International Journal of Technology

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Research Article

Injectable Alginate-Collagen Hydrogel with Propolis: A Potential Cardioprotective Biomaterial

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Abstract: Injectable hydrogel has become a viable therapeutic approach for the non-invasive treatment of myocardial infarction. Although natural and synthetic injectable hydrogel has been investigated, it typically exhibits a deficiency in cardioprotective characteristics. Therefore, this study aims to develop and characterize an injectable alginate-collagen hydrogel incorporated with propolis and crosslinked with calcium gluconate. The sample was developed by incorporating alginate, collagen, and varying quantities of propolis at 3%, 7%, and 10%. Subsequently, it was crosslinked with calcium gluconate to create a hydrogel. The rheology test results showed that jellification occurred at 37°C following the incorporation of 0.5% calcium gluconate, as evidenced by an elevation in storage modulus and a reduction in loss modulus. An increase in calcium gluconate facilitated crosslinking while concurrently leading to an increase in swelling degree of up to 17 times. This phenomenon was caused by the favorable water permeability of calcium ions. The results also showed that the elevation of propolis diminished the extent of swelling due to the enhancement of density. The elevated concentration of calcium gluconate reduced the degradation rate, but the increased amount of propolis accelerated the breakdown. The antioxidant activity assessed using DPPH assays showed that the percentage of inhibition increased along with the concentrations of propolis. Based on these results, propolis can be integrated into injectable hydrogel for potential therapeutic therapy of myocardial infarction.

Keywords: Alginate-collagen; Biomaterial; Injectable hydrogel; Myocardial infarction; Propolis

1. Introduction

Cardiovascular diseases (CVDs) are a leading cause of death globally, accounting for 17.8 million (32%) mortality cases annually (WHO, 2021). Among CVDs, myocardial infarction (MI) is a significant contributor, characterized by the death of cardiac cells due to prolonged oxygen deprivation following coronary artery occlusion (Franchina et al., 2020). Without timely intervention, MI can lead to myocardial necrosis, which causes heart dysfunction (Akbar et al., 2022). Consequently, effective treatments for MI are urgently needed to address the associated pathological changes, including inflammation and myocardial remodeling, which can progress to heart failure.

This work was supported by the Universitas Indonesia funded by Hibah PUTI 2023 NKB-501/UN2.RST/HKP.05.00/2023

https://doi.org/10.14716/ijtech.v16i3.7366
Received July 2024; Revised October 2024; Accepted February 2025

Hydrogels are 3-dimensional polymer networks capable of absorbing large amounts of water. These polymers have emerged as promising biomaterials for cardiac tissue engineering (Kamala et al., 2022) due to their soft and flexible structure, which mimics the extracellular matrix (Li et al., 2023a). In addition, their biodegradable and injectable properties enable minimally invasive delivery and targeted therapeutic effects (Bertsch et al., 2023; Park et al., 2019). Among various hydrogel-forming biomaterials, alginate and collagen (Gil-Cabrerizo et al., 2022; Puspitasari et al., 2022; Meng et al., 2021) stand out due to their biocompatibility (Fajarani et al., 2024) and mechanical properties. Alginate, derived from brown algae, forms strong, degradable crosslinked networks in the presence of calcium ions (Yamauchi et al., 2024; Hecht and Srebnik, 2016), while collagen provides thermosensitive properties conducive to tissue repair (Ahmad Raus et al., 2021; Moxon et al., 2019).

According to previous studies, propolis, a natural resinous substance produced by bees, has gained attention for its anti-inflammatory (Zhu et al., 2023; Dewi et al., 2022; Sabrina et al., 2022; Zulhendri et al., 2021; Pahlavani et al., 2020) and cardioprotective properties (Neto et al., 2022; Ahmed et al., 2017; Daleprane and Abdalla, 2013). The substance has also been reported to be rich in flavonoids and reduces inflammation by activating peroxisome proliferator-activated receptor gamma (PPAR-γ), making it a promising therapeutic agent for MI (Wang et al., 2022; Braakhuis, 2019). A previous study (Pangesty et al., 2024) showed that hydrogel patches facilitated a controlled release mechanism of propolis, which is beneficial for sustained therapeutic effects in MI treatment. However, the application of hydrogel patches requires invasive surgical procedures, limiting their clinical utility and patient compliance. To address the limitation, there is a pressing need to develop an injectable hydrogel system that combines the therapeutic benefits of propolis, including controlled release properties, with the practically minimally invasive administration. The system is expected to enable precise delivery to the infarcted area, promote cardiac tissue repair, and enhance the feasibility of MI therapy in clinical settings. Therefore, this study aims to develop and characterize an injectable alginate-collagen hydrogel incorporated with propolis and crosslinked with calcium gluconate. The effects of varying propolis and calcium gluconate concentrations on hydrogel properties, including flowability, degradation, swelling, and antioxidant capacity, were systematically evaluated to optimize its potential as a cardioprotective agent.

2. Methods

Sodium alginate was acquired from Sigma Aldrich (Saint Louis, USA). Types 1 and 3 collagen powder was obtained from Doctor's Best (California, USA). In addition, *Geniotrigona thoracica* and *Heterotrigona itama* propolis extract were produced by Kebun Efi (North Sumatera, Indonesia). Calcium gluconate 10% was obtained from PT Ethica Industri Farmasi (West Java, Indonesia). Phosphate-Buffer Saline (PBS) (pH 7.2-7.4) was purchased from Sigma Aldrich (Saint Louis, USA).

2.1. Hydrogel Fabrication

The hydrogel was prepared according to the procedure described by Gil-Cabrerizo et al. (2022) with some modifications, as displayed in Figure 1. Sodium alginate (10 mg/mL) was dissolved in PBS at 80°C with constant stirring at 600 rpm for 2 hours. Subsequently, 0.05 grams of collagen was added to the solution, and the mixture was heated at 60°C, then stirred at 500 rpm for 1 hour, resulting in a final collagen concentration of 0.5 mg/mL. The propolis was then added to the mixing solution at different concentrations of 0%, 3%, 7%, and 10%, which was stirred until homogenous. Finally, calcium gluconate at concentrations of 0%, 0.125%, 0.25%, and 0.5% was added dropwise while stirring to ensure homogeneous distribution. The final composition of each hydrogel was presented in Table 1.

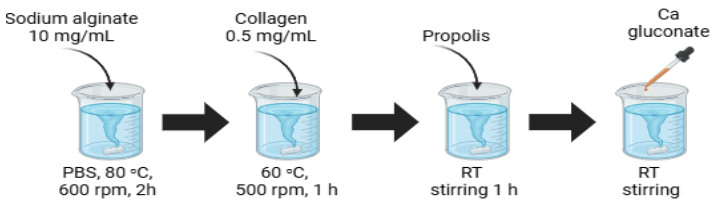


Figure 1 Fabrication illustration of injectable hydrogel

Table 1 Composition of the fabricated hydrogels

Variations	Sodium Alginate (mg/mL)	Collagen (mg/mL)	Propolis (%)	Calcium Gluconate (%)
0 P	10	0.05	0	0 0.125 0.250 0.500
3 P			3	0 0.125 0.250 0.500
7 P			7	0 0.125 0.250 0.500
10 P			10	0 0.125 0.250 0.500

2.2. Characterizations

2.2.1. Rheology

The hydrogel before and after the addition of calcium gluconate was subjected to rheological testing using a Discovery Series Hybrid Rheometer (DHR) with a cylindrical spindle. In addition, the isothermal time sweep dynamic rheological test parameters were carried out at 37°C. A 10 mL sample was subjected to a rotary compressive load with an angular velocity of 10 rad/s, 1% strain for 15 minutes. The data obtained were modulus changes, namely storage modulus (G') and loss modulus (G''), as a function of time. In this testing, the tested samples were those with the optimum jellification results.

2.2.2. Swelling Testing

Swelling tests were performed to assess the adsorption capability of the hydrogel samples. Before the test, the initial dry mass of the samples was determined by drying for 6 hours at 60°C in a dehydrator to remove moisture content. The anhydrous hydrogel samples were subsequently submerged in PBS at 37°C for durations of 30, 90, and 120 minutes. Subsequently, the samples were reweighed to determine their final mass (Tang et al., 2020a). The swelling ratio was calculated using the following formula in Equation 1.

$$Swelling\ Ratio = \frac{(W_t - W_0)}{W_0} \quad (1)$$

The data from this testing was plotted on a graph showing the relationship between time and the swelling ratio.

2.2.3. Degradation Time

The hydrolytic degradation of the hydrogel was performed by soaking the sample in PBS at 37°C. Degradation testing was carried out by monitoring for 7 days. Mass was measured on days 1, 3, 5, and 7. Samples could be weighed before being put into PBS (W_0) and at predetermined time intervals (W_t). Mass reduction was calculated using Equation 2 (Pangesty et al., 2024).

$$Weight\ loss\ (\%) = \frac{(W_0 - W_t)}{W_0} \times 100\% \quad (2)$$

The data from this testing was plotted on a graph showing the relationship between time and the weight loss percentage.

2.2.4. Antioxidant Activity

The antioxidant activity was measured using 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) assays. Screening was carried out with the DPPH assay method. A total of 40 µL of sample extract was reacted with 40 µL of DPPH methanol solution (0.1 mM) in a 96-well plate. The mixture was then homogenized and incubated at 25℃ for 30 minutes. Subsequently, the absorbance was measured with a spectrophotometer at a wavelength of 517 nm. The DPPH radical scavenging activity of the sample extract was measured through mg ascorbic acid equivalent per gram of sample. A standard calibration curve was performed in the range of 0 to 50 µg/mL. The percentage of inhibition was calculated using the following formula

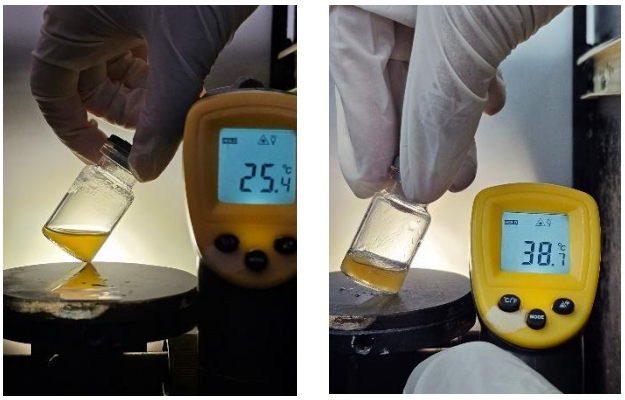
$$\% \text{ inhibition} = ((A0-A1)/A0)/100\% \quad (3)$$

Where A0 was the absorbance of the control and A1 was the absorbance of the sample.

3. Results and Discussion

3.1. Rheology

Figure 2 shows the tilting test result of the hydrogel solution before and after the incorporation of calcium gluconate. Initially, the alginate-collagen-propolis mixture exhibited a viscous liquid, as evidenced by its ability to flow when the container was tilted (Figure 2a). Upon the addition of calcium gluconate at 37°C, the solution underwent a significant transformation into a gel-like state, with no observable flow (Figure 2b).



(a) (b)

Figure 2 Tilting test result of the hydrogel (a) before and (b) after the calcium gluconate addition.

This phase transition was attributed to the process of ionic gelation, wherein calcium ions (Ca^{2+}) from calcium gluconate interacted with the carboxylate groups on the alginate and collagen backbone, forming a robust crosslinking network structure (Wang et al., 2025;Yamauchi et al., 2024; Hu et al., 2021; Andersen et al., 2015), as demonstrated by the reaction in Figure 3. Ionic gelation was a well-established mechanism for crosslinking alginate, enabling the liquid-to-gel transition critical for hydrogel formation. The physiological temperature of 37°C further supported this reaction, making it particularly relevant for biomedical applications. Furthermore, the presence of collagen enhanced the biocompatibility (Montalbano et al., 2018) and the propolis within the matrix potentially contributed to the structural stability (Pangesty et al., 2024) and bioactivity (Sulaeman et al., 2022; Widjiastuti et al., 2020).

A dynamic time sweep test was performed at 37°C to evaluate the rheological behavior before and after the addition of calcium gluconate. According to the physical form of the hydrogel samples (data not shown), it was found that the 0.5% calcium gluconate variation had the optimum sol-gel transformation. Therefore, the rheology test was executed using 0.5% calcium gluconate hydrogel. Figure 4a revealed that the loss modulus (G″) was consistently higher than the storage modulus (G′), indicating that the solution remained in a liquid-like state before the incorporation of calcium

gluconate. This behavior was a characteristic of viscous fluid, where energy dissipation dominated over elastic energy storage (Stojkov et al., 2021).

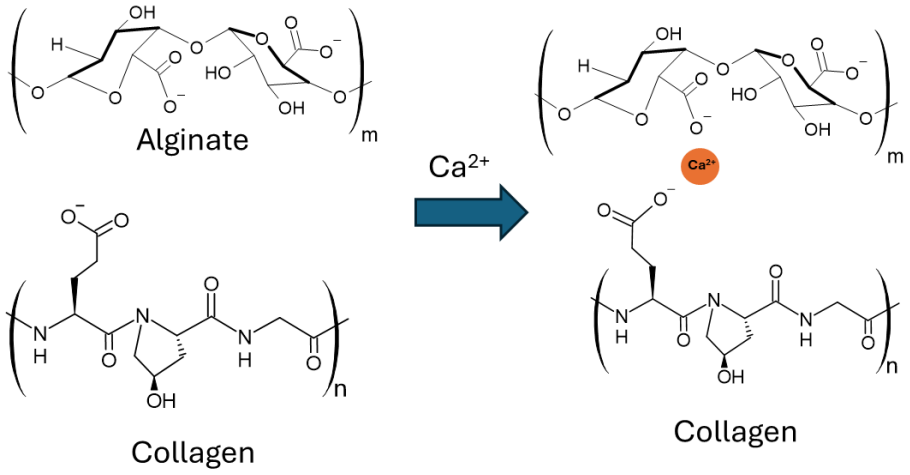


Figure 3 The chemical reaction of ionic gelation between alginate and collagen by Ca^{2+}

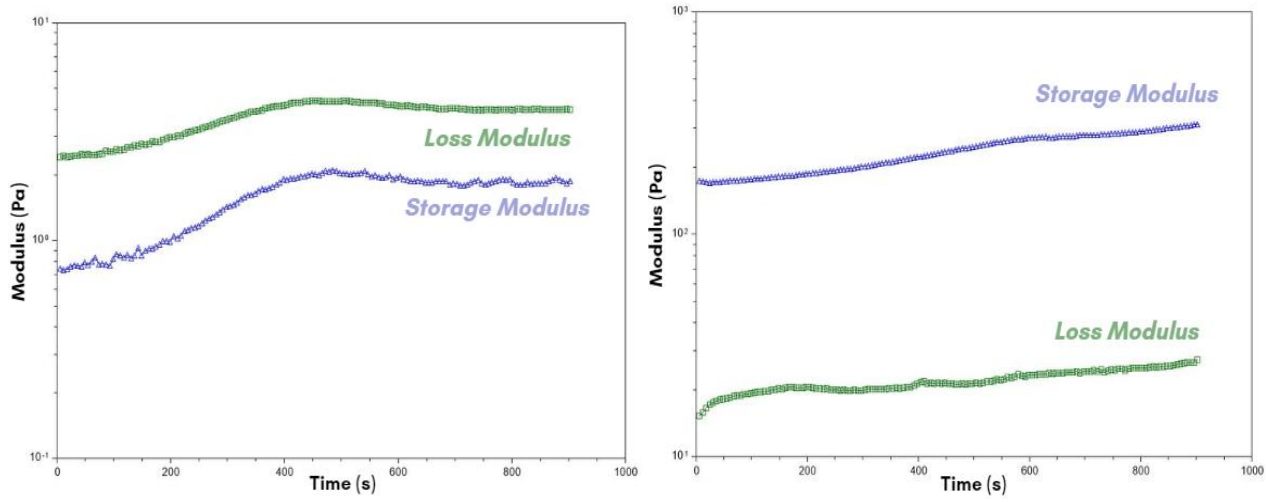
Figure 4b shows a pronounced increase in the storage modulus (G′) relative to the loss modulus (G″) following the addition of calcium gluconate, signifying the transition from a liquid to a gel state. The higher G′ values indicated the formation of a solid-like structure, as the elastic component became dominant due to the crosslinking process. As stated earlier, this transformation was a direct result of ionic gelation (Guo et al., 2016).

The observed shift in the rheological profile (G′ and G″) emphasized the sol-gel transformation induced by ionic crosslinker (calcium gluconate) at physiological temperature (37°C). This was particularly relevant for the development of injectable hydrogels, as the transition ensured localized gelation at the target site without premature solidification during injection. Such behavior was consistent with previous findings that emphasized the importance of tunable sol-gel properties for biomedical applications (Malektaj et al., 2023; Cuomo et al., 2019; Yang et al., 2013).

3.2. Swelling Behaviour

To analyze the influence of hydrogel's composition on the swelling ability, the dried samples were immersed in PBS at a physiological pH of 7 for 30, 90, and 120 minutes. Figure 4 demonstrated that an increase in calcium gluconate concentration correlated with an elevated swelling ratio of the hydrogel. For instance, hydrogel without propolis (0P) with a calcium gluconate concentration of 0.125% (Figure 5a) exhibited a swelling ratio of 2.5 in 30 minutes but increasing the calcium gluconate concentration to 0.25% (Figure 5b) and 0.5% (Figure 5c) improved the swelling capacity by approximately 13-fold and 5-fold, respectively. This was against the hypothesis that the increase of calcium gluconate could reduce the swelling behavior due to the crosslinking effect. However, this finding was consistent with a study conducted by Yamauchi et al. (2024), observing that the swelling ratio of the alginate hydrogel rose as the calcium quantity increased. The study further explained that the hydrogel containing a high mass of calcium had a high permeability in saline. As a result, a rise in calcium concentration could also increase the swelling ratio.

The higher propolis concentration addition caused a decrease in the swelling ratio regardless of the calcium gluconate concentration. For instance, Figure 4c shows that the addition of propolis concentration to 3%, 7%, and 10% reduced the swelling ratio to 5.5, 4.5, and 2 folds, respectively, in the first 30 minutes. This was possible because of the intermolecular and intramolecular crosslink density due to the incorporation of propolis, resulting in a hard gel and reducing polymer stretching during the soaking. The results were based on a study conducted by Pangesty et al. (2024) showing that the incorporation of propolis reduced the swelling degree of PVA hydrogel.



(a) (b)

Figure 4 Time dependence of storage modulus G′ (blue) and loss modulus G″ (green) of the hydrogel (a) before and (b) after the calcium gluconate addition of 0.5%

In general, all hydrogels displayed burst swelling within the first 30 minutes, followed by a steady increase in swelling ratio after 30 minutes. When it was assumed that the swelling process controlled the propolis release mechanism, then the propolis was released in a regulated manner when the hydrogel chain swells.

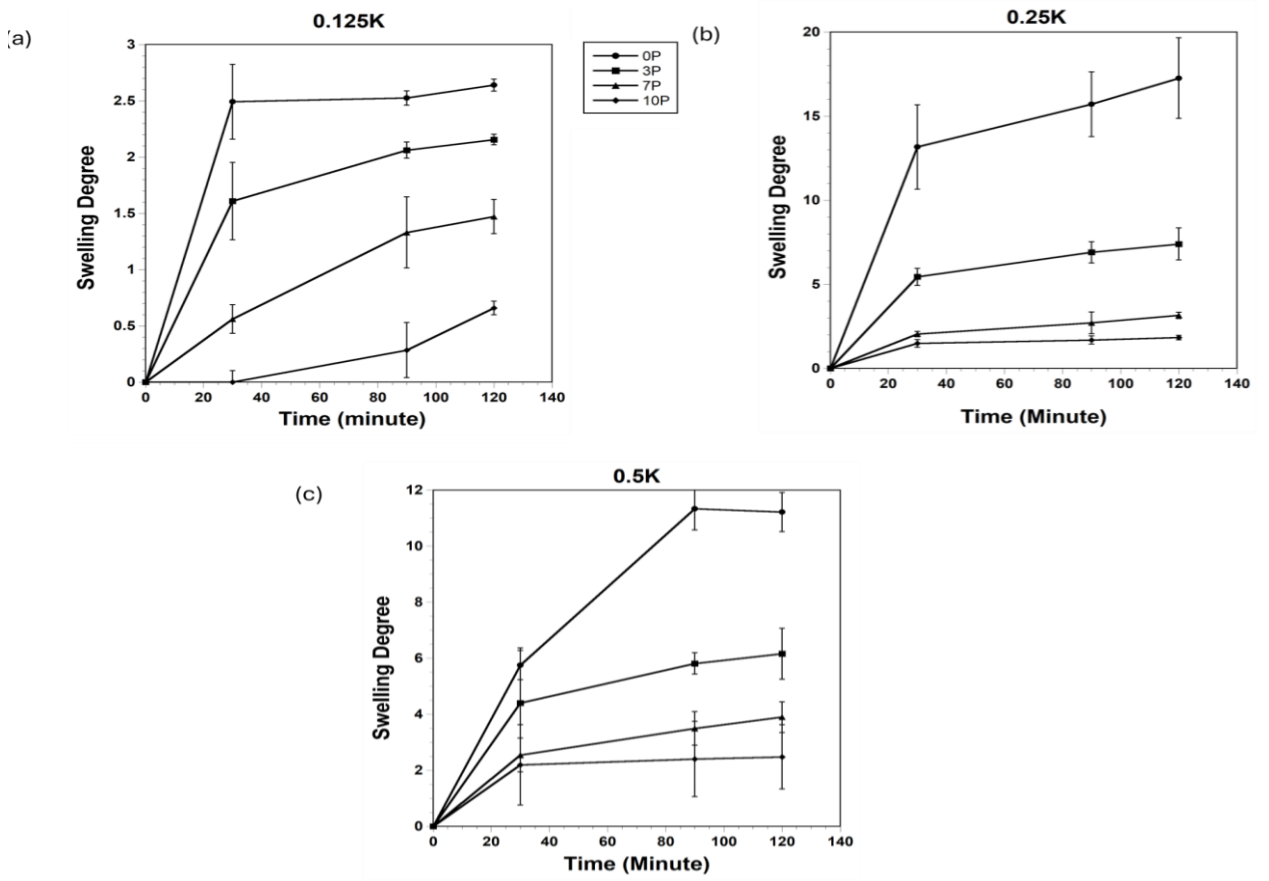


Figure 5 Swelling degree of the dried hydrogel with variation concentration of a) 0.125%, b) 0.25%, and c) 0.5% calcium gluconate

3.3. Degradation Behaviour

Figure 6 indicated that the degradation rate was influenced by calcium gluconate. The hydrogels with a higher concentration of calcium gluconate exhibited enhanced crosslinking. Hydrogels 0P 0.25K degraded almost completely in 1 day, as shown in Figure 6b. Simultaneously, with the increased concentration of calcium gluconate to 0.5% (Figure 6c), the degradation process decelerated, resulting in a residual weight of 65% after one day. Moreover, the deterioration was influenced by the concentration of propolis. The quantity of propolis could elevate the degradation mass. For instance, as shown in Figure 6c, the hydrogels containing 10% propolis (3P) underwent 35% weight loss within one day, while those with decreasing percentage of propolis to 7% and 3%, the hydrogels retained approximately 85% and 92%, respectively, of their mass on the same day. These occurrences could arise due to insufficient calcium gluconate concentration to facilitate the crosslinking of polymer chains when propolis concentration increased. The crosslink strength diminished due to excessively high concentrations of propolis (Krisanti et al., 2020).

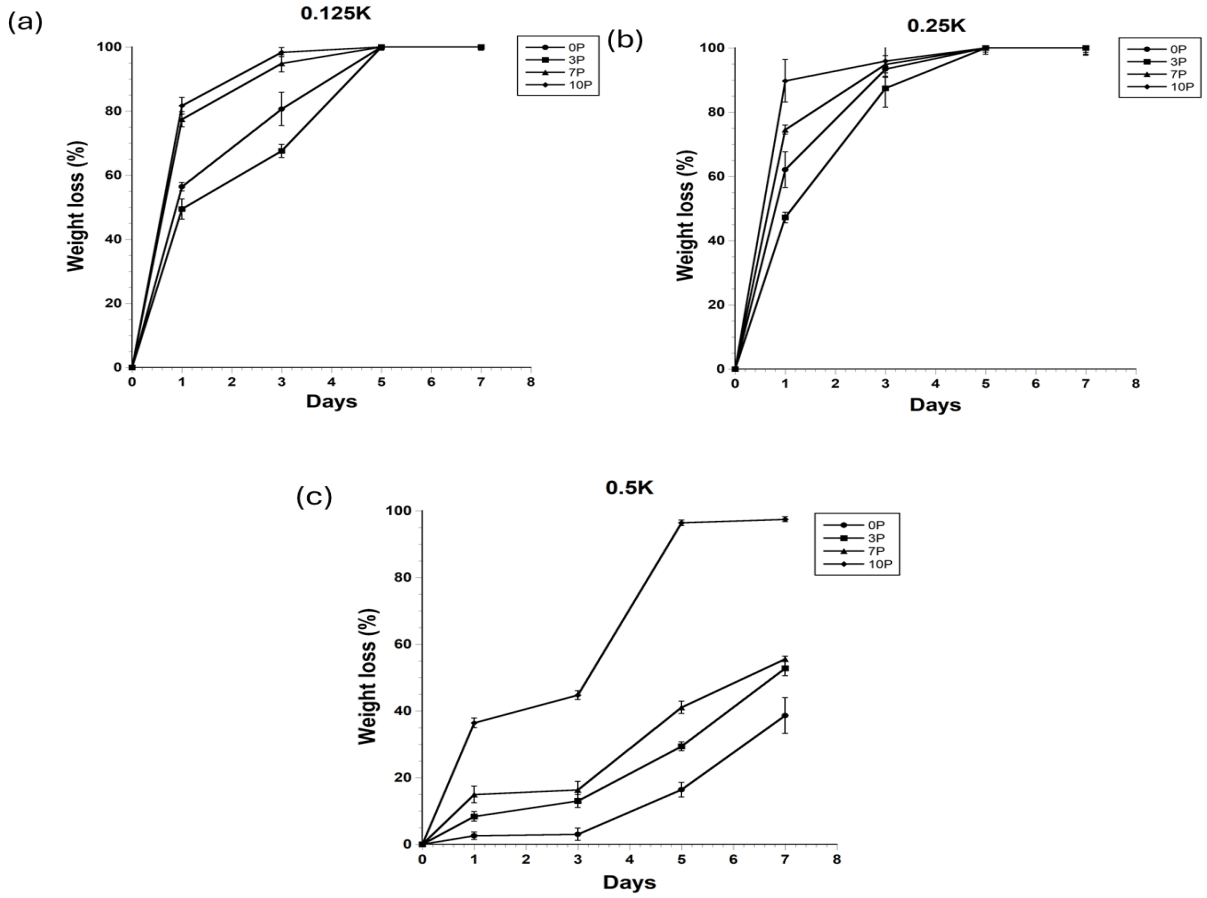


Figure 6 The weight loss data of the propolis incorporated hydrogel with various concentrations of (a) 0.125 %, (b) 0.25 %, and (c) 0.5 K calcium gluconate after hydrolytic degradation

3.4. Antioxidant Activity

The antioxidant capacity of the propolis incorporated-hydrogel was evaluated through a DPPH assay, which was a stable and purple free radical that functioned widely to determine antioxidant capacity and presented an absorption band at 517 nm. The method used was based on the reduction of stable free radicals DPPH (Šuran et al., 2021). In the presence of antioxidants, it could reduce free radicals because of its nature which donated protons or hydrogen (Li et al., 2023b). Antioxidant testing data in this study could be observed in Table 2.

As shown in Table 2, increasing the concentration of propolis led to a higher percentage of inhibition. This indicated that higher concentrations of propolis enhance the sample's ability to

inhibit free radicals, reflecting stronger antioxidant properties (Tang et al., 2020b). Antioxidant properties, in turn, were associated with cardioprotective effects, such as reducing the spread of infarction areas in the heart (Khan et al., 2021).

Table 2 Inhibition percentage result of antioxidant testing

Hydrogel	% Inhibition
3 P	16.87 ± 0.38
7 P	26.55 ± 1.04
10 P	29.76 ± 3.72

These findings were consistent with previous studies on the bioactive properties of propolis. For instance, Hossain et al. (2022) emphasized the immunomodulatory and anti-inflammatory effects of propolis. Similarly, Neto et al. (2022) demonstrated the cardioprotective properties of propolis in mice with myocardial infarction.

4. Conclusions

In conclusion, this study successfully developed the injectable alginate-collagen hydrogel by adding a therapeutic agent, propolis, that had the potential for MI therapy. The rheology test confirmed that gelation occurred at 37°C with the incorporation of 0.5% calcium gluconate, indicated by an increase in storage modulus and a decrease in loss modulus. Higher concentrations of calcium gluconate promoted crosslinking, resulting in a swelling degree of up to 17 times, attributed to the high water permeability of calcium ions. However, increasing propolis concentrations reduced the swelling degree by enhancing the hydrogel's density. Calcium gluconate also reduced the degradation rate of the hydrogel, while higher concentrations of propolis accelerated its breakdown. Furthermore, antioxidant activity, evaluated using DPPH assays, showed that the percentage of inhibition increased with higher propolis concentrations. This study demonstrated that propolis could be successfully incorporated into injectable hydrogels, offering potential as a therapeutic strategy for myocardial infarction treatment.

Acknowledgements

This was funded by the Directorate of Study and Development, Universitas Indonesia under HIBAH PUTI 2023 (NKB-501/UN2.RST/HKP.05.00/2023).

Author Contributions

Azizah Intan Pangesty: Conceptualization, Methodology, Supervision, Funding acquisition, Writing – original draft, Writing – review & editing.
Raajwa Ayudhia Kamila: Investigation, Data curation, Formal analysis, Visualization, Writing – original draft.
Audrey Cessnawati Putri Bengu Schlumbergerina: Investigation, Sample preparation, Data curation.
Muhammad Daneth Faizurrizqi: Validation, Formal analysis, Writing – review & editing.
Renanda Wafi Fakhri: Investigation, Software, Data analysis, Visualization.
Sunarso Sunarso: Resources, Supervision, Project administration.
Myrna Nurlatifah Zakaria: Conceptualization, Resources, Review & editing.
Lutviasari Nuraini: Methodology, Supervision, Review & editing.
Winda Azwani: Investigation, Laboratory support, Data acquisition

Conflict of Interest

The authors declared that there were no conflicts of interest associated with this publication. The study was conducted independently. No personal or professional relationships exist that could have influenced the work reported in this manuscript.

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