Preparation and characterization of collagen-ciprofloxacin HCL membranes produced using gamma irradiation as a candidate for wound dressing

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Abstract. Wound and bacterial infections are often found after periodontal surgery. Collagen has an important role in healing wounds. The purpose of this study is to produce a membrane film which has been antibacterial activity so that it can be developed as a candidate for periodontal wound dressing. Collagen from bovine tendons synthesized with the acidic method to obtain dry collagen. Dry collagen then dissolved to get a 2% collagen concentration and then mixed with ciprofloxacin HCl 0.1% (w/v) and poured on polyacrylic molds. The membrane is then irradiated by gamma rays irradiation at varying doses of 0, 15 and 25 kGy respectively. The irradiated membrane then characterized by its physicochemical properties in the form of functional group analysis, membrane pore size, water absorption, water vapor transmission, tensile strength and percent release of ciprofloxacin HCl. The result of the characterization is that the membranes didn’t show any significant changes in the functional group but show any significant changes in size membrane pores, water absorption, water vapor transmission rate, tensile strength, and percent release of ciprofloxacin hydrochloride after gamma rays irradiation.

1. Introduction
Periodontal disease is a gingival inflammatory condition that can cause damage to the periodontium tissue and the alveolar bone supporting the teeth [1]. Treatment of periodontal disease can be either surgical or non-surgical. Surgery will usually cause periodontal injury which needs to be covered with a material called periodontal dressing [2]. A periodontal dressing is a wound dressing that is used to cover and protect the wound surface from surgical procedures. The main requirements of periodontal dressing must be non-toxic, easily sterilized, adequate mechanical properties, strong, elastic and biocompatible [3]. Fiber that can be used for periodontal dressing varies, one of which comes from nature, namely collagen. Collagen is one type of protein which acts as the main component of skin and connective tissue consisting of \( \alpha \) polypeptide chains bound to each other in the form of helix rod structures [4]. Collagen is a fibrous protein that gives strength and flexibility to tissues and bones. Collagen can be used as a raw material in the food, cosmetics, biomaterial, and biomedical film making industries. Collagen plays an important role at every stage of the wound healing process.
because it has the ability, among others, as a hemostasis agent, interaction with platelets, controlling fluid exudation and encouraging the process of fibroplasia [5].

The clinical application of periodontal dressing is to apply the drug locally to the wound area. The addition of antibiotics one of which ciprofloxacin hydrochloride can be done to treat bacterial infections. Ciprofloxacin hydrochloride (Ciprofloxacin-HCl) is a second-generation antibiotic derived from fluororoquinone. Ciprofloxacin works by inhibiting the growth of gram-positive and gram-negative bacteria and can inhibit pathogenic bacteria that cause periodontal disease [6]. Gamma-rays irradiation is one of the sterilization methods that can be used to sterilize polymer membranes. This type of energy source has also been utilized further for the process of crosslinking and degradation of monomers or polymers. The advantages of gamma-rays irradiation include a relatively fast process, no residue is left and the irradiation dose can be adjusted as needed [4]. The dose of gamma-rays irradiation used for sterilization purposes is 15 kGy or 25 kGy [7].

Based on the description of the things above, the synthesis of collagen from bovine tendons will be mixed with the antibiotic ciprofloxacin HCl to form a membrane. The membrane is irradiated by gamma rays at a dose of 15 kGy and 25 kGy. The membrane is then characterized in the form of functional group analysis with Fourier Transform Infrared (FTIR) spectrophotometer, pore size with the Scanning electron microscope, water absorption, water evaporation rate, tensile strength and elongation at break and percent release of ciprofloxacin HCl.

2. Materials and method

2.1. Materials

Collagen used was collagen extracted from cattle tendons obtained from Tapos Abattoir, Cibubur, East Jakarta. Ciprofloxacin HCl used is obtained from PT. Mutifa, North Sumatra. Glacial acetic acid made by Merck. EMB from Merck. Other chemicals quality pro analysis.

2.2. Collagen Extraction from Bovine Tendons

The bovine tendons obtained are washed with tap water until clean and then cut into small cubes. Then the tendon was soaked with 0.7 M acetic acid and crushed with a hand blender. Furthermore, the collagen gel formed was coagulated with 4M sodium chloride and neutralized with phosphate buffer on a magnetic stirrer at 350 rpm. The collagen is then put into the freezer at -30 °C for 12 hours. Furthermore, frozen collagen is dried with a freeze dryer to remove water crystals for 3 days [8].

2.3. Manufacture of Collagen-Ciprofloxacin HCl Membranes

The dried collagen after freeze-dried is weighed as much as ± 2 grams and dissolved with 0.7 M acetic acid to obtain a 2% concentration of collagen. Collagen is stirred using a magnetic stirrer then mixed with 0.1% ciprofloxacin HCl. The mixture is stirred for ± 60 minutes until homogeneous then 20 ml of mixture is poured into a 7.5 x 7.5 cm polycrylic mold and dried at room temperature (28.8 °C) for ± 7 days. The dried membrane is then packed in polyethylene plastic and irradiated with gamma rays with varying doses of 15 and 25 kGy at a dose rate of 4.8 kGy / hour [9].

2.4. Functional Group Analysis Using Fourier Transform Infrared (FTIR) Spectrophotometer

Collagen - ciprofloxacin HCl membrane is cut to the size of a stainless steel cup. Potassium bromide (KBr) powder is inserted into a stainless steel cup then the cut membrane is placed on top of KBr powder. The infrared spectrum is measured in the region of wave numbers 4000-400 cm-1 [9].

2.5. Collagen-Ciprofloxacin HCl Membrane Water Absorption Test

Testing is based on the ASTM D570-98 procedure. The ciprofloxacin HCl collagen membrane from gamma irradiation results is cut to a size of 15x10 mm, then dried in an oven at 40 °C for 24 hours. The membrane is then put into 50 mL physiological sodium chloride at room temperature and soaked with a variation of immersion time of 1, 5, 15, 30, 60, 120, 360 and 1440 minutes. After soaking the membrane is placed on filter paper and weighed as the final weight [11].
2.6. Measurement of Collagen-Ciprofloxacin HCl Membrane Water Vapor Rate

This test follows the ASTM E96 standard [12]. Collagen-ciprofloxacin HCl membrane variation of gamma-rays irradiation dose of 0, 15 and 25 kGy is cut to the size of the surface of the cup and then placed on the surface of the cup that has been filled with 50 mL triple distilled water so that all holes are completely closed and the membrane does not touch with water. The cup which has been covered by the membrane is put into the oven at 37 °C for 48 hours and the rate of evaporation of water is calculated every 24 hours. The rate of evaporation of water against the membrane is calculated as follows [12]

\[
\text{Water vapor rate} = \frac{G}{t \times A}
\]

Where G is the difference from the weight of the cup before being put into the oven with the weight of the cup after being put into the oven, t is the time required for the change in weight of the cup and A is the surface area of the cup covered by the collagen-ciprofloxacin HCl membrane.

2.7. Measurement of Tensile Strength and Endurance of Collagen-Ciprofloxacin HCl Membrane

Collagen-ciprofloxacin HCl membranes with various doses of gamma irradiation of 0, 15 and 25 kGy were printed using a dumbbell tool to obtain a standardized form of measurement. The dumbbell shape membrane then measured its thickness using a Mitutoyo micrometer. The membrane is then measured by the tensile strength and elongation at break using the Instron 5944 tensile test tool at a speed of 10 mm/minute. Tensile strength is calculated using the formula [9]

\[
\text{Tensile strength, } \sigma \text{ (MPa)} = \frac{F}{A}
\]

Elongation at break = \[
\frac{(L - L_0)}{L_0} \times 100\%
\]

Where F is the loading and A is the membrane width times the membrane thickness, L is the extension of break and L0 is the length of the membrane area before breaking.

2.8. Percent Release of Ciprofloxacin HCl from Collagen Membranes

Collagen-ciprofloxacin HCl membranes resulting from gamma irradiation were immersed in a physiological sodium chloride solution at 100 mL at 37 °C and put into the incubator shaker. Observations are made every time 0.5; 1; 2; 3; and 6 hours. At each measurement time, the sample was pipetted as much as 5 mL of solution. Uptake is measured using a UV-Vis spectrophotometer at the maximum wavelength obtained [13].

3. Result and discussion

The FTIR spectrum of collagen-ciprofloxacin HCl membrane gamma irradiation at a varied dose of 0, 15 and 25 kGy is presented in Figure 1. Infrared spectra show the ionic bond of ciprofloxacin HCl to collagen as evidenced by the appearance of peaks in the gamma irradiation dose variation of 0, 15 and 25 kGy at wavenumbers 3538 cm\(^{-1}\), 3579 cm\(^{-1}\), and 3579 cm\(^{-1}\) respectively which is stretching the -OH carboxylate group from ciprofloxacin HCl and also shows the presence of -NH groups derived from amide A collagen. The characteristics of collagen are also shown by the appearance of peaks at wave numbers 2956 cm\(^{-1}\), 2946 cm\(^{-1}\), and 2928 cm\(^{-1}\), which shows amide B, while the characteristics of ciprofloxacin HCl are indicated by the peak at wavenumbers 1683 cm\(^{-1}\), 1685 cm\(^{-1}\), 1691 cm\(^{-1}\), which
shows the C = O quinolone group specific to the ciprofloxacin HCl group. It also appeared the peak at wave number 1269 cm\(^{-1}\) in all three variations of gamma-rays irradiation doses which showed the presence of amide III groups derived from collagen and -CF groups derived from ciprofloxacin HCl. The presence of amide III can be used to confirm the triple helix structure of collagen, which indicates that gamma-rays irradiation with a dose of 15 kGy and 25 kGy does not integrate collagen into gelatin which is proven by the presence of a triple helix structure [4]. The spectra for ciprofloxacin HCl both before and after gamma-ray irradiation showed no change or shift in functional groups and still showed specific functions of ciprofloxacin HCl. This is following Mohizea's research [14] which says ciprofloxacin HCl functional groups do not change after gamma-rays irradiation.

![Infrared spectra of collagen-ciprofloxacin HCl membrane after gamma-rays irradiation at a dose (a) 0 kGy, (b) 15 kGy, (c) 25 kGy](image)

**Figure 1.** Infrared spectra of collagen-ciprofloxacin HCl membrane after gamma-rays irradiation at a dose (a) 0 kGy, (b) 15 kGy, (c) 25 kGy

The results of pore measurements using SEM on collagen-ciprofloxacin HCl membranes at varied gamma irradiation doses of 0, 15 and 25 kGy using SEM are presented in Figure 2. From the results of SEM micrographs, it can be seen that the collagen-ciprofloxacin HCl membrane resulting from gamma-rays irradiation at a dose of 25 kGy has the largest pore size of 904.4 nm followed by the collagen-ciprofloxacin HCl membrane with a gamma-rays irradiation dose of 15 kGy with a pore size of 750.1 nm. The smallest pore was shown by the collagen-ciprofloxacin HCl membrane without irradiation (0 kGy) with a pore size of 469 nm. This shows that the greater the dose of gamma-rays irradiation, the larger the pore size of the collagen-ciprofloxacin HCl membrane.
Figure 2. SEM micrograph of collagen-ciprofloxacin HCl membrane gamma-rays irradiation dose (a) 0 kGy, (b) 15 kGy, (c) 25 kGy

Water absorption is a ratio of the weight of the membrane in a state of absorbing water to the dry weight of the membrane. The measurement of water absorption is one of the main parameters used to express the amount of water absorbed by the membrane. The relationship of the ability of the collagen-ciprofloxacin HCl membrane to absorb water affected by variations of gamma-rays irradiation doses is presented in Table 1.

Based on the curve, it is seen that the higher the dose of gamma-rays irradiation given, the higher the water absorption capacity. This might be due to the standard structure of collagen consisting of glycine, proline, and hydroxyproline which are polar functional groups but are located within the structure so they cannot be in direct contact with water, in this study physiological sodium chloride solution. When exposed to gamma-rays irradiation, the hydrogen bond that binds the -NH group with O = C in the peptide bond may break, causing new absorption sites to form which results in increased absorption [9]. This result is also supported by pore size data measured using SEM which states that the greater the gamma-rays irradiation, the greater the membrane pore, thus explaining the greater water absorption also due to the larger pore size.

Table 1. Water absorption of collagen-ciprofloxacin HCl membrane after gamma-rays irradiation at doses of 0 kGy, 15 kGy and 25 kGy

| Time (minutes) | Water Absorption (%) |
|---------------|----------------------|
|               | 0 kGy                | 15 kGy                | 25 kGy                |
| 1             | 12.05 ± 10.06        | 51.51 ± 10.66         | 85.72 ± 6.38          |
| 5             | 47.84 ± 27.44        | 52.61 ± 14.02         | 142.98 ± 26.05        |
| 15            | 48.22 ± 10.15        | 52.83 ± 11.08         | 148.76 ± 16.43        |
| 30            | 70.26 ± 38.72        | 130.07 ± 14.72        | 154.23 ± 34.31        |
| 60            | 98.58 ± 21.46        | 131.71 ± 6.04         | 158.13 ± 32.21        |
| 120           | 118.47 ± 3.93        | 137.92 ± 7.03         | 158.56 ± 13.65        |
Based on the data, the greatest water absorption is produced by a membrane with a variation of 25 kGy gamma-rays irradiation dose with a soaking time of 1440 minutes which is 230.84% followed by a membrane variation of 15 kGy doses of 163.06% and the last is a membrane dose variation of 0 kGy in the amount of 144.31%. According to Ratnawati et al. [15], the water absorption value of wound dressing must be in 200-500%, however from these results indicate that only 25 kGy gamma-rays membranes which close to this value. It means that 15 kGy and non-irradiated membranes have not met yet this requirement.

Collagen-ciprofloxacin HCl membrane with 25 kGy gamma-rays irradiation dose has the largest evaporation rate followed by 15 kGy gamma-rays irradiation membrane and 0 kGy respectively. The higher the dose of gamma-rays irradiation, the higher the rate of membrane drying. Based on statistical results, there was no significant difference in the collagen-ciprofloxacin HCl membrane variation in gamma-rays irradiation doses of 0, 15 and 25 kGy so it can be said that gamma-rays irradiation did not affect the rate of water evaporation. The greater the rate of evaporation as the dose of gamma-rays irradiation increases can be caused by the greater pore size of the membrane. The larger the pore size, the greater the ability of gas to get in and out. Another effect that causes the greater rate of evaporation is the presence of gamma-rays irradiation allowing the termination of peptide bonds in collagen which results in the more tenuous physical bond of collagen [4]. The required water evaporation rate for the wound dressing membrane is 279 g/m².h while the largest evaporation rate obtained by the collagen-ciprofloxacin HCl membrane is 198.78 g/m².h which means that the result does not meet the requirements. This can be caused by the membrane of collagen-ciprofloxacin HCl that is too thin. Membranes have thicknesses of ± 0.0167 mm while thickness requirements range from 1-3 mils (0.0254 - 0.0762 mm) [16].

The results of the measurement of the rate of evaporation of water against collagen-ciprofloxacin HCl membrane samples are shown in Figure 3.

![Figure 3](attachment:image.png)

**Figure 3.** Effect of gamma-rays irradiation doses 0, 15, and 25 kGy on the rate of evaporation of the collagen-ciprofloxacin HCl membrane.

The results of testing the tensile strength and collagen-ciprofloxacin HCl membrane are shown in Figure 4.
Figure 4. Effect of gamma-ray irradiation dose 0, 15, and 25 kGy on the tensile strength of the collagen-ciprofloxacin HCl membrane

Based on the graph, it appears that the higher the dose of gamma-rays irradiation given, the tensile strength of the collagen-ciprofloxacin HCl membrane decreases. This result may be due to a bond-breaking caused by exposure to gamma-rays irradiation. Breaking of these bonds causes the breakdown of inter and intramolecular -H-O-H hydrogen bonds in collagen [17]. Moreover, it can also be caused by irradiation breaking the peptide bonds of collagen, resulting in a decrease in the tensile strength of the collagen-ciprofloxacin HCl membrane [14]. The results of the collagen breakdown membrane test for ciprofloxacin HCl are shown in Figure 5.

Figure 5. Effect of gamma-rays irradiation dose 0, 15 and 25 kGy on elongation at break of collagen-ciprofloxacin HCl membrane

Similar to the results of tensile strength, the presence of gamma-rays irradiation at 15 and 25 kGy dose variations cause the extension of the collagen-ciprofloxacin HCl membrane to break up. This can be caused by the effect of gamma irradiation on collagen peptide bonds. Based on the results of the static analysis for tensile strength and elongation at the break it does not show any significant difference, thus it can be concluded that gamma-rays irradiation does not affect the tensile strength and elongation of collagen-ciprofloxacin HCl membrane breaks.
The ciprofloxacin HCl release test was used to determine the cumulative amount of the drug released from the collagen membrane. The curve of the effect of gamma-rays irradiation dose on the percent release of the collagen membrane is presented in Figure 6.

![Figure 6](image-url)

**Figure 6.** Effect of gamma-rays irradiation on percent release of ciprofloxacin HCl

Based on the curve, it can be seen that the largest percent release of ciprofloxacin HCl is produced by a membrane of collagen-ciprofloxacin HCl with a dose of 25 kGy gamma rays by 100% followed by a membrane dose of 15 kGy by 90.11% and finally the membrane dose is 0 kGy by 87.11%. The membrane releases ciprofloxacin HCl until 100% is achieved at the time of the release of 6 hours. The curve also shows the release of drugs in large concentrations in the early minutes, known as the phenomenon of burst effect. This phenomenon may be caused by a large number of drugs on the membrane surface so that more drugs are released [18].

The curve also shows that the greater the dose of gamma-rays irradiation given, the greater the percent of release. This might be due to the basic structure of ciprofloxacin HCl in crystal form which changes to the amorphous shape when exposed to gamma-rays irradiation [14]. It is known that the amorphous form has a higher solubility than the crystalline form so that the resulting release is also greater. Based on statistical analysis, there is an effect of gamma-rays irradiation on the percent release of ciprofloxacin HCl. Based on Tukey’s posthoc analysis, there was a significant difference between the membrane variation of the dose group 0 kGy with 25 kGy, and the dose of 15 kGy with 25 kGy, whereas in the variation of the dose group 0 kGy with 15 kGy there was no significant difference. These results are consistent with research by Bozdag et al [19] which states that greater gamma-rays irradiation given influence to the changes of pore size. It is due to a polymer chain breakdown that leads to a disturbing pore network.

**4. Conclusion**

Gamma-rays irradiation does not change the chemical structure of collagen and ciprofloxacin hydrochloride but gamma-rays irradiation can change the pore size of collagen and the shape of ciprofloxacin HCl crystals resulting in changes in water absorption and percent release of membranes. The higher the dose of gamma-rays irradiation, the greater the absorption of water (144.31 to 231%) and the percent release of the membranes (87.11 to 100%).
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