Effect of Gamma-ray Irradiation on Bacterial Penetration Power of Chitosan/Collagen Blend Membranes for Wound Dressing

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Abstract

The aim of this study is to investigate the effects of gamma-ray irradiation on the bacterial penetration power of chitosan/collagen blend membranes. For this study, commercially available chitosan, and collagen extracted from bovine tendon were made into solution using a modified acid-solution method. This solution is then used to prepare the chitosan, collagen and chitosan/collagen blend membranes via solution casting on a plastic plate. Each of the membranes are exposed to gamma-ray irradiation (0, 15 or 25 kGy), placed on an agar medium and immediately inoculated with Staphylococcus aureus. The bacterial penetration power of the irradiated specimens are determined by inspecting the growth of colony-forming units on the agar medium. Observations of the bacterial penetration power of each type of membrane are as follows: the collagen membranes displayed colony-forming units on the agar medium, the chitosan membranes had the colony-forming units encircling the membranes, while the chitosan/collagen membranes showed spots of colony-forming units. After exposure to the gamma-ray irradiation, a reduction in the colony-forming units was observed for each of the membranes. In addition, increasing the dose of the gamma-ray irradiation was shown to reduce the number of the colony-forming units on the agar medium for each membrane type.

In conclusion, the collagen membranes were able to prevent penetration of S. areus, whereas, the membranes containing chitosan were penetrable to the bacteria. The number of the colony-forming units shown in the agar medium depended on the the gamma-ray doses.

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Introduction

An increase in the number of patients suffering from wounds and burns over the recent years has been linked to the passage of bacteria through dressing membranes and has become a potential avenue of wound contamination. To prevent bacterial passage through a wound dressing, the wound should be covered with a suitable biomaterial. Wound dressings prepared from biomaterials are studied intensively because they can offer alternatives to traditional wound dressings. Traditional wound care products tend to address the wound's macroenvironment, including moist wound environment control, fluid management, and controlled transpiration of wound fluids. The newer class of biomaterials and wound-healing agents, such as collagen and growth factors, targets specific defects in the chronic wound environment with the possibility that they benefit the wound healing process at a biochemical level[1-4]. Recently, attention has been focused on employing natural polymers for medical applications, such as gelatin,[5-7] hyaluronic acid,[8-10] collagen[3,11-13] or chitosan .[14,15]

Chitosan is derived from chitin, through a deacetylation process to obtain a linear structure of N-acetyl glucosamine polysaccharide, which is found in the exoskeletons of marine crustaceans such as shrimps and crabs, as well as in insects and the cell walls of fungi.[16,17] Apart from these,

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there are many factors present in the chitosan molecule that can influence its antimicrobial properties, (which can be beneficial in biomedical applications) and the physical state of the chitosan, e.g. whether it is present in the form of hydrogels, powders, films, membranesmicro/nanoparticles and coatings. According to past reports, the most important medical application of chitosan's makes use of its capability to act as an artificial skin for humans with extensive burns[18] and as wound dressings.[14] The use of the chitosan was found to promote wound healing and induce cell migration and proliferation.

Collagen is the major protein of the extracellular matrix mostly found in connective tissue. Collagen is usually originated from bovine,[19] avian ,[20] or porcine[21] connective tissue, as collagen is the most abundant protein found in mammals. Among the above mentioned natural polymers, collagen in its natural state is abundantly available, for instance, as a by-product in the meat industry. Due to its negligible immunogenicity, excellent biocompatibility, mechanical stability and its ability to be involved in all 3 phases of the wound-healing cascade, studies have examined the use of collagen, for example, in periodontal reconstruction and wound dressing. Collagen can be prepared in forms of its binary or ternary blends; one of the reasons for using chitosan/collagen blends as a candidate for wound dressing is due to its potential to have extraordinary properties.

For effective crosslinking of proteins and polysaccharides such as , chitosan/collagen blends to improve their mechanical properties, physical methods including UV and gamma-ray irradiation have been applied. [22-24] On the other hand, the use of non-sterilized dressing is associated with the risk of infectious disease transmission. As the dressing comes in contact with the open wounds, it needs to be perfectly sterile to avoid the transmission of disease. Gamma radiation is the most reliable and effective method for sterilization of medical and health care products[22], although very few studies consider gamma-ray irradiation techniques as solvent free and environmentally processes.[24-26] According to ISO 11137, gamma-ray irradiation of 15–25 kGy, should be used for the sterilization of biomedical devices. However, relatively high irradiation doses lead to the degradation of polysaccharides such as chitosan or collagen due to the cleavage of the glycosidic bonds.[27]

Therefore, testing the bacterial penetration power of wound dressing chitosan/collagen blend membranes with gamma-ray irradiation is necessary as membrane dressings should serve as a barrier between the wound and microbes from the outside environment. This study aims to investigate the effect of gamma-ray irradiation on the bacterial penetration power of chitosan/collagen blend membranes.

Materials and methods

Specimen preparation

Chitosan powder, with 90% degree of deacetylation, was purchased from Kimia Farma (Jakarta, Indonesia), and used without further treatment. Whereas, bovine tendons were obtained from a local market in Jakarta, Indonesia. Staphylococcus aureus was obtain from Microbiology Laboratorium of Faculty of Medicine - Universitas Indonesia and *tryptic soy agar* from Merck (Darmstadt, Germany). The reagents used were glacial acetic acid (100% purity) and sodium chloride (Merck, Darmstadt, Germany).

Chitosan, collagen and chitosan/collagen membrane preparation

Chitosan membranes were prepared following the method used by Montoya MHU, *et al*[28]. In brief, chitosan dispersions were prepared in 0.7 M acetic acid aqueous and stirred at 700 rpm for approximately 2h at room temperature (25±1 °C) and allowed to stand without stirring for 12h. To obtain a chitosan membrane, the resulting solution was cast onto a plastic plate (15x10 mm^2) and dried at room temperature for 48h. These dried chitosan membranes were peeled off and were immediately kept in a polyethylene plastic bag prior to using. Collagen was extractedfrom the bovine tendons the modified acid-soluble method as prepared by Ramasamy P and Shanmugan A[29] and Gopinath [30]. For preparation of the collagen and chitosan/collagen blend membranes, with a mixture with equal parts of chitosan and collagen for approximately 12h at room temperature (25±1 °C). The collagen and the blends were then made into membranes using the method described above.

Irradiation Applications

Prior to irradiation, each membrane was packaged under in polyethylene film to prevent oxidation during the process and maintain an aseptic environment. The membranes (chitosan, collagen and chitosan/collagen) were then irradiated. The irradiation process was conducted using a linear electron accelerator of gamma-rays generated from a Co-60 source carrying 2.2 MeV of energy at a dose rate of 0.002 kGy/sec. The required doses were 15 and 25 kGy at room temperature (25±1 °C). The un-irradiated membranes that were used as controls.

Bacterial penetration test

Three round agar plates, 90 mm in diameter, were filled with *tryptic soy agar* medium. Each membrane was sterilized using of irradiation of 0, 15 or 25 kGy. Then membrane samples with diameters of 19±1 mm were placed on the agar of each plate. Subsequently, the sample was inoculated with a suspension (0,1 ml) of an overnight culture of *Staphylococcus aureus* (10^8 bacteria/ml) in its center. After one night of incubation at 37°C, the membranes were removed and the agar below the membranes were checked immediately for bacterial penetration power by observing any growth of colony-forming units on the agar medium.

Results

After one night of incubation, the bacterial penetration power of the irradiated membranes are displayed in Figure. 1, 2 and 3.

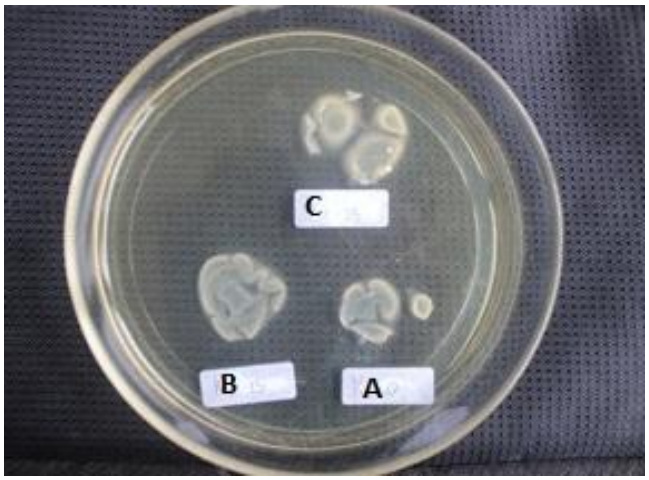

Figure 1. Bacterial penetration power in chitosan membranes with gamma-ray irradiations doses of (a) 0 kGy, (b) 15 kGy, and (c) 25 kGy showing the colony-forming units on the agar medium.

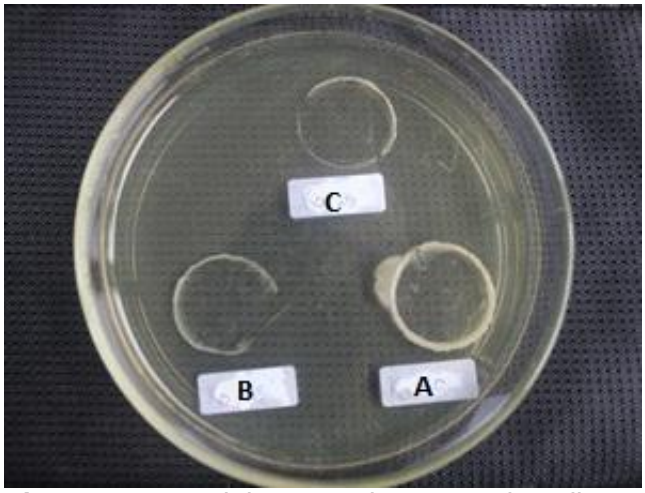

Figure 2. Bacterial penetration power in collagen membranes with gamma-ray irradiations doses of (a) 0 kGy, (b) 15 kGy, and (c) 25 kGy showing the colony-forming units on the agar medium.

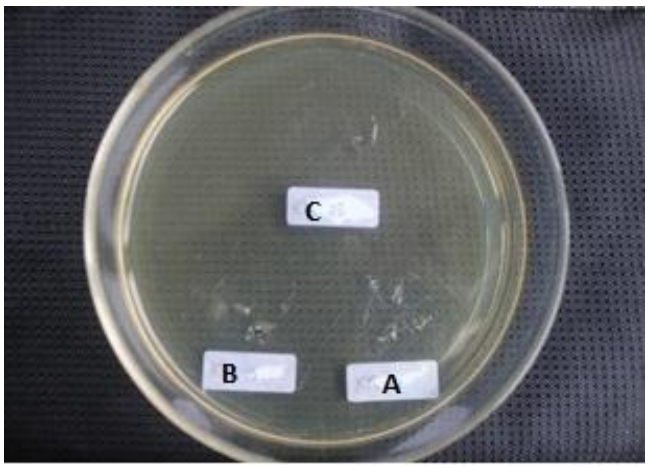

Figure 3. Bacterial penetration power in chitosan/collagen blend membranes with gamma-ray irradiations doses of (A) 0 kGy, (B) 15 kGy, and (C) 25 kGy showing the colony-forming units on the agar medium.

As seen in Figure. 1, the agar medium with chitosan membranes irradiated by (A) 0 kGy, (B) 15 kGy, and (C) 25 kGy showed colony-forming units, which indicate that they were penetrable by *S. areus*. The collagen membranes showed colony-forming units around the circumference of each membrane sample, as can be seen in Figure 2. When the membranes were removed, however, no colony-forming units on the agar medium below the membranes were seen. In terms of the blend membranes of chitosan/collagen, in Figure. 3, very small numbers of penetrable colony-forming units were detected on the agar medium. In general, the

agar medium that contained un-irradiated membranes (A) showed more colony-forming units than those irradiated with 15 kGy (B), and those exposed to 25 kGy (C) showed the fewest colony-forming units.

Discussion

There were visible colony-forming units on the agar mediums after removal of the membranes, with irradiation gamma ray doses of 0, 15 or 25 kGy (Figure. 1). This probably due to the acidic nature (pH <7) of the agar medium which may have caused swelling of the sensitive chitosan, as chitosan is soluble at pH values of lower than 5.5. The acidic conditions may have damaged the chitosan membrane , enabling the inouculated *S.aureus* bacteria to freely penetrate the ruptured chitosan membrane and grow on the agar medium as colony-forming units.

Absence of growth below the collagen membranes (Figure. 2) showed that the bacteria was unable to penetrate the membranes. This can be explained by the braid peptide bond occurring within the collagen structure that gives it a relatively dense membrane. Collagen could also have be crosslinked by the gamma-ray irradiation. Crosslinks are introduced by the bonding of radicals.[31] Therefore, the colony-forming units occurred outside the membranes with a circular shape which followed the circumference of the membrane.

The blend membranes also displayed colony-forming units on the agar medium (Figure. 3), although they were relatively small in number. This can be explained by the braid peptide bond mentioned above that gives the collagen a relatively dense membrane with crosslinks between the chitosan and collagen. This made the membrane impenetrable to colony forming units. In addition, scanning electron micrographs obtained from the chitosan/collagen blend membranes irradiated with doses of 15 and 25 kGy showed pore sizes of around 297-344 nm and 826 nm, respectively, as was shown in our previous study. As *S. aureus* is of around 800-1,000 µm in size, it may have been difficult for the bacterium to penetrate through the membranes. Nevertheless, a few colony-forming units were still seen showing that the bacteria was able to penetrate the blend membranes. This was likely due to the chitosan content in the blend membranes that were easily damaged from contact with the acidic environment of the agar medium. Similarly, the chitosan membrane was probably more easily ruptured when in contact with the agar medium.

The typical decreased vertical facial height of this patient resulted from the congenital.

Conclusions

It is concluded that the collagen membranes demonstrated the ability to prevent the bacterial penetration power of *S. aureus*, whereas the chitosan membranes were penetrated by the *S. areus.* The chitosan/ collagen blend membranes showed minimal penetration of the *S. areus*, as shown by the occurrence of colony-forming units on each agar medium. When gamma-rays were applied, the higher doses resulted in fewer colony-forming units on the agar medium.

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Declaration of Interest

The authors report no conflict of interest.

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