Effect of Different Preparation Conditions On The Properties of Bamboo Fiber-Based Bioactive Composite Membrane

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Effect of different preparation conditions on the properties of bamboo fiber-based bioactive composite membrane

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Abstract

A novel nano-hydroxyapatite/bamboo fiber (n-HA/BF) bioactive composite membrane was obtained by a simple casting technique. The membrane forming mechanism and the effects of different forming membrane methods, drying methods and n-HA amounts on the properties of n-HA/BF membrane were investigated by Fourier Transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), scanning electron microscopy (SEM), contact angle, electromechanical universal tester, *in vitro* soaking in simulated body fluid (SBF) and *in vitro* cell culture experiment. The results demonstrated that the n-HA dispersity in BF matrix was not affected by the preparation condition, however, the morphologies of membrane were determined by the different preparation conditions owing to different hydrogen bond shrinkage. Moreover, the hydrophilicity of the composite membrane was improved under the condition of the membrane formation in oven, freeze drying and high addition content of n-HA, and the mechanical properties of composite membrane depended on n-HA content. *In vitro* soaking behavior indicated that the degradability and bone-like apatite deposition could be controlled by different preparation conditions. And the cell proliferation experiment showed that the n-HA/BF composite membranes obtained under different preparation conditions were all non-toxic. The above results indicated that the n-HA/BF composite membrane could be obtained by a simple casting technique, and the properties could be controlled by adopting different preparation conditions, which would have a great promising as guide bone tissue regeneration (GBR) membrane, and the study would provide a new application for BF in biomedical field.

Keywords: Bamboo fiber; nano-hydroxyapatite; composite membrane; degradation

Introduction

Guided bone regeneration (GBR) membrane is commonly used to augment defect area, where

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the membrane was placed on the bone defect area as barrier membrane to create a singular space, so as to prevent epithelial cells from growing into the defect, and permit the proliferation of osteoblasts and new bone formation for bone tissue regeneration (Jiang et al., 2015; Niu et al., 2021; Li et al., 2020; 2015). Ideally, GBR membrane should possess appropriate mechanical properties, space-retention ability, biocompatibility and biodegradability (Lee et al., 2016; Yu et al., 2020; Hoornaert et al., 2016). To obtain the satisfactory GBR membrane, natural biodegradable polymers has become a research hotspot owing to the better biocompatibility and biodegradability, compared with the synthetic polymers (Prajatelistia et al., 2021; Ma et al., 2019; Mora-Boza et al., 2020; Bierhalz and Moraes, 2017; Pappu et al., 2019; Gurunathan et al., 2015; Weng et al., 2020).

Bamboo fiber (BF) is a natural fiber extracted from bamboo, which has high strength, biodegradability and low cost. Therefore, BF is usually used as a reinforcing agent for polymers (Khalil et al., 2012; Liu et al., 2012; Phuong et al., 2019; Long et al., 2019; Zuo et al. 2019). In our group previous study, we have systematically studied and concluded that BF had remarkable reinforce effect on the n-HA/poly (lactide-co-glycolide) (n-HA/PLGA) composite (Li et al., 2015; Jiang et al., 2017; 2018; 2019). Moreover, BF has been used to reinforce membrane by electrospinning (Chingakham et al., 2020; Cai et al., 2018). In viewing of the fact that the casting membrane had denser structure and simple preparation method, which would display better mechanical strength and slower degradation than electrospinning membrane (Oksana et al., 2020). Therefore, we studied the reinforce effect of carboxylated BF on chitosan-based casting membrane via ionic crosslinking (Tang et al., 2020). However, the preparation procession of carboxylated BF was too tedious. In addition, in the aboved study, BF was usually added in the

64 primeval state fiber form as reinforcement, which would be adverse for dispersion, so it would be
65 expected to explore a novel polymer membrane by a simple and green processing.

66 BF could be directly dissolved into homogeneous solution and obtained casting membrane,
67 whereas, as we know, pure BF membrane lacks osteoconductivity, which would be
68 detrimental to guide bone tissue regeneration, while nano-hydroxyapatite (n-HA) was similar to
69 with inorganic component of natural bone, so it was normally added into polymeric matrix as
70 nanofiller to endow polymers with better biological performance (Zhao et al., 2018; Muhammad
71 et al., 2017). In our previous study, we found BF could replace other polymers to develop
72 n-HA-based nanocomposite by co-precipitation method, and it displayed a promising to be used
73 as bone materials (Ma et al., 2020). However, similarly, whether BF could be replaced other
74 polymers to obtain n-HA/BF composite membrane by solution blending method, and whether the
75 different preparation conditions including different forming membrane methods, drying methods
76 and n-HA amounts would bring about different effect on the properties of n-HA/BF membrane,
77 and whether it would be used as GBR membrane, which were all not be reported and it was
78 worth exploring.

79 Based on these, in this work, we attempt to study the fabrication of the n-HA/BF composite
80 membrane by casting method, and the effects of different preparation conditions on the properties
81 of the n-HA/BF composite membranes, including forming film methods (in air and in oven),
82 different drying methods (in air, in oven, in freeze dryer) and different n-HA content (10%, 20%,
83 30% and 40%), were studied by Fourier Transform infrared spectroscopy (FT-IR), X-ray
84 diffraction (XRD), scanning electron microscopy (SEM), contact angle, electromechanical
85 universal tester, Moreover, the degradation behavior of the composite membrane was

investigated by soaking in simulated body fluid (SBF). Finally, the cell culture experiment was carried out. The main purpose of the work is to demonstrate the feasibility of the fabrication of the n-HA/BF composite membrane by a simple casting method, so as to provide a new GBR membrane by making full use of natural biomass resources.

Experiment Section

Materials

Bamboo fiber (BF) was provided by Zhejiang A&F University, whose size was Φ (0.03-0.2) mm $\times$ (6-10) cm. Dimethylacetamide (DMAc, AR) and LiCl (AR) were purchased from Aladdin. $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (AR), $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$ (AR), P_2O_5 , and NaOH (AR) were all from Tianjin Fengchuan Chemical Reagent Technologies Co. Ltd.. Other agents were analytical grade.

Preparation of the n-HA/BF composite membranes

Bamboo fiber was dissolved in DMAc with the concentration of 1.3 wt%. A certain amount of n-HA was dispersed in DMAc by ultrasonic treatment, which was slowly added into bamboo fiber solution with the magnetic stirring, and the evenly dispersed mixture solution was poured on the clean and dry glass plate, and the glass rod with two copper rings was applied to control the thickness of the membrane. Then, the glass plate covered with n-HA/BF mixture solution was put in air or in oven to form film, afterword, it was dried in air, in oven or freeze drying, thus, n-HA/BF composite membrane with 20% n-HA were obtained by six methods, which was noted as air-air, air-oven, air-freeze drying, oven-air, oven-oven and oven-freeze drying, respectively. Moreover, n-HA/BF composite membrane obtained by air-air method with different n-HA contents of 10 %, 20 %, 30 % and 40 % n-HA were prepared in the similar procedure.

Characterization of the composite membranes

108 The appearance of membranes obtained by the two different forming membrane methods of
109 air-oven and oven-oven were given as examples, which was taken by normal digital camera.

110 Thermo Nicolet 670 spectrometer was used to analysis the Fourier transformation infrared
111 (FTIR) of samples, and the collected spectrum range was 600~ 4000 cm^{-1} .

112 The phase analyses of samples were carried out by X-ray diffraction (XRD) (a Rigaku
113 Corporation X-ray diffractometer) with Cu-K α radiation, the scanning speed of 5°/min at 40 kV
114 and 45 mA, the range of $2\theta=10\sim70^\circ$.

115 The morphologies of samples treated with the gold sputtering were observed by scanning
116 electron microscopic (SEM, S-520, Hitachi, Japan).

117 The contact angles of samples were measured with Rotating drop interfacial tensiometer
118 (TX500TM, Kono, USA). The sample was put on the slide, and the distilled water was dropped
119 onto the surface of the liquid by stop drop method, then the water drop on the sample was
120 observed.

121 The tensile strengths of samples with the size of 0.2 mm $\times$ 4 mm $\times$ 60 mm were measured by
122 electromechanical universal testing machine (WDW-20, China) with the speed of 5 mm/min with
123 60 % relative humidity at room temperature, and the mean value was calculated based on the five
124 parallel samples of each specimen.

125 In vitro degradation of the composite membranes

126 The degradation of n-HA/BF composite membrane *in vitro* was studied by soaking in SBF,
127 whose ion concentration was very similar to that of human plasma, and it was obtained according
128 to the following procedure, that is, NaHCO₃ (0.350 g), NaCl (7.996 g), KCl (0.224 g), K₂HPO₄ ·
129 3H₂O (0.228 g), CaCl₂ (0.278 g), Na₂SO₄ (0.071 g), MgCl₂ · 6H₂O (0.305 g) were dissolved in

130 deionized water in order, and buffer with trimethylolmethylaminomethane (6.057 g) and
131 hydrochloric acid to adjust the solution to physiological pH 7.40 at 37 °C ($\pm 0.5^{\circ}\text{C}$). The samples
132 were taken out from SBF at 2, 4, 6 and 8 weeks, and filter paper was used to absorb surface
133 residual washing water. The water absorption and water loss were as follows.

134
$$\text{Weight loss rate } \text{\%} = (w_1 - w_3) / w_1 \times 100\%$$

135
136
$$\text{Water absorption } \text{\%} = (w_2 - w_3) / w_3 \times 100\%$$

137
138 The original weight of the sample was W_1 , and the wet weight and the dry weight was noted
139 as W_2 and W_3 , after being entirely dried after soaking, respectively.

140 *In vitro* cell biocompatibility of the composite membranes

141 Bone mesenchymal stem cells (BMSCs) were used to primarily assess *in vitro* cells viability,
142 which was isolated according to the related literature ([Hoseini et al., 2015](#); [Ye et al., 2019](#)). The
143 samples with the thickness of 0.2 mm and diameter of 6.0 mm were cleaned with 75 % ethanol
144 solution, sterilized under ultraviolet lamp. The treated samples were placed in a 96-well plate
145 with the density of 4000 cells/well without disturbed in an incubator for 3 hours, then an
146 additional 1 mL culture medium was added into each well.

147 The cell proliferation was evaluated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-
148 tetrazolium bromide) assay ([Priyadarshini et al., 2018](#); [Shakeri et al., 2014](#); [Hivechi et al., 2021](#)).
149 At designated time of 1, 2 and 3 days, the medium for the cell-seeded materials were discarded,
150 and 100 μL MTT solution with 3 mg/mL was added, incubated at 37 °C in an air atmosphere
151 containing 5 % CO_2 for at least 4 hours, and 100 μL DMSO was added to dissolve the formazan
152 crystals. Then the 200 μL purple solution was absorbed and transferred into a new 96-well plate,
153 and the optical density (OD) values of the solution were measured in microplate reader (Synergy

154 HTX, BIOTEK, USA) at 495 nm.

155 **Results and discussion**

156 **Characterization of surface-modified n-HA**

157 **Appearance of the membranes**

158 [Fig. 1](#) displays the appearance of membrane, it can be seen that the membrane formed in air had
159 smaller area and thicker than that formed in oven, which was mainly caused from the different
160 hydrogen bonds between the bamboo fiber molecules. In air, the bamboo fiber was easy to
161 absorb the water, which would make the hydrogen bond shrinkage in the bamboo fiber, thus the
162 bamboo fiber was gelled and DMAC/LiCl solution precipitated from the edge, resulting in
163 thicker film and smaller area. While in oven, DMAC solvent was easy to volatilize and the
164 hydrogen bond between the bamboo fiber molecules was destroyed, and the bamboo fiber has
165 been formed before shrinkage, resulting in thinner film and larger area.

172 **Fig.1. Membrane appearances formed by different conditions. (a) in oven, (b) in air.**

173 **IR analysis**

174 [Fig. 2](#) is the FT-IR spectra of different n-HA/BF composite membrane obtained by different
175 methods and different n-HA contents. The characteristic peaks of 2920 cm^{-1} and 2846 cm^{-1}

corresponded to the tensile vibration of C-H bond on methylene of BF existed in n-HA/BF composite membrane. In addition, the characteristics peaks at 3567.2 cm^{-1} attributed to the tensile vibration of OH^- and the peaks at 1095 cm^{-1} , 604 cm^{-1} and 565 cm^{-1} of PO_4^{3-} were related to n-HA (Chesley,et al., 2020). The peak position did not transfer obviously when n-HA was added into BF, which indicated that n-HA was simply blended with BF without chemical change. Compared with the membrane formed in oven and in air, the intensity of the peak at 1044 cm^{-1} of the film formed in oven was markedly weakened, which was caused by less substance in the unit area for the thinner film formed in the oven. Additionally, for the different n-HA/BF composite membrane with different n-HA contents, there was no obvious difference for the characteristics peak.

Fig.2. FTIR spectra of samples. (a)n-HA, (b)BF, (c)Air-Air, (d)Air-oven, (e)Air-freeze drying, (f) Oven-air, (g)Oven-oven, (h)Oven-freeze drying, (A)10% n-HA/BF, (B)20% n-H A/BF, (C)30% n-HA/BF, (D)40% n-HA/BF.

XRD investigation

To further understand the phase structure of n-HA/BF membrane, XRD pattern is shown in Fig. 3. Obviously, the amorphous peak at 20.5° was the peak of bamboo fiber (Guimaraes, et al., 2015), marked with “♦”, and the peaks at 25° and 31° of n-HA were found, noted as “♣”. The crystallization peak position of n-HA in the composite membrane did not change, indicating that the two components of n-HA and BF were only blended. Similarly, the crystallization peaks of Fig.3 (f), (g), (h) of n-HA were also obviously weaker than those of Fig.3 (c), (d) and (e) formed in air. This was because the content of n-HA in the membrane formed in the oven was less than that in the membrane formed in the oven, which leads to the weak crystal peak in the spectrum. With the increase of n-HA content, the characteristic peak and its crystallinity of n-HA in different membrane increased, which also confirmed that n-HA and BF were only blended without chemical reaction.

Fig. 3 XRD of samples. (a) n-HA, (b)BF, (c) Air-Air, (d) Air-oven, (e) Air-freeze drying, (f) Oven-air, (g) Oven-oven, (h) Oven-freeze drying, (A) 10% n-HA/BF, (B) 20% n-HA/BF, (C) 30% n-HA/BF, (D) 40% n-HA/BF.

SEM observation

Fig. 4 shows the morphologies of n-HA/BF composite membranes obtained by different methods. From the SEM micrographs, it can be seen that white particles existed in the composite membranes, which showed the existence of n-HA. However, there was subtle difference for the dispersion of n-HA particles in the membrane, and the n-HA was relatively more uniform in oven (Fig.4(d), (e), (f)) than in air (Fig.4(a), (b), (c)), which was caused by the different hydrogen bond between molecules during the forming membrane of bamboo fiber. In oven, the solvent of DMAC was evaporated at high temperature, and the fiber morphology had been fixed before the hydrogen bond between bamboo fiber molecules shrinking, which would bring out better dispersion of n-HA.

Fig. 4 SEM of samples. (a)Air-Air, (b)Air-oven, (c)Air-freeze drying, (d)Oven-air, (e)Oven-oven, (f)Oven-freeze drying, (A)10%HA/BF, (B) 30%HA/BF, (C)40%HA/BF.

However, for the three different dry methods, the surfaces were compact without porous

structures, and there was no obvious difference for the surface in air and in oven, which indicated that drying method in air or in oven had little effect on the membrane morphology after bamboo fiber molding, and the compact structure could effectively prevent the invasion of connective tissue. While for the freeze-drying method, there was some closed pores on the surface of freeze-dried membrane (Fig.4(c), (f)), which was originated from the pores left by the sublimation of water molecules, and the porous structure was conducive to cell adhesion for guided bone regeneration. Additionally, for the composite membranes with different n-HA contents, the white particles gradually increased on the surface of the membrane, but there was no obvious agglomeration phenomenon, and there displayed excellent interface between n-HA and BF, which indicated that the hydrophilic n-HA and BF had good compatibility.

Contact angle measurement

Fig.5. Contact angle of the n-HA/BF composite membranes obtained by different methods. (a) Air-Air, (b) Air-oven, (c) Air-freeze drying, (d) Oven-air, (e) Oven-oven, (f) Oven-freeze drying, (A) 10%-n-HA/BF, (B) 30% n-HA/BF, (C) 40% n-HA/BF.

To further make clear the hydrophilicity of the n-HA/BF composite membrane, the contact angle of the n-HA/BF composite membranes were tested with rotating drop interfacial tensiometer by dropping distilled water onto the liquid surface, and the results are shown in Fig. 5. As expected, the contact angle values of all n-HA/BF casting membranes were less than 90 °, which proved that n-HA/BF casting membranes were hydrophilic membrane. Comparing with the membranes obtained by different methods, we found that the membranes obtained by the same forming membrane method had a similar contact angle value, while the freeze-dried membrane possessed lower contact angle because of the rough membrane surface with the porous structure (Fig.5(c)), and the stronger hydrophilicity would be more useful for cell adhesion and proliferation (Dhinasekaran, et al., 2021). However, for the same dried method, the membrane formed in oven displayed lower contact angle than that of the membrane formed in air, and the reason was that the hydrogen bond between the film-forming molecules was destroyed and the surface tension was reduced when the membrane was dried in oven. In addition, for the n-HA/BF composite membranes with different n-HA contents, the contact angle gradually decreased with the increasing of n-HA, which showed that the higher n-HA content brought about better hydrophilicity of the membrane.

Tensile strength test

Fig. 6 shows the tensile strengths of the n-HA/BF composite membranes obtained by different methods. We found that membrane formed in air had higher mechanical strength than that in oven. Comparing to the membrane formed in air (Fig.6 (a), (b), (c)), the tensile strength of membrane dried by freeze-drying was the worst, and the tensile strength of the membrane dried in air was improved by 69.64%, and dried in oven was increased by 51.7% than dried by

freeze-drying, respectively. Likely, for the three membranes formed in oven (Fig.6(d), (e) and (f)), the tensile strengths of the membranes dried in air and in oven were improved by 23.89% and 5%, respectively. The reason was that the freeze-drying membrane was porous structure, and the forming membrane or dry membrane in oven caused the destruction of intermolecular hydrogen bond during molding, resulting in thinner membrane and larger area, so as to display lower tensile strength. For the n-HA/BF composite membranes with different n-HA contents, the tensile strength increased at first but decreased with the increasing of n-HA content, and the 20% n-HA/BF composite membrane displayed the highest tensile strength, which was accord with the nanoparticles filler reinforce principle (Yadav, et al., 2020), and the mechanical strength could meet the application requirement of GBR membrane (Castro, et al., 2018).

Fig.7 Weight loss rate of samples in SBF.

***In vitro* degradation and cell culture**

Weight loss of composite membrane after degradation

Fig.7 shows the weight loss rate of n-HA/BF composite membrane. It can be seen that the weight loss rate of the composite membrane first decreased and then increased within 8 weeks. During

the first 4 weeks, the weight loss rate was negative continuously, which indicated that the mass of composite membrane did not decrease but increased after immersion, meaning that the amount of apatite deposited on the surface of the membrane was greater than the mass of degradation, so that the total mass of composite membrane was greater than that before immersion. Similarly, the weight loss rate of 4-8 weeks was negative, but showed an upward trend, indicating that the membrane possessed greater degradation. Comparing to the different composite membranes, the weight loss rate of the membrane obtained by oven-freeze drying method changed the most among the membrane different methods, which indicated that bone like apatite adhered most owing to the porous structure, and the much apatite deposition would have better biological activity. Moreover, for the composite membranes with different n-HA contents, the higher the content of n-HA, the greater the negative value of weight loss rate, indicating that the total weight was more than the original weight, which was mainly caused by the more new apatite deposited, and it was further proved that the higher the content of n-HA was more favorable for the bone-like apatite deposition ([Kumar et al., 2014](#)).

Water absorption of composite membrane after degradation

[Fig.8](#) gives the water absorption of n-HA/BF composite membrane. The results showed that the water absorption of n-HA/BF composite membrane had a similar trend during the immersion process, that is, the water absorption increased slightly at the initial degradation, and then decreased greatly from 2 weeks to 4 weeks, which was caused by the bone-like apatite deposition with the extension of soaking time. Moreover, the freeze-dried membrane displayed the lowest water absorption, and the main reason was that the porous structure had the fastest degradation, which would produce much micropore and bring more apatite deposition, and it could be proved

by the weight loss result. Similarly, among the composite membrane materials with different n-HA content, the higher BF content, the higher water absorption showed, which was related to the water absorption of BF. In a word, the water absorption results indicated that the n-HA/BF composite membrane had good degradation and water absorption performance.

Fig.8. Water absorption rate of samples in SBF.

SEM observation of composite membrane after degradation

[Fig. 9](#) shows the surface morphology of n-HA/BF composite membrane after soaking in SBF for 8 weeks. It can be found that new bone like apatite was produced on the surface of the n-HA/BF composite membrane. However, comparing to the different composite membranes, the composite membrane formed in oven had less bone like apatite (seen [Fig.9 \(a\), \(b\), \(c\)](#)) than the membrane formed in air (seen [Fig.9 \(d\), \(e\), \(f\)](#)). The reason was that the hydrogen bond between molecules in cellulose shrank, and n-HA particles were wrapped in the inner part of the membrane when the membrane was formed in oven. While the composite membrane was formed in the oven, the intermolecular hydrogen bond was broken, and n-HA particles were dispersed on the surface of

the membrane, which was more conducive to the deposition of bone-like apatite on the surface of the membrane, and the bone-like apatite would help to improve interface between the tissue-implant and its surrounding tissues (Zhu, et al., 2020).

For the composite membrane with different n-HA contents of 10%, 30% and 40% n-HA (seen Fig.9(A), (B), (C)), respectively. From the 10% n-HA/BF composite membrane, it can be seen that there were pores on the surface of bamboo fiber (Fig.9(A)), which indicated that bamboo fiber could be degraded during soaking. With the increase of n-HA content, the more bone-like apatite was deposited, which further demonstrated that the n-HA in composite membrane had good induce bone like apatite deposition, and the results were consistent with the previous analysis.

Fig. 9. SEM micrographs of samples after soaking for 8 weeks. (a) Air-Air, (b) Air-oven, (c)Air-freeze drying, (d) Oven-air, (e) Oven-oven, (f) Oven-freeze drying, (A)10% n-HA/BF, (B) 30% n-HA/BF, (C)40% n-HA/BF.

MTT test of cell culture

The results of MTT cell proliferation of BMSC cultured on the surface of n-HA/BF composite membrane for 1, 2 and 3 days are shown in Fig. 10. It can be seen that the OD value of each sample increased significantly with the extension of culture time, indicating that cells could normally grow and proliferate on different membrane surfaces. In addition, the OD values of freeze-dried composite membrane were significantly higher than those of the membrane dried in air or in oven, which further indicated that the porous structure of freeze-dried composite membrane was more conducive for cell proliferation and had good biocompatibility (Ai, et al., 2021). Comparing to the n-HA/BF membranes with 10%, 20% and 30%, the OD value enhanced significantly with the increase of n-HA content and the extension of culture time. The results showed that the cells proliferated rapidly on the surface of the membrane, suggesting that the n-HA/BF composite membrane had good biological properties (Bee et al., 2019). This was consistent with the previous *in vitro* immersion analysis.

Fig. 10. MTT value of cell culture on sample surface.

Conclusions

398 In this study, the n-HA/BF composite membrane was successfully prepared by a simple casting
399 technology, and the effects of different forming membrane, drying methods, and different n-HA
400 contents on the composite membrane were studied. The results confirmed that there was no
401 chemical bonding between n-HA and BF components, however, the membrane formed in air was
402 thicker than that in oven, because the hydrogen bond shrinkage made the BF gel and
403 DMAC/LiCl solution precipitated from the edge in air. Thus, the tensile strength of the
404 membrane formed in air were higher than that of in oven, especially, the 20 % n-HA/BF
405 composite membrane was the highest, but the tensile strength of freeze-dried composite
406 membrane was poorest because of the porous structure. The contact angle test of the composite
407 membrane showed that the composite membrane was hydrophilic, and the freeze-dried
408 membrane brought better hydrophilicity owing to the rough surface and the small surface tension.
409 SBF soaking results indicated that n-HA/BF composite membrane displayed different
410 degradability, and the higher n-HA content in composite membrane displayed better bone-like
411 apatite deposition. The cell proliferation results showed that the composite membrane had no
412 cytotoxicity. This study would provide a new way for developing a novel GBR membrane based
413 on the utilization of natural BF.

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Graphical Abstract

In this manuscript, bamboo fiber (BF) was firstly chosen to prepare nano-hydroxyapatite/BF (n-HA/BF) composite membrane replacing other polymers by casting method, and the membrane forming mechanism and the effects of different forming membrane methods, drying methods and n-HA amounts on the corresponding n-HA/BF membrane were investigated. Results demonstrated that n-HA dispersity in BF matix was not affected by the preparation condition, however, the morphologies of membrane was determined by the different preparation conditions owing to different hydrogen bond shrinkage. Moreover, the hydrophilicity of the composite membrane was improved under the condition of the membrane formation in oven, freeze drying and high addition content of n-HA, and the mechanical properties of composite membrane depended on n-HA content. *In vitro* soaking behavior indicated that the degradability and bone-like apatite deposition could be controled by different preparation conditions. And the cell proliferation experiment showed that the n-HA/BF composite membranes obtained under different preparation conditions were all non-toxic. The above results indicated that the n-HA/BF composite membrane could be obtained by a simple casting technique, and the properties could be controlled by adopting different preparation conditions, which would have a great promising as guide bone tissue regeneration (GBR) membrane, and the study would provide a new application for BF in biomedical field.

Membrane appearances formed by different conditions. (a) in oven, (b) in air.

SEM micrographs of samples. (a)Air-Air, (b)Air-oven, (c)Air-freeze drying, (d)Oven-air, (e)Oven-oven, (f)Oven-freeze drying, (A)10%HA/BF, (B) 30%HA/BF, (C)40%HA/BF.