Production and Characterization of Membranes from Collagen of Carp Scales (Cyprinus carpio)

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Abstract

The development of barrier membranes with non-mammalian fish collagen material for Guided Bone Regeneration (GBR) in the treatment of alveolar bone defects due to periodontitis, has the advantage of low risk of infectious disease transmission in tissue regeneration. This study aimed to produce and characterize a barrier membrane derived from carp scale collagen material.

This study conducted the manufacture of collagen membranes with formula 1 consisting of 3% carp scale collagen, 2% chitosan and 7.5% polyvinyl alcohol (PVA), and formula 2 consisting of 3% carp scale collagen and 7.5% PVA by solution mixing and film printing. Membrane characterization with water absorption test, mechanical test, SEM and EDS characterization, and Fourier transform infrared (FTIR) spectroscopy test.

This study found a significant difference in water absorption between the two membranes; the elastic modulus, tensile strength and breaking strain of membrane formula 1 are 0.38 Mpa, 7.82 N/mm2 and 2.51%, smaller than membrane formula 2 which are 1.00058 Mpa, 21.95 N/mm2 and 23.69%; SEM image of membrane formula 1 shows surface porous and bonding between elements is better than formula 2; EDS results and map sum spectrum of membrane formula 1 showed detected elements of C (59%), O (40%), Ca (0.6%), and Si, Al and Mg, while formula 2 detected elements of C (59.7%), O (40.1%), and Si and Ca; FTIR spectra showed that both membranes have tri helical functional groups as a requirement for barrier membranes.

Periodontal regenerative surgery barrier membrane based on carp scale collagen can be effectively produced with desired properties.

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Introduction

Chronic periodontitis is an infectious disease caused by bacteria that leads to inflammation and gradual destruction of the tissues that support the teeth, notably the alveolar bone.[1] Guided Bone Regeneration (GBR) represents a modern remedy that rectifies bone deficiencies, enhancing the form and performance of the periodontium tissue.[2-5] Regeneration involves replacing and reconstructing damaged or missing body parts with cells and tissues that share the same form and function.[4] In the context of dental surgery, guided bone regeneration (GBR) necessitates a barrier membrane to regulate gum tissue and bone growth at the site of bone deficiency.[6] The function of the barrier membrane in GBR is to obstruct fibroblasts from migrating apically to the bone deficiency site and facilitate the growth of bone precursor cells.[2-5]

Membrane collagen, composed mainly of type I collagen and typically derived from

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mammals like pigs or cows, serves as a common barrier material in GTR/GBR.[6] However, due to the possibility of transmitting bovine spongiform encephalopathy to humans, the use of bovine collagen has been reevaluated.[7] In recent times, fish collagen sourced from shark and salmon skin has garnered considerable interest due to its low risk of infectious disease transmission.[8]

Collagen from fish is a viable source of raw materials meeting industry criteria, considering the potential use of by-products typically unutilized for consumption. As such, collagen production on commercial scales should not interfere with fish availability as regular nutrition for the community, ultimately helping to keep prices low. These criteria make using fish scales a promising resource.[9] Fish scales are abundant in organic compounds, such as collagen and ichtylepidin, with a protein content of 41-84%.[8] Nagai et al's research indicates that fish scales contain 70% water, 27% protein, 1% fat, and 2% ash.[8]

Fish scales are a largely underutilized waste product.[9] Fish scales, which are obtained in large quantities from the fish fillet industry, can serve as a source of collagen. However, the increase in fish production inevitably leads to an increase in fish waste in the form of both skin and scales.[9] A significant issue that arises is the lack of processing facilities for fishery waste, particularly for fish skin and scales.[9]

Indonesia is abundant in fish products, including the popular carp (Cyprinus carpio). Carp (Cyprinus carpio) is a common freshwater fish in Indonesia, particularly in West Java.[10,11] Studies have shown that carp are successfully cultivated in fast-moving waters.[10-13] These fish have high collagen content, with collagen from carp scales similar to that found in mammals.[10,14] This potential alternative collagen source could replace mammalian collagen for membrane production.[10,14,15] Multiple studies have confirmed the high collagen content in carp (Cyprinus carpio) scales.[10,11,13-15]. Research has investigated the application of fish collagen for membranes to treat alveolar bone defects in cases of periodontal disease.[16] One study utilized collagen from white snapper fish scales as a fundamental material for producing barrier membranes.[17] However, the deep sea habitat of this fish species poses challenges in obtaining it.[16,17] The freshwater fish, carp (Cyprinus carpio), is extensively and easily cultivated in Indonesia, particularly in West Java.[10-13] The physicochemical properties of collagen derived from carp scales are similar to mammalian collagen, making it a potential substitute for mammalian collagen as a membrane manufacturing source.[10,14,15] Carp scales (Cyprinus carpio) contain collagen, which is crucial in the process of wound healing and hemostasis.[14,15]

It is worth considering the exploitation of carp scale collagen as a replacement for mammalian collagen in future research.

Materials and methods

This study presents a methodology for the production of collagen membranes from carp scales (Cyprinus carpio). The research is descriptive, and the two formulations involved are formula 1, which consists of a 3% concentration of carp scale collagen, 2% concentration of chitosan, and 7.5% concentration of polyvinyl alcohol (PVA), and formula 2, which comprises 3% carp scale collagen and 7.5% concentration of polyvinyl alcohol (PVA). Both formulations underwent several tests, including tensile testing, water absorption testing, FTIR analysis, SEM, and EDS. These experiments were performed at the Laboratory of PT Biomedical Technology Indonesia, which is a subsidiary of PT BLST Holding Company of IPB.

Materials required for producing collagen membranes include carp scales (Cyprinus carpio), 0.9 N saline solution, 1N NaOH, 5% polyvinyl alcohol, 1N CH3COOH, potassium bromide, surfactant, and chitosan.

The production of carp scale collagen membranes necessitates a homogenizer mixer, petri dishes, acrylic molds measuring 75mm x 75mm x 50mm, as well as laboratory glassware or tubes.

The mechanical properties of said membranes were measured through the use of both a Shimadzu AGS-X series Universal Testing Machine (UTM) with a capacity of 10 kN, as well as Scanning Electron Microscopy (SEM) via a Thermofisher Quanta 650 SEM with Oxford Instruments 15 Xplore EDS. High vacuum SEM is utilized to achieve magnifications of 1000x, 2000x, 5000x, and 10,000x with a spot size of 4.0 and a high voltage of 2.0 kV. Additionally, Fourier Transform Infrared (FTIR) spectroscopy is employed using a Tensor 37 instrument with a

MIR light source and DTGS detector, and 32 scans are performed at a resolution of 4cm-1. Additionally, Fourier Transform Infrared (FTIR) spectroscopy is employed using a Tensor 37 instrument with a MIR light source and DTGS detector, and 32 scans are performed at a resolution of 4cm-1.

The process for producing a collagen membrane using carp scale collagen involves two separate formulations. Functional group testing with FTIR, water absorption and mechanical testing with UTM, morphology testing with SEM, and EDS testing were performed on both membrane formulations.

The first formula is to make a 3% collagen solution in acetic acid and homogenize it. Making a 2% chitosan solution. Making polyvinyl alcohol (PVA) solution with a concentration variation of 7.5%. Mixing 3% collagen solution + 2% chitosan + 7.5% PVA, with a ratio of 1:1:1 followed by a homogenization process. Pour the composite into an acrylic mold, then dry at room temperature until dry for 3-4 days. Soaking the dried membrane in NaOH solution for 1 hour. Wash the membrane until base-free (neutral pH). Freezing the membrane in a deep freezer before drying by lyophilization for 24 hours. Drying the membrane by lyophilization with a Freeze Dryer.

Description:
A: 3% carp scale collagen + 2% chitosan + 7.5% polyvinyl alcohol (PVA) membrane
B: 3% carp scale collagen+7.5% polyvinyl alcohol (PVA) membrane

Figure 1. The results of the Carp Scales Membrane (Cyprinus carpio).

The second formula procedure is to make a 3% collagen solution in acetic acid and homogenize it. Making a 2% chitosan solution. Making PVA solution with a concentration variation of 7.5%. Mixing 3% collagen solution with 7.5% polyvinyl alcohol PVA, in a ratio of 1:1 followed by a homogenization process. Pour the composite into an acrylic mold, then dry at room temperature until dry for 3-4 days. Soaking the dried membrane in NaOH solution for 1 hour. Wash the membrane until base-free (neutral pH). Freezing the membrane in a deep freezer before drying by lyophilization for 24 hours. Drying the membrane by lyophilization with a Freeze Dryer.

Statistical Analysis

Data were presented solely in a descriptive manner, providing mean values and standard deviations of triplicate measurements. Additionally, the mechanical test evaluation, SEM, EDS, and FTIR results were described in qualitative terms.

Results

The production of collagen membranes from samples A and B of Cyprinus carpio carp scales is presented in Figure 1.

Sample	Number	Mean (%)	Std. Deviation	P-value
A	3	289,26	22,51	0,011*
B	3	385,13	29,70	0,013*

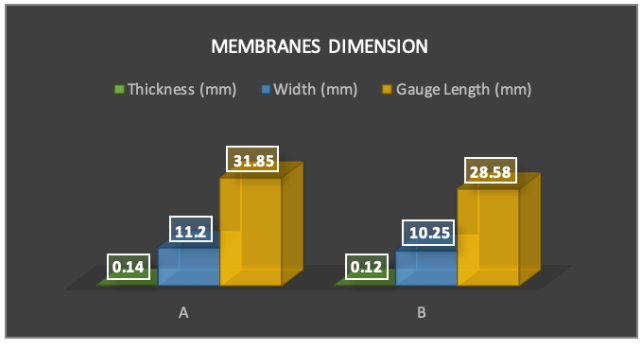
Table 1. Water Absorbency of Carp Scales Membrane (Cyprinus carpio).
Description:
F count = 0.596
P < 0.05 is significantly different
A: 3% carp scale collagen + 2% chitosan + 7.5% polyvinyl alcohol (PVA) membrane
B: 3% carp scale collagen+7.5% polyvinyl alcohol (PVA) membrane.

The water absorbency of the membrane was tested three times for each sample. Table 1 presents the membrane absorption test results for samples A and B, indicating a significant difference between them with a 95% confidence level, as the p-value is less than 0.05.

The measurements of the membrane of carp (Cyprinus carpio) scales are illustrated in Figure 2. Sample A exhibits a thickness of 0.14mm and a width of 11.2mm, whereas sample B has a thickness of 0.12mm and a width of 10.25mm.

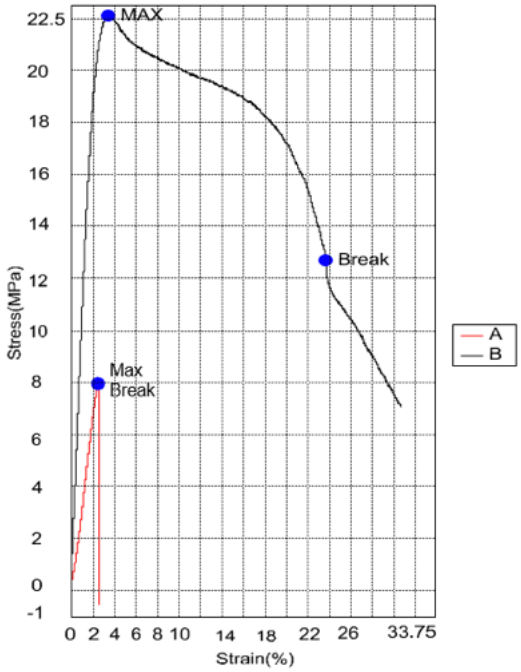
The graph in Figure 3 displays the results of mechanical testing on samples A and B using a universal testing machine. Sample A demonstrates an elastic modulus of 0.38 MPa, with a maximum tensile strength of 7.82 N/mm² and a strain to break of 2.51%. On the other hand, sample B has an elastic modulus of

1.00058 MPa with a maximum tensile strength of 21.95 N/mm² resulting in a strain to break of 23.69%. The maximum force that sample A can receive is 12.26 Newtons, whereas sample B can handle up to 27 Newtons.



Description:
A: 3% carp scale collagen + 2% chitosan + 7.5% polyvinyl alcohol (PVA) membrane
B: 3% carp scale collagen+7.5% polyvinyl alcohol (PVA) membrane

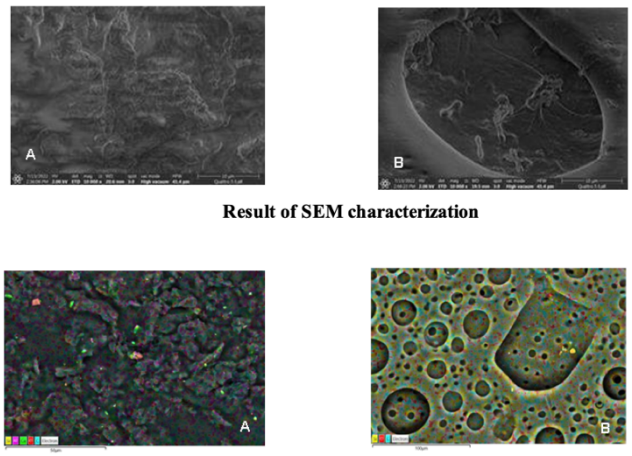
Figure 2. Dimensions of Carp Scales Membrane (Cyprinus carpio).



Description:
A: 3% carp scale collagen + 2% chitosan + 7.5% polyvinyl alcohol (PVA) membrane
B: 3% carp scale collagen+7.5% polyvinyl alcohol (PVA) membrane

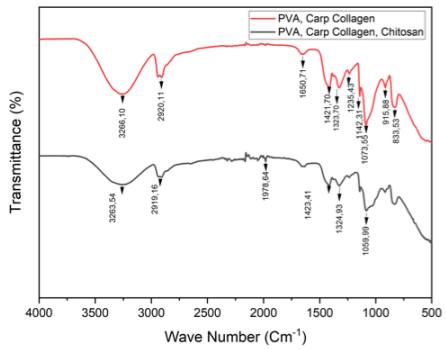
Figure 3. Graph of the elongation of the membrane break of carp scales (Cyprinus carpio).

SEM test results (Figure 4 upper) of sample A reveal crystals with an oval shape that are interconnected and agglomerated, whereas sample B exhibits an unconnected, spherical crystal membrane. EDS analysis (Figure 4 lower) detected the presence of C, O, Ca, Si, Al, and Mg in sample A, but only C, O, Si, and Ca in sample B. The Map Sum Spectrum findings reveal that sample A consists of 59% carbon, 40% oxygen, and 0.6% calcium, alongside minimal amounts of silicon, aluminum, and magnesium. Likewise, sample B (Figure 8) contains 59.7% carbon, 40.1% oxygen, and trace amounts of silicon and calcium.



Description:
A: 3% carp scale collagen + 2% chitosan + 7.5% polyvinyl alcohol (PVA) membrane
B: 3% carp scale collagen+7.5% polyvinyl alcohol (PVA) membrane

Figure 4. SEM and EDS characterization test results of Carp Scales Membrane (Cyprinus carpio) with 1000x magnification.



Description:
A (black line) : 3% carp scale collagen + 2% chitosan + 7.5% polyvinyl alcohol (PVA) membrane
B (red line) : 3% carp scale collagen+7.5% polyvinyl alcohol (PVA) membrane

Figure 5. FTIR Spectrum Results of Carp Scales Membrane (Cyprinus carpio).

The findings from the infrared spectra are presented in the FTIR curve graph (Figure 5). The absorption curves of amide A and amide B are visible with sample A showing absorption between 3263.54 - 2919.16 cm^{-1}, and sample B displaying absorption from 3266.10 - 2920.11 cm^{-1}. Another group, amide I, is identified with sample A remaining unidentified, whereas sample B shows an absorption region of

1650.71cm^{-1}. Additionally, the absorption region of amide II is observed in sample A at 1423.41 cm^{-1} and in sample B at 1421.70cm^{-1}. The final distinct absorption range corresponds to amide III, with sample A showing an absorption at 1059.99cm^{-1} and sample B at 1235.43cm^{-1}.

Discussion

Collagen can be sourced from discarded fish skin, scales, and bones, which can be easily extracted and purified.[18] Fish scales contain various elements, including proximate, calcium, chitin, alkaloids, benedict, bluret, and ninhydrin.[8] The carp's body is predominantly covered by elasmoid scales anchored in the dermis pocket, layered or overlapping.[19]

Fish collagen offers numerous advantages, such as high safety (without the risks of swine foot and mouth disease, bovine spongiform encephalopathy, or bovine transmissible spongiform encephalopathy), high absorbability, low cost, and biocompatibility.[20] These properties make fish collagen suitable for various applications in fields such as health food, cosmetics, and biomedicine.[20] A study analyzed Cyprinus carpio fish scales and determined that 22.1% to 23.9% of the scales' weight comprised protein with low fat and carbohydrate content.[21]

The production of membranes in this investigation involves creating a mixture of collagen, the primary base material obtained from *Cyprinus carpio* (carp) scales, with a 3% concentration of chitosan and 7.5% polyvinylalcohol (PVA) crosslinker. To ensure optimal mechanical properties, the quality of intermolecular interactions between the polymer components incorporated in this combination determines the mixing process.[22]

The thickness of collagen membranes can vary depending on their intended use and application.[23-27] In tissue engineering applications, collagen membranes with a minimum thickness of around 0.1 to 1 mm are viable. A membrane's thickness of approximately 0.2 to 1 mm can provide the necessary structural support for cell and tissue growth, particularly in bone regeneration procedures such as gap closure and bone augmentation.[23] Collagen membranes are commonly used due to their suitability. [23,26,27] This thickness helps in maintaining the required space for bone regeneration, as evidenced by various sources.[26-27]

The elasticity modulus, which demonstrates the tensile strength of the 3% carp scales, 2% chitosan, and 7.5% PVA membrane, is less than 1 MPa. Previous research suggests that this is because the primary constituent elements are not solely derived from natural materials.[22] As a general guide for collagen membranes from fish scales, some studies have shown that the tensile breaking strength of collagen membranes can range from 1 to 20 MPa, depending on factors such as the type of fish, production method, and membrane processing. Membranes within this tensile strength range may have numerous applications in tissue engineering or wound care.[18, 28] Collagen membrane's elastic modulus can vary greatly, ranging from 0.1 to 2 GPa. The modulus of elasticity measures a material's ability to bounce back to its original shape when subjected to a force.[18,28] This range accurately depicts the various levels of elasticity found in collagen membranes.[18,28]

Chemicals such as aldehydes, glutaraldehyde, and polyvinylalcohol can be used as crosslinkers to enhance the mechanical and chemical properties of collagen membranes.[29] Gamma irradiation is employed to sterilize the collagen membrane.[18,30] A sterilization process using 25kGy gamma irradiation was employed in this study.[30]

Scaffolds need to possess high porosity and interconnected porous structures to aid in cell attachment, proliferation, and differentiation.[31] In the current study, SEM analysis illustrated that the collagen and chitosan components were mixed uniformly, and the size of pores on the surface of the collagen membrane was approximately 30 µm. The study's findings align with Mighri et al's (2015) research, in which chitosan-coated collagen membranes were created and analyzed. Scanning electron microscope (SEM) imagery demonstrated surface variations on the chitosan-coated membranes, with pore sizes ranging between 20 and 50 µm.[22] Different results were presented in a study carried out by Rajam et al, 2014, which indicated that a Scanning Electron Microscopic (SEM) analysis of the surface pores of a collagen-chitosan scaffold (COL-CS) showed that they were well interconnected with an average diameter of 75-150 microns.[31]

Carp-scale collagen, chitosan, and PVA membranes reveal the presence of Si, Al, and

Mg through EDS testing. This can be attributed to the substrate used, which contains these elements. The presence of Calcium (Ca) in collagen, which consists only of amino acids (C, H, and O), is unexpected. This membrane may have been altered to include components beyond pure collagen.

EDS can analyze the chemical elements present within the collagen membrane. EDS analysis can offer insight into the membrane's chemical makeup, including the presence of carbon (C), oxygen (O), nitrogen (N), and any other elements that may be found in the membrane, such as calcium (Ca) from the bone collagen source or those arising during the manufacturing process.[32,33] Through the application of EDS, important details regarding the membrane's composition can be gleaned, particularly if certain elements are added during production or the membrane derives from a source abundant in specific elements. Additionally, this analysis permits the identification of any additional elements present in the membrane that may improve its functionality. This can aid comprehension of the physical and chemical properties of collagen membranes in diverse applications, including tissue engineering and medicinal products.[32,33]

Conclusions

The results of the testing indicate that collagen membranes comprising 3% collagen derived from carp scales, 2% chitosan, and 7.5% polyvinyl alcohol (PVA) possess an elastic modulus of 0.38 Mpa and a tensile strength at break of 2.51%. Additionally, the water absorption rate of the membranes was found to be 289. These findings demonstrate the potential of these membranes for use in biomedical applications. 27%; The collagen-based membranes that meet the minimum requirements have a triple helix formation, as confirmed by FTIR analysis. SEM images reveal that the crystals have oval shapes and are interconnected and agglomerated. The EDS composition of elements includes C, O, and Ca, as well as Si, Al, and Mg. These results are superior to those obtained from testing membranes with 3% collagen from carp scales and 7.5% polyvinyl alcohol (PVA). A barrier membrane for periodontal regenerative surgery can be efficiently manufactured using collagen derived from carp scales, possessing the desired properties.

Declaration of Interest

The authors report no conflict of interest.

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