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Study on poly(vinyl alcohol)/carboxymethyl-chitosan blend film as local drug delivery system

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Abstract The distinguishable films composed of poly(vinyl alcohol) (PVA) and carboxymethyl-chitosan (CMCS) were prepared by blending/casting method, and loaded with ornidazole (OD) as local drug delivery system. In vitro test, the blend films showed pH-responsive swelling behavior and moderate drug release action, and also exhibited a little antimicrobial activity against *E. coli* and *S. aureus* strains. Those characteristics of CMCS/PVA blend films were essentially governed by the weight ratio of CMCS and PVA. Increasing the content of PVA in blend film would decrease swelling and decelerated the drug release. However, increasing the content of CMCS would enhance the antimicrobial activity. The biocompatibility and bioactivity of the blend film were also evaluated using rabbit blood and Wister rats. This blend drug system was of no hemolysis, no toxicity to rat periodontia and no cytotoxicity to the rat muscle. After subcutaneously implanting the blend drug films in Wister rat, the systems kept a good retention at the application site and maintained high drug concentration in long time (5 days) which was longer than the period of drug released in vitro (160 min).

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Introduction

Local drug delivery system for treatment of some diseases received considerable attention during the past several decades due to its significant merits. It could achieve predictable and reproducible release of agents into a specific environment over an extended period, and also created a desired environment with optimal response, minimum side-effects and prolonged efficacy [1, 2]. An ideal formulation should exhibit ease of delivery, a good retention at the application site and a controlled release of agents. Cyril et al. [3] developed a high crosslinked amylose starch matrix as a sustained antimicrobial delivery system for local prevention or treatment of osteomyelitis. Marie et al. [4] used nonionic cellulose ethers as potential carrier systems for the delivery of local anesthetic agents to the periodontal pocket. Eve et al. [5] prepared a new type of chitosan (CS) thermosensitive hydrogel for the sustained release of paclitaxel at tumor resection sites in order to prevent local tumor recurrence. Other synthetic and natural materials were also made into various formulations. In all formulations prepared with biomaterials used in this field, micro-particles [6], bioadhesive gels [7] and films [8] were widely applied and deeply investigated.

CS got much application in medical field [9–11] due to its favorable properties such as biocompatibility, biodegradability, easily forming gels and films. Carboxymethyl-chitosan (CMCS) derived the reaction of chloroactic acid and CS in alkaline condition [12]. Compared to CS, some characteristics of CMCS were more excellent due to assimilating the carboxyl group. It could easily dissolve in neutral water solution. The

biocompatibility of CMCS was improved and the antimicrobial activity was also strengthened [13, 14].

Poly(vinyl alcohol) (PVA) films was known to possess high tensile and impact strength, high tensile modulus, and excellent resistance to alkali, oils and solvents [15]. It was also a biological friendly polymer due to its biocompatibility and appropriate mechanical properties [16]. PVA blends could be cast as films and applied as biomedical materials such as dialysis membranes, wound dressing, artificial skin, cardiovascular devices and as vehicles to release active substances in a controlled manner. Cast films of PVA and PVA combined with natural polymers like collagen, hyaluronan, gelatin or deoxyribonucleic acid [17, 18] had already been studied for medical purposes. Moreover, PVA was used extensively for pharmaceutical purposes in tablets and hydrogels containing bioactive drugs in controlled release systems [19].

Based on above consideration, an attempt was tried to prepare an excellent local drug delivery system using CMCS and PVA polymers. In this study, CMCS/PVA composite films with varied weight ratio were prepared using blending/casting method. Furthermore, Ornidazole (OD) was selected as model drug because it was effective in treatment of susceptible protozoal infections and prophylaxis of anaerobic bacterial infections [20]. Some tests were carried out to detect the characteristics of PVA/CMCS/OD films as local drug delivery system.

Materials and methods

Materials and animals

CMCS (Mη: 199.6 kDa, substituent degree of carboxymethyled: 0.93) was made by our laboratory from reaction of CS and chloroactic acid. OD was supplied by Xi'an Bodyguard pharmaceutical Co, Ltd (China). PVA1750 was purchased from Sigma (USA).

Wistar rats used in this study were conducted according to NIH guidelines and with the approval of the University of Mississippi Medical Center's Animal Care Committee on the use and care of animals.

Preparation of films

The process of preparing the films and their corresponding drug films (loaded OD) via blending/casting was shown in Fig. 1. The PVA/CMCS blend films with varied weight ratio were: P1C2 ([PVA]/[CMCS] = 1/2), P1C1 ([PVA]/[CMCS] = 1/1) and P2C1 ([PVA]/[CMCS] = 2/1). The free PVA film and CMCS film were also prepared meantime. For the drug films, 10 μg/mm^2 of OD was loaded in.

Morphological study of the films

Transparence was checked by UV-vis spectrophotometer (Hewlett Packard 8453, Palo Alto, CA). The films were cut into quadrate pieces (1 × 3 cm^2) and adhibited on the euphotic surface of the colorimetric utensil. The transmittances of films were measured every 50 nm band from 300 nm to 800 nm.

Microstructure was investigated by using scanning electron microscopy (JSM-840, Japan). The films were gently rinsed with distilled water and then air-dried in an incubator at 50 °C for 24 h. Samples were gold-coated for conductance before scanning.

Hemolysis test of the films

Anticoagulate rabbit blood was prepared according to Moreau's method [21]. Film pieces were dipped into of physiological saline (10 mL) and preserved in attemperator at 37 °C for 30 min to prepare 1% tested

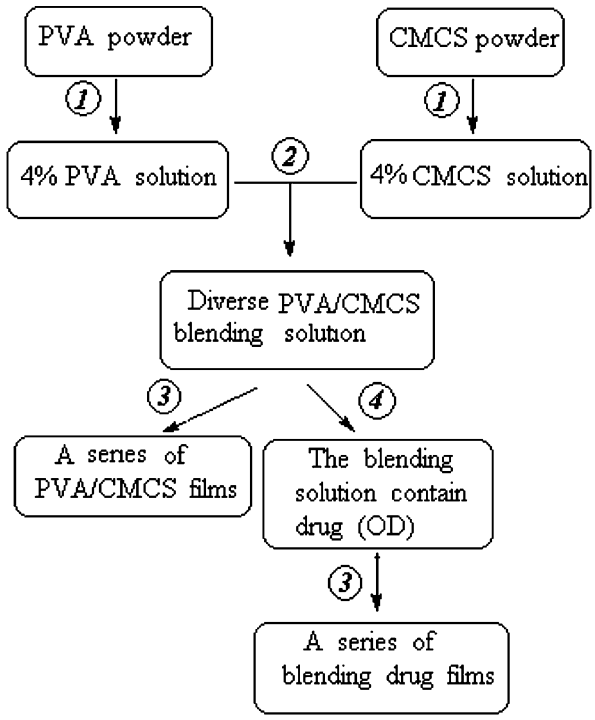

Fig. 1 Preparation of the PVA/CMCS blank films and drug films

suspensions, then 0.2 mL of anticoagulate rabbit blood was added in. After being maintained at 37 °C for 1 h, the suspensions were centrifuged at 1,000 rpm for 10 min and absorbance of supernatant was checked using spectrophotometer at 545 nm. For the positive control and negative control, rabbit blood was added into distilled water and physiological saline, respectively. The hemolysis index was calculated by formula (1)

$$HI = \frac{Dt - Dnc}{Dpc - Dnc} \times 100\% \quad (1)$$

where *HI* was the hemolysis index, *Dt* was the absorbance of tested suspensions, *Dnc* was the absorbance of negative control, *Dpc* was the absorbance of positive control.

Swelling behavior of the films

Dried films were initially cut into about 10 mm diameter disks and stained to facilitate visualization by immersion in 1 mg/mL of acridine orange solution for 60 s. Next, the films were washed and re-dried on a PTFE surface to give yellow films. The diameters of the dried film disks (*Di*) were measured using a vernier caliper. The dyed, dried disks were then placed in the interspace of two glass plate and added citrate buffer solution with pH values ranging from 2.4 to 8.0. Diameters of the swollen disks (*Ds*) were again measured at predetermined time intervals. The swelling degree (*SW*) was defined as the bulk of absorbed water per bulk of dried disk, and was calculated using the formula (2).

$$SW = \left(\frac{Ds}{Di}\right)^3 \quad (2)$$

In vitro drug release of drug films

Dried drug films were cut into 10 mm diameter disks. Each disk was placed in a conical flask, which contain 100 mL solution (pH 7.4 PBS or pH 3.5 citrate buffer). Then it was joggled at 37 °C in a constant-temperature shaker. At certain time, 1 mL of buffer solution was taken out and equivalent of blank buffer was complemented. The drug concentration released into the buffer was detected by UV as a function of time. The wave-lengths used for the detection of drugs were 318 nm. In this test, 90% drug released from the films was considered as drug releasing completely because of the weight loss of drug in the course of films prepared.

Antimicrobial test of PVA/CMCS blend system

Four concentrations of PVA/CMCS blend film extracts were prepared by dipping different weight films into 10 mL of distilled water at 70 °C for 48 h. The extracts were added to Muller Hinton broth (MHB) to prepare different concentrations of extracts culture media. The media contained extracts was autoclaved and poured plate for using. One loop bacteria strain (*E. coli* and *S. aureus*) was inoculated in MHB and subcultured for 12 h. Then the bacteria culture was stepwise diluted with autoclaved water until 0.1 mL such diluted bacterium culture contained about 100–300 cells. The diluted bacteria suspension (0.1 mL) was spreaded on the MHB agar plate (the test contained extracts but the control didn't contain) and then incubated at 37 °C for 24 h. The antimicrobial ability was expressed with the ratio of colony numbers in the test plate and in the control plate.

Toxicity to periodontium

Extracts were prepared by dipping 3 g PVA/CMCS/OD films in 10 mL of physiological saline at 70 °C for 48 h. Formaldehyde solution (0.1 g/mL) and sterilized physiological saline were also prepared. Twenty-four Wistar rats were averagely divided into three groups. The test group was daubed the extracts of drug film on rats' periodontia every twice a day up to fortnight. Simultaneously, the positive group was daubed formaldehyde solution and the negative group was daubed the physiological saline in the same way. Two weeks later, periodontia of rat were excised and fixed in formalin. Specimens were embedded in methylmetacryla sequentially. The plastic embedded specimens were cut into serial sections of 5–10 mm thickness, mounted on StarFrosts glass slides (Engelbrecht, Ederm.unde, Germany) and air dried [22]. Histological sections were stained with hematoxylin and eosin and evaluated with respect to cellular response. The specimens were viewed using an inverted phase contrast microscope (Axiovert 10, Opton, Germany) at 40× magnification. Images from the microscope were acquired using a camera (CCD color camera; Hitachi, Japan).

Subcutaneous implanting of drug film and in vivo drug release

Twenty-six wistar rats were randomly divided into two groups: test group 16 and control group 10. All rats were sheared a 4×4 cm^2 bare spot on its back after

being carried out anesthesia, then a pouch was made in the subcutaneous tissue. Sterilized blend drug films (4×8 mm^2) were implanted into the subcutaneous tissue of test group. For the control group rats, entwisted silk suture (10 cm) was implanted. Subsequently, the pouch and the skin incision were closed. After determined intervals of time, the rats were killed and the capsule tissues samples were excised. The implanted films were rinsed with PBS and then were freeze-dried prior to coating gold and observation using SEM. The excised surrounding tissues were fixed with formalin and prepared histological sections like Sect. 2.8. Those histological section samples were observed using inverted phase contrast microscope. The 10 test group rats and all the control group rats were used for this histological study.

Other 6 test group rats were used to study the drug release in vivo. At determinate time, the closed pouch was sliced off, and the tissue fluid around the embedded films was dipped with slender filter papers under the germfree condition. Weight of absorbed tissue fluid was got by checking the weight of the filter paper pre-dipped and post-dipped. The absorbed filter papers were then soaked in quantitative PBS at 37 °C for 20 h. OD concentration in the PBS was measured using UV-spectrophotometer at 318 nm posterior to centrifuging.

Results and discussion

The appearance of films

Thickness of all the films was 200 μm on average ($n = 6$). CMCS films exhibited yellow translucent appearance and PVA films were clear and colorless. But once PVA blend with CMCS, transmittance of films decreased. This was shown in Fig. 2.

SEM images were obtained to characterize the surface of the blend films which was shown in Fig. 3. The condensed CMCS film (Fig. 3a) and PVA film (Fig. 3b) had flat and featureless surfaces images. Heterogeneous surface was found at PVA/CMCS blend film (Fig. 3c). It might be caused by the microphase separation of PVA molecules or CMCS molecules. CMCS microdomains dispersed within PVA matrix, which was similar to the configuration of cellulose/CMCS blend films [14]. It meant that mechanical blending still reserved properties of the both components. The surface image of blend films loaded OD was showed in Fig. 3d. The crystalloid drug powder was homogeneously dispersed in the blend films.

The hemolysis test of films

Table 1 showed the result of hemolysis test. Hemolysis index of films ranged from 0.836 to 4.626. According to the criterion of ISO 10993 [23], the upper limited value of hemolysis index was 5. Thus all blank films and drug films had no hemolysis.

Swelling behavior of films

Figure 4 showed the swelling behavior of films investigated as a function of time at pH 7.4 media. All the films swelled rapidly and reached equilibrium within 30 min CMCS film swelled fastest, it even began to dissolve into fragments when retaining in the buffer only 10 min. Moreover, the equilibrium swelling degree of the films decreased with the increase of PVA content in the films. The order of swelling capability was: CMCS > P1C2 >P1C1 > P2C1 > PVA.

To characterize of the blend films response to the change of pH, films were equilibrated in aqueous media (pH value range from 2.4 to 8.0) for 30 min. Results were shown in Fig. 5. The blend films exhibited pH-responsive swelling behavior like CMCS film. Low swelling degree was found at acidic condition, but high swelling degree was found at high pH conditions. This could be attributed to the electrostatic attraction or repulsion between ion groups of the CMCS in different pH environments [24]. Molecules' shrinking or loosing resulted that the swelling degree of films diversified. This pH-responsive swelling behavior was demonstrated in Fig. 6. Minimum swelling degree was found at pH 4.8 because it was the isoelectric point (IEP) of CMCS. The number of $-NH_3^+$ was equal to that of $-COO^-$, and thus the swelling degree was lowest. However, increasing PVA content decreased the

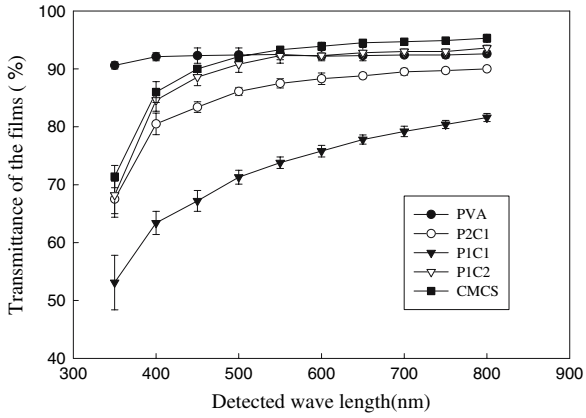

Fig. 2 Diaphaneity measuring of single CMCS, PVA film and blend films

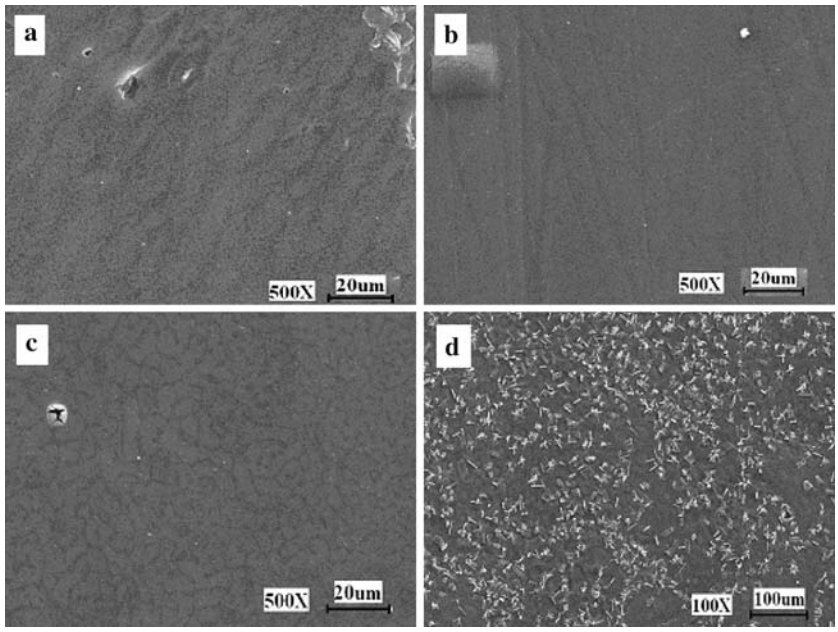

Fig. 3 SEM pictures of the films: the CMCS film (**a**), the PVA film (**b**), the PVA/CMCS blank film (**c**) and drug film (**d**)

pH-responsive swelling capability. Especially for PVA film, its swelling degree (around 2.25) was hardly affected by the pH value of solution.

In vitro drug release

The release profiles of OD from films in PBS (pH 7.4) were depicted in Fig. 7. The results indicated that almost 100 wt.% of OD from CMCS films was released within 60 min, but only 30% of OD from PVA drug film in the same interval. For the blend films, the drug release rate was moderate (faster than the release rate of PVA film but slower than that of CMCS film). These were caused by the bursting effect of the swelling molecule of CMCS and obstruct effect of PAV networks. Swelling exposed the drug particles to the flow of dissolution medium and hastened the dissolution of the drug, but PVA networks caused the matrix configuration relatively compact at buffer media. The order of drug release velocity of the various films was CMCS > P1C2 > P1C1 > P2C1 > PVA which accorded with the result of swelling kinetics. Time of drug released completely from five formulations was compared in Fig. 8. Drug released completely from PAV film need about 350 min which was much longer than that of CMCS film (20 min). In addition, time of drug released completely from the three blend drug films was intervenient, P2C1(220 min) > P1C1(160 - min) > P1C2 (80 min).

Result of release profiles of OD from P1C1 film in different pH solutions (PBS and citrate buffer) was shown in Fig. 9. The release rate of OD from blend system at pH 7.4 was faster than that at pH 3.5. This phenomenon would be attributed to the pH-responsive swelling effect of blend films. Although Zhao et al. [25] speculated that there were large extents of hydrogen bond between PVA and CMCS in the interpolymer complexes, it was still supposed that compact polyion complex between ammonium ion and carboxylate ion in CMCS instead of hydrogen bonding was formed at pH 3.5 and resulted a slow drug release rate comparing with the drug release rate at pH 7.4.

Antimicrobial activity of the blend systems

Figure 10 showed that extracts of PVA/CMCS blend film inhibited the growth of both *E. coli* and *S. aureus* strain. The inhabitation increased with the increase of

Table 1 The hemolysis of films

The sort of films	Hemolysis index (%)				
	PVA	P2C1	P1C1	P1C2	CMCS
Blank films	2.351 ± 0.323	2.687 ± 0.621	0.917 ± 0.133	0.836 ± 0.213	2.351 ± 0.166
Drug films	4.626 ± 0.521	3.456 ± 0.131	3.654 ± 0.211	2.461 ± 0.197	1.565 ± 0.243

Results were shown on mean ± SD ($n = 6$)

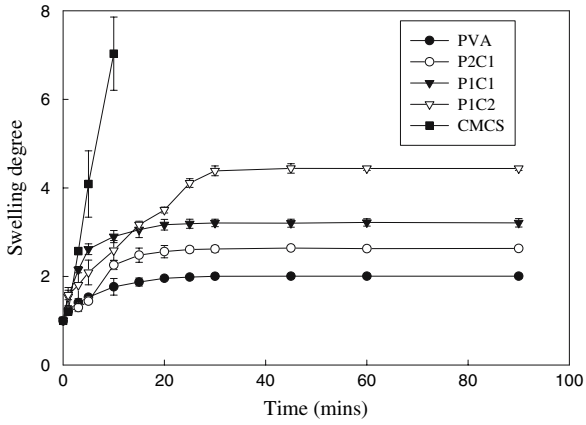

Fig. 4 Swelling kinetics of the films in PBS (pH 7.4)

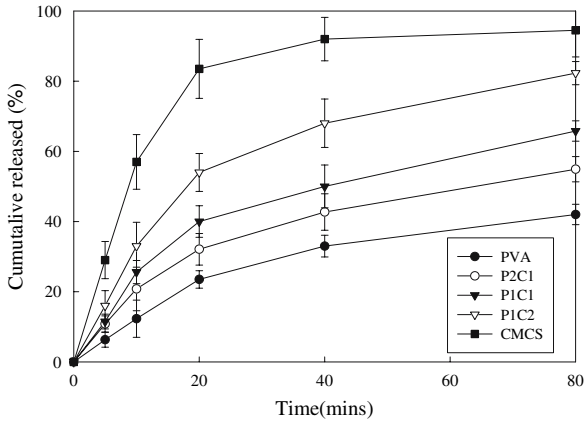

Fig. 7 The release curves of OD from the films in PBS (pH 7.4)

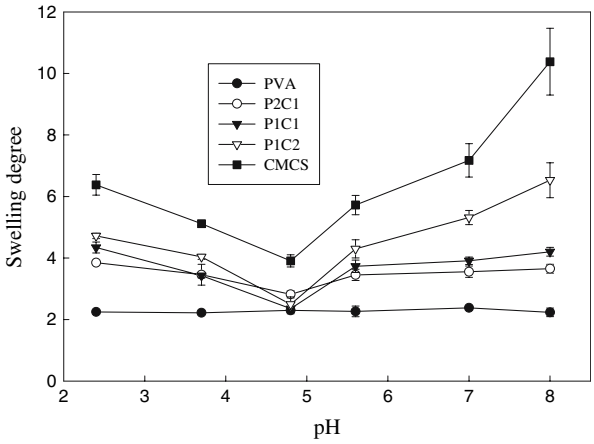

Fig. 5 Swelling degree change of films in different pH buffer solutions

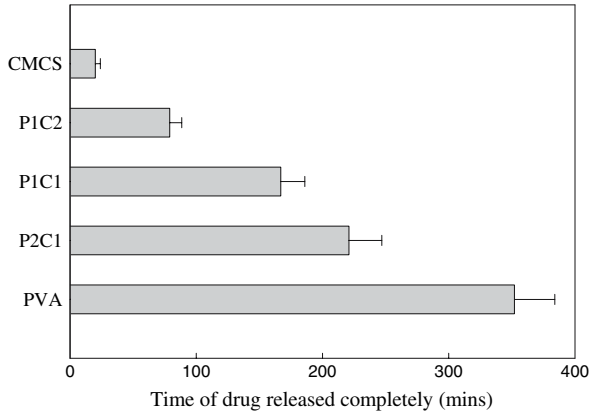

Fig. 8 Time of drug released completely from the five formulations

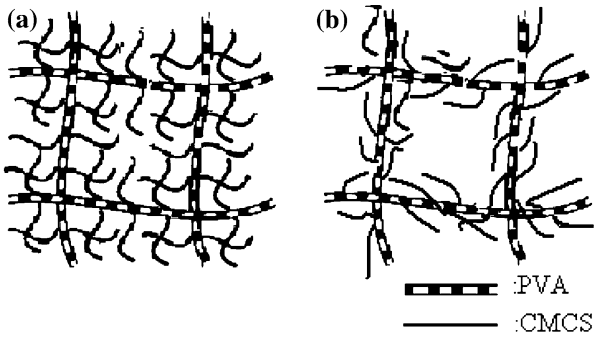

Fig. 6 pH-responsive swelling mechanism of PVA/CMCS blend film: (**a**) state in low pH condition and (**b**) state in high pH condition

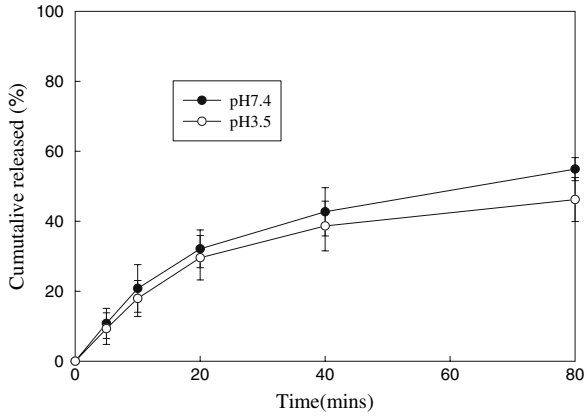

Fig. 9 The released curves of OD from the P1C1 drug film in different pH buffer solutions

CMCS' content in culture media. For *S. aureus*, the increasing inhibited trend was not notable at high concentration of CMCS. But for *E. coli*, the inhibited effects at high concentration of CMCS (>1.0%) was much stronger than that at low concentration. Other papers [26] also reported that CMCS had strong resistance to bacteria. So it was believed that bacteria wouldn't thrive in this system and it was unnecessary to

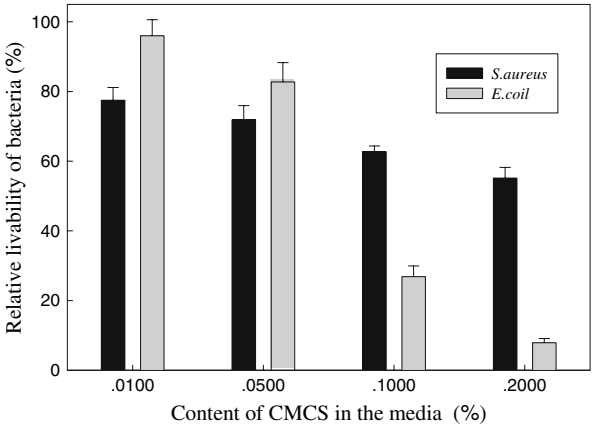

Fig. 10 The antimicrobial performance of the extracts of PVA/CMCS blend film

incorporate other antibacterial agents [27] in this blend system used for medical device.

Toxicity test to periodontium

One important intention for designing PVA/CMCS blend films was treating periodontitis which was a frequent and potentially severe complication [28]. Film loaded drugs was an excellent candidate for the treatment of oral periodontitis. This formulation embedded in the periodontal pocket could offer the palliative effects and potentially delivery drug [8]. PVA/CMCS/OD system could be potentially used for treating periodontitis if it was improved no toxicity to periodontium. We examined the toxicity of extracts of this system to the periodontium of Wister rats. After persistent administering dosage to fortnight, all the test and negative control periodontia were in good conditions. No hyperemia, no turgidity, no debaucjed and no ulceration changes were found on the periodontia. But the positive control periodontia became turgescent and cankered after only being administered 2 days and these symptoms became more severely with the administered time delayed. Figure 11 showed the result of the toxicity test observed using an inverted phase contrast microscope. The periodontia operated with the physiological saline (Fig. 11a) and extracts of PVA/CMCS drug film (Fig. 11c) was in natural and didn't display any cellular inflammatory responses. But the periodontium responses to formaldehyde solution (Fig. 11b) was acute. Epithelium exhibited abnormal hyperplasia, dermis was rich in polynuclear and macrophage inflammatory cells, and the infiltration of polynuclear cells and macrophages was severe.

Tissue-implanted reaction associated with drug release in vivo

The PVA/CMCS/OD film and entwisted silk suture were subcutaneously implanted in rats to analyze the inflammatory tissue response from 1 to 4 weeks by evaluating the cellular response and capsule thickness. All the implants were encapsulated by fibrous connective tissue after implanting only 1 week. The tissue around the implants exhibited hyperemia and tumefaction, especially for control wounds. Cutaneous ulcers were observed in both treated (2/10) and control

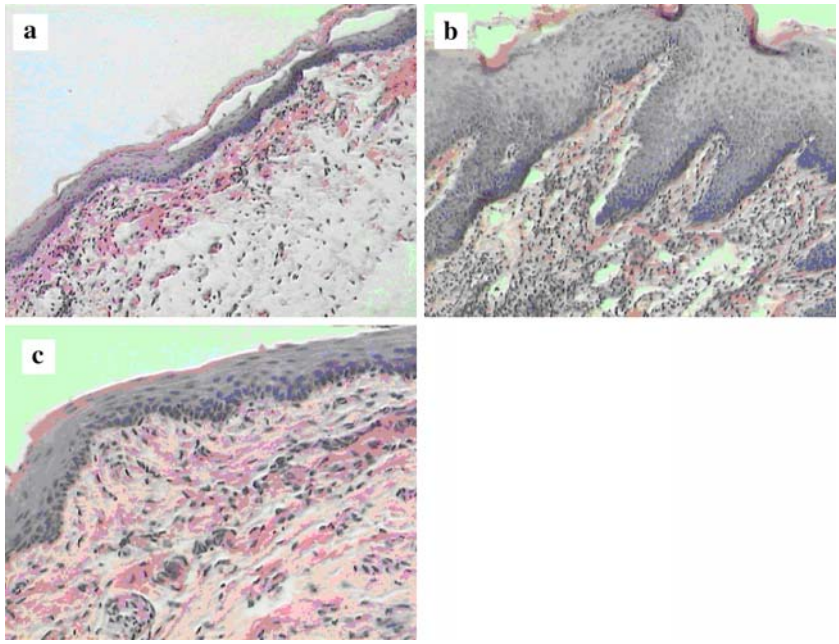

Fig. 11 The photomicrographs of rat periodontia dealt with the extracts of PVA/CMCS blend drug film (**a**), 0.1 g/mL formaldehyde solution (**b**) and sterilized 0.9% NaCl solution (**c**)

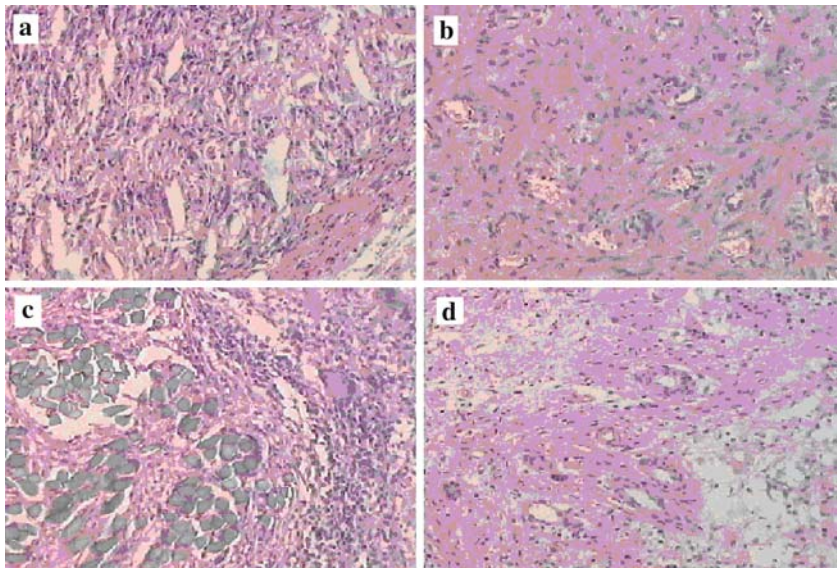

Fig. 12 The photomicrographs of tissue response to PVA/CMCS drug films (**a**, **b**) and entwisted silk suture (**c**, **d**) implants in the subcutaneous tissue after 1 and 4 weeks, respectively

groups (3/10). Granulation tissue areas were evident in all samples. At 2 weeks, re-epithelialization was complete in all cases, fibrosis was reported in 19/20 cases. The thickness of the fibrous capsule around the PVA/CMCS drug film implants attenuated significantly after 2 weeks. Figure 12 showed tissue response to PVA/CMCS blend drug films and entwisted silk suture implants in the subcutaneous tissue. The implants were encapsulated by fibrous connective tissue consisting of fibroblasts, inflammatory cells and collagen fibers. But the number of granulocytes and lymphocytes decreased with the time prolonged. Meanwhile, the number of macrophages and giant cells was also decreased. Granulocytes and lymphocytes were hardly found in any investigated samples after 4 weeks, but fibroblasts were equal in all groups regardless of treatment and time point.

The blend film would be eroded and degraded during subcutaneous implantation. Figure 13 showed the change of the PVA/CMCS drug film implanted only 1 week. Plentiful cells and tissue granula were adhesived on the specimen excised (Fig. 13a). Most of granula might be fibroblasts, inflammatory cells and collagen fibers. But it was very difficult to find macrophages and giant cells which always concerned with severe immune reaction. Alveolate pore microstructure (Fig. 13b) of PVA/CMCS drug films was exhibited after wiping off the tissue granula. This might be resulted from diffluence of CMCS components and release of drug crystal particles.

The pouch and capsule inwraped film could provide fitting environments for studying drug release in vivo. Drug released in local or site specific of individual could effectively cure some diseases, such as periodontitis, tumors, colonitis [1–3]. Investigating the drug release of PVA/CMCS/OD system in local region could provide essential indexes. So the drug release in the incision pouch of rats was also measured associating with the subcutaneous implantation. Results were shown in Table 2. The concentration of OD in the tissue fluid around the implanted films reached max (303.13 μg/mL) at the first day, then it was decreased to

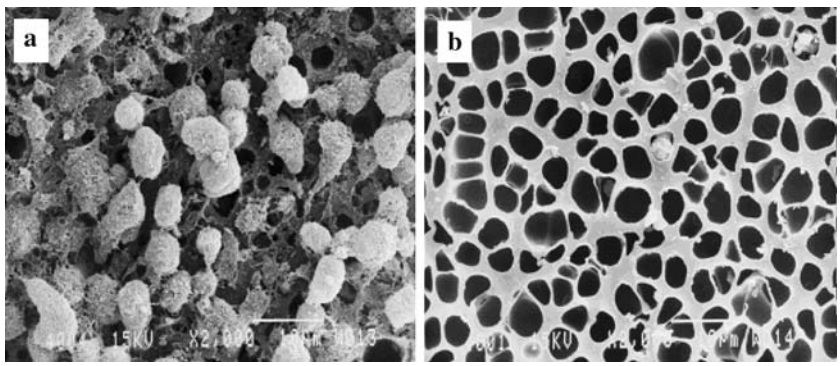

Fig. 13 SEM pictures of the change of PVA/CMCS blend drug film pre-wiping off the tissue (**a**) and pro-wiping off the tissue (**b**)

Table 2 OD release from the PVA/CMCS blend drug as subcutaneous implantation in rats

The tested animals	Drug concentration at determined time (μg/mL)		
	1 day	2 days	5 days
1	278.24	120.06	50.92
2	318.74	167.1	59.95
3	326.74	183.07	–
4	286.73	108.11	42.76
5	320.12	167.14	60.81
6	288.21	112.73	33.03
Mean value	303.13	143.04	49.49

143.04 μg/mL at the following day, but it still reserved about 49.49 μg/mL of OD after implanting 5 days. This was entirely different with the release profile of PVA/CMCS drug film in vitro. We speculated that it was correlated with the drug diffusing through the capsule and substance conveying of blood circulation in the local region.

Conclusion

It concluded from the above study that some characteristics of PVA/CMCS blend films could be controlled by varying the content of PVA or CMCS. Low swelling degree and slow drug release rate enhanced by increasing the PVA component in the system. But excellent biological properties (such as antibacterial activity and biocompatibility) could be achieved by increasing the content of CMCS.

Results of the in vitro and in vivo studies showed that CMCS/PVA blend drug film was an excellent candidate for local drug delivery system. It had neither hemolysis to rabbit blood nor toxicity to rat periodontium. It could offer some antimicrobial activity against infection and had potential to delivery therapeutic compounds. The test of subcutaneous implantation also indicated that the insertion of PVA/CMCS drug film in the surgical wounds did not promote any adverse effect. Over a long period of time, it is expected that the PVA/CMCS drug film would be absorbed and the wound would be cicatrized by new forming tissues eventually.

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