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A guided bone regeneration membrane composed of hydroxyapatite and polyurethane

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A hydroxyapatite (HA,10 wt%) and polyurethane (PU) composite as a guided bone regeneration (GBR)membrane was obtained from the polycondensation or polyaddition of diisocyanates and hydroxyl groups by a solvent evaporation method. The structure and properties of the membrane were investigated by XRD, IR, SEM, water absorption, wettability and a cell culture test *in vitro*. The results show that hydroxyapatite particles were dispersed uniformly in the polyurethane matrix and interfacial bonding between hydroxyapatite and polyurethane was formed. The hydroxyapatite/polyurethane membrane has good hydrophilicity and the surface pores can promote cell adhesion and growth. MTT (3-{4,5-dimethylthiazol-2yl}-2,5-diphenyl-2H-tetrazolium bromide) assay and light microscopy observation indicate that the hydroxyapatite/polyurethane membrane demonstrates excellent biocompatibility. The hydroxyapatite/polyurethane membrane will hopefully be selected as a guided bone regeneration and tissue engineering.

Key words: Hydroxyapatite, Polyurethane, Composite membrane, Characterization, Biocompatiblity.

Introduction

The guided bone regeneration method is a well-established therapy to treat bone defects in sites where limited mechanical loading exists, for example, in some cranial and maxillofacial areas and in dental applications. In recent years, increasing attention has been paid to composite guided bone regeneration membranes made of biodegradable and biocompatible synthetic or natural polymers and calcium phosphate [1]. As we all know, hydroxyapatite ($HA,Ca_{10}[PO_4]_6[OH]_2$) is the main mineral component of natural bone and has good biocompatibility, osteoconductivity and bioactivity [2-4], thus it is suitable for making the guided bone regeneration membranes. However, the brittleness of hydroxyapatite limits its applicability. Polymers are more flexible than ceramics. The properties of polymers can be varied to a large extent by changing the structure of the polymer, such as with polyurethane. Polyurethane has displayed excellent physical and chemical properties and good biocompatibility and has been widely used in biomedical fields [5]. In the preparation of polyurethane based on castor oil [6-9], castor oil was used to replace expensive polyols. Castor oil with an average hydroxyl functionality of 2.7 is a type of vegetable triglyceride [9]. The trihydroxyal castor oil can react with diisocyanates to generate urethanes.

In this study, we attempt to mix polyurethane and hydroxyapatite powders to synthesize hydroxyapatite/polyurethane composite membranes by a solvent evaporation method, in which hydroxyapatite powders offer the composite biocompatibity, bioactivity and osteoconductivity and polyurethane enhances mechanical properties. The micropores on the surface of the membrane can provide a free flow of nutrients and infiltrated cells. At the same time, these pores can also be large enough to allow the attachment and proliferation of cells to the formation of a functional tissue or organ [10-12]. The results of this paper can provide some insights and scientific data in the area of biocompatible and bioactive hybrided guided bone regeneration membranes for human tissue engineering.

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Materials and Methods

Materials

Castor oil, toluence 2,4-diisocyanate, 1,4 butane diol and acetone were from Chengdu Chemical Agent Co. Ltd, China, and of AR grade. Castor oil was dried at 80 ºC in a vacuum for 2 h. The other chemicals were used without further purification. Hydroxyapatite was synthesized in our laboratory [13], and ground after being oven-dried and sieved to 250-mesh.

Methods

Castor oil (30 g) was added in a three-necked flask with stirring, then dropped in the toluence 2,4-diisocyanate under a nitrogen atmosphere at a molar ratio of NCO/OH = 4/3 and reacted for 30 minutes at 60 ºC, then 1,4 butane diol of 5 ml was added and reacted for 30 minutes. Meanwhile, acetone was added to prevent the agglomeration

of the reactant. Finally, hydroxyapatite powders were compounded for 5 h. The hydroxyapatite/pulyurethane membrane was prepared on a culture dish after the acetone was evaporated at 40 °C for 12 h in a vacuum and the residual monomers and uncross-linked polymer were extracted in ethanol for 2 h.

Characterization

X-ray diffraction (XRD, Philips XRD analyzer) was used to detect the phase structure of the composite using Cu Ka radiation (40 kV, 30 mA). Fourier transformation infrared spectrometry (FT-IR, Nicolet 170SX) was used to determine the phase compositions of the composite in the wave length region of 4000-500 cm^{-1}. Scanning electron microscopy (SEM, JEOL, JSM-5900LV) was employed to observe the surface morphology of the composite.

The mechanical properties of the hydroxyapatite/ polyurethane membrane were evaluated by tensile tests with an electronic universal material testing machine (AG-10TA). Samples were prepared in a dumbbell shape with a size of 25.0 mm × 6.0 mm × 0.1 mm and the data were determined as the average value of 5 specimens. All the tests were conducted at a crosshead speed of 10 mm/minute at room temperature. Percentage strain (λ)was measured by $\lambda = [(l - l_0)/l_0] \times 100\%$, where l was the total extension after tension and l_0 was the gauge length (25.0 mm) before tension. The tensile strength was recorded at the ultimate fracture. Dried membranes were prepared as 5 parallel-samples (5.0 mm × 5.0 mm), and weighed on an electronic balance noted as W_1, then immersed into the de-ionized water at room temperature, and weighed after surface water had been removed with filter paper after 24 h, giving the wet weight (W_2). Therefore, water absorption was calculated through the formula: $\Omega = (W_2 - W_1)/W_1 \times 100\%$. The wettability of these membranes were measured by Contact Angle Determinator(JY-82) at room temperature by dropping 1.5 ml of de-ionized water onto the surface of specimens. Five random fields per sample were selected and the average value of five tests.

Cell culture test

Osteoblast-like cells of MG63 were cultured in a humidified atmosphere of 5% CO_2 at 37 °C. Cells were cultured in Dulbecco's modified Eagle's medium(DMEM, Sigma company) and supplemented with 10% foetal bovine serum and 2% antibiotics(200 mg/ml penicillium and 200 mg/ml streptomycin). The culture media was changed every alternate day. MG63 cells were used for 10-20 tests.

The hydroxyapatite/polyurethane membrane was made 10 mm × 10 mm and sterilized by ethylene oxide gas for 24 h, then placed into a 24-well plate and the cells were seeded into the well plates at a density of 2×10^4 cells/well. All the cells were cultured at 37 °C in a humidified atmosphere with 5% CO_2. The medium was changed every 3 days.

MTT assay

The proliferation of MG63 cells on hydroxyapatite/ polyurethane membranes was determined using MTT (3-{4,5-dimethylthiazol-2yl}-2,5-diphenyl-2H-tetrazolium bromide) assay. The medium was removed and MTT solution (200 μL, 5 mg/mL, Sigma) was added to each well after 1, 4, 7and 11-days and incubated at 37 °C in a fully humidified atmosphere with 5% CO_2 in air for 4 h to the formation of formazan crystals. After the culture medium was aspirated off and DMSO (dimethylsulfoxide) (150 ml/well) was added into each well, the well plate was left on a shaking platform for 30 minutes so as to dissolve purple formazan granules. The optical density of the solution was read on a microplate spectrophotometer at a wavelength of 570 nm. The analytical assays were performed and at least 4 wells were randomly taken for examination each time. Data were analyzed statistically using SPSS for windows (SPSS Inc. version 11.5 Chicago, U.S.A.) at a 95% significance level ($p < 0.05$) to determine the statistical significance between experimental groups.

Results and Discussion

XRD analysis

Figure 1 shows the XRD pattern of the composite

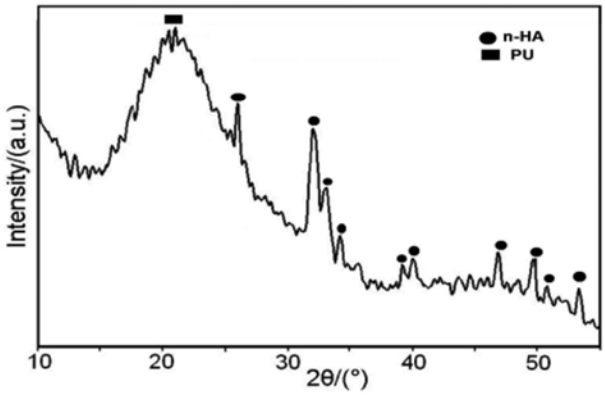

Fig. 1. XRD pattern of the HA/PU membrane.

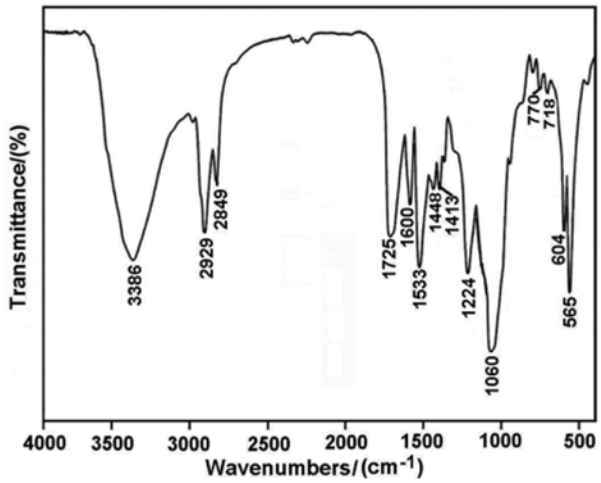

Fig. 2. IR spectrum of the HA/PU membrane.

membrane. It can be seen that the polyurethane had a widened characteristic peak because polyurethane is a type of high molecular weight polymer in a combination of crystalline and non-crystalline states. The crystalline peaks of hydroxyapatite were located at $2\theta = 25.9^{o}$, 31.9^{o}, 33^{o}, 34^{o}, 40^{o}. The results show that the crystallinity of hydroxyapatite decreased in the composite, but hydroxyapatite was still in a state of poor crystallization.

IR analysis

The IR spectrum of the composite is shown in Fig. 2. It can be seen that the absorption peak at 3571 cm^{-1}, representing bending vibration of -OH disappears, which means that linkage has been formed between the -OH of hydroxyapatite and -NCO group. The peaks at 3386 and 1725 cm^{-1} belong to -NH and -C = O stretching vibration separately, indicating that -OH and -NCO group have reacted and formed a urethane. Then excess -NCO reacts with a -NH group of the urethane to form a substitute urea which further reacts with -NCO and forms a biuret. So the adsorption peak of the biuret group appears at 1533 cm^{-1}. Peaks at 2929 and 2849 cm^{-1} are the assymmetry stretching vibrations of $-CH_2$ and the peak at 1224 cm^{-1} is the bending vibration of $-CH_3$. Peaks at 1448 and 1413 cm^{-1} belong to $-CO_3^{2-}$ and peaks at 565 and 1060 cm^{-1} are from the $-PO_4^{3-}$ of hydroxyapatite. These results indicate that the hydroxyapatite/polyurethane membrane has good homogeneity and chemical bonding between the inorganic and organic phases.

SEM observations

The SEM images of the composite (Fig. 3) indicate many micropores appeared on the surface and the diameters of pores were from 0.4 μm to 1.5 μm. These micropores can allow the ingrowth of fiber tissues and osteointergration. Meanwhile, these pores enlarge greatly the surface area, which is in favor of the adsorption of proteins, and also can enhance the adhesion and proliferation of osteogenic cells and accelerate the periosteal growth [14]. Therefore, using a solvent evaporation method might be a promising way to obtain guided bone regeneration membranes with micropores.

Mechanical test and wettability

The tensile strength and percentage strain (Table 1) both decreased with the addition of hydroxyapatite. Because castor oil based-polyurethane is an excellent elastomer, its tensile strength and percentage strain are very high [15]. But the tensile strength and percentage strain of the composite containing 10 wt% of hydroxyapatite were reduced due to inorganic powders blocking polyurethane hydrogen bond action in the material. However, the mechanical properties of the composite may match well with those of human natural guided bone regeneration membrane [16].

Table 1. Contrast of the PU and HA/PU membrane

Membrane	Tensile strength (MPa)	Percentage strain (%)	Water absorption (%)	Contact angle (°)
PU	80.0	108.9	1.18	116.7
HA/PU	65.7	90.3	22.58	61.4

Wettability is one of the important factors for biomaterials, involving the quantity and quality of protein adsorption, cell attachment and proliferation on the materials surface [17]. The water absorption and contact angle of polyurethane and hydroxyapatite/polyurethane membranes were tested and are given in Table 1. The polyurethane-based castor oil has good mechanical properties and hydrophobicity. The increasing cross-linked density makes molecules connect tightly and prevents water molecules from diffusing into the bulk. Simultaneously, the increase of the surface energy makes water soaking decrease. With the hydroxyapatite added, the hydroxyapatite/polyurethane membrane has a micro-phase separation structure of hydrophilicity-phobicity, its water absorption is much higher than that of the polyurethane membrane. Increasing the hydrophilicity of the surface can promote the adherence of proteins, the cell attachment and differentiation of histiocytes, which is fit for a guided bone regeneration repair material.

Cell culture

Cell culture tests in vitro were used to evaluate both cytotoxicity and cytocompatibility of the hydroxyapatite/polyurethane membrane. The composite with MG63 cells was studied to determine the cell's reproduction and differentiation. Fig. 4 shows the MG63 cells attach onto the hydroxyapatite /polyurethane membrane after 1- and 7-day culture. We can see that after 1-day culture,

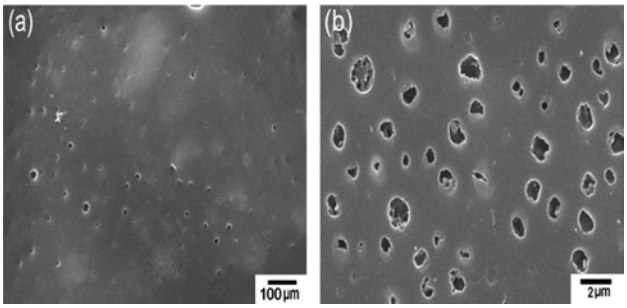

Fig. 3. SEM images of the HA/PU membrane at different magnifications.

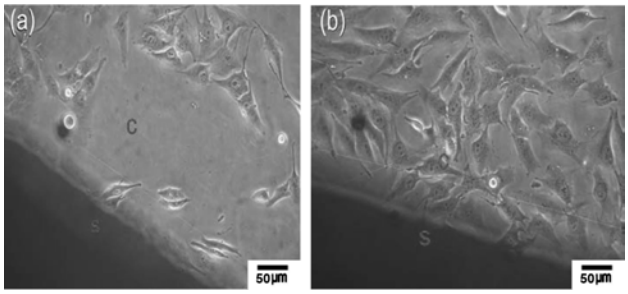

Fig. 4. Inverted microscope images of the MG63 (denoted as C) cultured in vitro on HA/PU membranes (denoted as S) for 1 day (a) and 7 days (b).

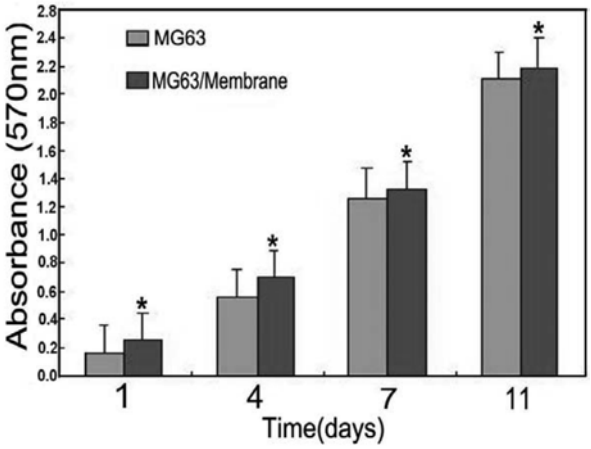

Fig. 5. MTT assays for proliferation of MG63 and MG63 cultured on HA/PU membranes for various incubation periods under the same culture conditions. Error bars represent means ± SD for n = 4. *p < 0.05.

cells exhibited fusiform, and the cell matrix decreased. Some cells had their morphology stretched and proliferated. After 7 days, the number of MG63 increased greatly and interconnected through generous pseudopodiums among the cells and formed a great quantity of cell matrix and silky fibers. These observations indicate that a porous hydroxyapatite/polyurethane membrane allows the diffusion of molecules and shows better biocompatibility with an enhanced cells affinity in vitro. Obviously, the composite has no negative effect. Adherence of cells on the surface of materials is a complicated process, including many factors; from a cytobiological point of view, such as cell metabolism, cell contacting periods with materials, cell hydrophobicity and surface charge, while in terms of tissue engineering such as physical, chemical and geometric properties of material surfaces, hydrophilicity-phobicity of the material etc. [18]. Hydroxyapatite/polyurethane membrane with a good hydrophililicity is favorable for the adsorption of proteins [19] and the porous structure can promote the proliferation of cells, adherence and increase the contacted surface area.

MTT test is one of important methods to evaluate the biocompatibility and cytotoxicity of biomaterials. Proliferation of MG63 on hydroxyapatite/polyurethane membrane was also assessed in vitro (seen in Fig. 5). During the 11-day culture, the cell numbers increased gradually, but the growth rate of MG63 on hydroxyapatite/polyurethane membrane is higher than that of the MG63 contrast group. The results indicate that the hydroxyapatite/polyurethane membrane is biocompatible and non-cytotoxic in vitro.

Conclusions

Hydroxyapatite/polyurethane membranes prepared by solvent evaporation are homogenous and display good wettability. The linkage between the inorganic and organic phases endows the membrane with excellent mechanical properties close to those of natural periosteum. The micropores can provide a micro-environment for the ingrowth of cells and tissues. The hydroxyapatite/polyurethane membrane has good biocompatibility and shows no cytotoxicity.

Acknowledgements

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References

1. S. Liao, F. water and Y. Zhu, Dental Materials 20 (2006) 1026-1034.
2. E.D. Case, I.O. Smith and M.J. Baumann, Materials Science and Engineering A 390 (2005) 246-254.
3. H. Ehrilich, B. Krajewska and T. Hanke, Journal of Membrane Science 273 (2006) 124-128.
4. H.K. Hockin and C.G. Simon, Journal of Orthopaedic Research 22 (2004) 535-543.
5. G.T. Howard, International Biodeterioration & Biodegradation 49 (2002) 245-252.
6. H. Yegneh and M.R. Mehdizadeh, European Polymer Journal 40 (2004) 1233-1238.
7. B.Z. Ramos, V. Soldi, E.L. Senna and R. Borsali, Macromol. Symp. 229 (2005) 234-245.
8. H.Q. Xie and J.S. Guo, European Polymer Journal 38 (2002) 2271-2277.
9. N.B. Tran, J. Vialle and Q.T. Pham, Polymer. 38 (1997) 2467-2473.
10. N.P. Ziats, K.M. Miller and J.M. Anderson, Biomaterials 9 (1988) 5-13.
11. M. Borkenhagen, R.C. Stoll, P. Neuenschwander, U.W. Suterand and P. Aebischer, Biomaterials 19 (1998) 2155-2165.
12. W.J. Kao, Biomaterials 20 (1999) 2213-2221.
13. W. Jie and L.Y. Bao, Europe Polymer Journal 40 (2004) 509-515.
14. K.L. Burg, S. Porter and J.F. Kellam, Biomaterials 21 (2000) 2347-2359.
15. P. Siddaramaiah, P. Mallu and A. Varadarajulu, Polymer. Degradation and Stability 63 (1999) 305-309.
16. C.C. Verheyen, J.K. Wijn and C.A. Blitterswijk, J. Biomed Mater. Res. 26 (1992) 1277-1296.
17. E. Hans, Biomaterials 19 (1998) 397-406.
18. E. Lieb, J. Tessmar and M. Hacker, Tissue Eng. 9 (2003) 71-84.
19. C.Z. Jiang, J. Mater. Sci. Mater. Med. 17 (2006) 1297-1130.