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Phosphorylcholine modified chitosan: Appetent and safe material for cells

Sheng Meng [a], Zongjun Liu [b], Wei Zhong [a,*], Qihong Wang [c], Qiangguo Du [a,*]

[a] Department of Macromolecular Science and The Key Laboratory of Molecular Engineering of Polymers, Ministry of Education, Fudan University, Shanghai 200433, China
[b] Department of Cardiology, Putuo Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai 200062, China
[c] Department of Neurosurgery, Huashan Hospital, Fudan University, Shanghai 200040, China

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Abstract

In this paper, the synthesis, characterization, and properties of a novel biodegradable polymer with improved hemocompatibility is reported. It was synthesized by combining chitosan with phosphorylcholine (PC) groups through a heterogeneous reaction process. The structure of the obtained polymer was confirmed using solid state ^{13}C NMR, ^{31}P NMR, and elemental analysis. The phosphorylcholine modification increased water absorption while it decreases bovine serum albumin (BSA) adsorption of chitosan. Some biological properties of the resulting polymer (PC-chitosan) were tested and compared with native chitosan. The hemocompatibility of PC-chitosan was estimated using full human blood. Better resistance to coagulation was observed during the blood contacting process, with prolonged activated partial thromboplastin time (APTT). Material-cell interaction was evaluated using human umbilical vein endothelial cells (HUVECs). Good biocompatibility and cytophilicity of PC-chitosan was observed with less influence to cell differentiation and multiplication.

Keywords: Phosphorylcholine; Chitosan; Anti-adhesion; Cell culture

1. Introduction

Polysaccharides obtained from nature have recently been used in the field of biomedical engineering nowadays for their good biocompatibility and biodegradability.

Sometimes it is important for the material surface to be hemocompatible, especially for those endovascular implants which are in direct contact with blood. The phosphorylcholine modified materials have been proven to have good anti-blood-coagulation properties because of their cell membrane-like structures (Nakaya & Li, 1999). Ishihara and his team (Ye, Watanabe, Iwasaki, & Ishihara, 2002) designed a hemocompatible filtration membrane system by blending cellulose acetate (CA) with 2-methacryloyloxyethyl phosphorylcholine (MPC) copolymers. In spite of the better water and solute permeability in comparison with an unmodified CA membrane, the blended membranes had much better blood compatibility as well as lower protein-adsorption. This was attributed to the MPC moieties in the copolymer, which offered biomembrane-like zwitterionic phosphorylcholine (PC) groups to the surface of the blended membrane.

Chitosan, derived from chitin, which has a chemical structure similar to glycosaminoglycan in the extracelluar matrix (ECM) and has been shown to be non-toxic and non-anaphylactic *in vivo* with good biocompatibility, biodegradability and bioactivity (Cima et al., 1991). Chitosan has been utilized in tissue regenerative therapy. It was reported that chitosan plays a critical role in cell attachment and growth (Zhu, Zhang, Wu, & Shen, 2002). Chitosan

* Corresponding authors. Tel.: +86 021 65642392; fax: +86 021 65640293.
E-mail address: weizhong@fudan.edu.cn (W. Zhong).

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itself can promote vascular endothelial growth through inducing fibroblasts to release interleukin-8, which is involved in migration and proliferation of fibroblasts and vascular endothelial cells (Lahiji, Sohrabi, Hungerford, & Frondoza, 2000). However, chitosan is also a blood-coagulant material: it is widely used as wound dressing for both its hemostaticity and cell affinity. This thrombus-generating character restricts its application in endovascular devices which often require both hemocompatibility and endothelial cell affinity.

To improve the surface properties of chitosan, especially it's anti-protein-adsorption and anti-platelet-adhesion properties, Zhu and his coworkers prepared MPC-bonded chitosan (Zhu, Wang, Yuan, & Shen, 2002) and MPC-bonded butyrylchitosan (Zhu, Shan, Yuan, & Shen, 2003) through the Michael addition of MPC to the amino groups of chitosan. Subsequent cell adhesion tests indicated that cell attachment could be easily controlled by adjusting the concentration of MPC bound to chitosan in the membrane. The MPC-bonded chitosan and butyrylchitosan both showed excellent hemocompatibility in blood clotting and platelet adhesion assays.

In another study, Zhu et al. used benzoyl-chitosan (BCS) which is soluble in organic solvents to prepare phosphorylcholine-BCS using 2-chloro-1,3,2-dioxaphosphospholane (COP) through a homogeneous solution reaction (Zhu, Zhang, & Shen, 2003). Subsequent blood-clotting and platelet adhesion assay results indicated that PC-BSC had good anticoagulant properties. Nevertheless, the benzoyl groups remaining in PC-BCS may be harmful to cell growth and multiplication compared to unmodified chitosan.

In this current paper, another type of phosphorylcholine-modified chitosan (PC-chitosan) was synthesized in a relatively easier heterogeneous process. The hemocompatibility and cytophilicity of PC-chitosan were evaluated *in vitro*. With its adscititious anti-adsorption properties, PC-chitosan seems to be a very interesting polysaccharide biomaterial, especially for endovascular applications.

2. Experimental

2.1. Materials

Medical grade Chitosan powder, with a degree of *N*-deacetylation of 78.6% and a viscosity of 90 cp was purchased from Shanghai Freemen International Trading Co., Ltd. The powder was screened through a 100 mesh sieve and dried *in vacuo* at 40 °C overnight before use. 2-chloro-2-oxo-1,3,2-dioxaphospholane (COP) was synthesized according to the methods of Lucas and Edmenson (Lucas, Mitchell, & Scully, 1950; Edmundson, 1962). The structure of the resulting compound was characterized using a GC-MS instrument (Voyager), which also determined the purity of COP (96.95%). All the other raw materials and solvents were dried and purified according to standard methods.

2.2. Modification of chitosan

Chitosan was suspended in a mixture of triethylamine (TEA) and 100 mL of dried THF in a flask purged with nitrogen. The solution of COP in 50 mL THF was slowly added dropwise through a pre-dried dropping funnel with magnetic stirring for about 3 h. Different ratios of chitosan/COP were compared. All these operations were performed at −15 °C. Then the system was slowly heated to room temperature and stirred for another 2 h before filtration to remove the solvent from the intermediate COP-Chitosan.

Then 60 mL dry acetonitrile saturated with trimethylamine (TMA) was added to another round-bottom flask with COP-Chitosan. The solution was slowly heated to 65 °C under magnetic stirring for 48 h. After that, the reaction product was carefully heated to drive out the residual TMA into H_2SO_4, and then filtered. The precipitated phosphorylcholine chitosan (PC-chitosan) was collected, carefully washed several times with pure water and dried *in vacuo* until it reached constant weight.

2.3. Characterization

^{13}C NMR and ^{31}P NMR spectra were recorded on the InfinityPlus 300 solid state nuclear magnetic resonance (NMR) machine using tetramethylsilane (TMS) as the standard for ^{13}C NMR and $NH_4H_2PO_4$ as the standard for ^{31}P NMR. The total phosphorus content of the resulting PC-chitosan was measured using an Inductively Coupled Plasma Atomic Emission Spectrometer (HITACHI P4010). An Energy Dispersive X-ray Detector (EDX) attached to a FEI Quanta 200 Scanning Electron Microscope (SEM) was used to evaluate the phosphorus content and its distribution on the PC-chitosan film surface as well as in the middle of the film cross-section.

2.4. Water absorption test

PC-chitosan film (prepared by casting from 1% acetic acid aqueous solution) was immersed into pure water or phosphate buffering solution (PBS, pH = 7.2) at 37 °C for 24 h. To prepare the sample, its weight was carefully quantified after drying *in vacuo* at 60 °C. The equilibrated degree of hydration (H_{eq}) of the modified chitosan saturated with water was determined by the following equation:

$$H_{eq} = (\text{weight of water in the sample}/\text{weight of the dry sample}) * 100\%.$$

2.5. BSA adsorption test

Bovine Serum Albumin (BSA) was selected to evaluate the anti-protein-adsorption property of the resulting PC-chitosan. BSA (BR) was purchased from the China Medicine (Group) Shanghai Chemical Reagent Corporation.

The sample films, prepared by casting from 1 wt% acetic acid aqueous solutions followed by drying in a 50 °C oven, and were pre-washed by Tris–HCl buffer solution (pH = 8.0) before being soaked in the BSA solution (2.5 mg/mL) for different periods of time at 37 °C. The amount of adsorbed protein was calculated by evaluating the decrease of the BSA content in the solution using the UV absorption method. A UNICO UV-2000 Spectrophotometer was used to measure UV absorbance of these solutions at 280 nm.

2.6. Hemocompatibility evaluation

Human blood serum obtained from healthy volunteers was mixed with 0.2 mL Trisodium Citrate (109 mmol/L) and transferred into plates coated with Chitosan and PC-chitosan, respectively, for a contact time of 1 h. Quartz was used as the control. Plasma was prepared by centrifuging the treated blood at 3000 rpm for 15 min. Then full human plasma activated partial thromboplastin time (APTT), prothrombin time (PT) and thromboplastin time (TT) of Chitosan and PC-chitosan was measured on a automatic Sysmex CA-1500 using DADE BEHRING Actin, DADE BEHRING Thromburel's and DADE BEHRING Test-Thrombin Reagents. Difference were evaluated, for significance with a paired t-test with $p < .03$.

2.7. Cell culture

HUVECs isolated from vein sources are likely candidates for clinical harvest and cell seeding applications (Prachi, Diane, John, & Christine, 2001). The HUVECs (purchased from Nanjing University of Traditional Chinese Medicine) were cultured in a growth medium (EGM) consisting of 10% new born calf serum (NCS) and DMEM. The HUVECs were grown in the six-well plates coated with chitosan and PC-chitosan, respectively, and incubated at 37 °C and 5% CO_2. The cell culture grown in the polystyrene six-well plates (Orange) was used as control. HUVECs, which had grown for 24 h on different materials, were imaged with a Leica DM IRB Microscope equipped with a Leica DC100 Camera and the number of cells was counted manually. The cell cycles were analyzed using a FACS Calibur (BECTON DICKINSON) with CycleTEST™ PLUS DNA Reagent Kit (BECTON DICKINSON). The results are expressed as mean ± standard deviation (SD). Differences were evaluated, for significance with a paired t-test with $p < .05$.

3. Results and discussion

3.1. Sample characterization

It is well known that chitosan and its hydrophilic derivatives cannot dissolve in organic solvents; and zwitterionic phosphorylcholine terminal groups may cause a physical crosslinking effect (Nederberg, Bowden, Nilsson, Hong, & Hilborn, 2004). PC-chitosan can not dissolve in normal deutered organic solvents. Thus, ^{13}C solid-state NMR was used to characterize the structure of PC-chitosan. This effect of zwitterionic terminal groups may offer a potential application for self-assembling nano-scaled drug loading particles or coatings in the future. Five main peaks can be found in the ^{13}C NMR spectrum of the original chitosan (see Fig. 1). The numbering of the carbon atoms is shown in Scheme 1. The peak at 24.2 ppm was attributed to the methyl groups in the remaining acetyl groups of chitosan. The peak at 59.5 ppm is assigned to both the peak of the C6 atom in the hydroxymethyl group and the peak of the C2 atom located in the carbonic ring binding to the amide group is shifted to 58.5 ppm because of the changes of chemical environment after phosphorylcholine modification. The peak at 76.5 ppm represents the C3 atom in the carbonic ring directly linked to the hydroxyl group, which is shifted to 75.6 ppm after modification. A successful bonding of phosphorylcholine to chitosan could be proved by the changes in chemical displacements of the C nucleus. While the peak at 105.1 ppm represented C1 and the peak at 84.0 ppm was the combination of C4 and C5, both did not show any differences in chitosan and PC-chitosan. Unfortunately, we cannot find the signals for the C nucleus in phosphorylcholine because of its relatively low content and because it overlapped with other strong C peaks.

As illustrated in Fig. 2, besides the spinning sidebands, the ^{31}P NMR spectrum of PC-chitosan showed two peaks located at 17.9 and 12.3 ppm, respectively. The former peak refers to P atoms in the phosphorylcholine groups covalently bonded to hydroxyl groups and the latter peak refers to P atoms of phosphorylcholine groups covalently bonded to amino groups in chitosan. No other peaks were found, indicating that the phosphorylcholine groups were all bonded to chitosan molecules as we anticipated.

The phosphorus content in PC-chitosan was measured by ICP; the phosphorus contents at different places on

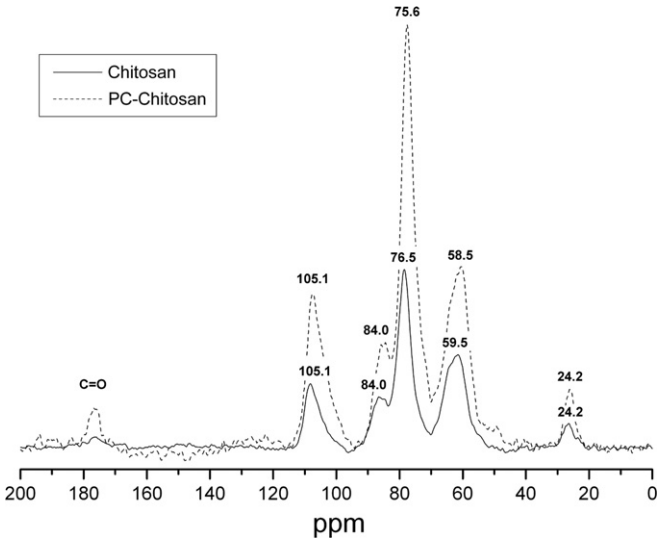
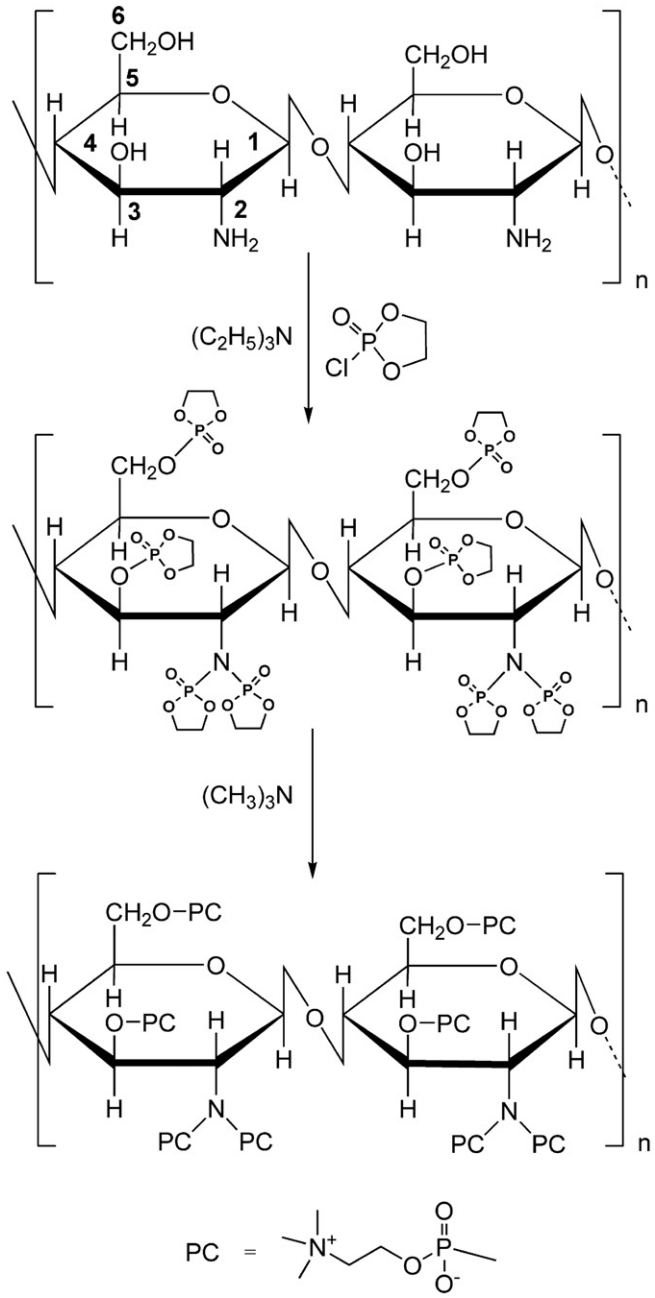


Fig. 1. ^{13}C solid NMR of Chitosan and PC-chitosan.



Scheme 1. Synthesis of PC-chitosan.

the cross-section of the sample films were measured by SEM-EDX. As shown in Table 1, the phosphorus content of the modified polymer increases with the increasing ratio of COP/chitosan in the reaction, but not proportionally. Though chitosan can react with COP only at the solid/liquid interface in such a heterogeneous system, the reaction is proved to be effective because there are substantial amount of phosphorylcholine groups covalently bonded to chitosan chains. Furthermore, the phosphorylcholine groups could move to the membrane surface during film casting or during the coating process from the aqueous acetic acid solution due to their strong hydrophilicity, which results in much higher phosphorus content at the film surface than

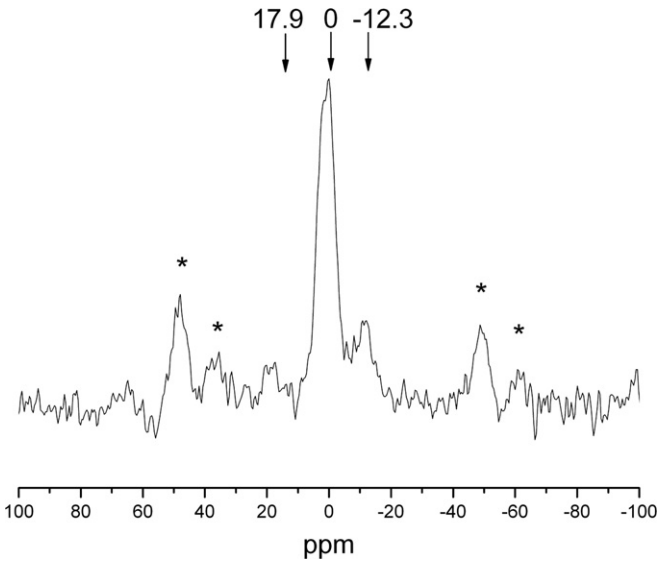


Fig. 2. ^{31}P solid NMR of PC-chitosan, * for spinning band. (Sample 2# was used).

Table 1
Phosphorus content in PC-chitosan samples

	In feed			Phosphorus content (wt%)		
	Chitosan (g)	COP (g)	TEA (g)	Holistic[a]	Surface[b]	Section[b]
PC-chitosan #1	3.0	5.2	5.1	0.55	0.71	0.41
PC-chitosan #2	3.0	10.3	10.1	0.64	0.94	0.32

[a] Estimated by ICP.
[b] Estimated by EDX.

in the middle of the cross-section. The sample PC-chitosan #2, with higher phosphorus content, was selected for the subsequent testing of its biological properties.

3.2. Water absorption test

Chitosan is hydrophilic but with a relatively low equilibrium hydration degree (H_{eq}) due to its semi-crystalline structure, and, for the same reason, it is soluble only in diluted acid. The solubility of PC-chitosan is similar to native chitosan. The chitosan powder used in this work can absorb about six times its weight of distilled water by. On the other hand, the H_{eq} of the PC modified chitosan almost doubled under the same conditions, as shown in Fig. 3. PC-chitosan swelled quickly in water and turned into a hydrogel, The H_{eq} values of PC-chitosan with different phosphorus contents was listed in Fig. 3. Moreover, it can also be seen from Fig. 3 that the water absorption property of PC-chitosan was not strongly affected by changing distilled water to PBS, a material which is different from many other polyelectrolytes. This can be an advantage for *in vivo* applications. The increased H_{eq} value of the modified chitosan is probably a result of the strong

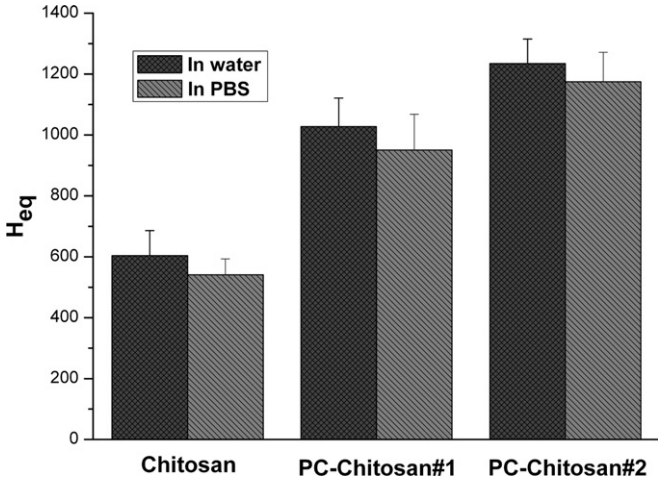


Fig. 3. H_{eq} of Chitosan and PC-chitosan in distilled water and PBS.

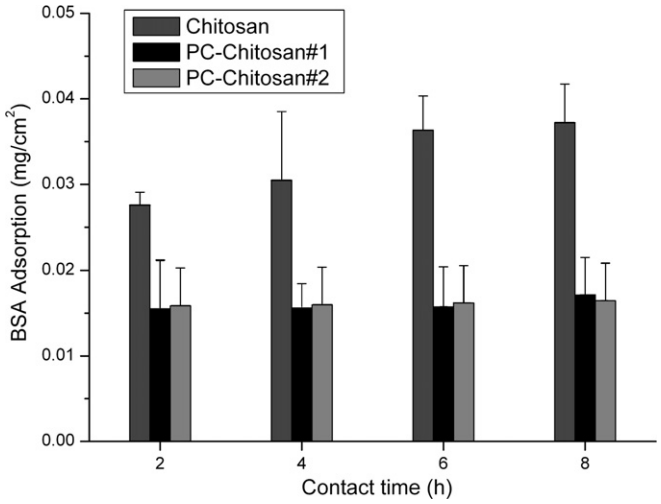


Fig. 4. BSA adsorption on Chitosan and PC-chitosan surfaces.

hydrophilicity of the phosphorylcholine groups and the damage to the crystalline structure of chitosan during the modification process. This offers great potential for PC-chitosan to be used as hydrogel implants such as embolic agent, in tissue engineering or as a controlled release material.

3.3. Protein adsorption test

The interaction between material and protein is a key factor for the biocompatibility of medical appliances. A material's interaction with proteins mediates its interactions with living cells. Reducing the amount of protein adhered the surface maybe one effective way to improve the performance of biomedical materials. The reduction of protein adsorption to the material surface could effectively reduce activation of the immune system, which could consequently prevent or delay the initial coating of foreign implants with components that may lead to phagocytosisacute and clotting (Nederberg, Bowden, & Hilborn, 2004). It has been reported that a hydrophilic surface is good for anti-non-specific protein adsorption (Lu, Lee, & Park, 1991). The modification of phosphorylcholine groups offers chitosan a more wettable surface, and as a result, compared to the unmodified chitosan samples, it can be found from Fig. 4 that fairly small amounts of BSA were adsorbed on the PC-chitosan samples. Furthermore, even after contacting for 8 h, BSA adsorption on PC-chitosan remained at a low level, while the amount of BSA on native chitosan samples kept increasing with time. The phosphorylcholine modification was thus proved to be efficient against long-term protein adsorption. The low protein-adsorption characteristic of PC-chitosan may lead to low coagulation activity which is essential for blood contacting applications.

3.4. Hemocompatibility test

Full blood APTT, PT and TT are often used to evaluate material hemocompatibility. As shown in Fig. 5, longer

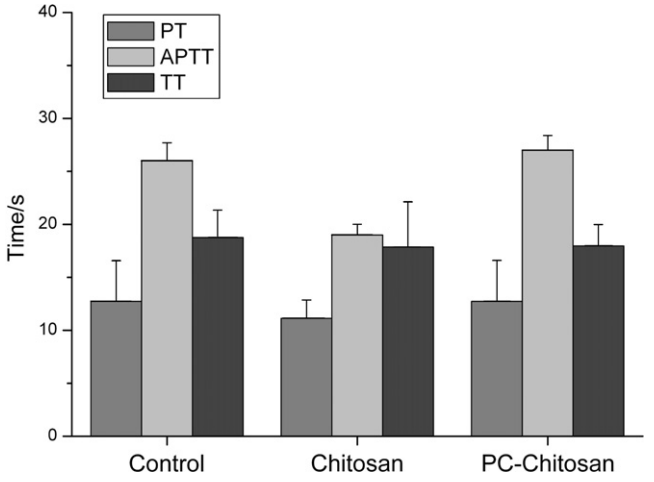


Fig. 5. APTT, PT and TT of Chitosan and PC-chitosan (Quartz used as the control), $p < .03$.

human full blood APTT and PT were observed in the case of PC-chitosan, compared with neat chitosan. This indicates better hemocompatibility, which is mainly due to low protein adsorption, the first step of coagulation. The TT value distinguished little difference between Chitosan and PC-chitosan, for it was only sensitive to heparin or heparin-like substances (Gerhards, 1991). Chitosan is commonly regarded as a hemostatic material, and has already been used as wound dressing. In our study, the hemostaticity of chitosan has been well controlled by the modification of the phosphorylcholine group. This result agrees well with the H_{eq} value and the BSA adsorption data, because a hydrophilic and low-protein adsorption surface is never favorable for aggradation and activation of the coagulation-related proteins in blood.

3.5. Cell affinity test

For a further study of the biocompatibility of PC-chitosan, cell culture and cell cycle tests were performed using

HUVECs. The microscope images (magnification 100×) of HUVECs grown on chitosan and PC-chitosan are shown in Fig. 6, from which it can be seen that most of the cells are well attached to the surface and closely arrayed maintaining their original shape.

The counted cell number showed little variation for the three coating materials we used. In the case the of control, the growth of HUVECs reached the value at $20{,}010 \pm 665\ cm^{-2}$, while in the case of chitosan it raised to $22{,}040 \pm 1622\ cm^{-2}$, probably due to the direct interaction of the cationic polymer molecules with the negatively charged cell membrane (Borchard et al., 1996). PC-chitosan showed a slightly lower value ($19{,}200 \pm 1410\ cm^{-2}$), which was likely caused by interference with the cell attachment on the material surface by the anti-protein-absorption phosphorylcholine groups. It seems that chitosan performed a little better. However, from the shapes of the cells and the cell numbers it is still difficult to tell the difference in cytophilicity between those samples (Fig. 7).

FACS was then used to analyze the cell cycles of HUVECs on the materials. From previous reports (Schafer, 1998; Wilk et al., 1998), it is known that the control of cell cycle is linked directly to an estimation of biocompatibility of different materials. This information has been used in the study of interactions between materials and cells. In all the samples tested, no exceptional DNA diploid (diploid: 100%) was found, indicating that both PC-chitosan and chitosan had no mutagenicity. The content of the cells in S phase, which refers to the cells synthesizing DNA, can be used as an indicator for the acceleration effect of the multiplication pace of cells. From Fig. 8, it can be seen that the S phase content increased in the other: control < PC-chitosan < chitosan. Also, the ratio of S phase/$G_0 - G_1$ showed that both chitosan and PC-chitosan enhance the cell growth in $G_0 - G_1$ entering S phase, and thus accelerated cell differentiation and multiplication. It could then be conclude that both chitosan and PC-chitosan performed better than the tissue culture treated polystyrene (control) in the HUVEC culture test.

The phosphorylcholine modified chitosan retains the affinity for cells with a much better hemocompatibility compared to neat chitosan. It has potential application for endovascular implants, with fast endothelial cell covering as well as anti-thrombus property.

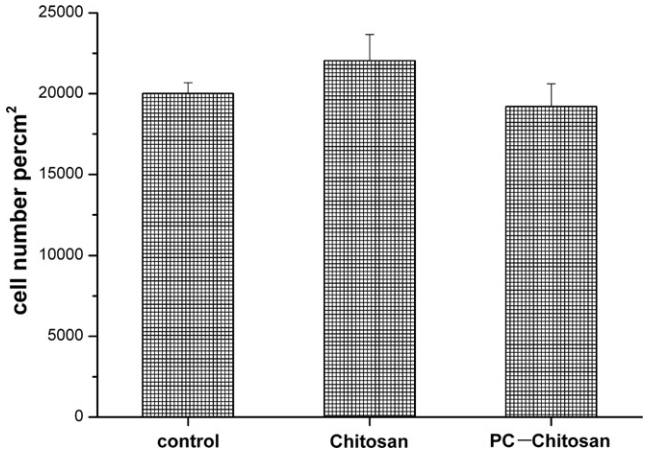


Fig. 7. Comparison of HUVECs growths on tissue culture treated polystyrene (control), chitosan and PC-chitosan for 24 h.

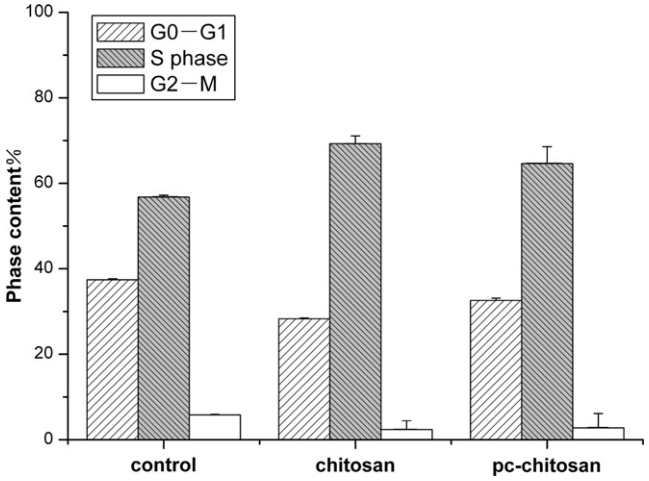


Fig. 8. Comparison of HUVECs' multiplication cycles on tissue culture treated polystyrene (control), chitosan and PC-chitosan.

Chitosan is a positive charged polymer with amino side groups. As a result, is easy for protein and cell adsorption through static interaction. But in the blood contacting cases, the blood corpuscles are also easily adsorbed, and even worse, when activated on the chitosan surface, may finally lead to clotting. In our study, parts of the amino side groups of chitosan are replaced with the more hydrophilic and biomimetic phosphorylcholine groups. Both the

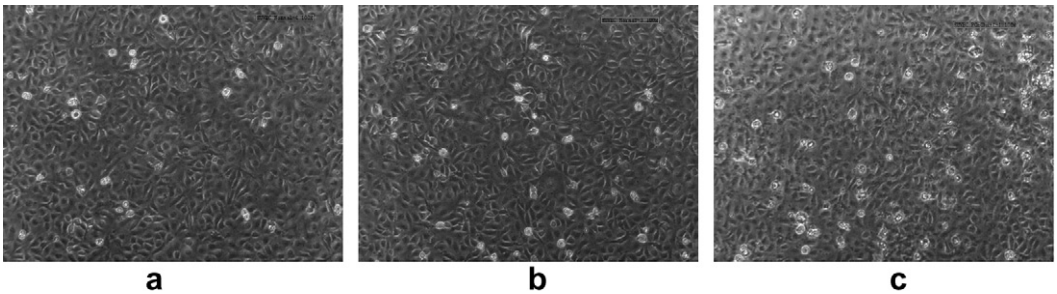


Fig. 6. Microscope images (100×) of HUVECs growth on (a) tissue culture treated polystyrene (control), (b) chitosan and (c) PC-chitosan for 24 h.

reduction of amino side groups and addition of phosphorylcholine groups offer chitosan a remarkably lower protein-adsorption surface, which leads to better hemocompatibility. Furthermore, the remaining amino side groups both on the membrane surface and in the membrane still keep their appetency for cell attachment.

4. Conclusions

In this work, phosphorylcholine modified chitosan was synthesized through a simple and efficient heterogeneous process. The resulting product, PC-chitosan, showed a more hydrophilic hydrogel characteristic, better anti-protein-adsorption capability, improved hemocompatibility, as well as similar cell affinity and safety, compared with native chitosan. This unique material may have potential applications for long-term implants and drug delivery systems that are in direct contact with tissue and blood.

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References

Borchard, G., Lueben, H. L., de Boer, A. G., Verhoef, J. C., Lehr, C. M., & Junginger, H. E. (1996). The potential of mucoadhesive polymers in enhancing intestinal peptide drug absorption 3. Effects of chitosan-glutamate and carbomer on epithelial tight junctions in vitro. *Journal of Controlled Release, 39*, 131–138.

Cima, L. G., Vacanti, J. P., Vacanti, C., Ingber, D., Mooney, D., & Langer, R. (1991). Tissue engineering by cell transplantation using degradable polymer substrates. *Journal of Biomechanical Engineering, 113*(2), 143–151.

Edmundson, R. S. (1962). Oxidation of cyclic phosphorochloridites. *Chemistry & Industry, 42*, 1828–1829.

Gerhards, H. (1991). Low-dose calcium heparin in horses – plasma heparin concentrations, effects on red-blood-cell mass and on coagulation variables. *Equine Veterinary Journal, 23*(1), 37–43.

Lahiji, A., Sohrabi, A., Hungerford, D. S., & Frondoza, C. G. (2000). Chitosan supports the expression of extracellular matrix proteins in human osteoblasts and chondrocytes. *Journal of Biomedical Materials Research, 51*, 586–595.

Lucas, H. J., Mitchell, F. W., & Scully, C. N. (1950). Cyclic phosphites of some aliphatic glycols. *Journal of the American Chemical Society, 72*(12), 5491–5497.

Lu, D. R., Lee, S. J., & Park, K. (1991). Calculation of solvation interaction energies for protein adsorption on polymer surfaces. *Journal of Biomaterials Science – Polymer Edition, 3*(2), 127–147.

Nakaya, T., & Li, Y. J. (1999). Phospholipid polymers. *Progress in Polymer Science, 24*, 143–181.

Nederberg, F., Bowden, T., & Hilborn, J. (2004). Synthesis, characterization, and properties of phosphorylcholine functionalized poly epsilon-caprolactone and charged phospholipid analogues. *Macromolecules, 37*(3), 954–965.

Nederberg, F., Bowden, T., Nilsson, B., Hong, J., & Hilborn, J. (2004). Phosphorylcholine introduces dual activity in biomimetic ionomers. *Journal of the American Chemical Society, 126*(47), 15350–15351.

Prachi, D., Diane, H. A., John, R., & Christine, E. S. (2001). Vascular graft endothelialization: Comparative analysis of canine and human endothelial cell migration on natural biomaterials. *Journal of Biomedical Materials Research, 56*(4), 545–555.

Schafer, K. A. (1998). The cell cycle: A review. *Veterinary Pathology, 35*, 461–478.

Wilk, A., Orth, J., Lomb, M., Fuhrmann, R., Kienapfel, H., Griss, P., et al. (1998). Biocompatibility analysis of different biomaterials in human bone marrow cell cultures. *Journal of Biomedical Materials Research, 40*(2), 301–306.

Ye, S. H., Watanabe, J., Iwasaki, Y., & Ishihara, K. (2002). Novel cellulose acetate membrane blended with phospholipid polymer for hemocompatible filtration system. *Journal of Membrane Science, 210*(2), 411–421.

Zhu, A. P., Shan, B., Yuan, Y. L., & Shen, J. (2003). Preparation and blood compatibility of phosphorylcholine-bonded O-butyrylchitosan. *Polymer International, 52*(1), 81–85.

Zhu, A. P., Wang, S. Q., Yuan, Y. L., & Shen, J. (2002). Cell adhesion behavior of chitosan surface modified by bonding 2-methacryloyloxyethyl phosphorylcholine. *Journal of Biomaterials Science – Polymer Edition, 13*(5), 501–510.

Zhu, A. P., Zhang, J., & Shen, J. (2003). Preparation and anticoagulant property of phosphorylcholine-terminated o-benzoylchitosan derivative. *Journal of Applied Polymer Science, 88*(2), 489–493.

Zhu, A. P., Zhang, M., Wu, J., & Shen, J. (2002). Covalent immobilization of chitosan/heparin complex with a photosensitive hetero-bifunctional crosslinking reagent on PLA surface. *Biomaterials, 23*, 4657–4665.