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Short communication

Antibacterial activity of konjac glucomannan/chitosan blend films and their irradiation-modified counterparts

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ABSTRACT

Antibacterial activity of glucomannan/chitosan (KGM/CHI) blend films and their irradiation-modified counterparts were tested. The KGM/CHI blend films after irradiation showed good antibacterial effects against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Antibacterial activity of the blend films increased with the increase of the content of chitosan. After different dose irradiation, there was no obvious change in the antibacterial effects against *St. aureus* of the blend films, but the antibacterial effects against *E. coli* and *P. aeruginosa* increased significantly.

1. Introduction

Biomedical polymeric materials have been playing an important role in the field of functional polymer materials, and chitosan has been widely applied in the medical materials for its biocompatibility and other special functions (Allan & Hadwiger, 1979; Blanco, Gomez, Olmo, Muniz, & Teijon, 2000; Mi et al., 2001; Oh, Drumright, Siegwart, & Matyjaszewski, 2008). Chitosan has excellent broad-spectrum antibacterial property (Gao & Cranston, 2008; Rabea, Badawy, Stevens, Smagghe, & Steurbaut, 2003; Spasova, Manolova, Paneva, & Rashkov, 2004; Yeo et al., 2000). Previous studies have demonstrated that the molecular weight of chitosan significantly decreased after high dose of radiation (Matsuhashi & Kume, 1997), and chitosan degraded in both solid and solution state with radiation. When the dose of irradiation reached 500 kGy, degradation of solid state chitosan was similar to cellulose. This was caused by the fracture of β-1,4-glycosidic bond, which means main chain's rupture. However, irradiated chitosan showed better antibacterial property because the basic units structure of which were not changed (Matsuhashi & Kume, 1997; Yeo et al., 2000). Above all, the radiation dose and the deacetylation degree of chitosan were the major factors influencing the antibacterial activity of chitosan (Xuan Tham et al., 2001).

Konjac glucomannan (KGM) was a rich resource in west China. In our previous works, KGM based blend materials were successfully produced (Li & Xie, 2004) and the structure and performance of the materials were evaluated by modern macromolecular research methods (Li et al., 2011). In this paper, antibacterial effects and biocompatibility of the blend films and their irradiation-modified counterparts were primarily evaluated, and the research provided a theoretical foundation for the application of the biomedical film materials.

2. Materials and methods

2.1. Materials

Konjac glucomannan was extracted and purified from the tuber of *Konjac Amorphophallus* (Ye, Kennedy, Li, & Xie, 2006). Chitosan was purchased from Wuhan Tianyuan biomaterial Co. (Wuhan, China) whose degree of deacetylation was 85% according to the method of Jiang (2002). Viscosity-average molecular weight (Mv) of the chitosan was 1.68×10^6 according to the Mark–Houwink equation $[\eta] = 1.424 \times 10^{-3} Mv^{0.96}$ at 25 °C. The solutions of konjac glucomannan and chitosan with different mixing ratios [90/10, 80/20, 60/40 and 40/60 konjac glucomannan/chitosan (w/w)] were coded as KC1, KC2, KC4 and KC6, the same as the series of blend

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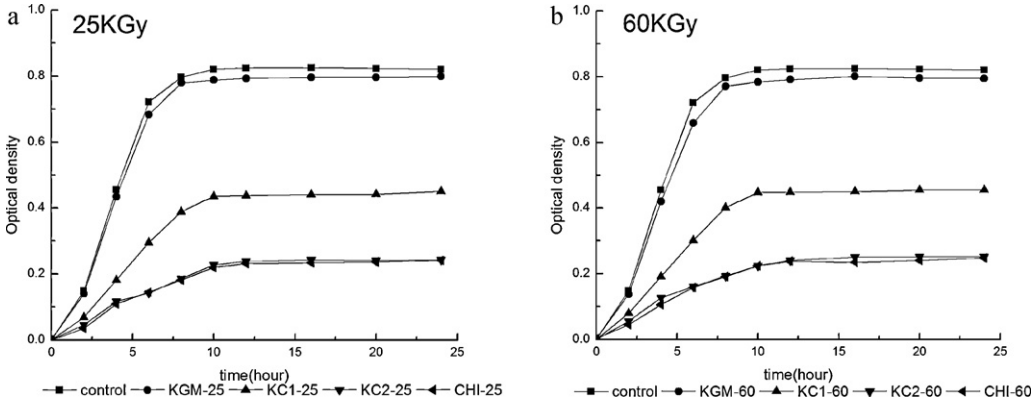

Fig. 1. Effect of the content of chitosan on antibacterial activities to *Staphylococcus aureus* of KGM/CHI blend films (25/60 kGy).

films. The films obtained from pure konjac glucomannan and chitosan was coded as KGM and CHI.

2.2. Preparation and irradiation of blend films

The preparation and irradiation of blend films followed the previously described procedure (Ye et al., 2006).

2.3. Preparation of bacterial suspensions for testing

The target kinds of bacteria were the primary pathogenetic bacteria in burn (*Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*). A ring of the rejuvenated colonies on the slant culture was taken by means of a wire and inoculated in flasks containing 150 ml nutrient broth (NB). Subsequently, it was incubated at 37 °C for 24 h with shaking. This mixed inoculum was inoculated as seeding solution. Every procedure was completed in bioclean environment.

2.4. Antimicrobial assay

The antimicrobial activity test of blend films was carried out by turbidimetry. Dried film (0.5 g) was put into liquid medium (5 ml), which contained 2% bacterial suspensions. The mixture was incubated at 37 °C for 48 h with shaking. Samples' optical density was measured at 650 nm every 2 h. The bacterial suspensions without films were enrolled as control group.

2.5. Acute toxicity test

The acute toxicity test of KC2-25 was evaluated according to Chinese standards (GB/T16886.1-2001).

3. Results and discussion

3.1. Effect of chitosan content on antimicrobial activity

Antimicrobial activity of KGM/CHI blend films and their irradiation-modified counterparts were shown in Figs. 1–3. With the increase of chitosan content, antibacterial activities of blend films against *St. aureus*, *P. aeruginosa* and *E. coli* improved significantly. When the blend films were irradiated by 25 and 60 kGy, samples still showed good antibacterial activities, even the content of chitosan accounted only for 10 wt.%. The results showed that irradiated blend films containing chitosan had excellent bactericidal action.

3.2. Effect of radiation dose on antibacterial activities of blend films

The changes of antimicrobial activity with different irradiation doses of KGM/CHI blend films were shown in Figs. 4–6. KGM film and its irradiation-modified counterparts had no inhibition on all three bacterium (Figs. 4a, 5a, 6a). As it is shown in Fig. 4, KC1 blend films showed a certain inhibition against the growth of *St. aureus* and KC2 and CHI blend films showed remarkable inhibition against *St. aureus*. KC1 blend films displayed a certain inhibition against the growth of *P. aeruginosa* after exposed to 40 kGy radiation. The

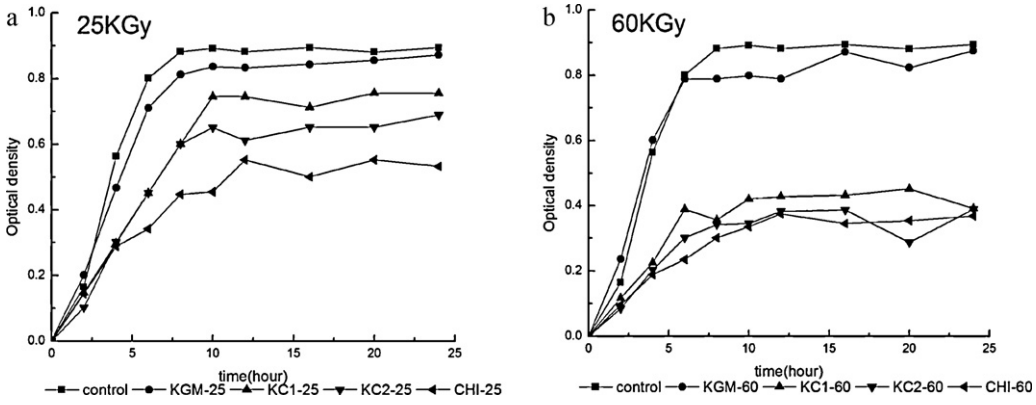

Fig. 2. Effect of the content of chitosan on antibacterial activities to *Pseudomonas aeruginosa* of KGM/CHI blend films (25/60 kGy).

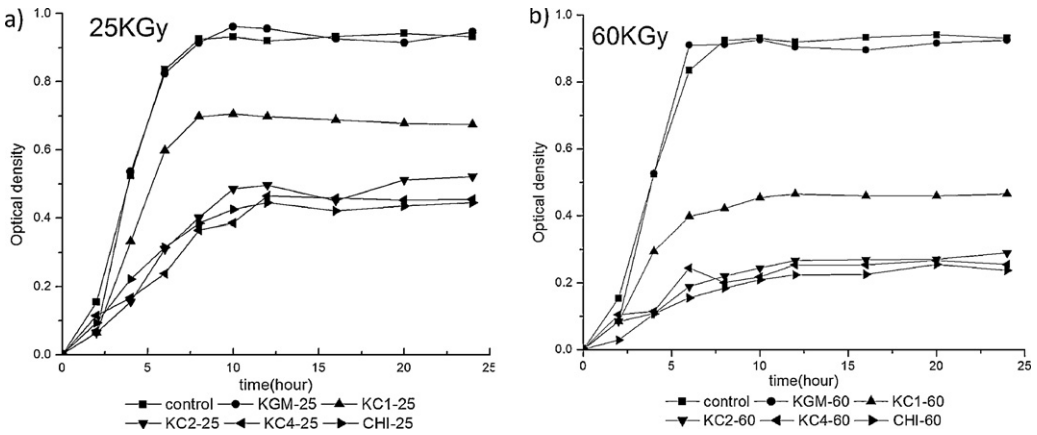

Fig. 3. Effect of the content of chitosan on antibacterial activities to *Escherichia coli* of KGM/CHI blend films (25/60 kGy).

antimicrobial activity of KC1 film to *P. aeruginosa* improved as the increase of the irradiation dose as well (Fig. 5b). KC2 and CHI films had faint bactericidal effect against *P. aeruginosa*, with or without 10 kGy irradiation. Whereas they had obvious inhibition effect on *P. aeruginosa* after exposed to 25 kGy radiation (Fig. 5c and d). As it is shown in Fig. 6b–e, antimicrobial activity against *E. coli* showed similar trends as *P. aeruginosa*. KC1, KC2, KC4 and CHI films had faint bactericidal effect against *E. coli* with or without 10 kGy irradiation. When irradiation dose increased to 25 kGy, their antimicrobial activity to *E. coli* improved significantly, and continued to enhance with the increase of irradiation dose.

We have studied the effect of gamma irradiation on the structure and mechanical properties of KGM/CHI blend films in previous work (Li et al., 2011). We found gamma irradiation could affect the crystalline structure of the blend film, sequentially influencing its microstructure, and finally brought about changes of its mechanical properties. The antibacterial effects enhanced with the increase of irradiation dose on the blend films, mainly due to the degradation of chitosan molecular chain.

Previous studies have shown that chitosan is a radiation degradation polymer. Its molecular weight decreased obviously after high dose irradiation, both in solid state and solution (Kume &

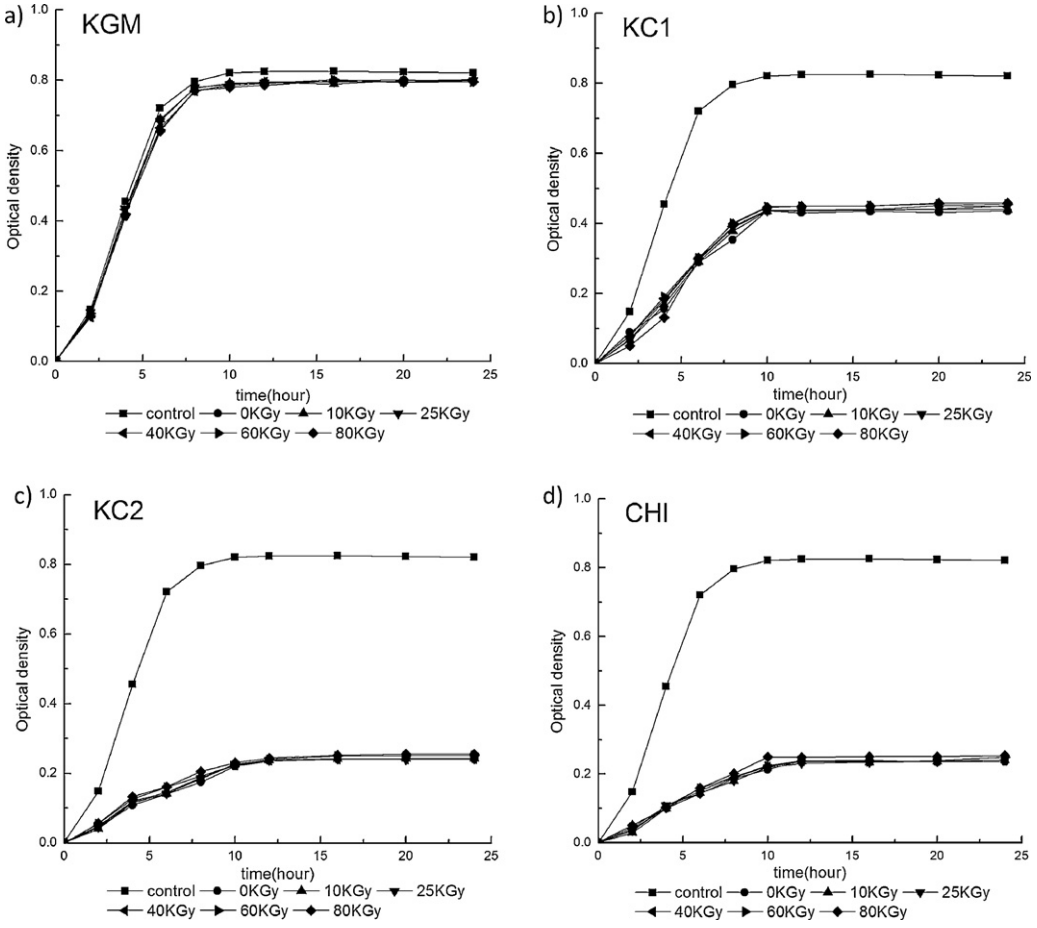

Fig. 4. Effect of radiation dose on antibacterial activities to *Staphylococcus aureus* of blend films.

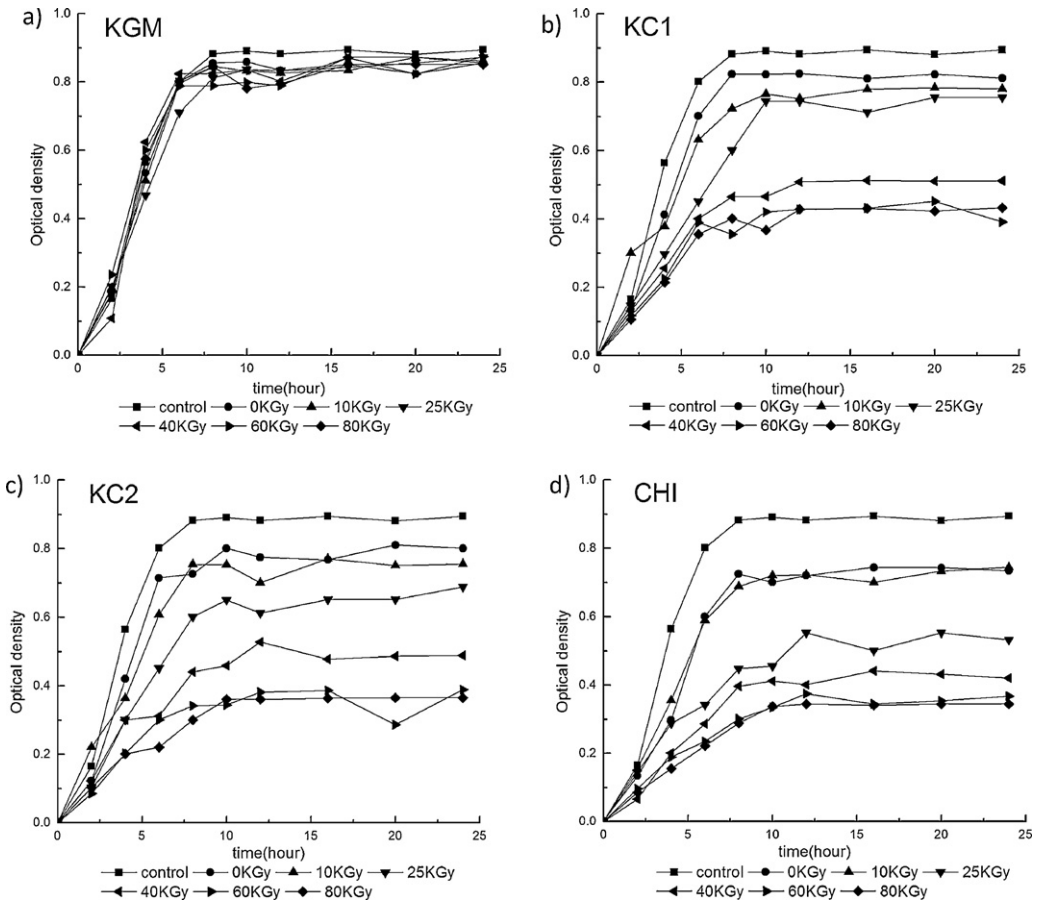

Fig. 5. Effect of radiation dose on antibacterial activities to *Pseudomonas aeruginosa* of blend films.

Takehism, 1982; Zhao et al., 2003). For gram-negative bacteria, small molecular chitosan could intrude into the microbial cell and adsorb intracellular electronegative cytoplasm, then disturb the normal cell physiological activities. Chitosan can also disrupt the barrier properties of the outer membrane of both gram-positive and gram negative bacteria. For *St. aureus*, the possible reason is chitosan formed a layer of membrane on the cells surface, which blocked the nutrients to enter into the cell. However, unlike gram-negative bacteria, small molecular chitosan could affect by intruding into the microbial cell. These provide a possible reason why inhibition effect of konjac glumannan/chitosan counterparts against *St. aureus* enhanced significantly after irradiation but no significant difference among different irradiation dose.

3.3. Statistical analysis of radiation dose influence on antibacterial activities of CHI and KC2 films

The data of KC2 and CHI in antimicrobial assay were analyzed by the statistical analysis system (Table 1). After irradiation, the antibacterial effects against *E. coli* and *P. aeruginosa* increased significantly.

It induced significant uptake of the hydrophobic probe 1-N-phenylnaphthylamine in gram-negative bacteria, interfering with the normal life activity of the cell firstly, and inhibited bacterial growth finally (Helander, Nurmiaho-Lassila, Ahvenainen, Rhoades, & Roller, 2001). After radiolysis, low molecular weight chitosan showed better effects. As a result, antibacterial effects against *E. coli*

Table 1
Contrast antibacterial activities of CHI and KC2.

Sample	Optical density (24 h)		
	Staphylococcus aureus	*Pseudomonas aeruginosa*	*Escherichia coli*
Control	0.821 ± 0.00002a	0.894 ± 0.00003a	0.931 ± 0.000004a
KC2-0	0.241 ± 0.00001e	0.801 ± 0.00007b	0.796 ± 0.00002b
CHI-0	0.236 ± 0.00003e	0.735 ± 0.00003c	0.733 ± 0.00001c
KC2-10	0.242 ± 0.00002de	0.756 ± 0.00002d	0.749 ± 0.00002d
CHI-10	0.240 ± 0.00002e	0.744 ± 0.00001e	0.701 ± 0.000004e
KC2-25	0.242 ± 0.000003de	0.689 ± 0.00001f	0.522 ± 0.00001f
CHI-25	0.241 ± 0.000002de	0.533 ± 0.00002g	0.445 ± 0.00006g
KC2-40	0.241 ± 0.00002de	0.489 ± 0.00001h	0.477 ± 0.000003h
CHI-40	0.241 ± 0.00004e	0.421 ± 0.00002i	0.399 ± 0.00001i
KC2-60	0.252 ± 0.00002bc	0.389 ± 0.00004j	0.290 ± 0.000001j
CHI-60	0.248 ± 0.00001cd	0.368 ± 0.00001k	0.238 ± 0.00003k
KC2-80	0.255 ± 0.00001b	0.365 ± 0.000001l	0.262 ± 0.00001l
CHI-80	0.254 ± 0.00001bc	0.345 ± 0.00002m	0.226 ± 0.000001m

Same letters within a column indicate no significant differences, and different letters within a column indicate significant differences ($p < 0.05$).

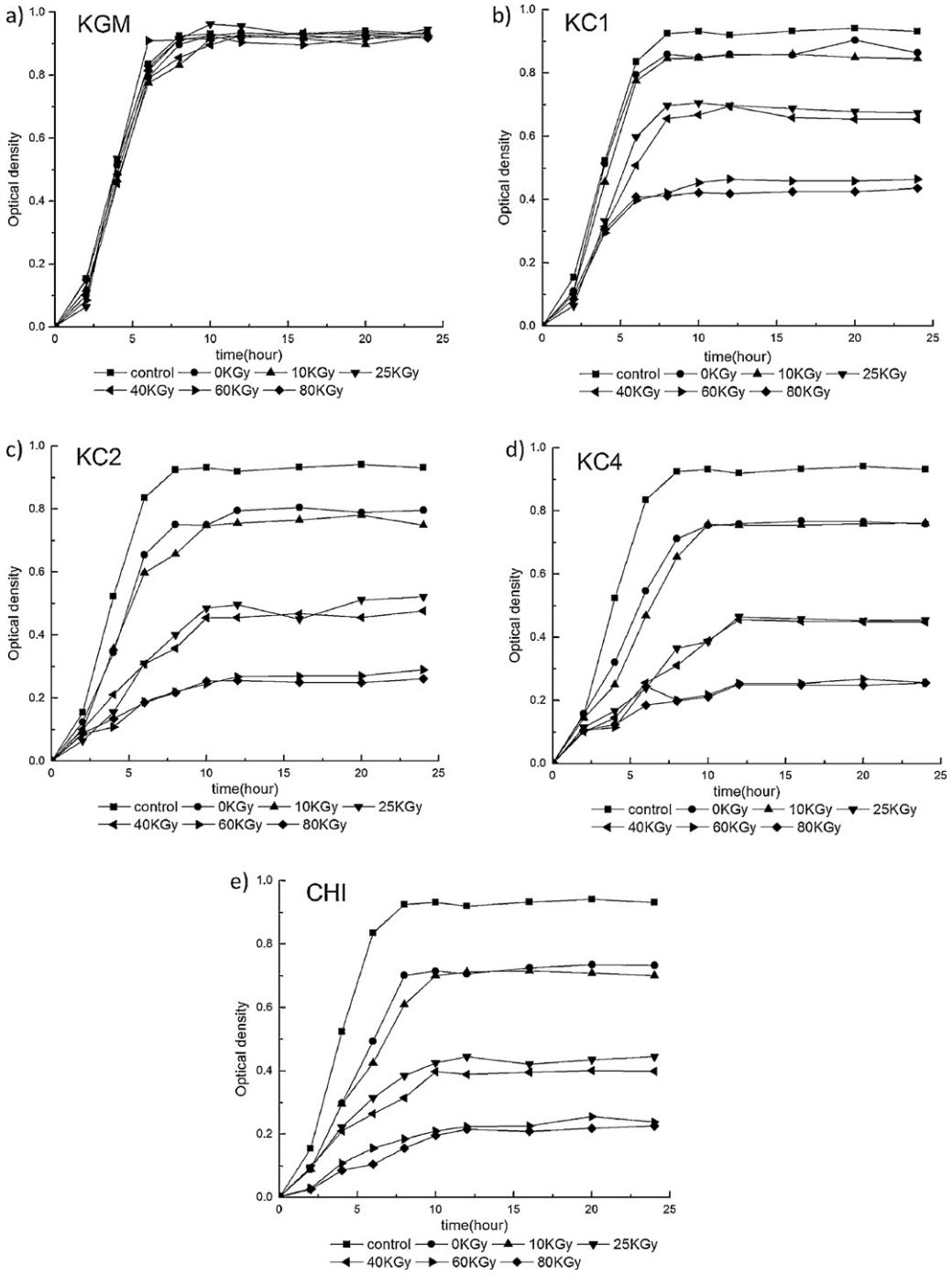

Fig. 6. Effect of radiation dose on antibacterial activities to *Escherichia coli* of blend films.

and *P. aeruginosa* increased significantly, indicating that low molecular weight chitosan have been produced through irradiation. For gram-positive organisms (*St. aureus*), chitosan adsorbed in microbial cells surface formed a layer of membrane, which blocked the nutrients to enter into the cell.

These results effectively revealed that KGM film mixed with CHI (KC2) improved the antimicrobial activity.

3.4. Biological evaluation of KC2-25 film

The safety of KC2-25 was evaluated according to Chinese standards (GB/T16886.1-2001). Toxicity of KGM/CHI blend films was investigated by cytotoxicity test, L929 cell morphology, skin irritation studies and sensitization test (Ye et al., 2006). The safety evaluation results showed that KC2-25 had no cytotoxicity, and it was safe according to the results of the test of the acute systemic toxicity. Skin irritation and sensitization tests showed that the skin original irritation and sensitization of KC2-25 was slight. The acute toxicity test showed that there were no death animal and non-toxic reaction in the test group which was injected KC2-25 physiological saline extract liquid after 4 h, 24 h, 48 h, and 72 h. Average weight of control group increased by 3.6300 ± 1.0791 g ($n = 10$). And average weight of test group increased by 3.6819 ± 1.1035 g ($n = 11$), which is greater than control group. Results suggest that acute systemic toxicity of KC2-25 is qualified.

Biological evaluation of KC2-25 film indicated that KC2-25 blend film could be used as a highly safe medical dressing.

4. Conclusion

Antibacterial activity of the blend films enhanced with the increase of the content of chitosan. After irradiation, there was no obvious change in the antibacterial effects against *St. aureus* of the blend films among different irradiation dose, but the antibacterial effects against *E. coli* and *P. aeruginosa* increased significantly. In KC2-25, due to the intense intermolecular hydrogen bonds between the KGM and CHI in addition to their good compatibility, the tensile strength of the film was prominently improved compared to the CHI film. The mechanical properties of the blend film were further improved by irradiation compared to the CHI film. At the same time, antibacterial activity against *E. coli* and *P. aeruginosa* also increased after the irradiation. The KC2-25 is a promising biomedical polymeric material thanks to its good mechanical properties, biocompatibility, safety and low cost.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2012.10.006.

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