

## **A bio-hybrid material with special water affinity made from polyvinyl alcohol, rice starch and silk fibroin**

P. Kuchaiyaphum<sup>1,2</sup>, R. Watanesk<sup>2</sup>, S. Watanesk<sup>2</sup> and T. Yamauchi<sup>1</sup>

<sup>1</sup>(Department of Materials Science and Technology, Niigata University, Japan)

<sup>2</sup>(Department of Chemistry, Faculty of Science, Chiang Mai University, Thailand)

**ABSTRACT:** A hybrid material composing of polyvinyl alcohol (PVA), rice starch (RS) and silk fibroin (SF) with opposite water affinity on each surface was successfully prepared. The hybrid material was prepared by placing PVA/RS/SF hydrogel onto glycerol-modified PVA/RS/SF film and leaving them to adhere. SF content in the hydrogels was optimized first by mixing various SF amounts to a mixture of PVA:RS (2:1 by weight). The 8.00 part per hundred of polymer (php) of SF was an appropriate concentration revealed by the maximum values of gel fraction and percent porosity. This SF content caused the blend to exhibit the highest water saturation and best mechanical properties. The glycerol-modified hydrophobic film with the same PVA/RS/SF composition was prepared and found that the water contact angle of the film had increased approximately three times after water soaking. The hybrid material was then prepared and characterized. As evidenced by physical appearance and SEM images, the interface between layers became homogeneous after 30 h layering time. Water contact angle values pointed out the hydrophilic nature of the hydrogel surface, while the film surface was hydrophobic. Coupling of the hydrogel with the film improved overall mechanical properties as indicated by stress and % elongation at break.

**Keywords** - bio-hybrid material, poly(vinyl alcohol), rice starch, silk fibroin

### **I. INTRODUCTION**

A hybrid material is defined as a material that consists of two constituents at nano level. Research and development of bio-hybrid materials for temporary skin covers or as wound dressing is becoming a subject of great commercial interest [1]. Wound dressing hydrogels are three-dimensional polymeric networks and are available in sheet form or as a spreadable viscous gel with their uniquely interesting properties such as easy replacement, transparency to allow healing follow-up, oxygen permeability, and so on [2, 3]. In recent years, interest in using the PVA hydrogel has significantly increased related to its high biocompatibility, hydrophilicity, harmlessness, sterility, transparency and the good gel forming capability [4-10]. Several methods have been used for preparing PVA hydrogels [11]. One of them is the successive freeze-thaw cycles which result in a physically cross-linked gel called as cryogel [12]. However, the gel degradation rate and oxygen gas permeability are not satisfactory for further applications. Thus, more improvement of these properties is required. One of the methods used to improving PVA properties is blending PVA with other materials. Natural polymers are usually preferred in biomaterials due to their excellent biological properties, including cell adhesion, mechanical properties similar to the natural tissue, biodegradability and biocompatibility [13]. Thus many researchers prepared PVA blends with various natural polymers such as alginate, chitosan and hyaluronic acid [14-16].

Rice starch (RS) and silk fibroin (SF) are two interesting natural polymers with several advantages that can be utilized for blending with PVA. SF has been widely used in many areas such as medical, pharmaceutical, cosmetic and agricultural applications in the forms of wound dressing, film matrices, hydrogels, etc. Recently, many researchers have reported the study of silk fibroin for various applications [17-22]. Regenerated SF can either be blended or chemically cross-linked with

other natural or synthetic polymers to form hydrogels with improved properties. SF composite gels have been prepared with PVA, gelatin, collagen, poloxamer-407, modified polyethylene glycol (PEG), N-isopropylacrylamide (NIPAAm), polyacrylamide, etc. [23-33]. Rice starch (RS), another renewable and biodegradable biopolymer, has been increasingly utilized in many applications, primarily because of its low cost and ease of availability [34]. Only a few studies on starch polymer blend hydrogels have been reported [35]. PVA/starch blend hydrogels were prepared by chemical cross-linking [36] and also irradiation technique [37]. Our previous work demonstrated that such prepared PVA/RS/SF film exhibited some improved properties such as lower water solubility, higher oxygen permeability and degradability compared to PVA itself [38, 39].

In this work demonstrates further surface properties improvement of the previously prepared PVA/RS/SF film using glycerol treatment for generating a hydrophobic film. Additionally, the PVA/RS/SF blends were used to prepare PVA/RS/SF hydrogels using the freezing/thawing method to increase porosity in the hydrogels. The optimization of the SF content for preparing both the hydrogel and the glycerol-modified film was done through by firstly blending a PVA:RS mixture (2:1 by weight) with various amounts of SF ranging from 2.67-13.3 php (part per hundred of polymer), and then the characterization of some hydrogel properties. Finally, depending on the proven self-healing property of PVA gels [40], a PVA/RS/SF hybrid material was prepared through the coupling of the prepared PVA/RS/SF hydrogel with the glycerol-modified PVA/RS/SF film by simply layering the hydrogel onto the glycerol-modified film, and leaving them at room temperature until completely adhered to each other. The surface properties of both layers of the hydrogel and the glycerol-modified film of the PVA/RS/SF hybrid material were determined through the water contact angle measurement. The prepared hybrid material has opposite water affinity on each surface. We presume that the opposite water affinity on each surface can give a bandage the advantage of isolating a wound from the external environment while protecting the wound bed from dehydration to facilitate the healing process.

## **II. EXPERIMENTAL**

### **II.1. Materials**

Silk waste was purchased from Jun Thai Silk Group Co., Thailand. Silk fibroin powder (SF) was prepared from silk waste, as the procedure stated by Moonsri *et al.* [41], without further characterization. RS (Era-Tab, Lot # T510405, Anal. # T0405, loss on drying 11.22%, residue on ignition 0.26 % and pH 5.49) was obtained from Erawan Pharmaceutical Research and Laboratory Company Co., Ltd., Thailand. PVA (99% hydrolyzed with a molecular weight average of 85,000-124,000) was purchased from Aldrich Chemical Co. Inc. USA. Glycerol (86-89% assay) was purchased from Fluka BioChemika, Germany. Hexane (95.0% purity) was purchased from Kanto Chemical Co. Inc. Japan. All other chemicals were analytical grade and distilled water was used throughout.

### **II.2. Preparation of PVA/RS/SF hydrogel**

A mixture of 10% w/v PVA and 5% w/v RS solutions (30 mL each) was stirred for 60 min at 80 °C before adding various amounts of SF (0.0200-0.1000 g) into each of the mixture (10 mL) at room temperature. After that the mixed solutions were stirred for 60 min more, then poured into the moulds (20×20×5 mm<sup>3</sup>) and processed for 7 cycles of freezing at -20 °C for 45 min and further thawing at 25 °C for 45 min. All obtained hydrogels of PVA:RS (2:1 by weight) with the SF contents ranging from 2.67-13.3 php were optimized for obtaining an appropriate SF content for further preparation of glycerol modified PVA/RS/SF film and PVA/RS/SF hybrid material.

### *II.2.1. Percentages of gel fraction and water absorption*

The gel fraction (G) of the PVA/RS/SF gels with various contents of SF was estimated [42] using equation (1). The dried hydrogel and the water-removed hydrogel were weighed. Water uptake of the hydrogel samples was measured at 25 °C. All dried samples were immersed in distilled water at different time intervals, then the swollen samples were removed and immediately weighed after blotting out excess water off the surface. The equilibrium water saturation ( $W_{eq}$ ) in the swollen samples was calculated [11] using equation (2).

$$G(\%) = \frac{W_2}{W_1} \times 100 \quad (1)$$

$$W_{eq} = \frac{W_3 - W_1}{W_3} \times 100 \quad (2)$$

where  $W_1$ ,  $W_2$  and  $W_3$  are the weight of dried hydrogel, the weight of the water-removed hydrogel, and the weight of the swollen sample, respectively.

### *II.2.2. Hydrogel porosity*

Percent porosity of the PVA/RS/SF hydrogels was measured by the liquid displacement method [43]. The porosity,  $\varepsilon$ , is defined as the total volume of the pores divided by the total volume of the porous sample. The sample was immersed in a known volume ( $V_1$ ) of hexane in a graduated cylinder for 10 min. Then the total volume of hexane was recorded again as  $V_2$ . Later on, the hexane imbibed sample was carefully removed from the cylinder and the remaining hexane volume was recorded as  $V_3$ . Porosity of the samples was calculated from equation (3).

$$\varepsilon = \frac{V_1 - V_3}{V_2 - V_3} \times 100 \quad (3)$$

### *II.2.3. Mechanical properties*

The stress at break and % elongation at break of five selected pieces of the PVA/RS/SF hydrogels were measured using a IMADA force measurement (MX2-500N), with a cross-head speed of 10 mm/min. The results were reported as the average values.

## **II.3. Preparation of glycerol modified PVA/RS/SF film**

A blended film of PVA/RS/SF modified with glycerol in aqueous medium was prepared as the procedure previously mentioned by Kuchaiyaphum *et al.* [39] with the optimal composition as the above mentioned in the PVA/RS/SF hydrogel preparation. A 1.67 g of glycerol was added into 100 mL of PVA:RS mixture (2:1 by weight) containing 8.00 php of SF to obtain the concentration of 20 % w/w and stirred for 40 min more. Then the mixture was casted in the moulds (10 cm diameter) and the solvent was evaporated at room temperature in a laminar flow hood for a day. After solvent evaporation, the glycerol-modified film was peeled off and soaked in water for various time intervals (15, 30, 60 and 90 min). The soaking time resulted in generating the glycerol-modified film to be more hydrophobic was selected so that this modified film could be used further for a hybrid material preparation.

#### *II.3.1. Film hydrophobicity*

The hydrophobicity of the film was evaluated from water contact angle measurement and it was measured by dropping 1  $\mu\text{L}$  of deionized water onto the glycerol-modified film surface using a KYOWA DM 300N Contact Angle Analyzer at 25 °C. The contact angles at 5 second were monitored and the average of at least three measurements was calculated. The remaining glycerol on the PVA/RS/SF films surface after soaking in water at various time intervals were also confirmed through functional group analysis using ATR spectroscopy. The ATR spectra of film samples were recorded with a FTIR-8400S spectrometer (SHIMADZU, Japan) in the range between 4000 to 650  $\text{cm}^{-1}$ .

#### *II.3.2. Mechanical properties*

Each dried glycerol-modified film sample was cut into small pieces ( $1 \times 5 \text{ cm}^2$ ) with a film thickness of  $0.060 \pm 0.010 \text{ mm}$ . The stress at break and % elongation at break of five selected pieces of the samples were measured in the same fashion as done with the hydrogels and the average values were reported.

### **II.4. Preparation and characterization of PVA/RS/SF hybrid material**

The PVA/RS/SF hybrid material was prepared by placing the PVA/RS/SF hydrogel ( $20 \times 20 \times 5 \text{ mm}^3$ ) on the glycerol modified PVA/RS/SF film ( $50 \times 50 \times 0.060 \text{ mm}^3$ ). The interface between the surfaces of the hydrogel and the glycerol-modified film was investigated after allowing them to adhere for various time periods. Then the cross-sectional morphology of the interface was observed using a Scanning Electron Microscope (SEM; JCM-6000, JEOL). For measuring the stress at break and % elongation at break, five selected pieces of the hybrid material were clamped across the bound interface and pulled parallel to the interface using IMADA force measurement (MX2-500N), with a cross-head speed of 10 mm/min. The results were reported as the average values. In addition, gel fraction and % porosity of the hybrid material were also determined using equations (1) and (3), respectively. Finally the surface properties on each surface of the hybrid material were investigated by measuring the water contact angle.

## **III. RESULTS AND DISCUSSION**

### **III.1. Preparation of PVA/RS/SF hydrogel**

#### *III.1.1. Percentages of gel fraction and water absorption*

The optimum conditions for preparing the PVA/RS/SF hydrogel can be evaluated from the gel fraction values and water absorption data shown in Table 1 and Fig. 1, respectively. Results in Table 1 demonstrate that the percentages of gel fraction of the PVA/RS/SF mixtures at various SF contents are in the range of 82-89 %. Within this range, it appears that the gel fraction values increase with the increase of SF content until reaching the maximum value at the SF content of 8.00 php. Afterward, the more SF is added, the lower the values of gel fraction of the hydrogel are observed. As the SF content increases, more SF molecules enter the network of PVA/RS blend to form gel. However after the maximum amount of SF is reached, the rest of the increased SF could not be part of the gel formation and is washed out later, thus lowering the gel fraction values.

Since water can be absorbed by the PVA/RS/SF hydrogels quite easily due to the hydrophilic nature of the hydrogel, so the extent of water absorption of the PVA/RS/SF hydrogels in Fig.

1 agrees with the result of the gel fraction that the largest water absorption occurs at the highest gel fraction.

### *III.1.2. Hydrogel porosity and mechanical properties*

From Table 2, the PVA/RS/SF hydrogels show similar trend of their porosity and mechanical properties on being SF dependent. The hydrogel with 8.00 php SF also exhibits the highest porosity which is agreeable with the gel fraction and water absorption values. Moreover, the maximum values of stress at break and % elongation at break in the presence of SF are also found when 8.00 php of SF is added. However, the stress at break and % elongation at break of the PVA/RS/SF hydrogels are lower than those of the PVA/RS hydrogel without SF. This can be justified by the generated porosity due to the addition of SF and because most of the silk materials developed from silk fibroin solution are weak and brittle [21].

From the above mentioned results, the optimal content of SF in the PVA/RS/SF hydrogel, with 2:1 weight ratio of PVA:RS, was therefore selected at 8.00 php. This optimal condition was used for preparing the glycerol-modified PVA/RS/SF film and also the PVA/RS/SF hybrid material with an opposite water affinity on each surface.

## **III.2. Preparation and optimization of glycerol-modified PVA/RS/SF film**

### *III.2.1. Film hydrophobicity*

From our previous study [39], hydrophobicity of the glycerol-modified films was successfully achieved by ethanol treatment. However, in order to avoid using an organic solvent, water was used to replace ethanol in enhancing hydrophobicity of the film. Then its hydrophobicity was investigated through the measurement of water contact angle after soaking the glycerol-modified film in water at various soaking times.

Results in Fig. 2 reveal that the glycerol-modified films become hydrophobic after soaking in water for 30 min and their hydrophobicity slowly increases at longer soaking time, evidenced by the increase of water contact angles. This is because the glycerol molecules interact with PVA, RS and SF molecules in the blend through hydrogen bonding between the hydroxyl groups of glycerol, PVA, RS and the amide groups of SF [44, 45]. However, some of the glycerol molecules remain unbound, causing more interaction of the hydroxyl groups of unbound glycerol molecules in the glycerol-modified film with water molecules in the water drop. Thus, the water drop would be attracted and adhered more to the surface of the film resulting in less contact angle which indicates less hydrophobic character. Once the films are soaked in water, water molecules are allowed to penetrate the blend and remove more of the unbound glycerol molecules. It was found that 60 min is long enough to remove such glycerol.

As the water contact angle of the glycerol-modified PVA/RS/SF film increases with the increase of soaking time, therefore in order to confirm the removal of glycerol, its content in the PVA/RS/SF films after soaking in water at various time intervals was followed from the ATR spectra shown in Fig. 3. Considering the –OH group stretching vibration in the region of 3600-3000 cm<sup>-1</sup>, after soaking the modified film in water for longer time, the spectra show more disappearance of –OH band indicating that more unbound glycerol molecules are removed from the films.

### *III.2.2. Mechanical Properties*

Mechanical properties in terms of the stress at break and % elongation of the glycerol-modified films after soaking in water at various soaking times are shown in Fig. 4. The increase in soaking time causes the increase of the film strength but decreases its flexibility. Since glycerol is a plasticizer, its function is to reduce the phase separation between PVA, RS and SF by forming hydrogen bonding between the blended materials so that elongation at break of the film is improved. After water-treatment, the amount of glycerol is reduced which causes the higher strength and less flexibility in the film.

However, at soaking times longer than 60 min, the film strength is decreased because the increasing soaking time allows more glycerol within the blend to be removed, in addition to the unbound glycerol molecules. From the above results, 60 min was considered to be appropriate for soaking the glycerol modified film in water so that it can be used further for preparing the hybrid material.

### **III.3. Preparation and characterization of PVA/RS/SF hybrid material**

#### *III.3.1. Physical appearance and interfacial morphology*

The photos in Fig. 5 show the interface between the PVA/RS/SF hydrogel and the glycerol modified PVA/RS/SF film within the PVA/RS/SF hybrid material after layering at various times. The two layers of the hybrid material are completely bound together after layering the hydrogel on top of the glycerol-modified film for 30 h in which there is no cleavage between the layers observed. This can be justified, considering the hydroxyl group of vinyl alcohol, the bonding process can be attributed to the formation of hydrogen bonding between PVA chains. It is easy to picture that the hydrogen bonds responsible for the bonding should essentially be those formed between PVA chains on both sides of the interface and/or those between PVA chains on one side and PVA chains diffusing across the interface from the other side when the two surfaces are brought into contact [40]. The cross-sectional SEM image in Fig. 6 also confirms the existing bound interface within the hybrid material after 30 h of layering. This image also reveals that the structures of the hydrogel layer appear to be porous whereas the blended film layer is more dense.

#### *III.3.2. Gel fraction, porosity and mechanical properties*

Result in Table 3 reveals that after coupling the PVA/RS/SF hydrogel with the glycerol-modified PVA/RS/SF film, the gel fraction and percent porosity of the PVA/RS/SF hybrid material do not change that much. However, the stress at break and % elongation at break of the hybrid material are a bit higher than those of the hydrogel itself, whereas, the elongation at break of the hybrid material is higher than that the glycerol-modified film. Hence, these results show the improvement of the mechanical properties of the PVA/RS/SF hybrid material indicating that the structure of the PVA/RS/SF hybrid material is stronger than that of the PVA/RS/SF hydrogel and more flexible than both PVA/RS/SF hydrogel and the glycerol-modified PVA/RS/SF film.

#### *III.3.3. Surface property and water absorption*

Fig. 7 depicts the water affinity behavior of the PVA/RS/SF hybrid material. As evidenced by a large value ( $110.4 \pm 3.4^\circ$ ) of water contact angle and water drop formation observed on the surface of the glycerol-modified film, this indicates the hydrophobic nature of the surface. On contrary, when water was dropped on the surface of the hydrogel layer, it was quickly absorbed, thus its water contact angle of the PVA/RS/SF hydrogel surface could not be detected. So the hydrogel layer of the hybrid material is very strongly hydrophilic. These results of the water affinity behavior of the prepared PVA/RS/SF hybrid material suggest an approach to produce a bio-hybrid material with opposite water affinity on its surfaces.

## **IV. CONCLUSIONS**

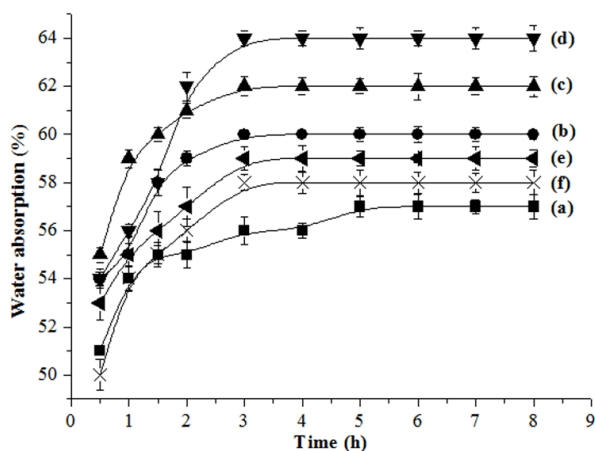
The PVA/RS/SF hybrid material with opposite water affinity on each surface was successfully prepared. The PVA/RS/SF hydrogel was coupled to the glycerol modified PVA/RS/SF film and leaving them bonded for 30 h at room temperature. The content of SF for preparing the layers of hybrid material was initially optimized from the specific properties of the PVA/RS/SF hydrogel. The addition of SF produced more porosity in the hydrogel. In addition, the water absorption, gel fraction and mechanical properties of the hydrogel were also dependent on the porosity of the hydrogel. The optimal



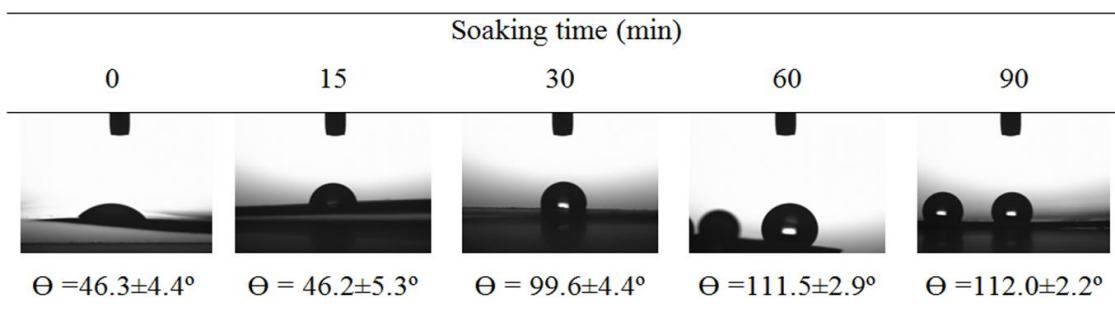
SF content was selected to be 8.00 php in which the gel fraction of the PVA/RS/SF hydrogel was about 89% after 7 cycles of freezing/thawing. The PVA/RS/SF film modified with glycerol became hydrophobic by simply soaking it in water. The hydrophobicity of the glycerol-modified film was slowly increased by increasing the soaking time up to 60 min as the excess glycerol was leached out. The obtained PVA/RS/SF hybrid material had better mechanical properties than the PVA/RS/SF hydrogel and the glycerol-modified PVA/RS/SF film itself. Results from the measurement of water contact angle on both surfaces of the hybrid material indicated that the surface of the PVA/RS/SF hydrogel layer was hydrophilic while the layer of glycerol-modified PVA/RS/SF film was hydrophobic with a water contact angle of about 110°.

## V. ACKNOWLEDGEMENTS

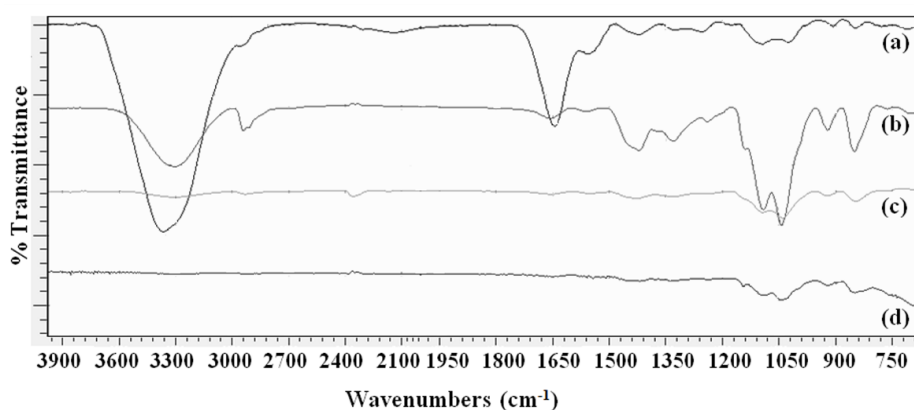
Financial support from Global Circus-Graduate School of Science and Technology, Niigata University and an Independent Administrative Institution, Japan Student Services Organization (JASSO), is gratefully acknowledged. Thanks to the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Office of the Higher Education Commission (OHEC), Ministry of Education, Thailand and also the Graduate Schools of both Niigata University and Chiang Mai University for their financial supports. A partial support from the National Research University Project under Thailand's OHEC, Materials Science Research Center, Faculty of Science, Chiang Mai University is also very appreciated.



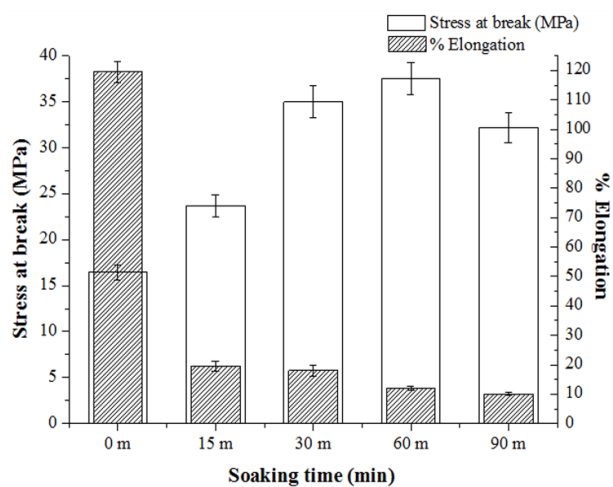
**Fig. 1.** Water absorption of (a) the PVA/RS hydrogel, and the PVA/RS/SF hydrogels with the SF contents of (b) 2.67 php (c) 5.33 php (d) 8.00 php (e) 11.7 php and (f) 13.3 php after freezing/thawing for 7 cycles.



**Fig. 2.** Water contact angles on the glycerol modified PVA/RS/SF films after soaking in water at different soaking times.

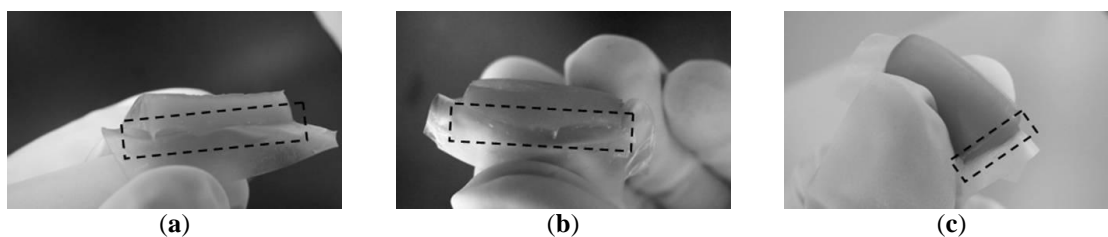


**Fig. 3.** ATR spectra of (a) the glycerol-modified PVA/RS/SF, and the glycerol-modified films after soaking in water for various time intervals of (b) 15 min, (c) 30 min and (d) 60 min.

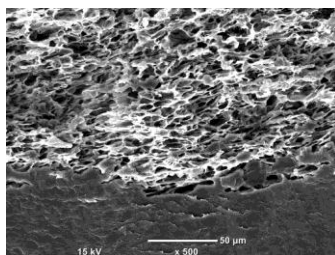


**Fig. 4.** Stress at break and % elongation at break of the glycerol modified PVA/RS/SF films after soaking in water.

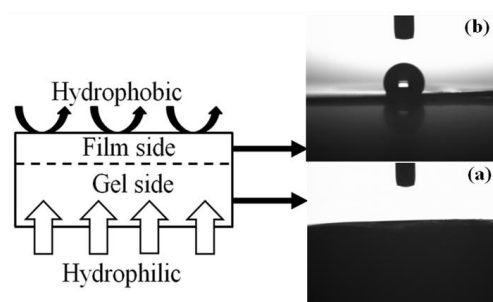




**Fig. 5.** Photos of the PVA/RS/SF hybrid material after layering at room temperature for (a) 18 h, (b) 24 h and (c) 30 h.



**Fig. 6.** Cross-sectional SEM images showing the interface within the PVA/RS/SF hybrid material after layering for 30 h.



**Fig. 7.** Demonstration of water affinity behavior of the PVA/RS/SF hybrid material and water absorption on each surface of the (a) PVA/RS/SF hydrogel and (b) glycerol modified PVA/RS/SF film, after complete adhering.

**Table 1.** Gel fraction (G) of PVA/RS/SF hydrogel with various SF contents.

	SF content (php) in the PVA/RS/SF hydrogel					
	0.00	2.67	5.33	8.00	10.7	13.3
% gel fraction	85.2±0.8	86.4±0.5	87.3±0.3	89.4±0.5	84.7±0.6	81.9±0.7

**Table 2.** Porosity and mechanical properties of PVA/RS/SF hydrogels with various SF contents.

SF contents (php) in the PVA/RS/SF hydrogel	Porosity (%)	Mechanical properties	
		Stress at break (MPa)	% Elongation at break
0.00	50.0±0.8	0.64±0.03	132.89±7.50
2.67	52.3±0.7	0.29±0.07	76.98±2.49
5.33	60.0±1.2	0.35±0.04	85.74±5.28
8.00	71.4±0.7	0.45±0.12	105.45±2.72
10.7	66.7±1.0	0.43±0.10	78.31±3.70
13.3	66.4±0.5	0.42±0.19	76.41±2.12

**Table 3.** Comparison of some properties of the PVA/RS/SF hydrogel, the glycerol-modified PVA/RS/SF film, and the PVA/RS/SF hybrid material at the same optimal composition of 2:1 weight ratio of PVA:RS and 8.00 php SF.

Properties	PVA/RS/SF		
	Hydrogel	Glycerol-modified film	Hybrid material
Gel fraction (%)	89.4±0.5	-	90.1±0.3
Porosity (%)	71.4±0.7	-	71.0±1.1
Mechanical properties			
- Stress at break (MPa)	0.45±0.12	37.53±1.75	1.7±0.1
- Elongation at break (%)	105.45±2.72	12.01±0.60	118.4±1.6

## REFERENCES

- [1] M.T. Razzak, S.P. Zainuddin Erizal Dewi, H. Lely and E. Taty, Sukimo, The characterization of dressing component materials and radiation formation of PVA-PVP hydrogel, *Radiat. Phys. Chem.*, 55, 1999, 153-165.
- [2] L.L. Lloyd, J.F. Kennedy, P. Methacanon, M. Paterson and C.J. Knill, Carbohydrate polymers as wound management aids, *Carbohydr. Polym.*, 37, 1998, 315-322.
- [3] S.K. Purna and M. Babu, Collagen based dressings-a Review, *Burns*, 26, 2000, 54-62.
- [4] J.S. Gonzalez, A.S. Maiolo, C.E. Hoppe and V.A. Alvarez, Composite gels based on poly(vinyl alcohol) for biomedical uses, *Procedia Mater. Sci.*, 1, 2012, 483-490.
- [5] J.O. Kim, J.K. Park, J.H. Kim, S.G. Jin, C.S. Yong, D.X. Li, J.Y. Choi, J.S. Woo, B.K. Yoo, W.S. Lyoo, J.A. Kim and H.G. Choi, Development of polyvinyl alcohol-sodium alginate gel-matrix based wound dressing system containing nitrofurazone, *Int. J. Pharm.*, 359, 2008, 79-86.
- [6] C.M. Hassan, J.E. Stewart and N.A. Peppas, Diffusional characteristics of freeze/thawed poly(vinyl alcohol) hydrogels: Applications to protein controlled release from multilaminate devices, *Eur. J. Pharm. Biopharm.*, 49, 2000, 161-165.
- [7] C.M. Hassan and N.A. Peppas, Structure and Morphology of Freeze/Thawed PVA Hydrogels, *Macromolecules*, 33, 2000, 2472-2479.
- [8] K. Pal, A.K. Banthia and D.K. Majumdar, Preparation and Characterization of Polyvinyl Alcohol-Gelatin Hydrogel Membranes for Biomedical Applications, *AAPS Pharm. Sci. Tech.*, 8 (1), 2007, 1-5.
- [9] G. Paradossi, F. Cavalieri and E. Chiessi, Poly(vinyl alcohol) as versatile biomaterial for potential biomedical applications, *J. Mater. Sci.-Mater. Med.*, 14, 2003, 687-691.
- [10] C.M. Hassan and N.A. Peppas, Structure and Applications of Poly(vinyl alcohol) Hydrogels Produced by Conventional Crosslinking or by Freezing/Thawing Methods, *Adv. Polym. Sci.*, 153, 2000, 37-65.
- [11] K.H. Syed, Gulrez, S.A. Assaf and G.O. Phillips, Hydrogels: Methods of Preparation, Characterisation and Applications, *Progress in Molecular and Environmental Bioengineering-From Analysis and Modeling to Technology Applications*,

- (2011), Prof. Angelo Carpi (Ed.), ISBN: 978-953-307-268-5, InTech.
- [12] R. Ricciardi, G. Mangiapià, F. Lo Celso, L. Paduano, R. Triolo, F. Auremma, C. De Rosa and F. Laupretre, Structural Organization of Poly(vinyl alcohol) Hydrogels Obtained by Freezing and Thawing Techniques: A SANS Study, *Chem. Mater.*, 17(5), 2005, 1183-1189.
- [13] F. Rosso, G. Marino, A. Giordano, M. Barbarisi, D. Parmeggiani and A.J. Barbarisi, Smart materials as scaffolds for tissue engineering, *Cell. Physiol.*, 203(13), 2005, 465-470.
- [14] Y.F. Tang, Y.M. Du, X.W. Hu, X.W. Shi and J.F. Kennedy, Rheological characterisation of a novel thermosensitive chitosan/poly(vinyl alcohol) blend hydrogel, *Carbohydr. Polym.*, 67, 2007, 491-499.
- [15] S.H. Cho, S.H. Oh and J.H. Lee, Fabrication and characterization of porous alginate/polyvinyl alcohol hybrid scaffolds for 3D cell culture, *J. Biomater. Sci. Polym. Ed.*, 16(8), 2005, 933-47.
- [16] S.J. Kim, S.G. Yoon, Y.M. Lee, H.C. Kim and S.I. Kim, Electrical behavior of polymer hydrogel composed of poly(vinyl alcohol)-hyaluronic acid in solution, *Biosens. Bioelectron.*, 19, 2004, 531-536.
- [17] C. Vepari and D.L. Kaplan, Silk as a biomaterial, *Prog. Polym. Sci.*, 32, 2007, 991-1007.
- [18] M. Okhawilaia, R. Rangkupanb, S. Kanokpanonta and S. Damrongsakkula, Preparation of Thai silk fibroin/gelatin electrospun fiber mats for controlled release applications, *Int. J. Biol. Macromol.*, 46(5), 2010, 544-550.
- [19] E. Wenk, H.P. Merkle and L.J. Meinel, Silk fibroin as a vehicle for drug delivery applications, *Control. Release.*, 150, 2011, 128-141.
- [20] L.P. Yan, J.M. Oliveira, A.L. Oliveira, S.G. Caridade, J.F. Mano and R.L. Reis, Macro/microporous silk fibroin scaffolds with potential for articular cartilage and meniscus tissue engineering applications, *Acta. Biomater.* 8 (1), 2012, 289- 301.
- [21] B. Kundu, R. Rajkhow, S.C. Kundu and X. Wang, Silk fibroin biomaterials for tissue regenerations, *Adv. Drug Deliv. Rev.*, 65, 2013, 457-470.
- [22] B. Kundu, N.E. Kurland, S. Bano, C. Patra, F.B. Engel, V.K. Yadavalli and S.C. Kundu, Silk proteins for biomedical applications: Bioengineering perspectives, *Prog. Polym. Sci.*, 39, 2014, 251-267.
- [23] X. Chen, W.J. Li, W. Zhong, Y.H. Lu and T.Y. Yu, PH sensitivity and ion sensitivity of hydrogels based on complex forming chitosan/silk fibroin interpenetrating polymer network, *J. Appl. Polym. Sci.*, 65, 1997, 2257-62.
- [24] G.D. Kang, J.H. Nahm, J.S. Park, J.Y. Moon, C.S. Cho and J.H. Yeo, Effect of poloxamer on the gelation of silk fibroin, *Macromol. Rapid Commun.*, 21, 2000, 788-91.
- [25] S.H. Kim and C.C. Chu, Synthesis and characterization of dextran-methacrylate hydrogels and structural study by SEM, *J. Biomed. Mater. Res.*, 49, 2000, 517-27.
- [26] M. Li, N. Minoura, L. Dai and L. Zhang, Preparation of porous poly(vinyl alcohol)-silk fibroin (PVA/SF) blend membranes, *Macromol. Mater. Eng.*, 286(9), 2001, 529-33.
- [27] M.K. Yoo, H.Y. Kweon, K.G. Lee, H.C. Lee and C.S. Cho, Preparation of semi interpenetrating polymer networks composed of silk fibroin and poloxamer macromere, *Int. J. Biol. Macromol.*, 34, 2004, 263-70.
- [28] E.S. Gil, R.J. Spontak and S.M. Hudson, Effect of beta-sheet crystals on the thermal and rheological behavior of protein based hydrogels derived from gelatin and silk fibroin, *Macromol. Biosci.*, 5, 2005, 702-9.
- [29] J.Y. Fang, J.P. Chen, Y.L. Leu and H. Wang, Characterization and evaluation of silk protein hydrogels for drug delivery, *Chem. Pharm. Bull.*, 54, 2006, 156-62.
- [30] E.S. Gil and S.M. Hudson, Effect of silk fibroin interpenetrating networks on the swelling and deswelling kinetics and rheological properties of poly(Nisopropyl acrylamide) hydrogels, *Biomacromolecules*, 1, 2007, 258-264.
- [31] Q. Lu, K. Hu, Q.L. Feng and F. Cui, Fibroin/collagen hybrid hydrogels with crosslinking method. Preparation properties and cytocompatibility, *J. Biomed. Mater. Res. Part A*, 1, 2008, 198-207.
- [32] B.B. Mandal, S. Kapoor and S.C. Kundu, Silk fibroin/polyacrylamide semi interpenetrating hydrogels for controlled drug release, *Biomaterials*, 30 (28), 2009, 26-36.
- [33] J. Kundu, L.A. Poole-Warren, P. Martens and S.C. Kundu, Silk fibroin/poly(vinyl alcohol) photocrosslinked hydrogels for delivery of macromolecular drugs, *Acta. Biomater.*, 8, 2012, 1720-1729.
- [34] J.F. Zhang and X.Z. Sun, Mechanical properties of PLA/starch composites compatibilized by maleic anhydride, *Biomacromolecules*, 5, 2004, 1446-1451.
- [35] K. Hashim, K.Z. Dahlan and N.M. Noordin, Hydrogel of sago starch/water-soluble polymers by electron beam irradiation technique. *International Symposium on Radiation Technology in Emerging Industrial Applications*, 2000, 79-80. International Atomic Energy Agency(IAEA)-SM-365.
- [36] K. Pal, A.K.Banthia and D.K.Majumdar, Preparation of Transparent Starch Based Hydrogel Membrane with Potential Application as Wound Dressing, *Trends Biomater. Artif. Organs*, 20(1), 2006, 59-67.
- [37] M. Zhai, F. Yoshii, T. Kume and K. Hashim, Syntheses of PVA/starch grafted hydrogels by irradiation, *Carbohydr. Polym.*, 50, 2002, 295-303.
- [38] P. Kuchaiyaphum, W. Punyodom, S. Watanesk and R. Watanesk, Composition optimization of polyvinyl alcohol/rice starch/silk fibroin-blended films for improving its eco-friendly packaging properties, *J. Appl. Polym. Sci.* 129, 2013, 2614-2620.
- [39] P. Kuchaiyaphum, T. Yamauchi, R. Watanesk, S. Watanesk, Hydrophobicity Enhancement of the Polyvinyl Alcohol/Rice Starch/Silk Fibroin Films by Glycerol, *Appl. Mech. Mater.*, 446-447, 2014, 360-365.
- [40] H. Zhang, H. Xia and Y. Zhao, Poly(vinyl alcohol) Hydrogel Can Autonomously Self-Heal, *ACS Macro. Lett.*, 1, 2012, 1233-1236.
- [41] P. Moonsri, R. Watanesk, S. Watanesk, H. Niamsup and R.L. Deming, Fibroin membrane preparation and stabilization by polyethylene glycol diglycidyl ether, *J. Appl. Polym. Sci.*, 108, 2008, 1402-1406.
- [42] S. Jolanta, K. Martins, D. Anda and T. Velta, Poly (Vinyl Alcohol) Cryo-Hydrogel Systems for Development of Biomaterials, *Polym. Sci.*, 58, 2009, 63-66.

- [43] N. Bhardwaj and S.C. Kundu, Silk fibroin protein and chitosan polyelectrolyte complex porous scaffolds for tissue engineering applications, *Carbohydr. Polym.* 85, 2011, 325-333.
- [44] S. Lu, X. Wang, Q. Lu, X. Zhang, J.A. Kluge, N. Uppal, F. Omenetto and D.L. Kaplan, Insoluble and flexible silk films containing glycerol, *Biomacromolecules*, 11, 2010, 143-150.
- [45] L. Dai, J. Li and E. Yamada, Effect of glycerin on structure transition of PVA/SF Blends. *J. Appl. Polym. Sci.*, 86, 2002, 2342-2347.