quirk, R.A., Howdle, S.M., Shakesheff, K.M., 2001. Growth factor release from tissue engineering scaffolds. J. Pharm. Pharmacol. 53, 1427–1437.

Yang, J., Chung, T.W., Nagaoka, M., Goto, M., Cho, C.S., Akaike, T., 2001. Hepatocyte-specific porous polymer-scaffolds of alginate/galactosylated chitosan sponge for liver-tissue engineering. Biotech. Lett. 23, 1385–1389.