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Original Article

Development of fish collagen-poly(vinyl) alcohol hydrogel using natural polymers for wound dressing

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Abstract

This study presents the development and characterization of *Pangasionodon hypothalamus* fish collagen-based polyvinyl alcohol hydrogels incorporated with natural polymers, konjac glucomannan (KGM), hyaluronic acid (HA) and carrageenan (CA) for potential wound dressing applications. Using a freeze-thaw method, COLKGM@PVA, COLHA@PVA and COLCA@PVA hydrogels were fabricated and evaluated for their physicochemical and biological properties. All hydrogels demonstrated excellent swelling capacity (>400%), appropriate water vapor transmission rates, and porosity levels favorable for wound healing. Mechanical properties showed COLCA@PVA had the highest tensile strength while COLHA@PVA offered superior flexibility. *In vitro* permeation studies revealed sustained amoxicillin release from COLHA@PVA and COLCA@PVA, aligning with first-order kinetics. Antibacterial assays confirmed that both drug-loaded and unloaded hydrogels exhibited inhibitory effects against *S. aureus* and *E. coli*, attributed to the inherent bioactivity of the incorporated natural polymers. Among them, COLCA@PVA showed the most balanced profile in terms of mechanical integrity, drug release and antimicrobial efficacy. These findings suggest that COL-based composite hydrogels, particularly COLCA@PVA, hold promise as multifunctional wound dressings with controlled drug delivery capabilities.

Keywords: *Pangasionodon hypothalamus*, collagen, natural polymer, wound dressing

1. Introduction

Wound healing is a multifaceted process involving intricate biological and physiological mechanisms. Effective wound management is essential for promoting tissue repair, preventing infections and enhancing patient outcomes. The complexity of this process hinges upon numerous factors such as wound size, location, and patient health and immune function, all influencing the speed and quality of healing (Anderson & Hamm, 2012). In recent decades, significant studies have been made in developing advanced wound dressings, leveraging naturally derived polymers like collagen, chitosan, cellulose, alginate, gelatin, konjac glucomannan (KGM), hyaluronic acid (HA) and carrageenan (CA), as well as their derivatives and combinations with synthetic polymers such as poly(vinyl alcohol) (PVA). These materials have been integrated into various formats including skin substitutes, hydrocolloids and hydrogel dressings (Shen, Shamshina, Berton, Gurau, & Rogers, 2010; Ubaid & Murtaza, 2018).

Among these, hydrogels are particularly promising due to their high water retention, biocompatibility, and flexibility, creating an optimal healing environment (Del Valle, Díaz, & Puiggalí, 2017). Collagen, a key component of the extracellular matrix, supports cell attachment and tissue regeneration (Moura *et al.,* 2014). Fish-derived collagen offers a safer alternative to mammalian sources, avoiding zoonotic risks and religious concerns. However, fish collagen hydrogels often lack mechanical strength (Rigogliuso, Campora, Notarbartolo, & Ghersi, 2023). PVA, known for its

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biodegradability and mechanical properties, is commonly used to reinforce collagen hydrogels (Sonker, Rathore, Teotia, Kumar, & Verma, 2019), though it also has limitations (Hago & Li, 2013). Therefore, to enhance performance, natural polymers like KGM, HA, and CA were incorporated into fish collagen-PVA hydrogels using a freeze-thaw method, which avoids toxic crosslinkers and improves structural integrity (Adelnia *et al.,* 2022). This study investigates the swelling behavior, porosity, WVTR, mechanical strength, drug release and antibacterial activity of these hydrogels, aiming to develop multifunctional wound dressings.

2. Materials and Methods

2.1 Materials

Pepsin-solubilized collagen (PSC) was extracted from *Pangasianodon hypophthalmus* (*P. hypophthalmus*) skin in the laboratory using standard reported techniques (Hukmi & Sarbon, 2018; Singh, Benjakul, Maqsood, & Kishimura, 2011). The *P. hypophthalmus* fish was purchased from a local fish farm in Kuantan, Pahang (Malaysia). Polyvinyl alcohol (PVA, Mw = 89000-98000, 99+% hydrolyzed) was purchased from Sigma (St. Louis, MO, USA). Acetic acid, ethanol and phosphate buffered saline (PBS) were purchased from Merck (Darmstadt, Germany). Carrageenan (CA) and konjac glucomannan (KGM) were purchased from Zhejiang Top Hydrocolloids CO (Zhejiang, China). Hyaluronic acid (HA) was purchased from the Eva Chem Sdn. Bhd (Selangor, Malaysia).

2.2 Extraction of fish collagen

2.2.1 Preparation of fish skin

The *P. hypophthalmus* skin was prepared by rinsing it with cold water (5-8°C) and then cutting it into 5 cm × 5 cm small pieces. These prepared skin samples were placed in polyethylene bags and kept at -20 °C until they were utilized (Singh *et al.,* 2011). The preservation duration in freezer (-20°C) was not more than one month.

2.2.2 Pretreatment of fish skin

To eliminate the non-collagenous protein, the produced fish skin was soaked in 0.1M NaOH at a 1:10 alkali solution ratio (w/v). Then the mixture underwent continuous agitation for 12 h at 4 °C, and the solution was replaced every 2 h. Subsequently, the processed skin underwent rinsing with cold water until the wash water reached a neutral or slightly basic pH (Singh *et al.,* 2011).

2.2.3 Extraction of pepsin-soluble collagen (PSC)

To obtain collagen from the skin of *Pangasianodon hypophthalmus*, the initial skin underwent a defatting process using 10% butyl alcohol at a solid-to-solvent ratio of 1:10 (w/v) for 6 days, with solvent changes every 12 h. After defatting, the skin was washed with deionized water and then immersed in a solution of 0.5M acetic acid and 0.05% pepsin (w/w) at a solid-to solvent ratio of 1:10 (w/v) for 24 h. The resulting mixture was filtered through two layers of cheesecloth and the residue was subjected to a repeat extraction under the same conditions, yielding crude collagen. The crude collagen was subsequently combined with a mixture of 0.05 M Tris (hydroxymethyl) aminomethane and 2.6 M NaCl (powder). After centrifugation for 15 min at 4,000 rpm, the pellet was dissolved in a minimal amount of deionized water over 24 h. PSC was produced by freeze drying the dialysate for 7 days. The yield of PSC was determined by calculating the proportion of the dry weight of extracted collagen in comparison to the wet weight of the initial skin used (Hukmi & Sarbon, 2018). Percentage yield of PSC was calculated from the following equation:

$$\text{Yield of PSC (\%)} = \frac{\text{Dry weight of collagen extract}}{\text{Wet weight of initial skin used}} \text{ X } 100$$

2.3 Preparations of hydrogels

2.3.1 Preparation of stock solutions

The study involved the preparation of *P. hypophthalmus* fish collagen PVA hydrogels blended with various natural polymers, including KGM, HA and CA. An 8% (w/v) PVA stock solution was prepared by heating distilled water to 60-100°C and gradually dissolving the PVA powder with constant stirring for 2-4 hours. A 4% (w/v) COL stock solution was prepared by dissolving the freeze-dried *P. hypophthalmus* collagen in distilled water with the addition of three drops of 0.5 M acetic acid and then stirring with a homogenizer until completely dissolved. 1.5% (w/v) solutions of KGM and HA were prepared by adding the powders directly to the fish collagen-PVA hydrogel mixture without mixing with deionized water. For CA, a 5% (w/v) stock solution was prepared by dissolving the powder in deionized water at 35°C with constant stirring. The composite hydrogels were prepared by combining the PVA, COL and natural polymer solutions in the desired ratios.

2.3.2 Formulation of hydrogels

The hydrogel was formulated by mixing 1.5% (w/v) COL with 2.0% (w/v) PVA via physical cross-linking (Table 1) (Azuri, Forid, & Maznah, 2023). The COLKGM@PVA hydrogel was prepared by adding 1.5% (w/v) KGM powder to the COL-PVA mixture and stirring for 30 min. Similarly, for COLHA@PVA and COLCA@PVA hydrogel, 1.5% (w/v) HA and CA were added and stirred until homogenized. The pH of the composite hydrogel has been adjusted to 7.4. The solution was ultrasonically defoamed for 20 minutes to eliminate the bubbles, then put onto Petri dishes for 1 h at room temperature and thereafter frozen at -80 °C for 1 hour, followed by three consecutive freeze-thaw cycles, each consisting of freezing at −20 °C for 18 hours followed by thawing at 25 °C for 6 hours (Qi *et al.,*2015). To prepare Amoxicillin loaded composite hydrogel, the drug has been mixed with polymer at final stage of mixing on preparing A@COLKGM@PVA, A@COLHA @PVA and A@COLCA@PVA composite hydrogels.

Table 1. Formulations of fish collagen-PVA hydrogels using natural polymers

Formulation	Concentration of fish collagen (%)	Concentration of PVA (%)	Concentration of natural polymers (%)
COLKGM@PVA	1.5	2.0	KGM: 1.5
COLHA@PVA	1.5	2.0	HA: 1.5
COLCA@PVA	1.5	2.0	Carr: 1.5

2.4 Characterization of hydrogels

2.4.1 Swelling behaviour

The swelling capacity of hydrogel was determined following reported methods with slight modification (Baniasadi, Madani, Ajdary, Rojas, & Seppälä, 2021). Briefly, hydrogels were cut into pieces of square shaped specimens with dimensions of 1.5 cm x 1.5 cm. Then, the hydrogels were weighted (M0) and immersed in 50 mL of PBS at pH 7 for 24 h. The sample was taken out at 1, 2, 4, 8, 12 and 24 h. Blotting the samples lightly using filter paper to removed any excess PBS on their surfaces. Following that, the samples were weighed (M1) immediately. Swelling ratio (SR%) was calculated from the following equation below.

$$\text{Swelling ratio} = \frac{(M1-M0)}{M0} \text{ X } 100$$

where M0 is the weight of dry hydrogel before immersed in PBS solution and M1 is the weight of swollen hydrogel after immersion in PBS solution.

2.4.2 Water vapor transmission rate

The WVTR of hydrogel was investigated using the following reported method with slight modification (Bahadoran, Shamloo, & Nokoorani, 2021). Briefly, for the WVTR of hydrogels, 8mL of deionized water was injected into a centrifuge tube. Then, the hydrogels were cut into irregular specimens that can fully cover the opening of the centrifuge tube and then parafilm was used to seal the gap between the sample and the centrifuge tube. This can prevent gas leakage which can cause deviation in result. These tubes were placed in the incubator set at 37 °C and with a humidity of 75% RH (Relative Humidity). All samples were weighed after 0, 1, 2, 4, 6, 8, and 24 h. The WVTR of hydrogel was calculated using the following equation:

$$\text{WVTR} = \frac{(M0-Mt)}{A \text{ x } M0} \quad g/m^2\ h$$

where M0 is the initial weight of water and hydrogel, Mt is the final weight of water and hydrogel, and A is the test area of hydrogel. Each assay was repeated three times. The values are reported as the mean ± standard deviation (n=3).

2.4.3 Porosity measurement

The porosity of hydrogel was determined by a modified reported method, called the liquid displacement method (Li *et al.,* 2024). Briefly, the lyophilized hydrogel has been cut into a rectangular shape, and the volume (V) was calculated by measuring the sample's dimensions (length, width, and height) with a Vernier caliper. Then, the dried hydrogel samples were immersed in absolute ethanol (ρ = 0.79 g/cm^3) for 12 h. At the 0 h and 12 h, the samples were taken out and weighed directly after the excess ethanol on the sample surfaces was blotted by using filter paper. The porosity was calculated using the following equation.

$$\text{Porosity} = \frac{M2-M1}{pV} \text{ X } 100$$

where M1 is the weight of the hydrogel before it is immersed in absolute ethanol, M2 is the weight of the hydrogel after it has been immersed in absolute ethanol, $\boldsymbol{\rho}$ is the density of absolute ethanol, and V is the hydrogel volume.

2.4.4 Mechanical characterization

Tensile strength (TS) and percentage of elongation at break (EB) of hydrogels were measured using Universal Tensile Machine at a stretching speed of 20 mm (about 0.79 in)/min (Azuri *et al.,* 2023). The load capacity is 200 N, with efficiency within ±1%. The hydrogels were cut into rectangles with a dimension of 80 mm (about 3.15 in) x 20 mm (about 0.79 in) and the thickness of the samples is measured using a Vernier calliper. Three samples are prepared for each formulation to calculate the TS at a minimum of three positions. Wet samples that were pre-soaked in PBS for 24 h were used in this mechanical test. Besides that, mechanical support was added on both sides of the samples to increase the friction between the test sample and clamps during stretching processes and prevent samples from being cut by the clamp. The percentage of elongation at break and tensile strength was calculated from the equation belows:

$$\text{Elongation break (EB)} = \frac{\text{Displacement at break}}{\text{Sample length}} \text{ X } 100$$

$$\text{Tensile strength (TS)} = \frac{\text{Fb Thickness}}{\text{Width}}$$

where Fb is the maximum force (N) on the sample during tensile fracture.

2.5 *In vitro* permeation study of amoxicillin from hydrogels

In vitro diffusion tests were conducted using a Microette-Hanson system (model 57-6AS9, USA), which consists of six Franz diffusion cells with a diffusion area of 1.767 cm² and a receptor chamber volume of 6.5 ml. The hydrogels loaded with Amoxicillin were placed in the donor compartment, while the receptor chambers were filled with PBS at pH 7.4. The system was maintained at a temperature of membrane, simulating skin, was positioned between the donor

and receptor compartments. Samples were collected at intervals of 0, 1, 2, 4, 8, 12, 24, 36, 48, 60, and 72 hours and analyzed using UV-Vis spectroscopy. Furthermore, to elucidate the release mechanism, the cumulative amoxicillin release data were fitted to four kinetic models: zero order kinetic (time-independent release), first order kinetic (concentration-dependent release), the Higuchi model (diffusion-controlled release) and the Koresmeyer-Peppas model (controlled release, non-Fickian diffusion) to better understand the release mechanism of amoxicillin from hydrogel as described by Eswaramma *et al*. (2017). The model exhibiting the highest correlation coefficient (R^2 value close to 1) was considered the best fit (Eswaramma *et al*., 2017; Zhou *et al*.,2025).

2.6 *In vitro* antibacterial assay

The antibacterial assay of hydrogels was performed using the disc diffusion method (Singh *et al*., 2011). The gram-negative bacteria *Escherichia coli* (*E. coli*) and gram-positive *Staphylococcus aureus* (*S. aureus*) have been used. The inoculums were prepared by cultivating two bacterial strains *E. coli* and *S. aureus* separately in sterilized flasks containing 50 mL of nutrient broth medium, followed by incubation at 37°C for 24 hours. The circular disc was submerged in the hydrogel samples and kept to swell the sample for 4hr. Sterilized growth agar media was carefully poured into sterilized Petri plates under the controlled conditions of a laminar flow environment and allowed to solidify fully. The inoculation of bacteria was accomplished using a sterile cotton swab. The plate was uniformly covered by streaking in a linear motion from the edge to the edge, with the swab being applied three times while rotating the plate by 60 degrees between each application. The tested samples Col@PVA, 2% PVA, COLKGM@PVA, COLHA@PVA, COLCA@PVA and amoxicillin loaded A@COLKGM @PVA, A@COLHA@PVA, A@COLCA@PVA composite hydrogel were placed onto inoculated nutrient agar surfaces on the Petri plates and incubated at 37 ◦C for 24 h. The antimicrobial activity was assessed through the presence or absence of a zone of inhibition surrounding the films, with all experiments conducted in triplicate to ensure the reliability of the results.

3. Results and Discussion

3.1 Collagen yield

Collagen was extracted from *P. hypophthalmus* fish skin, yielding 3.25% PSC. PSC retains bioactive components that support cell proliferation and tissue regeneration, making it suitable for wound healing applications. Its fibrillar structure enhances hydrogel stability and mechanical strength. Incorporating PSC into hydrogels may also promote anti-inflammatory effects and angiogenesis, highlighting its potential in tissue engineering and regenerative medicine (Zhang *et al*., 2023).

3.2 Hydrogel formation

The gelation of the polymer is a critical phase transition that occurs during the sol-gel process, characterized by a transformation from a liquid to a gel state. The status of the hydrogels after three freeze-thaw cycles is presented in Table 2, demonstrating the successful formation of COLKGM@PVA, COLHA@PVA, and COLCA@PVA hydrogels following this treatment. Figure 1 illustrates photographs of hydrogels, highlighting their morphology after the freeze-thaw cycles.

Table 2. The state of the hydrogels after 3 cycles of freeze-thaw

Hydrogel	Hydrogel forms after 3 cycles of freeze-thaw
COLKGM@PVA hydrogel	Formed hydrogel
COLHA@PVA hydrogel	Formed hydrogel
COLCA@PVA hydrogel	Formed hydrogel

3.3 Characterization of hydrogels

3.3.1 Swelling behaviour of the hydrogels

The swelling behavior of hydrogels was evaluated in PBS at 37 °C over 24 hours. The maximum swelling rates achieved by COLKGM@PVA, COLHA@PVA, and COLCA@PVA hydrogels were 521%, 1102% and 1238%,

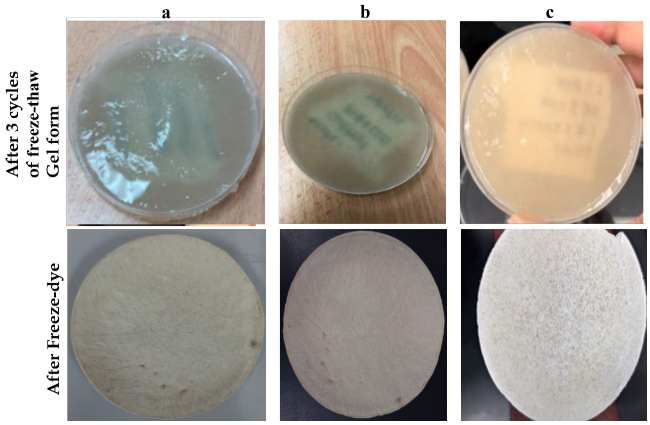

Figure 1. Hydrogels after 3 cycles of freeze-thaw. (a) COLKGM@PVA; (b) COLHA@PVA; and (c) COLCA@PVA

respectively. COLKGM@PVA showed rapid initial swelling within 2 hours, while COLHA@PVA and COLCA@PVA reached peak swelling at 8 hours. The high water absorption is attributed to hydrophilic groups (–OH and –COOH) in collagen chains (Mogoșanu & Grumezescu, 2014). Hydrogels with swelling ratios above 400% are considered suitable for wound dressings due to their ability to absorb exudate and facilitate drug release (Kamoun, Kenawy & Chen, 2017). All formulations exceeded this threshold, with COLCA@PVA demonstrating the most promising profile.

3.3.2 Water vapour transmission rate of hydrogels

The WVTR of the hydrogels decreased progressively over 24 hours (Figure 2B). Initial moisture release peaked within 2 hours at 111.71 g/m²/h (COLKGM@PVA), 154.21 g/m²/h (COLHA@PVA), and 19.24 g/m²/h (COLCA@PVA). After 24 hours, values had stabilized at 11.52, 14.16, and 1.19 g/m²/h, respectively. Hydrogels with WVTR within 75–96 g/m²/h are ideal for maintaining wound moisture balance (Jantrawut *et al.,* 2019). COLKGM@PVA and COLHA@PVA fall within or near this range, making them suitable for moderate to high-exudate wounds. The low WVTR of COLCA@PVA, likely due to its dense carrageenan network, suggests its use for low-exudate wound care.

3.3.3 Porosity measurement of hydrogels

Porosity plays a vital role in hydrogel performance, influencing water absorption, exudate retention and swelling behavior. Higher porosity enhances fluid uptake by increasing the contact area between the hydrogel matrix and surrounding fluids (Zhai & Wang, 2023). As shown in Figure 2C, COLCA@PVA exhibited the highest porosity (62.13 ± 2.60%), followed by COLHA@PVA (59.83 ± 2.16%) and COLKGM@PVA (52.92 ± 3.35%). Optimal porosity for wound dressings ranges from 40–82%, supporting moisture retention and nutrient diffusion (Demeter *et al.,* 2023). Compared to conventional synthetic hydrogels, which often require modification to improve porosity (Annabi *et al.,* 2010), the values observed here are favorable. The incorporation of fish collagen with CA, HA and KGM likely contributed to this enhancement. Notably, COLCA@PVA aligns with commercial carrageenan-based dressings (Cui *et al.,* 2022), reinforcing its potential for wound care applications.

3.4 Mechanical characterization of hydrogels

The mechanical properties of hydrogels were assessed via tensile testing. COLKGM@PVA and COLHA@PVA showed low tensile strengths of 0.10 ± 0.02 N/mm² and 0.108 ± 0.006 N/mm², respectively, while COLCA@PVA demonstrated significantly higher strength at 0.57 ± 0.05 N/mm² (Figure 2D). In terms of flexibility, COLHA@PVA exhibited the highest elongation at break (115%), followed by COLCA@PVA (105%); and COLKGM@PVA (35%) tended to be brittle. These differences might be attributed to polymer–polymer interactions and porosity.

The results align with previous studies on hydrogel-based wound dressings. For example, sodium fusidate-loaded hydrogels showed 0.1010 N/mm² tensile strength and 273% elongation (Jin *et al*., 2016), while honey-loaded chitosan/gelatin/PVA hydrogels had 1.51 kPa strength and 46%

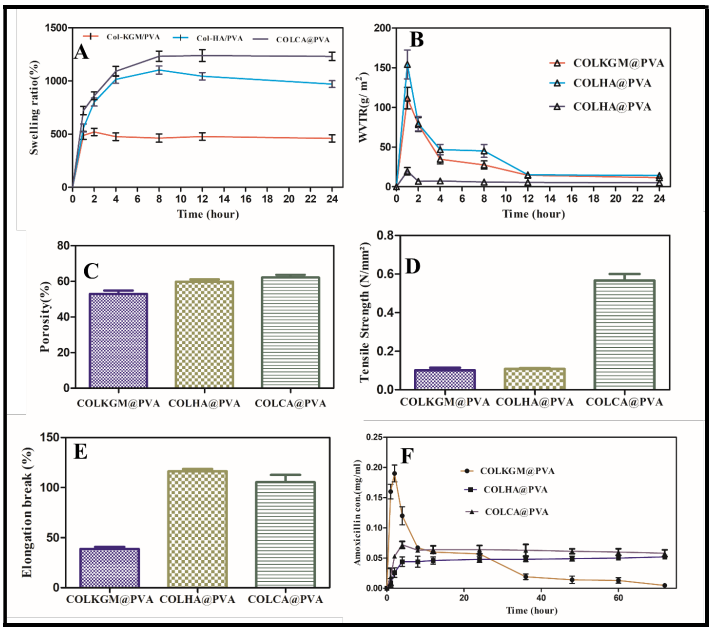

Figure 2. Characterization test of composite hydrogels. (A) Swelling ratio ; (B) WVTR ; (C) Percentage of porosity; (D) Tensile strength ; (D) Elongation at break; and (F) *in vitro* skin permeation study using Franz diffusion cell where cellulose membrane were used. Experimental data were analyzed with Prism software (GraphPad version 10.2.3) and are expressed as the mean ± SD (n=3). Statistical significance levels are indicated by *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

elongation (Shamloo *et al*., 2021). Zinc oxide-loaded alginate/PVA hydrogels reached 0.27 N/mm² and 160% elongation (You *et al*., 2014). Other studies reported tensile strengths ranging from 0.14–1.60 N/mm² and elongation between 16–273% (Abdollahi, Chamchangi, Raoufi, & Heidari, 2025; Zhang *et al.,* 2025). These benchmarks suggest that tensile strength within 0.10–1.60 N/mm² and elongation at break in 16–273% are suitable for wound dressing applications. The COLCA@PVA hydrogel meets these criteria, offering a strong balance of mechanical integrity and flexibility.

3.5 *In-vitro* permeation study of hydrogels

Amoxicillin release from fabricated hydrogels were assessed over 72 hours (Figure 2F). COLKGM@PVA showed the highest initial release (0.19 ± 0.014 mg/mL at 2 h) and then gradually decreasing over time. COLHA@PVA and COLCA@PVA peaked at 4 h (0.049 ± 0.004 and 0.072 ± 0.0057 mg/mL, respectively) and maintained steady concentrations up to 72 h, indicating sustained release. Kinetic modeling revealed poor fit for COLKGM@PVA. In contrast, COLHA@PVA and COLCA@PVA followed first-order kinetics ($R^2 = 0.969$ and 0.920), indicating diffusion-controlled, concentration-dependent release. These findings highlight COLHA@PVA and COLCA@PVA as promising candidates for controlled drug delivery, while COLKGM@PVA may be suitable for applications requiring rapid drug release.

3.6 *In-vitro* antibacterial assay of hydrogels

The antibacterial activity of COLKGM@PVA, COLHA@PVA, and COLCA@PVA hydrogels was tested against *S. aureus* and *E. coli* (Table 3, Figure 3). COLKGM@PVA showed the highest inhibition against *E. coli* (11.76 mm), while COLCA@PVA was most effective against *S. aureus* (9.33 mm), followed closely by COLHA@PVA (9.00 mm). These results indicate that the type of natural polymer influences antibacterial performance.

Amoxicillin-loaded COL@PVA hydrogels exhibited the strongest antibacterial effect confirming the efficacy of drug release. COLKGM@PVA showed no activity against *S. aureus* and plain PVA and COL@PVA lacked antibacterial effects. The observed activity in blank hydrogel is attributed to the bioactive properties of polymers (Neamtu *et al.,* 2022). Also, these polymers, combined with sustained drug release, enhance bacterial inhibition and drug bioavailability. COLCA@PVA and COLHA@PVA are particularly promising for wound dressing applications. Future studies may explore synergistic combinations to optimize both immediate and prolonged antibacterial effects.

Table 3. Length of inhibitory zone of *S.aureus* and *E. Coli*

Formulation	Length of inhibitory zone (mm)	
	S.aureus (*avg*)	*E. coli* (*avg*)
COLKGM@PVA hydrogel	0.00	11.67
COLHA@PVA hydrogel	9.00	8.33
COLCA@PVA hydrogel	9.33	9.33
2.0% PVA	0.00	0.00
COL/PVA hydrogel-loaded with amoxicillin	13.67	13.33
COL/PVA hydrogel	0.00	0.00

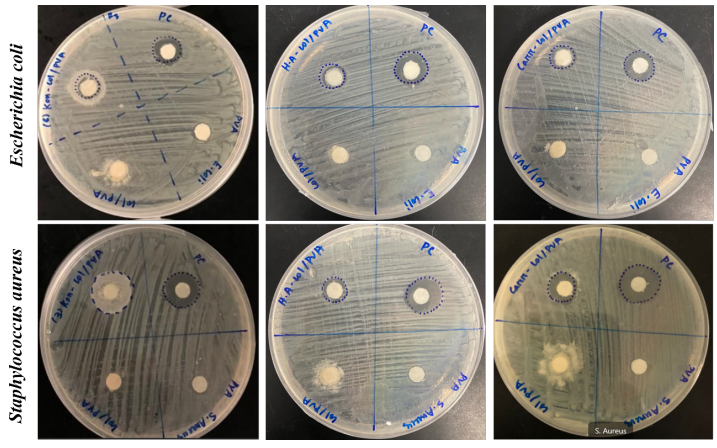

Figure 3. Photographs illustrate antibacterial effects by (a) COLKGM@PVA, (b) COLHA@PVA, and (c) COLCA@PVA hydrogels

4. Conclusions

This study successfully developed COLKGM@PVA, COLHA@PVA and COLCA@PVA hydrogels using a freeze-thaw physical crosslinking method, with fish collagen from *P. hypophthalmus* as the base material. COLCA@PVA and COLHA@PVA showed superior swelling properties, while COLKGM@PVA and COLHA@PVA demonstrated favorable WVTR for maintaining wound moisture. All hydrogels exhibited suitable porosity and mechanical strength, with COLCA@PVA offering the best balance of tensile strength and flexibility. Antibacterial assays confirmed that natural polymers contributed to antimicrobial activity and drug release studies revealed sustained amoxicillin delivery by COLHA@PVA and COLCA@PVA, following first-order kinetics. These findings highlight the potential of collagen-based composite hydrogels, particularly COLCA@PVA and COLHA@PVA, as multifunctional wound dressings with controlled drug delivery capabilities. Further *in vitro* and *in vivo* studies are recommended to validate their clinical applicability and optimize formulations for enhanced therapeutic outcomes.

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Author Contributions

Md Shaekh Forid: Data curation and Writing - Original draft. Muhammad Saupi Azuri: Software. Nur Ain Arina Bukhari: Methodology, Investigation. Fatin Ayu Kartika Mohd Suzaki: Methodology, Investigation. Nurul Fatihah Syazwana Mohamad Spari: Methodology, Investigation. Wan Maznah Wan Ishak: Conceptualization, Supervision, Reviewing and Editing.

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