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A homogenous CS/NaCMC/n-HA polyelectrolyte complex membrane prepared by gradual electrostatic assembling

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Abstract A homogenous membrane composed of chitosan (CS), sodium carboxymethyl cellulose (NaCMC) and nano hydroxyapatite (n-HA) was prepared by a gradual electrostatic assembling (GEA) method. The physical and chemical properties of the membranes with different n-HA contents and CS/NaCMC ratios were characterized by Scanning electron microscopy, Fourier transform infrared spectroscopy, X-ray diffraction and mechanical test. The schematic formation mechanism of the membrane was discussed. The results show that GEA is an effective method to prepare the polyelectrolyte complex (PEC) membrane, in which oppositely charged CS-NaCMC polysaccharides can assemble mildly and gradually through electrostatic interaction to form the membrane framework, while the filled n-HA crystals can regulate the structure stability of the composite membrane. The optimum preparation condition for the PEC membrane can be fixed to a content of 60 wt% n-HA, an equivalent amount of CS to NaCMC and a drying temperature of 60°C. The PEC membrane may have good prospect for guided bone regeneration.

1 Introduction

Polysaccharides are of great research and practical importance in biomedical field due to their good biocompatibility and desirable biodegradability [1, 2]. The biocompatibility is the most necessary condition for their use as biomaterials, while the biodegradability is advocated to avoid a second surgery for removal of the implants [3]. When two polysaccharides with opposite charges are mixed together in an aqueous solution, they will interact spontaneously to form polyelectrolyte complex (PEC). The formation of PEC is essentially a result of the electrostatic interaction between oppositely charged poly-ions. One of the major driving forces is the entropy gain associated with the release of counter-ions [4, 5].

Chitin and cellulose are two natural polysaccharides abundant in crustaceans [6], bacterium [7] and higher plants [8]. Their special derivatives which contain different types of functional groups, such as carboxyl and amino groups, have attracted wide and new interests as biomaterials in recent years. Chitosan (CS), which derives from the *N*-deacetylated derivative of chitin, is a linear polymer formed by glucosamine and *N*-acetyl-D-glucosamine units with β-(1-4) glycosidic bounds [9]. Due to its unique cationic character, CS has been extensively used to prepare ion-crosslinked hydro gels with polyanions [10], such as polyacrylic acid, alginic acid, dextran sulfate, pectin, xanthan, heparin, and sodium carboxymethyl cellulose (NaCMC) [11–13]. Carboxymethyl cellulose (CMC) is an anionic polymer in which the original H atom of cellulose hydroxyl group is replaced by carboxymethyl substituent ($-CH_2-COO^-$) [14]. It is an important biodegradable polysaccharide and usually used by its sodium salt NaCMC. With a similar molecular structure (Fig. 1), a perfect PEC can be formed between cationic CS and anionic NaCMC.

Based on their natural, renewable, nontoxic and biodegradable properties, CS/NaCMC PEC (microsphere or membrane) has been promising candidate for controlled drug-release system, protein separation and skin substitute

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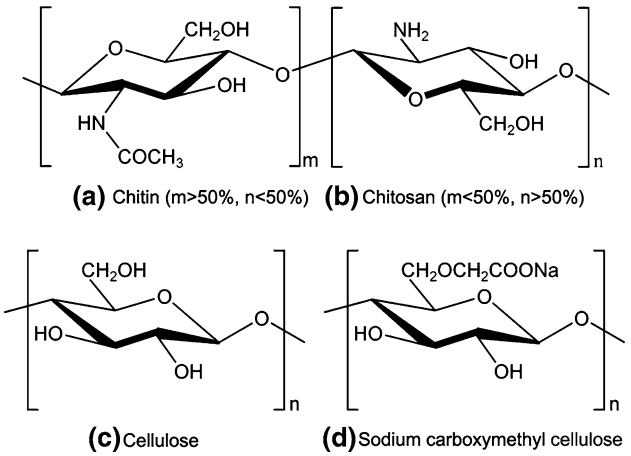

Fig. 1 Structure of chitin, chitosan, cellulose and sodium carboxymethyl cellulose

[15–17]. When CS solution and NaCMC solution are mixed together, strong electrostatic interaction happens immediately, of which polymer chains agglomerate and PECs precipitate quickly at the bottom [13]. Thus, by one-step mixing, it's hard to spread the PECs and form a homogenous membrane without any macroscopical phase separation. However, when the prepared PECs are dissolved in NaOH aqueous solution, a membrane can be produced by casting the alkaline solution on polysulfone ultra-filtration membrane [13]. Some cross-linking agents (such as glutaraldehyde) are used to help the membrane formation [18], but the additives may release toxic residue that is harmful to living organism. There are also bilayer and multilayer PEC films for special purposes [19, 20], but they are not real uniform PEC films of CS and NaCMC. These CS/NaCMC membranes are usually used for ion-exchange separation and few literatures give reports about their applications for guiding bone tissue regeneration.

For a guided bone regeneration (GBR) membrane, osteoconductivity is required, as well as good biocompatibility and biodegradability [21, 22]. It is known that CS and NaCMC are natural biodegradable polysaccharides with excellent bioadhesivity and biocompatibility [10, 23], but they lack osteoconductive capability. Nano hydroxyapatite (n-HA) is the main mineral component of natural bone. Due to its good biocompatibility and osteoconductivity, n-HA has been widely used for repairing bone defects or promoting bone tissue regeneration [24]. In this case, n-HA should be a good candidate to be introduced into the PEC system. Such a tri-component composite is expected to combine their advantages and develop a new GBR membrane.

In previous study, we used the layer-by-layer casting method to prepare a CS/NaCMC/n-HA membrane, which was expected to be used as a GBR membrane [25]. We assumed that the CS solution could penetrate into the n-HA/NaCMC liquid membrane by self-assembly of electrostatic action. However, later studies revealed that the membrane was in a double layer structure, and the electrostatic attraction was only restricted at the interface of the two layers. We also prepared CS/NaCMC/n-HA scaffold by a co-solution method in our previous work [26], but this method can not be applied to fabricate a membrane, because phase separation emerges severely during the membrane formation.

In this study, a gradual electrostatic assembling (GEA) method was used to prepare a homogenous CS/NaCMC/n-HA PEC membrane. In the procedure, the oppositely charged polymer chains of CS and NaCMC were controlled to assemble mildly and gradually without appearance of phase separation. For comparison, some other membrane samples were also made by solution blending method and layered casting method. The properties and formation mechanisms of membranes by different preparation methods were tested and discussed. The prepared PEC membrane is expected to be used as a barrier membrane for guiding bone tissue regeneration.

2 Materials and methods

2.1 Materials

Chitosan was from Haidebei Marine Bioengineering Co. (Jinan, China), $M_w \sim$ 200–250 kDa (DD = 95.41%). Sodium carboxymethyl cellulose was from Kelong Chemical Factory (Chengdu, China), $M_w \sim$ 550–600 kDa (Na-content of 6.5–8.5 wt%). n-HA slurry (24 wt% n-HA crystals in deionized water) was prepared by wet synthesis in our lab [27]. All other chemicals were analytical reagents from Kelong Chemical Factory (Chengdu, China).

2.2 Membrane preparation

2.2.1 Gradual electrostatic assembling

A certain amount of n-HA slurry and CS powder were added into NaCMC aqueous solution. After stirring for 2 h, n-HA crystals and CS particles were suspended and distributed evenly in the NaCMC solution. Then 2 vol% acetic acid solution was slowly dropped into the mixture under intense stirring. The pH value of mixing solution was kept stable between 5.0 and 5.2 and the temperature was controlled at 25–30°C. During the dropping process of acetic acid solution, CS particles gradually dissolved in NaCMC aqueous solution and interacted with surrounding NaCMC polymer chains by electrostatic action. Afterwards, the tri-component mixture was poured and spreaded on a glass plate. After standing for 20 min, a uniform gel formed on the glass. The gelatinous samples were air-dried

Table 1 The original amounts of three components, drying temperature (T_d) and drying time (t) for the preparation of CS/NaCMC/n-HA composite membrane (S-sample)

S	n-HA (g)	CS (g)	NaCMC (g)	T_d (°C)	t (h)	S	n-HA (g)	CS (g)	NaCMC (g)	T_d (°C)	t (h)
A1	0	2	2	40	45	F1	2	1	1	40	40
A2	0	2	2	50	35	F2	2	1	1	50	25
A3	0	2	2	60	22	F3	2	1	1	60	15
A4	0	2	2	70	16	F4	2	1	1	70	9
A5	0	2	2	80	10	F5	2	1	1	80	5
B1	0.4	1.8	1.8	40	45	G0	2.4	0.8	0.8	RT	48
B2	0.4	1.8	1.8	50	35	G1	2.4	0.8	0.8	40	30
B3	0.4	1.8	1.8	60	22	G2	2.4	0.8	0.8	50	10
B4	0.4	1.8	1.8	70	16	G3	2.4	0.8	0.8	60	9
B5	0.4	1.8	1.8	80	10	G4	2.4	0.8	0.8	70	7
C1	0.8	1.6	1.6	40	45	G5	2.4	0.8	0.8	80	5
C2	0.8	1.6	1.6	50	35	H1	2.8	0.6	0.6	40	10
C3	0.8	1.6	1.6	60	22	H2	2.8	0.6	0.6	50	6
C4	0.8	1.6	1.6	70	16	H3	2.8	0.6	0.6	60	3
C5	0.8	1.6	1.6	80	10	H4	2.8	0.6	0.6	70	3
D1	1.2	1.4	1.4	40	42	H5	2.8	0.6	0.6	80	2
D2	1.2	1.4	1.4	50	30	I1	3.2	0.4	0.4	40	7
D3	1.2	1.4	1.4	60	19	I2	3.2	0.4	0.4	50	4
D4	1.2	1.4	1.4	70	12	I3	3.2	0.4	0.4	60	3
D5	1.2	1.4	1.4	80	7	I4	3.2	0.4	0.4	70	2
E1	1.6	1.2	1.2	40	42	I5	3.2	0.4	0.4	80	2
E2	1.6	1.2	1.2	50	30	J1	2.4	0.533	1.067	60	8
E3	1.6	1.2	1.2	60	19	J2	2.4	0.640	0.960	60	8
E4	1.6	1.2	1.2	70	9	J3	2.4	0.960	0.640	60	9
E5	1.6	1.2	1.2	80	7	J4	2.4	1.067	0.533	60	9

at room temperature (RT) and dried in heating oven at 40, 50, 60, 70 and 80°C, respectively. To neutralize residual acetic acid, the dried composite membranes were immersed in 5 wt% NaOH solution for 24 h. Finally, the membranes were fully washed by deionized water and dried again at RT. Different weight ratios of three components (n-HA: 0, 20, 40, 60 and 80 wt%, CS:NaCMC = 1:2, 1:1.5, 1:1, 1.5:1, 2:1) were adopted to prepare membranes according to above steps (Table 1).

2.2.2 *Solution blending and layered casting for comparison*

Solution blending method was used to prepare the contrastive membrane (the amounts of three components are n-HA = 60 wt% and CS:NaCMC = 1:1): First, 2 wt% CS solution was obtained by dissolving CS powder in 2 vol% acetic acid solution. Then a certain amount of n-HA slurry was added into 2 wt% NaCMC aqueous solution and stirring for 2 h to form a binary mixture. Afterwards, 2 wt% CS solution was directly mixed into n-HA/NaCMC mixture under continuous stirring. The ternary blending mixture was poured onto glass plate and air-dried at RT. The dried composite was immersed in 5 wt% NaOH solution for 24 h and rinsed with deionized water, then dried again at RT.

Contrastive samples were also prepared by layered casting method which conforms completely to the given steps in our previous paper [25]. 2 wt% CS solution and n-HA/NaCMC mixture were prepared as the same steps mentioned in solution blending method. Differently, n-HA/NaCMC mixture was first poured onto glass plate to form a liquid membrane, and then the CS solution was cast slowly on the n-HA/NaCMC composite membrane. The layered solution was also air-dried at RT. For comparison, the amounts of three components are also n-HA = 60 wt% and CS:NaCMC = 1:1.

2.3 Characterization analyses

2.3.1 *Macroscopic observation*

The macroscopic observation of membranes prepared by different methods was recorded by camera at the same angle.

2.3.2 Scanning electron microscopy (SEM) observation

The cross-section and surface morphologies of the membranes were observed by SEM (JEOL JEM 5600LV, Japan) at 20 kV after surface gold sputtering. The cross-section was formed by brittle fracture of the membrane in liquid nitrogen.

2.3.3 Mechanical test

The mechanical properties of the membranes were tested by universal mechanical testing machine (AG-IC 50KN, Shimadzu Corporation, Japan) at RT. Before test, the membranes were immersed in deionized water for 2 h and then tailored to dumbbell-shaped standard samples. The test was conducted using a drawing speed of 10 mm min^{-1}. Five specimens were tested for each sample.

2.3.4 Fourier transform infrared spectroscopy (FT-IR) analysis

FT-IR spectra for the characteristic functional groups of the sample powders were recorded by a Perkin-Elmer 6000 FT-infrared absorption spectrometer (Nicolet Perkin-Elmer Co, USA) in a wave number range of 400–4000 cm^{-1}. The membranes were pulverized after soaking in liquid nitrogen for 30 min.

2.3.5 X-ray diffraction (XRD) analysis

The crystal structure and composition of n-HA, CS, NaCMC and their membranes were tested by XRD with X-ray diffractometer (DX-2500, Dandong, China) using Cu Kα radiation ($\lambda = 0.154184$ nm). Each sample was scanned at 2θ from 5 to 70° with a scanning rate of 3°min^{-1}.

3 Results and discussion

3.1 Macroscopic observation and formation mechanisms of membranes

Figure 2 shows the macroscopic morphologies of the samples prepared by three different methods. All the samples have the same weight ratio of the three components (60 wt% n-HA, 20 wt% CS and 20 wt% NaCMC) and air-dried at RT. In the method of gradual electrostatic assembling, the PEC mixture forms a uniform gelatinous composite at first, and the gel becomes a homogeneous membrane after water evaporation. There is no phase separation present among the three components as shown in Fig. 2(a1, a2). However, the ternary mixture can not form a membrane by solution blending method, the PECs aggregate into macroscopic particles or clusters (Fig. 2(b1, b2)). For layered casting method, because the CS solution does not penetrate into the underlayer n-HA/NaCMC liquid membrane, an upper transparent CS membrane layer adheres on the surface of n-HA/NaCMC membrane, forming a double-layered membrane (Fig. 2(c1, c2)).

A schematic membrane formation mechanism is presented in Fig. 3a. For gradual electrostatic assembling, n-HA crystals and undissolved CS powder are firstly dispersed in the NaCMC aqueous solution, i.e. in the interspace of

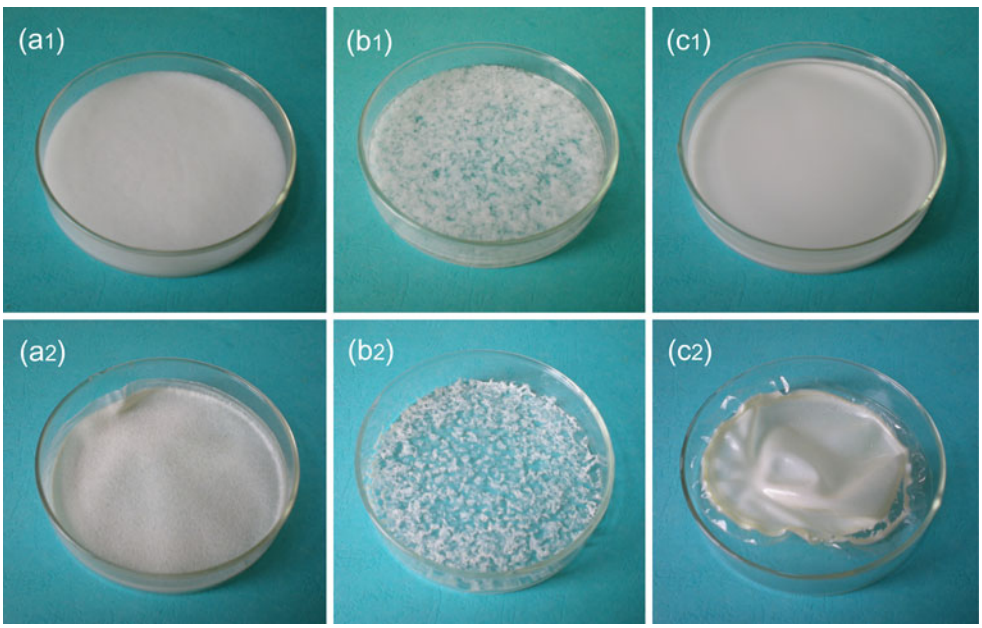

Fig. 2 Macrographs of membranes prepared by GEA (*a1*, *a2*), solution blending (*b1*, *b2*) and layered casting (*c1*, *c2*). (*a1*), (*b1*) and (*c1*) represent fresh mixture solution poured onto glass plate, while (*a2*), (*b2*) and (*c2*) denote the dried composite membranes

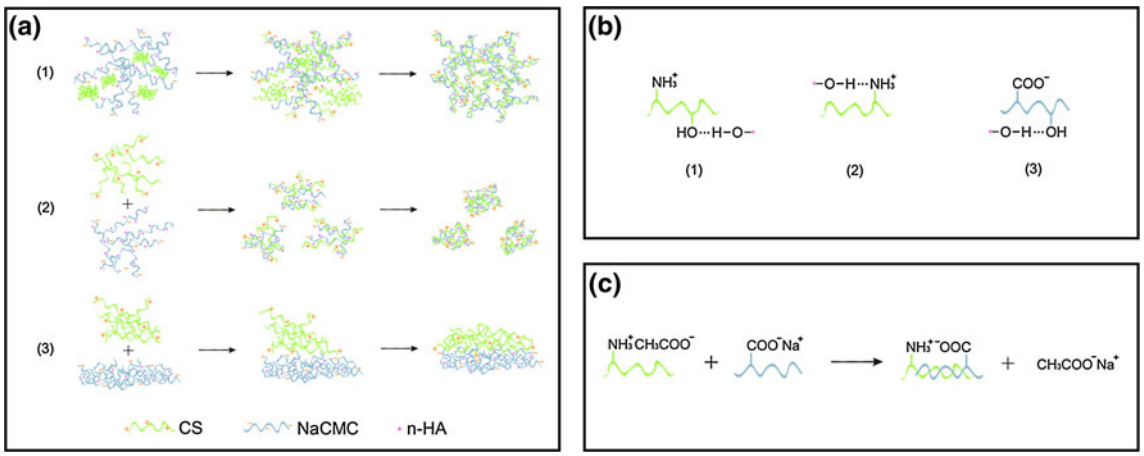

Fig. 3 The schematic diagrams of **a** formation mechanism of the membranes prepared by GEA (*1*), solution blending (*2*) and layered casting (*3*); **b** hydrogen bonding between n-HA and polyelectrolyte chains: hydroxyl group of CS with hydroxyl group of n-HA (*1*), amino group of CS with hydroxyl group of n-HA (*2*) and hydroxyl group of NaCMC with hydroxyl group of n-HA (*3*); **c** electrostatic interaction of CS with NaCMC

NaCMC chains. Then CS powder is gradually dissolved by adding acetic acid solution in the mixture solution. Meanwhile, the extended CS chains start to bond with surrounding NaCMC chains through electrostatic interaction and intermolecular hydrogen bonding, forming a polymer-matrix composite (Fig. 3a(1)), in which the n-HA crystals also form hydrogen bonding with the polymer chains and promote the stability of the composite structure. Just as Fig. 3b shows, hydrogen bonding may form by hydroxyl groups of n-HA with hydroxyl groups of CS, amino groups of CS and hydroxyl groups of NaCMC. In this way, the three components entwine into networks and produce a homogenous membrane. But it is quite different in solution blending process. While CS powder dissolves in acetic acid solution, polysaccharide chains will extend in the solution and the amino groups will become cationic by combining with hydrogen ions of acetic acid. Similarly, NaCMC extends its polymer chains in aqueous solution and exposes its anionic carboxylic ions. When CS solution blends with n-HA/NaCMC mixture solution, electrostatic interaction happens immediately between cationic groups of CS and anionic groups of NaCMC in a way of Fig. 3c, thus the polymer chains of CS and NaCMC aggregate and twist into composite particles or clusters with n-HA crystals inside (Fig. 3a(2)). As for layered casting procedure, the CS solution does not penetrate into the preformed underlayer n-HA/NaCMC liquid membrane, thus electrostatic attraction is only restricted at the interface of the two layers (Fig. 3a(3)).

3.2 SEM observation

Figure 4a shows the micrograph of the cross-section of CS/NaCMC/n-HA membrane prepared by gradual electrostatic assembling. It can be clearly seen that the three components have even distributed and united in a uniform network. The thickness of the membrane is about 160 μm. The membrane prepared by solution blending is shown in Fig. 4b, in which the three components seriously conglomerate in particles, resulting in an irregular and non uniform membrane shape. For the membrane prepared by layered casting, it is in layered structure as shown in Fig. 4c. The upper layer is CS, and the underlayer is n-HA/NaCMC mixture. There is a little infiltration of underlayer component into the upper CS layer at the interface.

In addition, the CS/NaCMC/n-HA composite membrane prepared by GEA is very stable when soaking in water, while both the particle-conglomerated membrane and the double-layered membrane collapse progressively into pieces in water. Therefore, based on the morphologic observation, GEA is the best way among the three methods for preparation of homogenous CS/NaCMC/n-HA composite membrane.

Figure 5 shows the surface of CS/NaCMC/n-HA membranes prepared by GEA with different n-HA contents (0, 20, 40 and 60 wt%, CS:NaCMC = 1:1). The membrane made of CS and NaCMC has a smooth surface, as shown in Fig. 5(a1, a2). With the increase of n-HA content in the system, the surface of membranes becomes rougher, and some irregular cavities can be clearly observed (Fig. 5(d2)). The enhanced surface roughness is favorable to cells attachment and adhesion, and the cavities and n-HA content are helpful to the growth of cells and osteoconduction of new tissues [28, 29].

3.3 Mechanical properties

The tensile strength and elongation rate of the GEA membranes are shown in Fig. 6. Figure 6a and b gives the tensile strength and elongation rate of membranes with

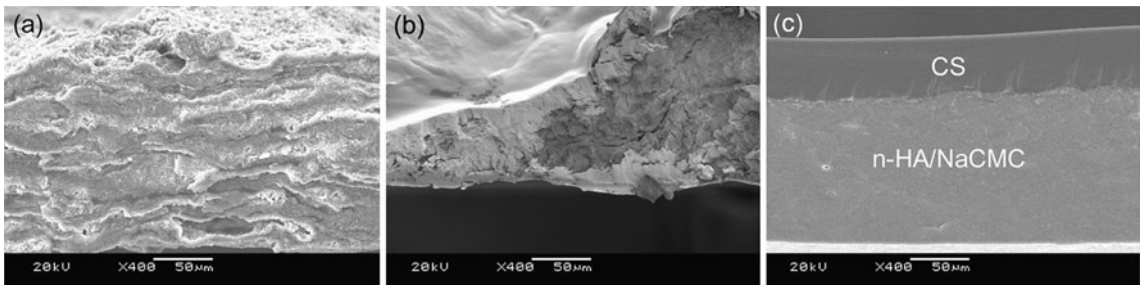

Fig. 4 SEM photographs of the cross-section of membranes prepared by GEA (**a**), solution blending (**b**) and layered casting (**c**)

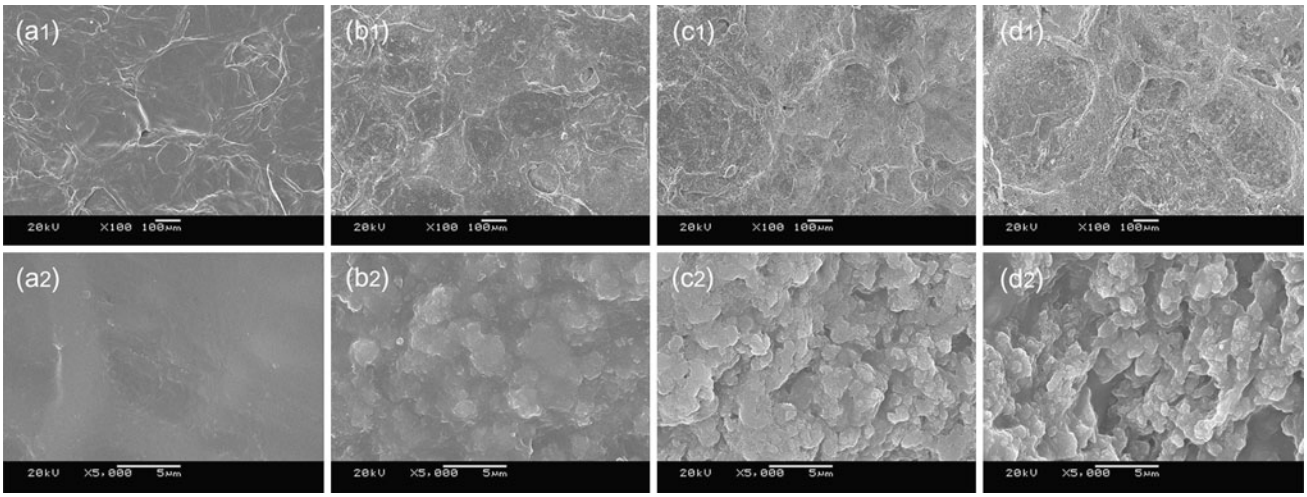

Fig. 5 SEM photographs of the surface of GEA membranes with a CS:NaCMC ratio of 1:1 and different n-HA weight contents. (*a1*), (*b1*), (*c1*) and (*d1*) represent n-HA content of 0, 20, 40 and 60 wt% respectively with a magnification of 100, (*a2*), (*b2*), (*c2*) and (*d2*) denote membranes of corresponding n-HA content with a magnification of 5000

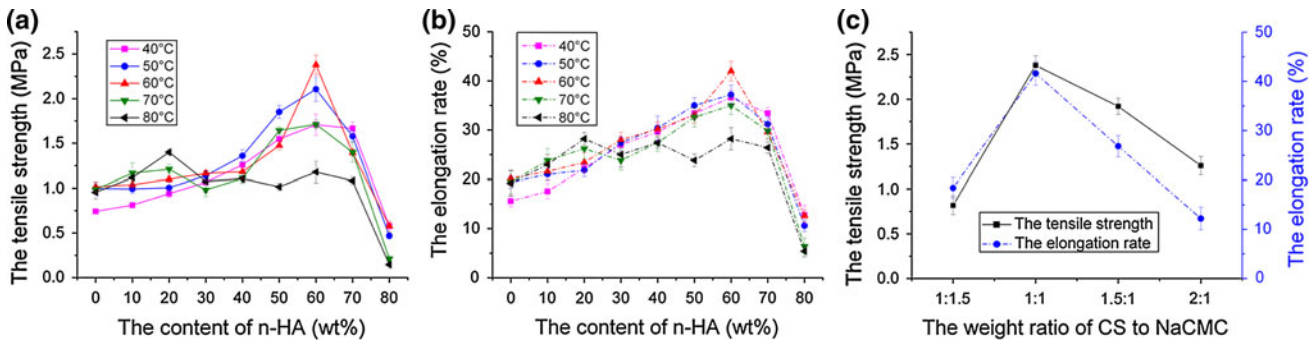

Fig. 6 The tensile strength and elongation rate of GEA membranes. **a**, **b** Represent membranes (CS:NaCMC = 1:1) with different n-HA contents and different drying temperatures, **c** denotes membranes (60 wt% n-HA and $T_d = 60°C$) with different weight ratios of CS to NaCMC

different n-HA contents (0–80 wt%) and drying temperature ($T_d = 40$, 50, 60, 70 and 80°C). All these membranes are in an equivalent amount of CS to NaCMC. It can be seen that when the drying temperature is between 40 and 70°C, the tensile strength and elongation rate exhibit an increase trend with the n-HA content up to 60 wt%, different from the fluctuation when the drying temperature is 80°C. In spite of the drying temperature, the tensile strength and elongation rate decrease sharply when the n-HA content further increases from 60 to 80 wt%. It indicates that an appropriate amount of n-HA can support the network of the composite membrane and promote the stability of the membrane structure. However, excessive n-HA content and drying temperature will also destroy the composite network. Among all the conditions, the composite membrane with a content of 60 wt% n-HA and dried

at 60°C holds the highest tensile strength of 2.38 ± 0.11 MPa and the elongation rate of 42.0 ± 2.0%.

Besides, when the content of n-HA is settled at 60 wt% and the drying temperature is 60°C, the tensile strength and elongation rate of membranes with different weight ratios of CS to NaCMC (CS:NaCMC = 1:1.5, 1:1, 1.5:1, 2:1) are shown in Fig. 6c. The membrane with a CS:NaCMC ratio of 1:2 is too fragile to get a test value. In Fig. 6c, the membrane with an equivalent amount of CS and NaCMC holds the highest tensile strength and elongation rate. The membrane with CS:NaCMC = 1:1.5 has the lowest tensile strength and the membrane with CS:NaCMC = 2:1 gives the lowest elongation rate. The result indicates that interactions, such as electrostatic interaction and hydrogen bonding, are present among CS, NaCMC and n-HA. It is the synergistic action of the three components that makes the excellent mechanical properties of the composite membrane. As a result, the optimum preparation condition for the PEC membrane can be fixed to 60 wt% n-HA content, an equivalent amount of CS to NaCMC and a drying temperature of 60°C.

As can be seen in Fig. 4c, the membrane prepared by layered casting is in a layered structure. It processes the mechanical property of both single CS membrane and n-HA/NaCMC membrane. Differently, although the GEA forms a homogenous three-component membrane, the polymer network continuity of the membrane is not as good as the single CS membrane, this may be one reason why the mechanical properties of the membrane prepared by GEA method are quite different from and lower than the membrane prepared by layered casting.

3.4 FT-IR analysis

Figure 7 shows the IR spectra of CS, NaCMC, n-HA and composites with different n-HA contents. The peaks at 1646 and 1324 cm^{-1} in the CS spectrum of Fig. 7e are characteristic peaks of amide I and III groups, respectively [30, 31]. The characteristic peak at 1599 cm^{-1} is for amino groups and the absorption bands at 1106 and 1031 cm^{-1} belong to the polysaccharide skeleton [32]. The NaCMC spectrum in Fig. 7f presents two peaks at 1643 and 1429 cm^{-1} that are assigned to the asymmetric and symmetric carboxylate vibration [31]. Comparing the spectrum of CS/NaCMC composite (Fig. 7a) with the spectra of CS and NaCMC, the characteristic peak of $–COO^-$ group at 1643 cm^{-1} shifts to lower wave number, and its intensity decreases a lot. It suggests that an inter polymer complex has formed between the two polysaccharides. For CS/NaCMC/n-HA composite (Fig. 7b–d), the peak around 1643 cm^{-1} further shifts to lower wave number with the increase of n-HA. This infers the occurrence of interaction among the three components. The hydroxyl groups of n-HA may form hydrogen bonding among the polysaccharide chains, which has effect on the electrostatic interaction between CS and NaCMC. In the spectrum of n-HA in Fig. 7g, the peaks at 1095, 1034 and 566 cm^{-1} belong to PO_4^{3-} groups, and the absorption peaks at 3568 and 632 cm^{-1} are attributed to the bending vibration of hydroxyl groups [33]. The characteristic peaks of n-HA are also observed in the spectra of CS/NaCMC/n-HA composites and the peak intensity increases with the n-HA content.

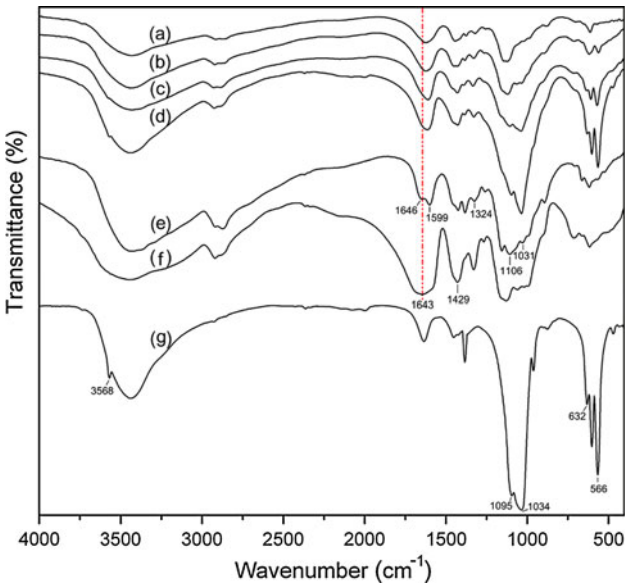

Fig. 7 FTIR spectra of GEA membranes with n-HA content of 0 wt% (*a*), 20 wt% (*b*), 40 wt% (*c*), 60 wt% (*d*) and spectra of CS (*e*), NaCMC (*f*) and n-HA (*g*)

3.5 XRD analysis

Figure 8 gives the XRD patterns of CS, NaCMC, n-HA and CS/NaCMC/n-HA composites with different n-HA contents. Comparing with the peaks around 20° of NaCMC in Fig. 8a and CS in Fig. 8b, it should be noted that the pattern of CS/NaCMC composite in Fig. 8c exhibits two split characteristic peaks at 20.2 and 22.3°, and the characteristic peak of CS at 11.5° still appears in the composite with a little shift to lower degree. The peak intensity of CS and NaCMC also largely decreases in their composite. The result indicates the occurrence of strong interaction [34, 35], i.e. strong electrostatic interaction between CS and NaCMC in the membrane. When n-HA is added in the CS/NaCMC system, the two split peaks are nearly absent as shown in Fig. 8d–f. This means the n-HA crystals can further interact with CS and NaCMC in the composite membrane, in a way of forming hydrogen bonding with the polyelectrolyte chains as shown in Fig. 3b. The characteristic peaks of n-HA in Fig. 8g are present in

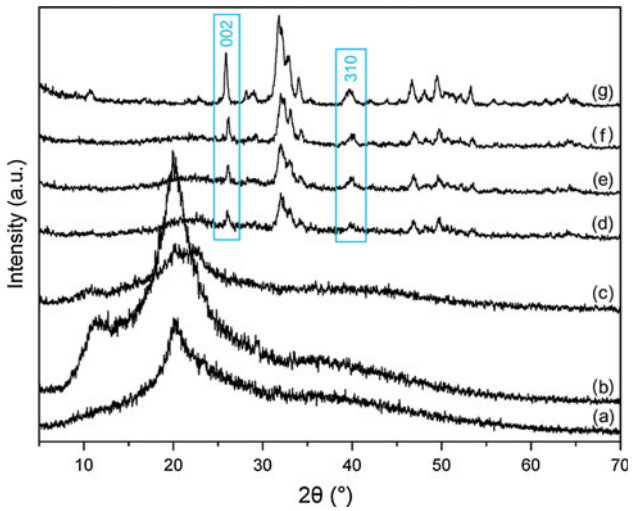

Fig. 8 XRD patterns of NaCMC (*a*), CS (*b*), GEA membranes with n-HA content of 0 wt% (*c*), 20 wt% (*d*), 40 wt% (*e*), 60 wt% (*f*) and pattern of n-HA (*g*)

CS/NaCMC/n-HA composites, except the intensity of these peaks decreases with the reduction of n-HA content.

The D_{hkl} values relating to the crystal size of n-HA in the long dimension (002) and the cross-section (310) were calculated using Scherer formula [36]: $D = K\lambda/(\beta_{1/2}\cos\theta)$, where K is a constant depending on crystal habit (here chosen as 0.89), λ is the wavelength of X-ray radiation (1.54184 Å), $\beta_{1/2}$ is the half width of the diffraction peak (rad) and θ (degree) is Bragg angle. The results show that the original crystal size of n-HA is 24.37 nm in (002) direction and 8.85 nm in (310) direction, and the crystal size of n-HA in the CS/NaCMC/n-HA composite (60 wt% n-HA) is 25.47 nm in (002) direction and 9.12 nm in (310) direction, keeping a similar level of crystal size. This means the polysaccharide chains or the polyelectrolyte chains have little influence on the structure of n-HA crystals.

4 Conclusion

A homogenous CS/NaCMC/n-HA PEC membrane was successfully prepared by a GEA method. The new composite membrane preparation method—GEA presented by this study is a great improvement on the preparation of such GBR membrane. The procedure includes dispersion, dissolution, electrostatic interaction and evaporation dehydration. Compared with solution blending and layered casting methods, the GEA method is outstanding for its excellent film forming ability, simple operation and good reproducibility. Furthermore, it does not introduce toxic cross-linking agent. With the addition of n-HA content in the membrane, the enhanced surface roughness and cavities are favorable for attachment, adhesion and growth of cells, and the n-HA crystals are helpful to the osteoconduction of new bone tissues. The prepared PEC membrane shows good mechanical property. Further investigation will be carried out on the in vitro cytocompatibility and in vivo membrane degradation and biological evaluation for guided bone regeneration.

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