To Control Floating Drug Delivery System in a Simulated Gastric Environment by Adjusting the Shell Layer Formulation

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Research article

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Posted Date: April 6th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-363794/v1

To Control Floating Drug Delivery System in a Simulated Gastric Environment by Adjusting the Shell Layer Formulation

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Abstract

Background:

Gastroretentive drug delivery system (GDDS) are novel systems that have been recently developed for treating stomach diseases. The key function of all GDDS systems is to control the retention time in the stomach. However, research into the bulk density or entanglement of polymers, especially regarding their effects on drug float and release times, is scarce.

Methods:

In this research, we prepared the floating core-shell beads carrying tetracycline. The ratio of chitosan and xanthan gum in the shell layer was changed to modify polymer compactness. Tetracycline was encapsulated in the alginate core.

Results:

Using scanning electron microscopy (SEM) techniques, we observed that the shell formulation did not change the bead morphology. The cross-sectional images showed that the beads were highly porous. The interaction between anionic xanthan gum and cationic chitosan made the shell layer dense, resisting to the mass transfer in the shell layer. Due to the high mass transfer resistance to water penetration, the longer float and delivery time were caused by the dense surface of the beads. The cell culture demonstrated that floating core-shell beads were biocompatible. Importantly, the beads with tetracycline showed a significant prolonged anti-bacterial effect.

Conclusion:

Research results proved that the floating and releasing progress of core-shell beads can be well controlled by adjusting the shell layer formulation that could promote the function of gastroretentive drugs.

Keywords: gastroretentive drug delivery, core-shell particles, floating beads, chitosan, xanthan gum, anti-bacterial effect

Introduction

Oral administration is the most common drug delivery method as it is multifunctional and convenient [1]. The size of drug carriers is usually adjusted to 1-2 mm, allowing the medicine in the stomach to pass through the pylorus and enter into the small intestine [2]. Advances in pharmaceutical techniques have enabled to release drugs in a specific position *in vivo* to lower toxicity, decrease side effects, and promote efficiency. Thus, the gastroretentive drug delivery system (GDDS) has been developed for treating stomach cancer, ulcer and infection. GDDS could keep drugs in stomach for a prolonged period to achieve a specific release, including several types: floating, mucoadhesive, expandable and rafting forming drug delivery systems.

The floating drug delivery system (FDDS) is also called a hydrodynamically balanced system (HBS). In FDDS, the drugs carriers float in gastric juice to ensure that drugs do not leave stomach shortly. It efficiently increases drug bioavailability by prolonging the release period. The variation of drug concentrations in blood is also decreased [3]. FDDS is a potential treatment for stomach and duodenum cancers. The density of FDDS carriers must be lower than those of the gastric juice and chymus. Therefore, medicines float and are slowly released in the stomach, compared with the convectional drug delivery methods.

Currently, most FDDS would not float for 2-8 h; however, the drug float and release periods need to be prolonged. To obtain improved drug float and release times, previous research studies have widely investigated control of the drug carrier materials. Kawashima [4, 5], Sato [6], Thanoo [7] et al. prepared hollow spheres for FDDS by using the emulsion-solvent diffusion method. These studies focused on controlling the carrier density by solvent diffusion. In particular, polymer porosity dominates the solvent diffusion rates that determining the carrier floating behaviors. Xiaoqiang, Choi

and El-Kamel et al. added gas-forming/generating agents in polymers to increase the porosity, and therefore, the floating properties [8-10]. Indeed, the porosity was influenced by the amount of gas-generating agents. According to prior researches, the gas generating agents and solvent diffusion would significantly affect the porosity in FDDS. In contrast, research into the bulk density or entanglement of polymers, especially regarding their effects on drug float and release times, is scarce.

In this research, we prepared the core-shell floating particles for GDDS. We used two polymers, chitosan and xanthan gum, as the shell layer, which are cationic and anionic, respectively. Adjusting the chitosan/xanthan gum ratio enabled us to adjust the polymeric entanglement, and allowed us to control the shell layer properties and structures in floating beads. Then, we studied how the shell layer affects the float, releae and biocompatibility properties.

Materials and Methods

Materials

Alginic acid sodium salt (medium viscosity), xanthan gum (from Xanthomonas campestris), $NaHCO_3$ (sodium bicarbonate powder), chitosan (low molecular weight), tetracycline (>98.0%) were purchased from Sigma-Aldrich (St. Louis, MO). $CaCl_2$ (calcium chloride) and HCl and acetic acid were purchased from J.T. Baker, Japan. Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS), penicillin, trypsin was purchased from Gibco Life Technologies (Thermo Fisher Scientific - TW). Kaighn's modification of Ham's F-12 medium was purchased from Manassas, VA, USA. MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) kit was purchased from Carlsbad, CA, USA. Distilled deionized (DI) water was used throughout the experiment.

Preparation of alginate floating beads containing tetracycline

Two solutions were prepared respectively for core-shell beads. One was the alginate solution for core, and the other was the chitosan/xanthan-gum solution for shell. 50 mg tetracycline and 200 mg alginate were mixed in 10 ml DI water, and stirred for 2 hours to obtain the tetracycline-alginate solution. 200mg $NaHCO_3$ was then added into alginate solution, and stirred at 500 rpm for 1 hour. On the other hand, chitosan solution was prepared by dissolving chitosan powders in 1 v/v% acetic acid aqueous solution, and xanthan gum solution was prepared by dissolving xanthan gum in DI water. After that, chitosan and xanthan gum solutions were mixed with different chitosan/xanthan-gum ratios of 4:1, 4:2, 4:3, 4:4 and 1:4, where $CaCl_2$ was also added as the concentration of 1 w/v%. The tetracycline-alginate solution was extruded through a 26-gauge needle into the continuously stirred chitosan/xanthan gum solution, and the solution with floating beads was continuously stirred for 15 minutes. After the beads were collected and washed with DI water, they were vacuum dried for 48 hours, and then stored in 4°C until used. The exact formulations of core and shell layer were described in Table 1.

FE-SEM (field emission scanning electron microscope) analysis

SEM (JEOL JSM-6390LV, Japan) images were taken to analyze the morphology of floating beads. The beads were dried at 4°C. After that, the bead samples were spread and fixed on the metal plate with double-sided carbon tape, and a gold layer was then coated on the sample surface under vacuum using an auto-sputter coater for 1minute under argon atmosphere. The beads morphologies were then observed with SEM. With SEM images, the bead diameters were measured by Image J software.

Swelling and floating test

The swelling percentage of beads was determined by measuring the extent of swelling of the polymer matrix in pH2 aqueous solution. The weight of dried beads was recorded. After the immersion in pH2 buffer for 1, 2, 4, 6, 8, 12 and 24 hours, water on bead surfaces was removed by filter paper and the weight beads were measured again. The swelling percentage was calculated by using the following equation.

$$\text{Swelling index } (\%) = \frac{\text{Weight of beads after swelling} - \text{Dry weight of beads}}{\text{Dry weight of beads}} \times 100\%$$

For the floating analysis, twenty dried beads were kept in pH2 buffer for 24 hours with continuous shaking. At 1st, 2nd, 4th, 6th, 8th, 12th and 24th hour, the floating percentage was calculated according to following equation:

$$\text{Floating percentage } (\%) = \frac{N_f}{N_f + N_s} \times 100\%$$

N_f : number of floating beads; Ns: number of settled beads

In vitro release and encapsulation efficiency of tetracycline

The dry beads were immersed in pH2 buffer in the dynamic gastric simulator [11]. After 2, 4, 6, 8, 12 and 24 hours, 10ml solution was taken out and analyzed by UV/VIS spectrophotometer at 266nm, followed by refilling fresh 10ml buffer.

In the analysis of encapsulation efficiency, beads were suspended in pH2 buffer with continuous shaking at 37 °C for 24 hours, where a part of encapsulated tetracycline was released. Then, the beads were completely broken by using ultrasonic shaker for 30 minutes, and the residual tetracycline would be completely released. After the solution was filtrated, UV/VIS spectrophotometer was applied to quantify tetracycline

in buffer by deducting the absorbance at 266 nm, allowing the determination of encapsulation efficiency as following equation.

$$\text{Encapsulation Efficiency (EE\%)} = \frac{\text{Actual loaded drug}}{\text{Amount of drug added}} \times 100\%$$

Cytotoxicity test

MTT assay was applied to evaluate the cytotoxicity of core-shell beads. In this experiment, a non-small lung cancer cell line (A549) was seeded on a 24-well plate with the density of 5×10^3 cells per well. As the cell monolayer were cultured to confluence, they were exposed to fluid extracts. The extracts were obtained by placing the core-shell beads in culture medium (0.2 g core-shell particles in 1 ml medium) for 24 hours at 37°C. Each fluid extract obtained was then applied to A549 monolayer, replacing the medium that had nourished the cells. The cells were then cultured with extracts for 1 day. After the cell culture, the metabolic activity of A549 was determined by MTT assay. The cells were incubated with 1 mg/mL of MTT for 4 hours. Then, the MTT was removed and the formazan crystals were dissolved with dimethyl sulfoxide for 30 min. Finally, absorbance values were read at 570 nm by using an automatic microplate reader (ELx800; Bio-Tek Instruments, Winooski, VT, USA).

Antibacterial testing

LB medium (from Creative Life Science Co., Ltd.) was applied for the culture of E. coli which was from Bioresources Conservation and Research Center (BCRC), Taiwan. The medium with cultured bacterial was added onto agar plate evenly,

followed by an overnight culture. After that, core-shell beads were added onto the agar plate, and the antibacterial effects were observed at various time points.

Results

Table 1 demonstrates the floating bead formulations. In this research, the mass ratio of chitosan to xanthan gum in the shell layer was adjusted to 4:1, 4:2, 4:3, 4:4 and 1:4, where the amounts of salts ($CaCl_2$ and $NaHCO_3$) and alginate in core were fixed.

The morphologies of core-shell particles were analyzed by using SEM as shown in Fig. 1(a). The beads were roughly spherical, with a diameter ranging from 1.5 to 2 mm. The particle diameters were analyzed using Image J and presented in Fig. 1(b). The formulation of the shell layer had a weak impact on the particle morphologies and diameters. The cross-sectional images of core-shell beads are presented in Fig. 2. Results show that the particles prepared in this research were highly porous.

Fig. 3 revealed the swelling ratios of core-shell particles with different immersion periods at pH 2. As shown in Fig. 3, all kinds of particles were gradually swelled during first 6 h and reached a steady state over the next 2 h. The highest swelling ratios at steady state were 150%-200%. Fig. 3 also shows that the particles are swelled faster and steady-state swelling ratios increase when the amounts of chitosan are much higher or much lower than the amounts of xanthan gum, such as chitosan:xanthan gum = 4:1 and 1:4. In contrast, the swelling ratios were relatively low with chitosan:xanthan gum = 4:3 and 4:4.

Fig. 4 presents the floating percentages of core-shell beads in the aqueous solution with pH 2 after immersion for different periods. Fig. 4 (a) shows that the floating percentage of chitosan beads decreased to 55% after 24 h when there is no core-shell

structure. Results in Fig. 4 (b) show that about 90% and 88% of core-shell particles would still keep their floating conditions after 8 and 24 h, respectively.

The encapsulation efficiency and releasing rate of tetracycline of floating beads are described in Figs. 5. There is no significant difference in the encapsulation caused by the ratios of chitosan and xanthan gum, as revealed in Fig. 5 (a). The release profile in Fig. 5 (b) has been evaluated in dynamic conditions.

The results in Fig. 6 identified the good biocompatibility of floating beads when the bead concentration was as high as 1.5 mg/ml, which contained 149.03 μg/ml tetracycline, 112.9 times higher than the effective concentration of tetracycline for a 60-kg person [11].

The anti-bacterial effects of floating beads are proved in Fig. 7. The obvious anti-bacterial rings are formed by applying beads with encapsulated tetracycline onto cultured *E. coli.* It was caused by the released tetracycline, and the core-shell beads without tetracycline did not result in any anti-bacterial ring (Fig. 7 (a), (b), and (c)). To identify the duration of the floating particles, we immersed the particles in a pH 2-buffer for 2 and 4 h before conducting the anti-bacterial experiments. The results in Figs. 7 (e) and (f) proved that the beads can efficiently suppress *E. coli* though there was a pre-release for 2 and 4 h. Compared with the beads without prerelease in Fig. 7 (d), the anti-bacterial effect did not decay, as shown in in Figs. 7 (e) and (f).

Discussion

The densities of chitosan and xanthan gum used in this research were approximately 0.3 and 1.5 g/cm^3, respectively. The bulk densities of blended chitosan and xanthan gum were approximately 1.47 g/cm^3 when the ratio was close to 1:1. After

get mixed, chitosan and xanthan gum demonstrated high density compared with their original values. Thus, we assume that interactions between chitosan and xanthan gum increase the density of the two components. The anionic polymer chains of xanthan gum and protonated chitosan exert high intermolecular forces because of the electro-affinity. Polymer interactions make the shell layer dense.

According to SEM images, the bead surfaces are dense, which would prolong floating periods due to the resistance of mass transfer in water penetration. The dense skin layer could be help prolong the release time of encapsulated drugs. The chitosan/xanthan-gum ratios did not significantly affect the porosity of core-shell beads.

Since the positive charges of chitosan and negative charges of xanthan gum would result in a strong polymer interaction. These interactions make the shell layer dense, forming a resistance of mass transfer in the shell layer. This hinders water penetration into particles, and therefore, the swelling ratio is low. This result in Fig.3 revealed that the particle swelling can be tuned by controlling the electro-statistical properties of the shell layer. Besides, the high swelling ratios of core-shell beads show that all the beads developed in this research are very hydrophilic. The hydrophilic particles can provide good encapsulation efficiency and release profile due to the high affinity between the drugs and particles.

The retention periods of FDDS in the stomach reported in previous studies were about 2-4 h [12]. The floating time of core-shell particles was much longer than the above residual periods, indicating the dense shell layer developed in this research can prolong the floating time of drugs in the stomach.

When the amounts of chitosan are much higher or much lower than xanthan gum (chitosan:xanthan gum = 4:1 and 1:4), the release of tetracycline is also higher than

those from chitosan:xanthan-gum=4:3 and 4:4. This is due to the strong interactions between positive chitosan and negative xanthan gum, which results in a dense shell layer. With the dense shell layer, the particle swelling is slow, and the tetracycline release is delayed due to the high resistance in mass transfer. The results supported that the prolonged release can be achieved by controlling the formulation of the dense layer in the core-shell floating beads.

The floating beads are proved to be biocompatible, and can carry effective antibiotics when they were applied. The released tetracycline from core-shell beads present clear antibacterial effects. This supports that the core-shell beads developed in this research can continuously delivery antibiotics for a certain period.

Conclusion

In this research, we developed core-shell floating beads for GDDS with porous alginate core and a dense chitosan/xanthan-gum shell layer. The compactness of the shell layer in floating beads was controlled by adjusting the ratios of anionic xanthan gum and cationic chitosan. When the chitosan/xanthan gum ratio was 4:3 and 4:4, the shell layer would be dense and would cause high resistance of mass transfer under the water penetration. Thus, a low swelling rate and a prolonged release was achieved. The experimental results proved the high biocompatibility of the floating beads, and the anti-bacterial effects of beads were also significant after the release for 4 h. This study proposed the method to modify the properties of shell layer, allowing the control of the swell and release behaviors of floating beads.

Abbreviations

GDDS: Gastroretentive drug delivery system

SEM: scanning electron microscopy

FDDS: floating drug delivery system

HBS: hydrodynamically balanced system

DMEM: Dulbecco's modified eagle medium

FBS: fetal bovine serum

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed in this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was financially supported by National Science Council, Taiwan (NSC, No. 108-2221-E-011-107-) and by National Taiwan University of Science and Technology (NTUST).

Authors' contributions

Ming-Hua Ho and Chen-Yu Kao designed this research. Yu-Tung Hsu performed the experiments. Ming-Hua Ho, Yu-Tung Hsu and Chen-Yu Kao wrote the manuscript. Ming-Hua Ho supervised the project and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

Acknowledgements

The authors would like to thank Precious Instrumentation Center of NTUST for the assistances in SEM set up.

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Figures

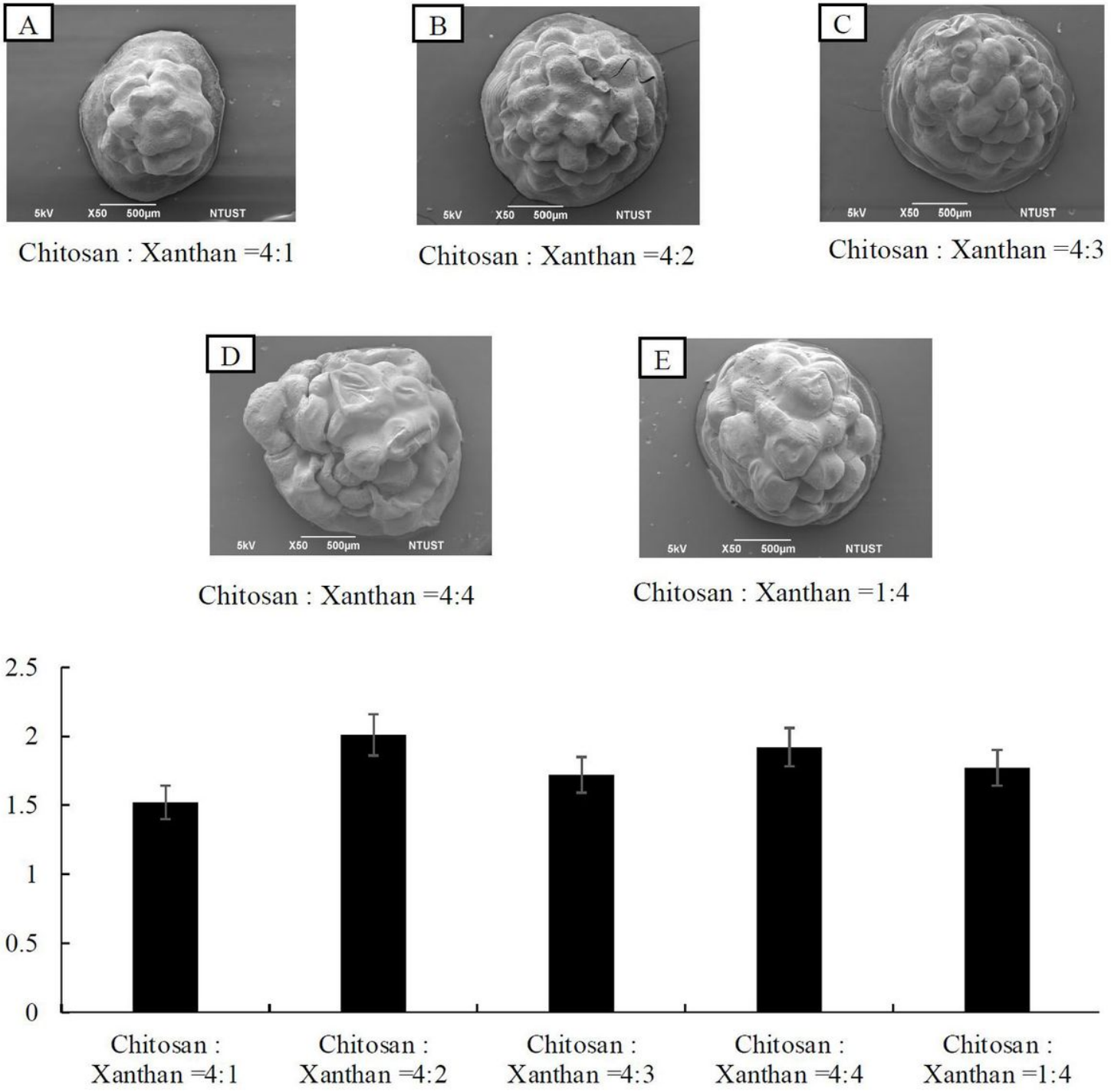

Figure 1

Morphologies of core-shell floating beads with various formulation in chitosan/xanthangum shell layer. (a) SEM images of floating beads. The chitosan : xanthan gum ratios are 4:1 (A), 4:2 (B), 4:3 (C), 4:4 (D) and 1:4 (E). (b) Diameters of beads with various formulations in chitosan/xanthan-gum shell layer

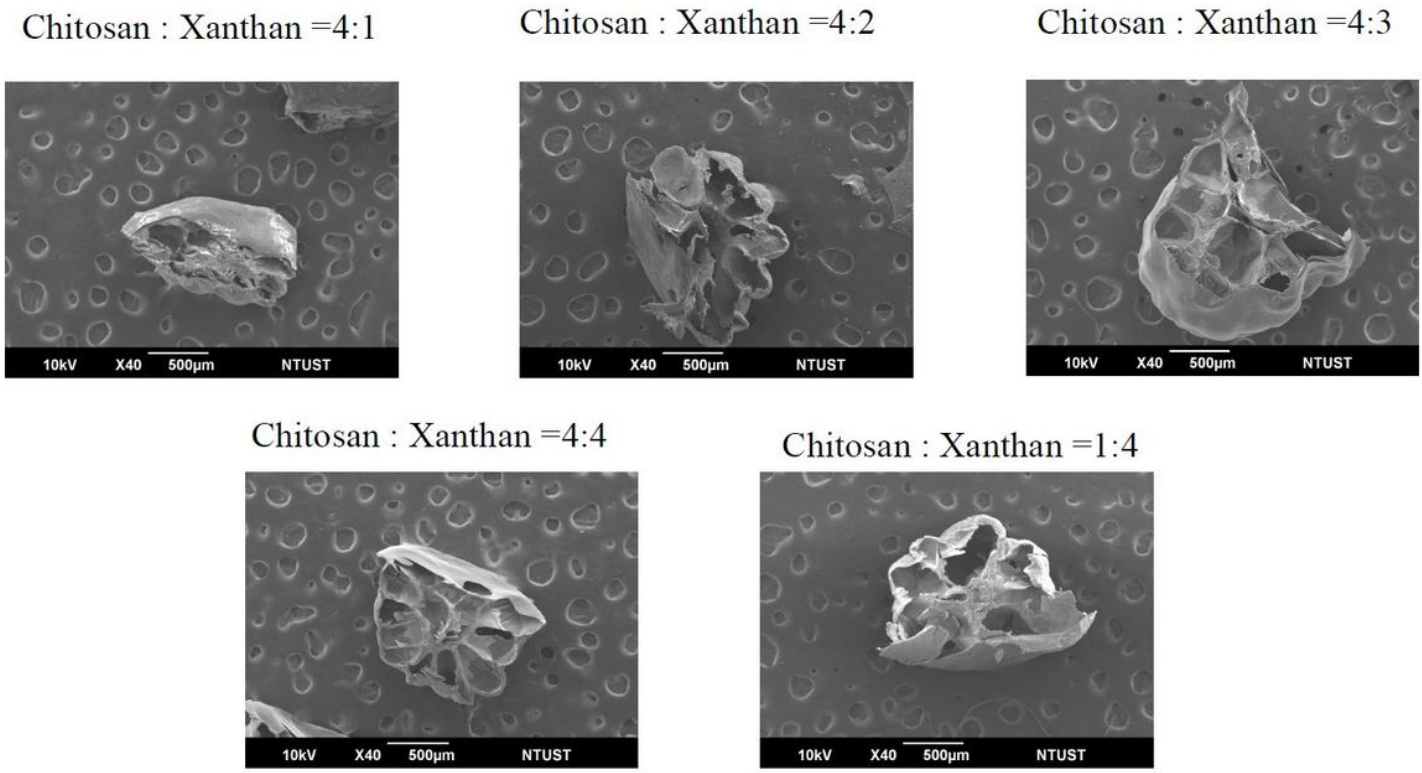

Figure 2

The cross-sectional images of floating beads with various formulation in chitosan/xanthangum shell layer

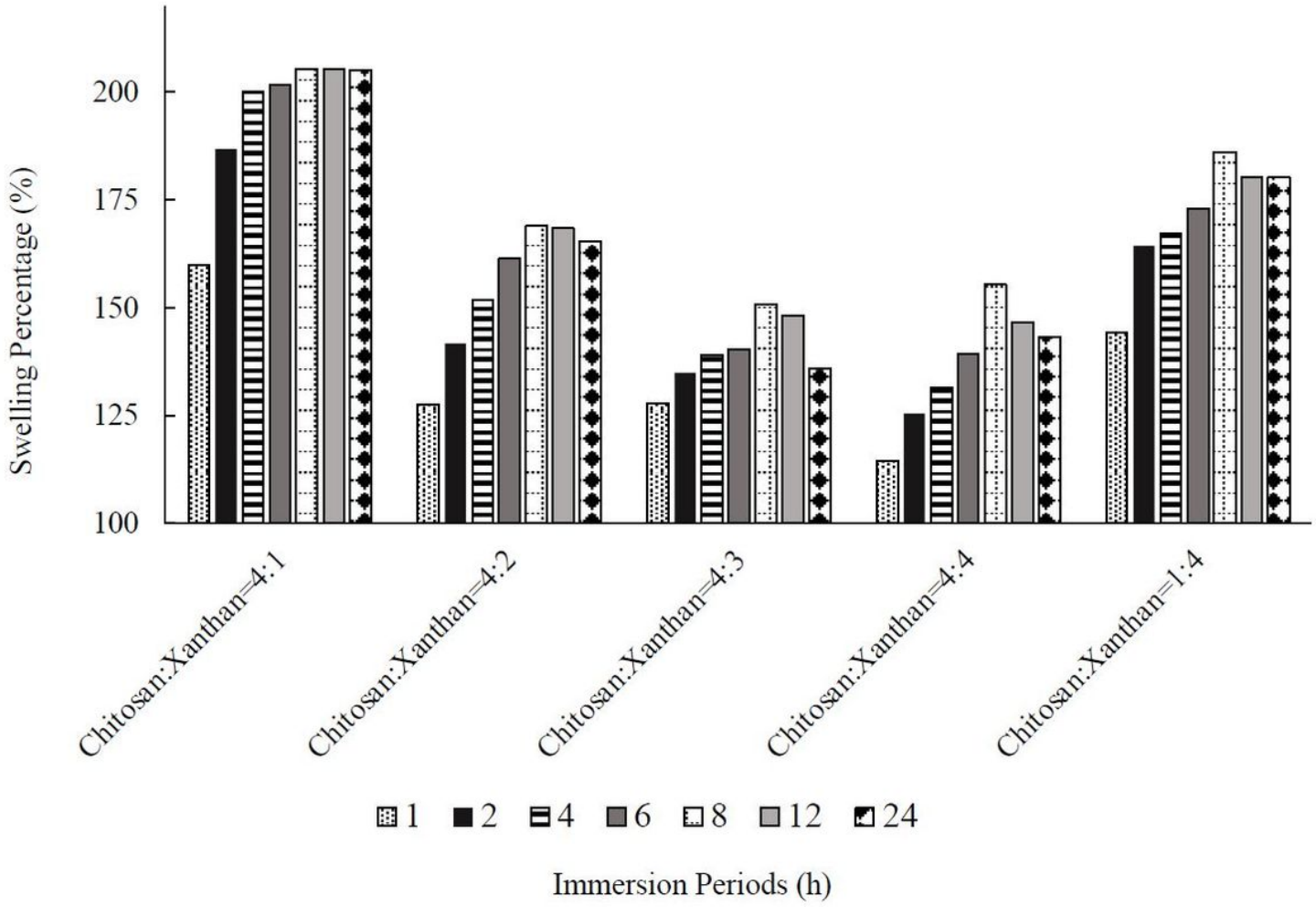

Figure 3

The swelling ratios of core-shell beads with various formulation in chitosan/xanthan-gum in pH=2 buffer. The times for immersion were 1, 2, 4, 6, 8, 12 and 24 hours.

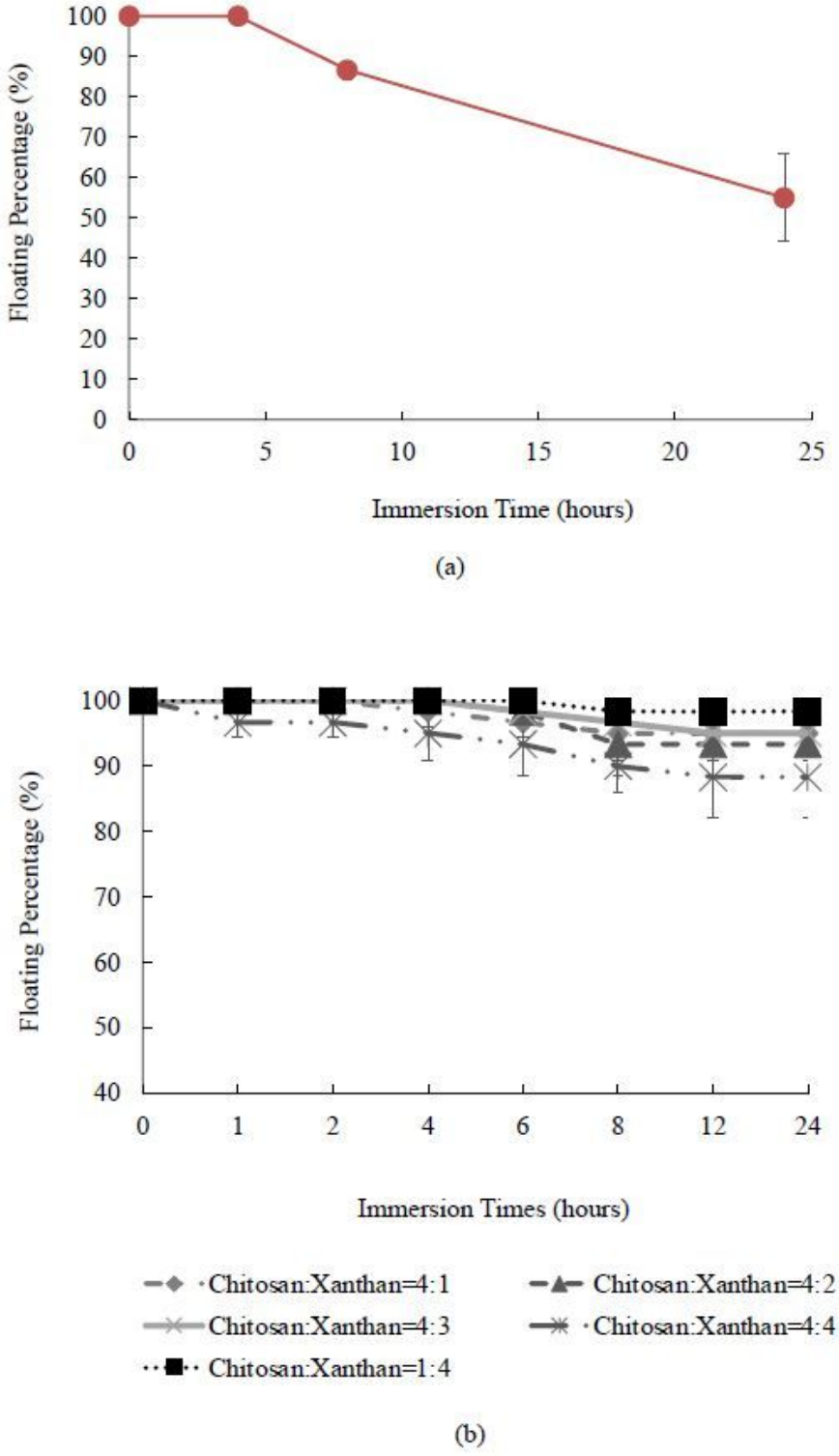

Figure 4

The floating percentage of (a) Chitosan beads without core-shell structure and (b) Coreshell beads with various chitosan/xanthan gum ratios

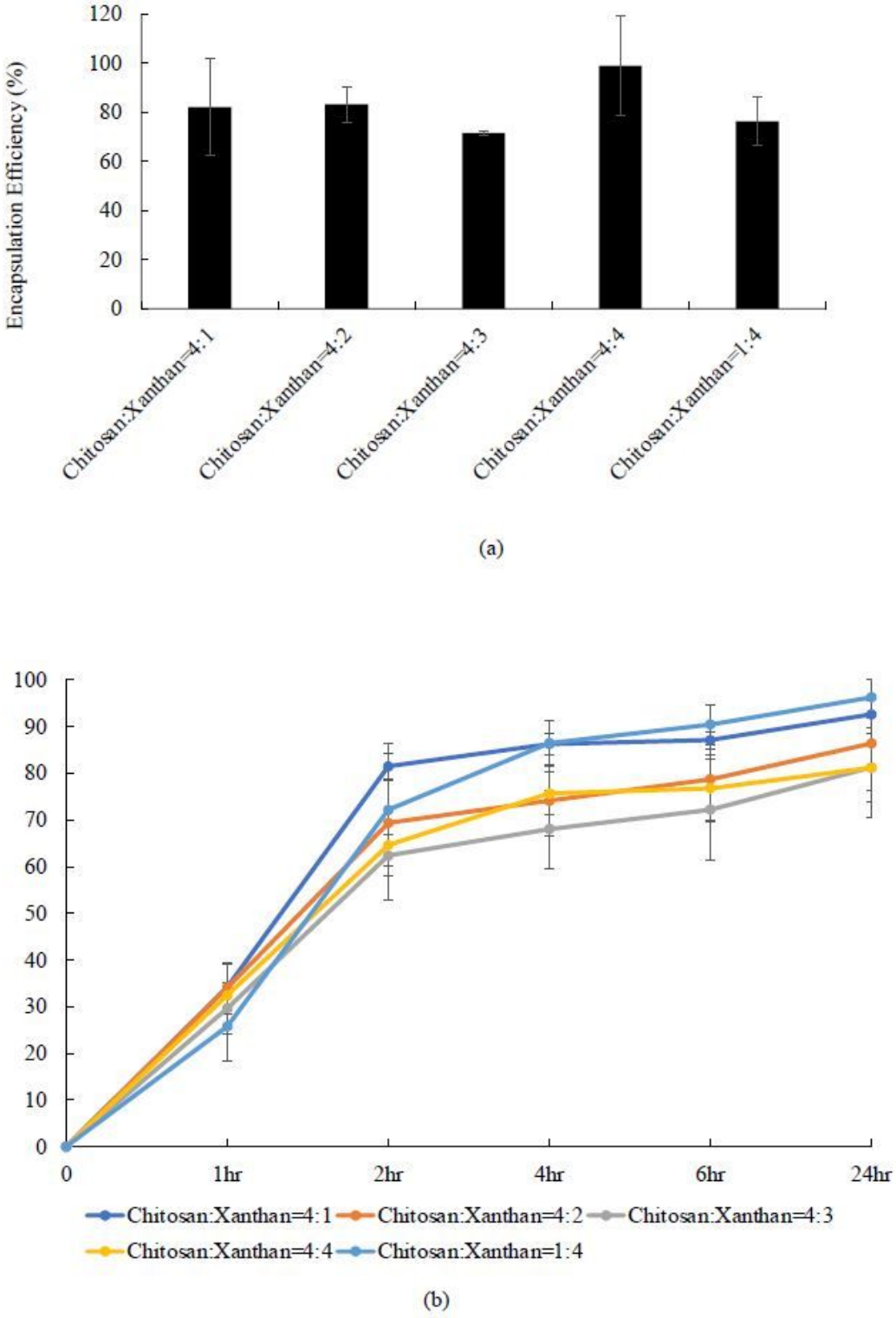

Figure 5

Encapsulation efficiency (a) and releasing profile (b) of tetracycline-alginate floating beads in pH=2 buffer

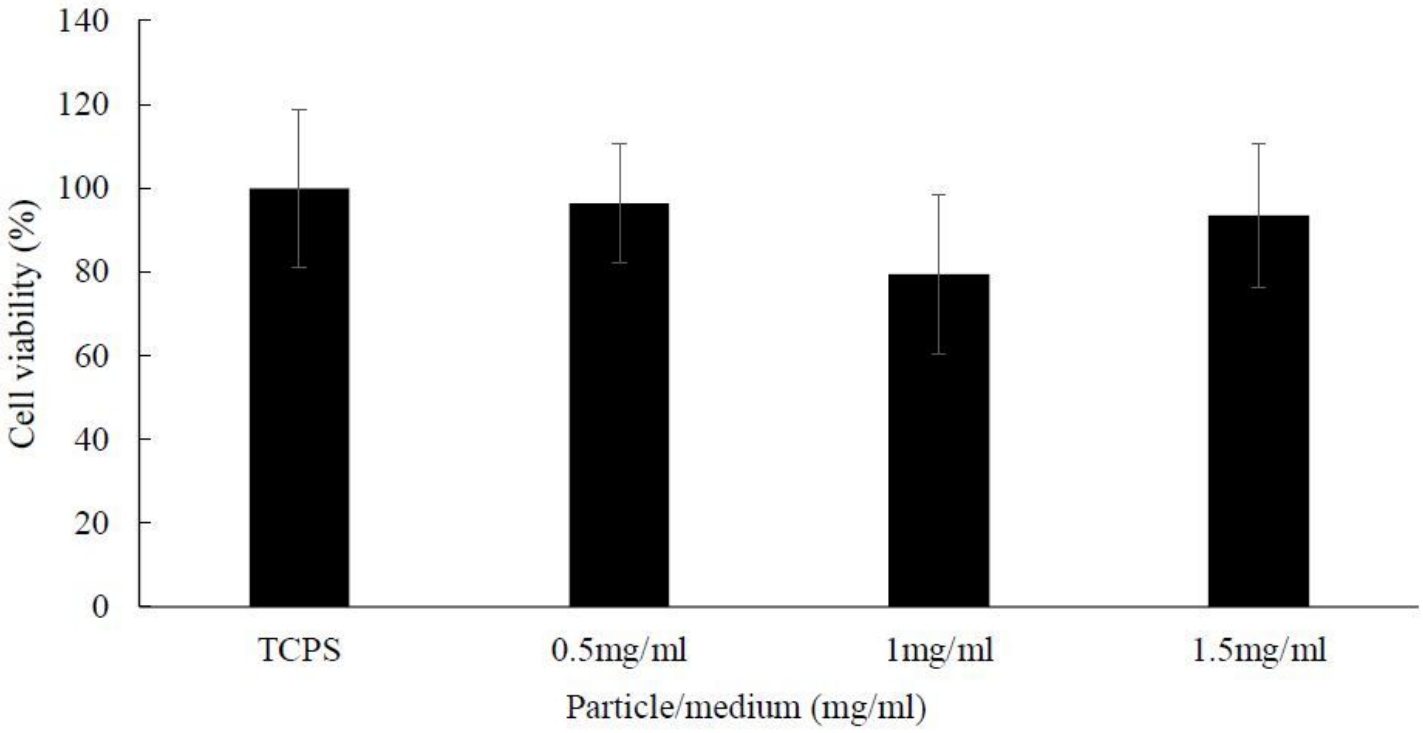

Figure 6

Biocompatibility of floating beads. The bead amounts in culture medium were 0.5, 1 and 1.5 mg/ml, respectively. TCPS was tissue culture polystyrene which was used as the controlled group. The culture period was 24 hours.

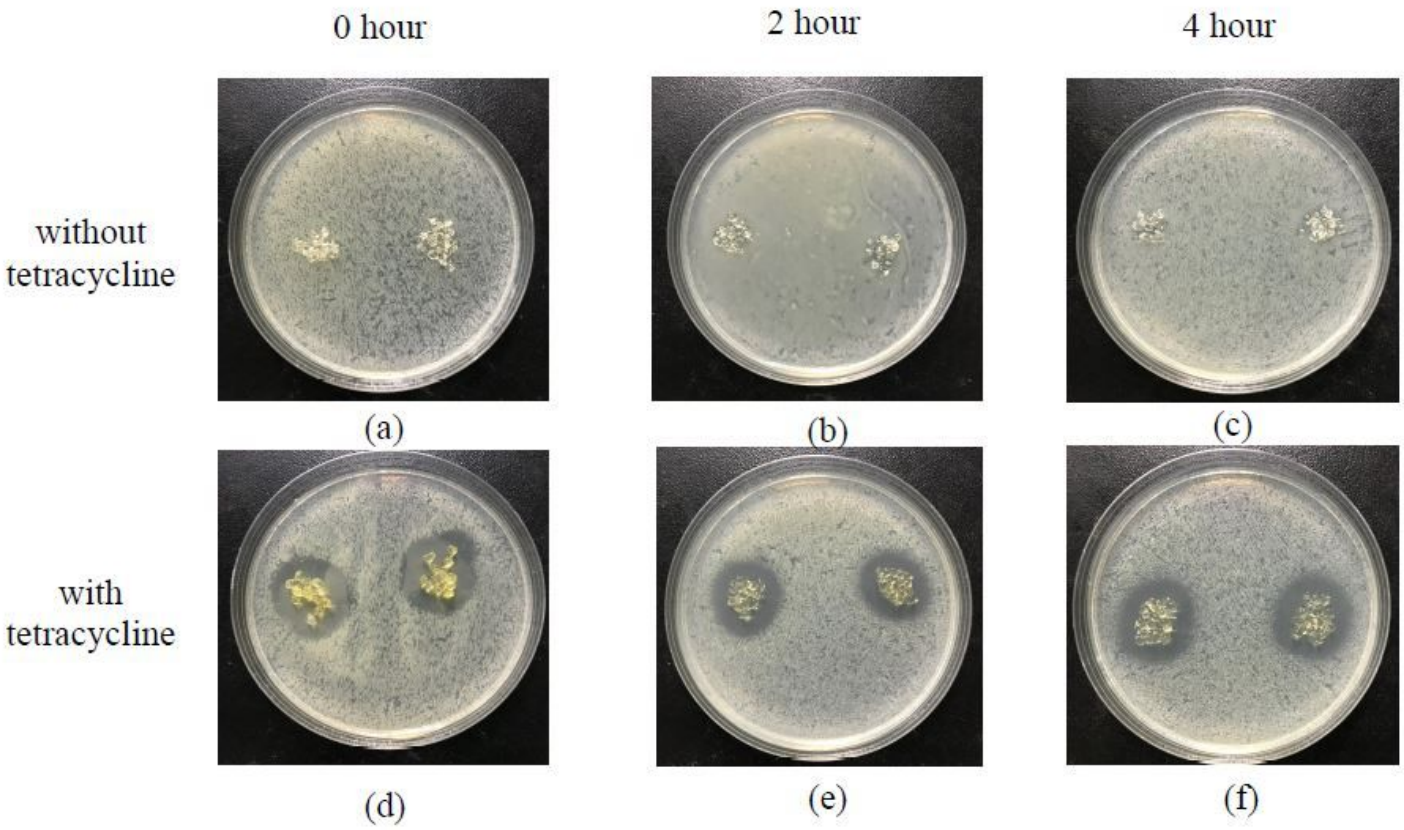

Figure 7

Antibacterial effects of floating beads with/without tetracycline for different immersion periods. (a), (b) and (c) are core-shell beads without tetracycline, and (d), (e) and (f) are beads with tetracycline. The immersion time before antibacterial test is 0 hour for (a) (d), 2 hours for (b) (e), and 4 hours for (c) (f). . The chitosan : xanthan gum ratios are 4:3

Supplementary Files

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