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Physical, Thermal and Morphology Properties af Polyvinyl Alcohol-Based Composite Enhanced with Collagen and Bromelain for Cartilage Application

Nur Syafiqa Amirah Izam[1], Nadirul Hasraf Mat Nayan[1]*, Mohd Syahir Anwar Hamzah[1]

[1]Department of Chemical Engineering Technology, Faculty of Engineering Technology, Universiti Tun Hussein Onn Malaysia – Pagoh Campus, 84600 Pagoh, Johor, Malaysia

*Corresponding Author Designation

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Abstract: This thesis reports the preparation of polyvinyl alcohol (PVA) film modified with collagen and 3% bromelain via solvent casting and surface entrapment method for cartilage application. The concentration of collagen used was varied from 0 to 2% w/v the hydrophilicity properties of the film was observed via swelling test and moisture absorption test. PVA/2%Col with 3% bromelain having good swelling properties in phosphate buffer saline (PBS) solution and have a good results of moisture absorption compared to other films. PVA/2%Col with 3% bromelain concentration was chosen as the optimum PVA/Collagen film and was further tested in order to observe its physical properties. The chemical interaction of the film was analyzed by Fourier Transfrom Infrared Spectroscopy (FTIR). Scanning Electron Microscope (SEM) testing also have been conducted to observe it surface morphology. Differential Scanning Calorimetry (DSC) testing, PVA/2%Col with 3% bromelain film give a better result compared to other films. Furthermore, for In-vitro Biomineralization testing using SEM, PVA/2%Col with 3% bromelain shows good results. These reports were clearly suggestive that biopolymers such as PVA can exhibit an increasing of physical properties by treated with collagen and bromelain. This new modified towards the PVA film can enhance its quality and decrease the limitations of application in treating cartilage application.

Keywords: PVA, Collagen, Bromelain, Solvent Casting, Entrapment Method, Cartilage.

Corresponding author: nadirul@uthm.edu.my

1. Introduction

There are several ways to tear cartilage which is in the joint area, constant stress can result in irreversible damage, which eventually leads to osteoarthritis, a painful condition that can only be treated with painkillers or joint replacement. The prevalence of tracheal and nasal cancer has grown due to an increase in cancer cases and also both commonly need for radical resection as part of aggressive treatment plans. Severe deformity of the ear and nose can result from congenital diseases such Treacher Collins syndrome and Aperts syndrome. Each of these clinical situations clearly entails significant damage to important skeletal cartilage, which is why there is a push for improvements in cartilage tissue engineering. To create constructions that successfully recreate the histology, dynamics, and shape of real tissues, tissue engineering integrates ideas from cell biology, engineering, and medicine. An appropriate scaffold is required that can offer a 3D environment for cell proliferation and adhesion. The essential qualities for the optimum scaffold are still up for debate, although they will probably vary depending on the kind and location of the cartilage that has to be built [1].

Natural polymers have qualities that resemble biological activities like cellular responses, but they have no control over structural aspects like fiber diameter. Synthetic polymers, on the other hand, provide for structural control. The signalling capability, on the other hand, reduces [7]. Animal collagen has been extensively explored as a biomaterial for nanofiber manufacturing. Other materials, such as alginate or aloe vera, have not been well investigated, despite the fact that they may be more beneficial than synthetic materials since they are less toxic to cells put in scaffolds. To correctly link to nanofibers and result in tissue creation suited for implantation, the product must contain the requisite qualities for cell proliferation and structuring [4]. PVA is a semi-crystalline, generally hydrophilic, nontoxic, and biocompatible polymer that has resistance, water solubility, gas permeability, and heat properties. The degree of hydrolysis has a direct impact on the resistance of the nanofiber. Because PVA is a particularly hygroscopic ingredient that retains a lot of water or humidity, it has a lower mechanical capability [10]. In the morphology and structure of nanofibers, variables such as voltage, surface tension, electrical conductivity, polymer molecular weight, and solvent volatility have a role. In this study, we will look at how solvent casting and entrapment method is used, as well as the factors that govern nanofiber synthesis, biomaterials, and applications in nanopharmaceuticals and scaffold manufacturing.

Collagen's main biomedical uses include biomaterials, particularly as carriers for drugs and genes, tissue engineering, absorbable surgical sutures, osteogenic and bone filling materials, hemostatic agents, immobilisation of therapeutic enzymes, and burn/wound cover dressings [11, 7, 14, 13, 2]. Collagen has a key function in all stages of wound healing, including hemostasis, inflammation, proliferation, and remodelling. It serves as a natural structural scaffold or substrate for the creation of new tissue. Endogenous collagen, which is composed of three lengthy amino acid chains with helicoidal shapes, is collagen generated by living things. Collagen is made up of polypeptide chains with the repeating pattern (Gly-X-Y)n, where X and Y can be any amino acid but are most frequently proline and 4-hydroxyproline [6, 12]. Aging, being exposed to UV light, and smoking all cause collagen to break down. It is crucial to find new sources of collagen for regenerative tissue applications since collagen degradation causes wrinkles, drooping skin, stiff joints, and dry skin [5, 3]. Wrinkles, sagging skin, stiff joints, dry skin, exposure to UV radiation, and tobacco use are all caused by collagen breakdown.

The pineapple plant, Ananas comosus, has long been used for medicinal purposes. Native cultures used it as a digestive aid and as a remedy for skin disorders. A compound called bromelain, which was found to be highly concentrated in mature pineapple stems has since been linked to the medicinal properties. Research on bromelain has been conducted for decades in Europe and Asia, and in recent years, it has been of interest in the United States. Although most of the available information comes from in vitro and animal studies or anecdotal evidence, rather than randomized, controlled clinical trials, bromelain has been shown to exhibit beneficial therapeutic effects while maintaining low toxicity and

producing few harmful or undesired side effects. In particular, bromelain is reported to have antiinflammatory, antiedematous, anticoagulant, and antimetastatic properties, and has also been shown to enhance antibiotic activity [9].

Because of its biocompatibility, low tendency for protein attachment, and low toxicity, PVA is used in a variety of biomedical application. PVA (polyvinyl alcohol) is a hydrogel-forming polymer that is neutral, synthetic, and water soluble. The chemical structure of PVA is quite simple, featuring a pendant hydroxyl group. It has recently become a hot topic in the domains of medication delivery and tissue engineering. However, various limitations of PVA, including as limited mechanical strength, excessive absorbency, and lack of cell-adhesive characteristic, necessitate further modifications for desired and focused applications. This work used a combined biomaterial of collagen and bromelain to overcome the limitations of known PVA due to its high hydrophilicity.

2. Materials and Methods

In this study, the mixing of PVA with Collagen and Bromelain for nanocomposite fabrication by using solvent casting and entrapment method were used. The testing methods that will be used to study the physical properties of the nanocomposite are degree of swelling test and moisture adsorption test followed by the experiment involve which is Fourier-transform infrared spectroscopy (FTIR) and Differential Scannning Calorimetry (DSC). The biocompatibility of the nanocomposite are also investigate used In-Vitro Biomineralization method.

2.1 Materials

Firstly, boiled the 50mL of deionized water at 95°C to 100°C with cover of aluminium foil to control the temperature. Then, 5g of PVA will be add to 50mL of deionized water and rapidly mixed using magnetic stirrer at 150°C to 160°C until it dissolve and become clear. 5mL of dimethyl sulfoxide (DMSO) added after the temperature drop a little bit and 1 w/v collagen will be added into the PVA solutions until it become clear. The flask will be kept under shaken conditions (250 rpm) at 30 minutes. The mixture is going to be collected for the film casting.

Table 2.1.1 The composition of raw material used in the PVA with collagen and bromelain.

Formulation / Material	Amount					
	A	**B**	**C**	**D**	**E**	**F**
Polyvinyl Alchohol (w/v%)	5	5	5	5	5	5
Collagen (w/v%)	0	1	2	0	1	2
Bromelain (w/v%)	0	0	0	3	3	3
Dimethyl sulfoxide (w/v%)	5	5	5	5	5	5
Distilled water (mL)	50	50	50	50	50	50

2.2 Methods

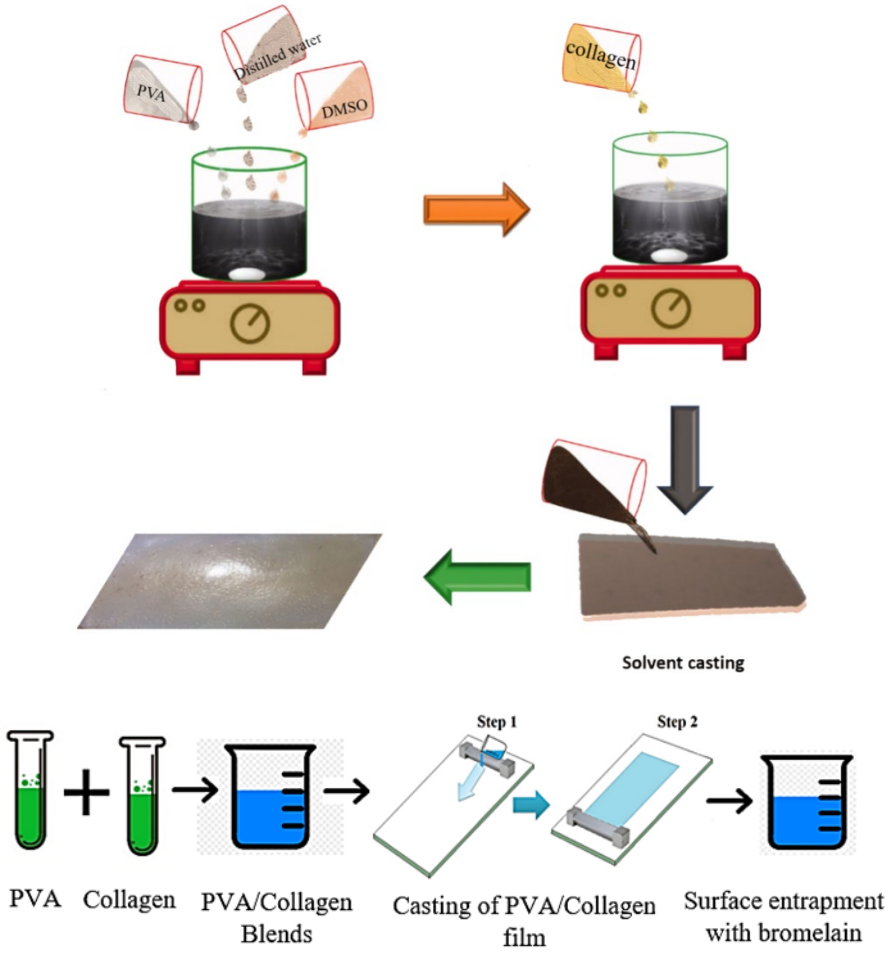

Figure 3.4.2: The flowchart of fabrication PVA with Collagen and Bromelain.

2.3 Surface entrapment of Bromelain

The fabricated films were then soaked into 3% of bromelain enzyme solution and kept into incubator at 37°C for 24 hours. After 24 hours, the film which undergoes the integral modification process are taken out and ready to be analysed. PVA/Collagen blends are ready to be tested for Characterization of PVA (Polyvinyl Alchohol) With Collagen and Bromelain.

2.4 Degree of Swelling

The dry of PVA samples is cut into 2.5 cm × 2.5 cm dimensions and submerged for 24 hours in 10 ml distilled water and 10 ml phosphate buffer saline (PBS). Wet scaffolds were wiped with filter paper to remove excess solution and weighed right away. Formula is used to compute the percentage of solution adsorption or degree of swelling (3.1).

$$(W_{sw} - W_D)/\, W_D \times\ 100\% \qquad (3.1)$$

2.5 Moisture Absorption

According to ASTM E 104-02 standards, the moisture absorption of the films is measure by placing 2.5cm x 2.5cm film samples in a desiccator (ASTM Standard E104-02, 2012). The desiccator's environment is regulated by silica gel beads. Following that, the weights of the samples will record at 12-hour duration, 24-hour duration, and then 48-hour duration. Equation (3.2) was used to compute the percentage of moisture absorption:

$$M_t(\%) = \frac{W_F - W_1}{W_i} \times 100\% \qquad (3.2)$$

2.6 Morphological Analysis

The surface morphology of PVA/collagen film was observed under an SEM (SU8000; Hitachi, Tokyo, Japan) at an accelerating voltage of10 or 15 kV. Prior to scanning under the SEM, the samples were sputter coated with a thin layer of gold for 30s with E-1030 ion sputter (Hitachi). An average of eight images was obtained at a 250-µm resolution power. To measure the average pore sizes, the image analysis was performed in triplicate. The diameter of porous matrix was measured.

2.7 Fourier Transform Infrared Spectroscopy

The presence of collagen in the PVA was confirmed by FTIR (PerkinElmer Frontier and Spectrum Two, Perkin Elmer) analysis. By creating an infrared absorption spectrum, FTIR testing is a useful instrument for identifying the kind of chemical bonds and functional groups in a molecule. Using a wave range of 400 to 4000 cm31, a PVA with a dimension of 1cm x 1cm was created for testing.

2.8 Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) technique provides information such as glass transition (Tg), melting (Tm) and crystallization (Tc) temperatures, in addition to the associated enthalpy for each process. DSC analyses were performed on a Shimadzu DSC 60 (Japan) calorimeter in the temperature range from 23 to 300 °C, at a heating rate of 10 °C min−1. Three samples were used to characterize each material in a short test times (10-30 minutes) in 5gram to 20gram of each sample.

2.9 In-Vitro Biomineralization

Immersion in simulated body fluid (SBF) solution was used to evaluate the hydrogel's in-vitro bioactivity. To analyse the growth of HA, the sample is submerged in 15 mL of the prepared SBF solution and put in an incubator at 37°C for 2 weeks. The sample was taken out of the SBF every 7 days and dried in the incubator for 24 hours at 50°C. The dried sample was then examined under a scanning electron microscope (SEM) for morphological alterations and hydroxyapatite (HA) growth.

3. Results and Discussion

The results and discussion section presents data and analysis of the study. This section can be organized based on the stated objectives, the chronological timeline, different case groupings, different experimental configurations, or any logical order as deemed appropriate.

3.1 Degree of Swelling

Swelling test was conducted in order to evaluate the swelling properties or degree of swelling for each film. The film samples were immersed in six solutions for 4 times to compare the ability of film to swell up to 6 hours, 12 hours, 18 hours and 24 hours. Figure 3.1 shows the bar chart percentage of swelling for PVA/collagen with bromelain film for each formulation.

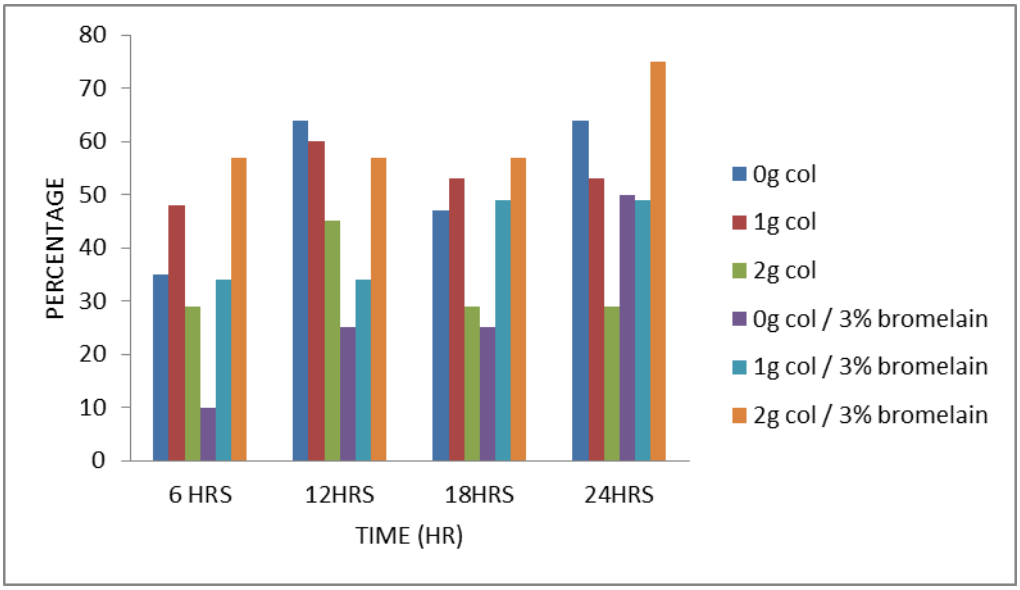

Figure 3.1 Percentage of swelling for PVA/collagen with bromelain film for each formulation.

Based on Figure 3.1, the film of PVA composite of 2%Collagen/3%Bromelain at 24 hours exhibited the highest degree of swelling compared to others. The results difference between PVA composite of 0%Col at 12 hours and PVA composite of 0%Col at 24 hours get the same results. Thus, PVA composite of 2%Col at 6 hours until 18 hours get a remain results. However, PVA composite of 0%Col/3%Bromelain at 6 hours get the lowest degree of swelling compared to others. PVA is very sensitive to water due to the presence of many hydroxyl groups. On the other hand, the collagen that contains abundant hydrophilic groups, so it swells in an aqueous solution.

In order to achieve the aim for treated cartilage by using PVA/Collagen with Bromelain film, the film used must have higher swelling properties, so the more the collagen, the absorbence will be increaase in the film. Other than that, swelling behavior considered the most important key parameters for cartilage application which control the release kinetics of water molecules from this scaffold. PVA composite of 2%Collagen/3%Bromelain at 24 hours have proven that having higher degree of swelling which suitable for being used for mention application.

3.2 Moisture Absorption

Moisture absorption test is to identify the amount of moisture absorb by the film. The weight has been taken with interval time of 6, 12, 18 and 24 hours, the results are as in Figure 3.2. Usually, the moisture absorption is related with the degree of swelling of the film. Depending on the results of swelling test, 2%Collagen/3%Bromelain at 24 hours have the best result because it was prove that this concentration have a highest absorption of PBS and that could help in cartilage application. For moisture absorption, 1%Collagen/3%Bromelain at 24 hours shows the increasing trend of percentage of absorption throughout the time intervals. 0%Collagen, 1%Collagen, 2%Collagen get a steady moisture at 6 and 12 hours, but it is increases for the next 12 hours. Moreover, 1%Collagen at 6 hours were the lowest percentage of moisture absorption among the others.

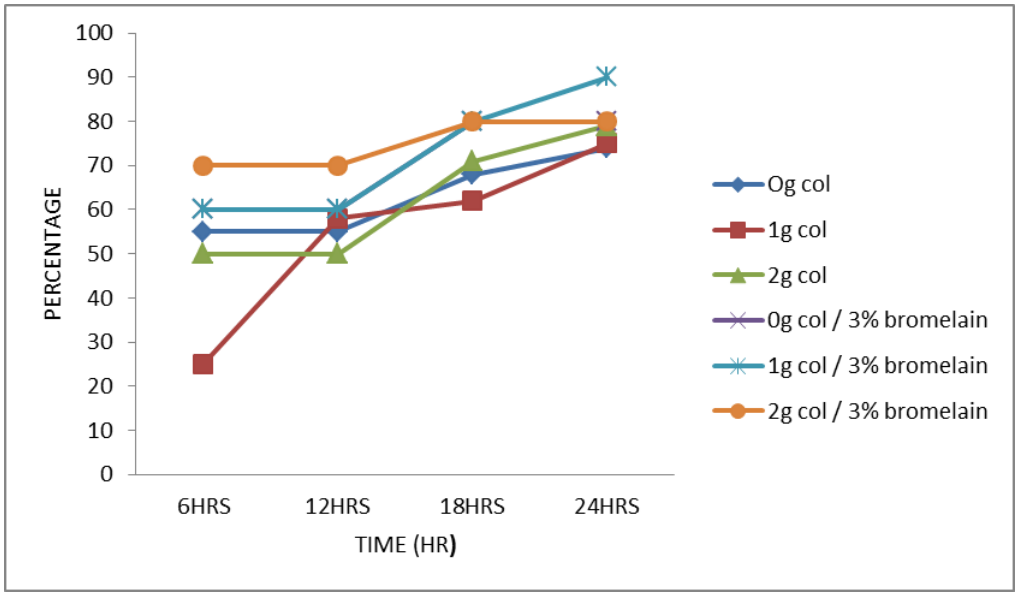

Figure 3.2 Percentage of moisture absorption for PVA/collagen with bromelain film for each formulation.

The capability of the film to absorb moisture are very important in cartilage application since it is had been used to help in would healing. Moisture absorption of Natural fiber affects the morphology of the natural fiber reinforced hybrid composites.

3.3 Selection of Excellent PVA/Collagen Formulation

The purpose of adding bromelain using entrapment method is it have strong preteolytic activity to neat PVA film to help treating in cartilage application. Based on swelling and moisture absorption test, shows that 2%Col with 3% Bromelain was the best characterization among the others because help to recovered the would healing faster. Moreover, it is because collagen acts as a natural structural scaffold and bromelian have 35% bromelain in a lipid base which helps in would healing. The optimum pH of bromelain is 5.5-8 and the optimum pH for healing the would is 7-8 and it is proven that 2%Col with 3% Bromelain was an excellent characterization.

3.4 Morphological Analysis

The surface morphology of PVA/collagen film was observed under an SEM (SU8000; Hitachi, Tokyo, Japan) at an accelerating voltage of10 or 15 kV. Prior to scanning under the SEM, the samples were sputter coated with a thin layer of gold for 30s with E-1030 ion sputter (Hitachi). An average of eight images was obtained at a 250-µm resolution power. To measure the average pore sizes, the image analysis was performed in triplicate. The diameter of porous matrix was measured.

Figure 3.4 SEM micrographs showing the morphological changes in (a) PVA/0%collagen, (b) PVA/1%collagen, and (c) PVA/2%collagen with bromelain. It can be seen from the figure that there is a change in surface morphology of PVA/Col and with the presence of 3% bromelain. SEM micrograph of native collagen has a smooth surface morphology. The average pore sizes of the composites range from few microns to several microns. This packing of collagen is due to the formation of H-bonds between the hydroxyl groups of PVA and collagen. It has been reported that PVA/collagen film show a pore size of 10µm.

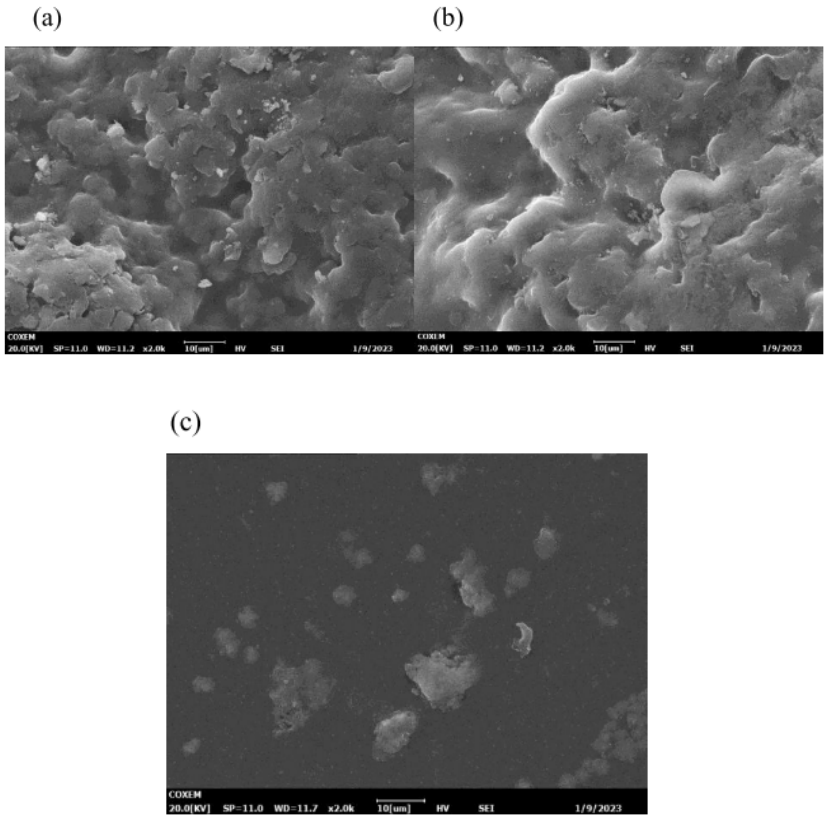

Figure 3.4 SEM micrographs showing the morphological changes in (a) PVA/0%Col, (b) PVA/1%Col, and (c) PVA/2%Col with bromelain.

However, PVA/0%collagen and PVA/1%collagen film demonstrated a rougher, irregular surface of collagen. While PVA/collagen in the presence of bromelain demonstrated a small appearance surface of collagen. This effect is mainly due to the presence of bromelain. A pre-requisite for developing collagen-based matrices is creating a porous matrix that can facilitate cells to be infiltrated into the matrix for biomedical applications in vitro and in vivo. The inherent porous matrix of collagen is often collapsible This gives an added advantage for the preparation of collagen-based biomaterials with a tunable porosity.

3.5 Fourier Transform Infrared Spectroscoopy (FTIR)

The characteristics of PVA observed in all six films spectrum, pure PVA film displays peak value of 3246.5 cm-1, followed by 3265.1 cm-1 for PVA/1Col film, 3261.4 cm-1 for PVA/2Col film, 3250.2 cm-1 for both pure PVA and PVA/Col enhanced with bromelain and finally PVA/2Col enhanced with bromelain at peak 3254 cm-1. This is because at wavenumber ranging from 3600 cm-1 to 3200 cm-1 indicates O-H stretching, which verified the presence of O-H bond group of PVA. Figure 3.5.1 shows the Fourier Transform Infrared Spectroscoopy (FTIR) spectrum of PVA/Collagen Films (a)PVA, (b)PVA/1Col, (c)PVA/2Col.

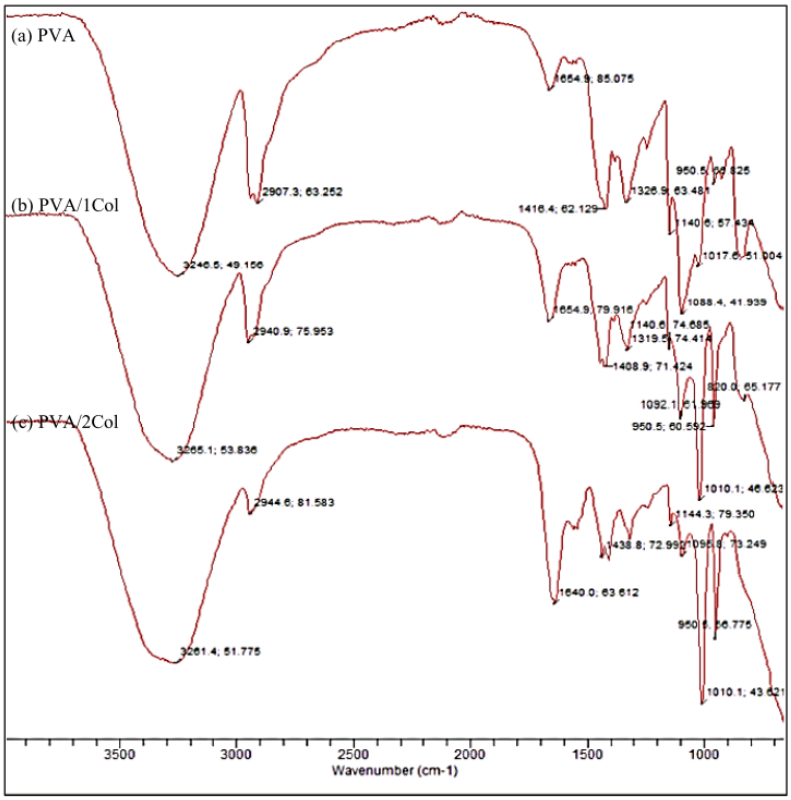

Figure 3.5.1 Fourier Transform Infrared Spectroscoopy (FTIR) spectrum of PVA/Collagen Films (a)PVA, (b)PVA/1Col, (c)PVA/2Col.

On the other hands, pure PVA shows reading at peak 2907 cm-1, 2940 cm-1 for PVA/1Col and 2944 cm-1 for PVA/2Col, pure PVA, PVA/1Col and PVA/2Col which enhanced with bromelain. Referring to the result and comparing it to previous literature, peak ranging from 3000-2840 cm-1 specified a medium stretching of C-H bonds came from alkene group.

Figure 3.5.2 shows the Fourier Transform Infrared Spectroscoopy (FTIR) spectrum of PVA/Collagen Films (a)PVA/Bromelain, (b)PVA/1Col Bromelain, (c)PVA/2Col Bromelain. In the case of bromelain, the specific activity data of the bromelain is shown in the FTIR spectrum that bromelain has OH and aliphatic primary amine overlapping at bands 3000–3500 cm-1 originates from N-H stretching vibration. At peak of 1654 derived from pure PVA and PVA/1Col film to 1640 cm-1 exhibited by PVA/2Col film complemented with all of which films enhanced with bromelain, it indicates a strong intensity of C=O stretching vibration coupled to N-H bending vibration which associated with amide I area, and amide II band appears from N-H bending vibration coupled to C-N stretching vibration of the peptide linkage.

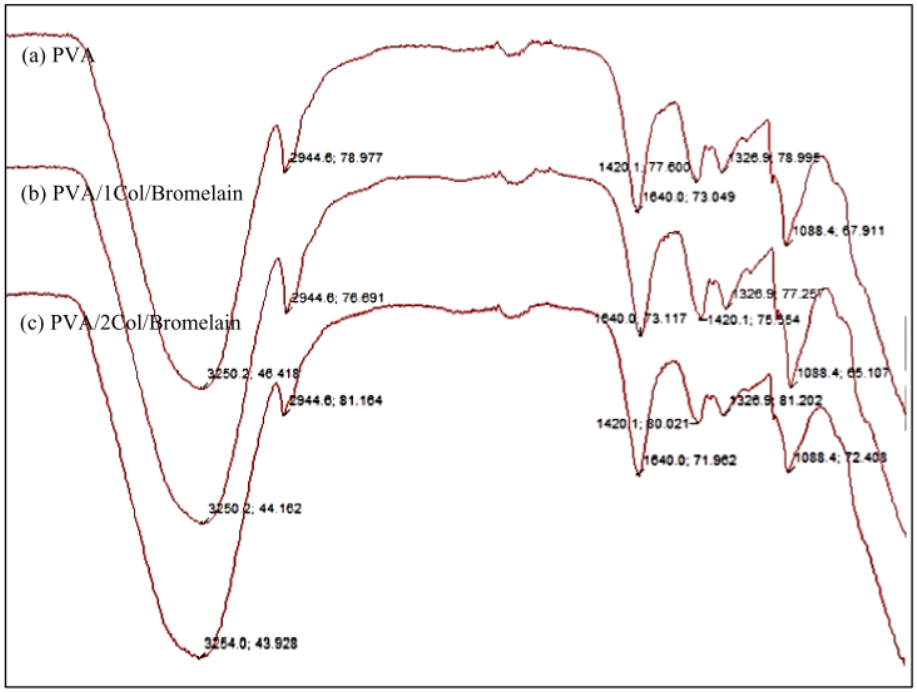

Figure 3.5.2 Fourier Transform Infrared Spectroscoopy (FTIR) spectrum of PVA/Collagen Films (a)PVA/Bromelain, (b)PVA/1Col Bromelain, (c)PVA/2Col Bromelain.

In the fingerprint region (600-1500cm-1), strong signal was found at about 1500cm-1 (informing aromatic ring). Vinyl-related compound was also found at about 1000cm-1. Thus, the functional group of collagen type I is 1620cm-1 to 1800cm-1 with C=O stretching. Amide III band for PVA/0Col, PVA/1Col and PVA/2Col with 3% bromelain was at 1420 to 1088cm-1. An amide III band of both collagens at 1420cm-1 revealed that both collagens did not denature during entrapment method.

3.6 Differential Scanning Calorimetry

DSC characterization had been carried out to get some information about transition temperatures of the prepared samples and it is one of the most convenient methods to determine miscibility and thermal properties of polymer blend (Cooper et al., 2000 ; Gill et al., 2010). The DSC curve shown in Figure 3.6.1 shows the curve of PVA/0%Col, Figure 3.6.2 shows the curve of PVA/2%Col and Figure 3.6.3 shows the cruve of PVA/2%Col with 3% bromelain.

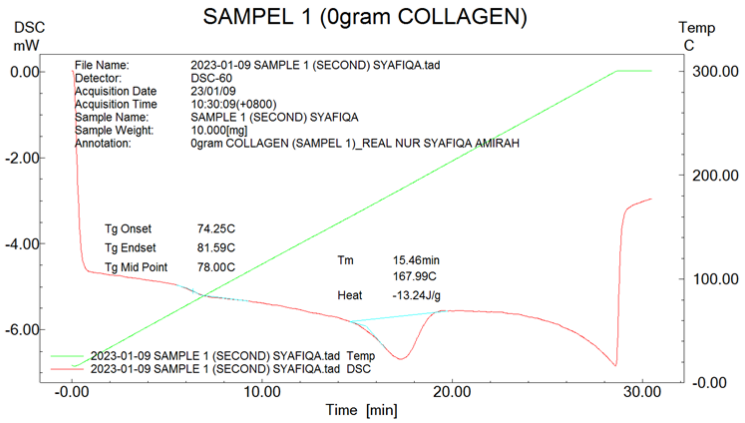

Figure 3.6.1 shows the DSC testing for 0%col of PVA.

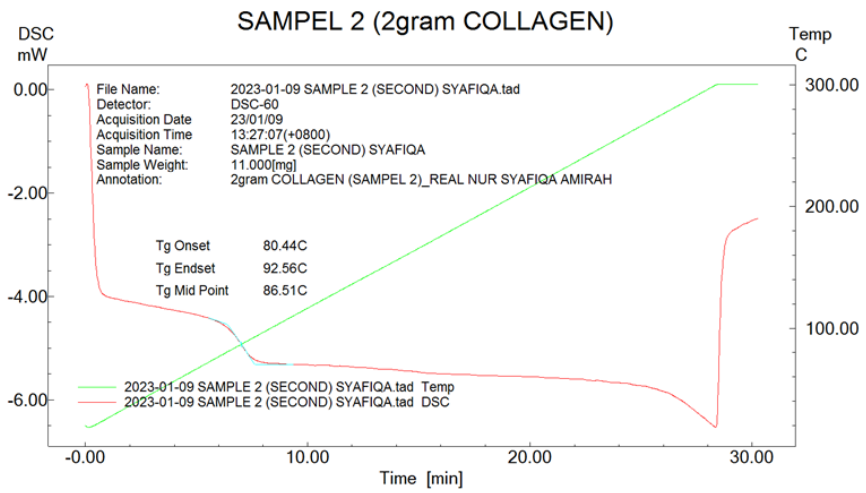

Figure 3.6.2 shows the DSC testing for 2%col of PVA.

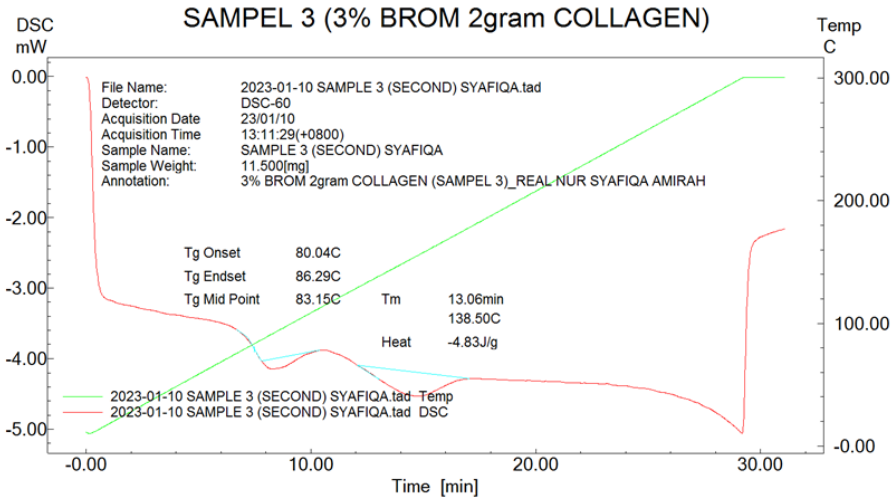

Figure 3.6.3 shows the DSC testing for 2%col of PVA with 3% bromelain.

It can be observed that DSC spectra of PVA, PVA/collagen film and PVA/2Col with 3% bromelain showed there are characteristic features of semicrystalline materials. But DSC spectra of PVA/2Col shows no characteristic features of semicrystalline. Based on Figure 3.6.1 that shows the curve of PVA/0%Col, the Tg is 80.04C and 86.16C which related to glass transition (Tg) that prior to endothermal peak of melting point (Tm). The Tg is the melting of disordered/amorphous region. The Tm is 167.99C and the heat is -13.24J/g. While for Figure 3.6.2 shows the curve of PVA/2%Col, it only get the Tg which is 80.44C and 92.56C, which shows there are no semicrystalline materials present in PVA/2%Col. Then, Figure 3.6.3 shows the cruve of PVA/2%Col with 3% bromelain, the Tg is 80.04C and 86.29C. Tm 138.50C and the heat is -4.83J/g.

Chemical cross linking within PVA/collagen blend by gamma radiation is proposed through a complex mechanism. The major mechanism is involving the immediate effect of irradiation i.e. radiolysis of water and formation of polymer free radicals (Pietrusha, 1990 ; Von Sonntag et al., 1995 ; Yang et al., 2008). These macroradicals mainly produce by indirect effect of radiation while OH radicals attack the polymers to produce the alpha-carbon radical peptide in case of collagen. For PVA, the OH radicals can either abstract an H atom in α-position to the OH group (70%) or at the neighboring methylene group (β-position, 30%). The main reaction that involved OH groups may be the underlying reason of strong O-H stretching band in IR spectra of irradiated film blend.

3.7 In-vitro Biominerarization

For the purpose of developing synthetic hybrid materials for the regeneration of human and dental tissues, in-vitro biomineralization testing was carried out to see whether synthetic biological mechanisms are capable of producing mineralized matrices that can be useful. Figure 3.7 shows that in the 0% collagen of PVA, 2% collagen of PVA, 2% collagen of PVA with 3% bromelain, the HA developed rapidly and inhabited the whole PVA/Collagen film surface. While the HA developed in a dispersed manner, there were areas of the 2% collagen with 3% bromelain of PVA/Collagen surface that were not cultured with HA.

In addition, the size of the HA crystal development on the 2% collagen with 3% bromelain PVA/Collagen is larger than that on the 2% collagen of PVA/Collagen. More ions and molecules can be adsorb on the apatic surface due to the larger surface area produced by smaller crystals (Azmi et al., 2016). To sum up, although the entrapment technique of bromelain has significantly changed the biocompatibility of the PVA/Collagen film, it is still capable of aiding in the synthesis of HA, which is the essential to accurately simulating a biological system.

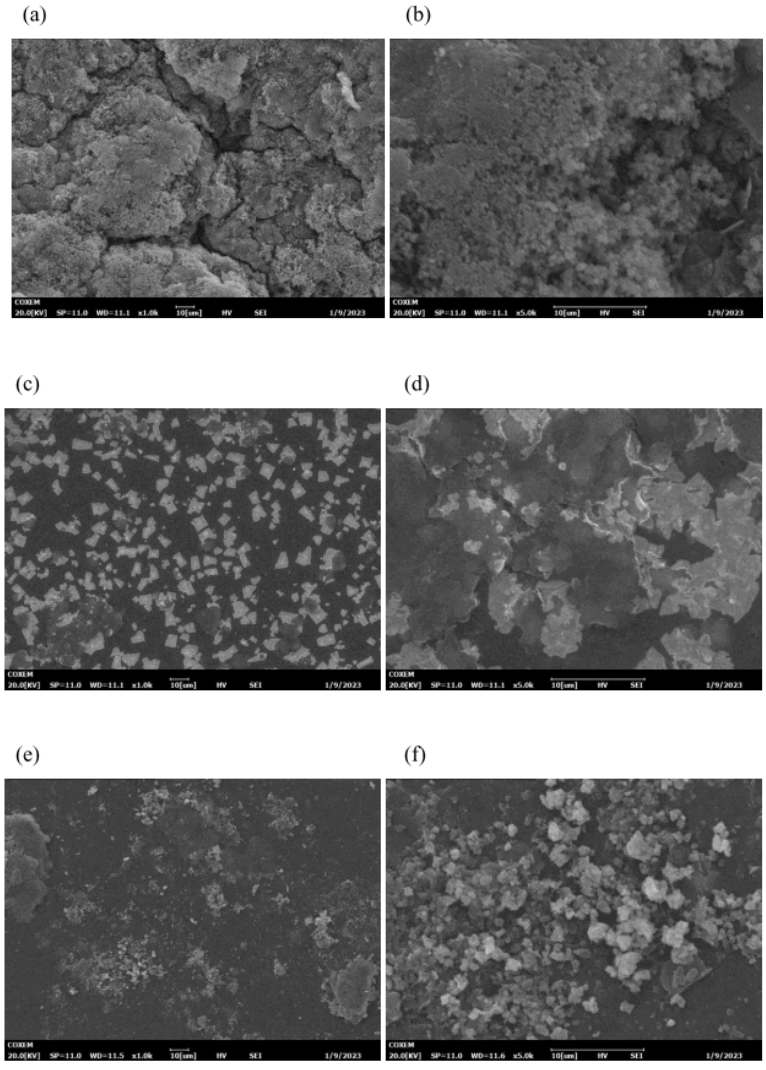

Figure 3.7 SEM image for a) 0%collagen of PVA at 1000x b) 0%collagen of PVA at 5000x c) 2%collagen of PVA at 1000x d) 2%collagen of PVA at 5000x e) 2%collagen with 3% bromelain at 1000x f) 2%collagen with 3% bromelain at 5000

4. Conclusion

This study provides scientific understanding on the collagen as filler material in order to improve the PVA properties in cartilage application. PVA/Col film and PVA/Col film with bromelain was successfully fabricated using simple techniques of solvent casting and entrapment method. PVA/2%Col with 3% bromelain concentration proved to be suitable for preparing PVA/Col film to help in treating cartilage application. This was proven by the samples having the best swelling capacity and moisture absorption. The FTIR results indicated the existence of intermolecular bond between CH3 in the PVA and NH2 of the collagen made up the amine linkages.

Morphology images by SEM revealed that the surface of the film become rougher with the addition of collagen. PVA/0%collagen and PVA/1%collagen film demonstrated a rougher, irregular surface of collagen. For DSC testing, PVA/collagen film and PVA/2Col with 3% bromelain showed there are characteristic features of semicrystalline materials. But DSC spectra of PVA/2Col shows no characteristic features of semicrystalline. For in-vitro biomineralization testing, it is proven that the size of the HA crystal development on the 2% collagen with 3% bromelain PVA/Collagen is larger than that on the 2% collagen of PVA/Collagen. More ions and molecules can be adsorb on the apatic surface due to the larger surface area produced by smaller crystals and the entrapment technique of bromelain has significantly changed the biocompatibility of the PVA/Collagen film.

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