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Preparation and Characterization of Hair Keratin/Gelatin Blend Films

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Abstract: Keratin solution was extracted from human hairs and used as subject for preparation of keratin/gelatin blend films. This study was aimed to explore the suitable method using for keratin extraction and extend to study the blend film properties. The blend films were prepared by simple evaporation method. After homogeneously mixed between keratin and gelatin solution at different ratios, the solution were placed on the plates and left in an oven at 40°C for 3 days. All of the films were then analyzed for their morphology, secondary structures and thermal properties by using SEM, FTIR and TGA, respectively. The result from SEM images showed that native keratin films have the highest rough surface compared to other films. In addition, the smooth surface of films gradually increased when the gelatin content increased. Keratin blending with gelatin showed structural changes, especially at the absorption bands of 3300-2900 cm^{-1} as well as the amide I, II and III regions. Moreover, thermal properties of the keratin films were enhanced by blending with gelatin. This study suggested that gelatin help to improve some properties of keratin while still remain its strength.

Key words: Gelatin, keratin, morphology, secondary structures, thermal properties

INTRODUCTION

Keratin is a structural protein and is typified by sulfur content. Generally, it is classified into α-keratin which was found in skin, wool, hoof, whale baleen and β-keratin is found in claw, scale, feather and beaks (Meyers *et al*., 2008). Now-a-days, feather is one important source of keratin (Joshi *et al*., 2007; Tamilmani *et al*., 2008). Over the past century, development of keratin-based biomaterials was gradually increased. This was due to keratins have unique biological activity and biocompatibility as well as self-assembled structures. Protein-based materials including collagen, albumin, gelatin, silk fibroin and gelatin have been wildly explored and applied in various fields as naturally-derived biomaterials (Rouse and Van-Dyke, 2010). Study on physical, chemical and biological properties of keratin has been published over three past decades (Yamauchi *et al*., 1996; Tanabe *et al*., 2002; Yamauchi *et al*., 2003; Fujii *et al*., 2004). On the other hand, researchers have been done to fabricate keratin-based biomaterials for tissue engineering and regenerative medicine (Apel *et al*., 2008; Sierpinski *et al*., 2008; Aboushwareb *et al*., 2009; Reichl, 2009). Moreover, keratin could be performed into various forms such as sponges (Kurimoto *et al*., 2003; Tachibana *et al*., 2005), scaffolds (Katoh *et al*., 2004; Peplow and Dias, 2004), fibers (Aluigi *et al*., 2007, 2008; Varesano *et al*., 2008) and films (Yamauchi *et al*., 1996, 1998). However, pure keratin was too fragile and had poor strength and flexibility (Fujii *et al*., 2004). Therefore, some studies have been focused on the optimization of the keratin properties by addition both natural (Lee *et al*., 1998; Lee and Ha, 1999; Lee, 2001; Tanabe *et al*., 2002; Yamauchi *et al*., 2003; Vasconcelos *et al*., 2008) and synthetic (Tonin *et al*., 2007) polymers. Gelatin, a derivative product of collagen, has been used to improve properties of hydrophobic materials like silk fibroin (Wilaiwan *et al*., 2010; Watcharin *et al*., 2009; Prasong, 2010).

At present little information about the effect of gelatin on the keratin was rarely occurred. In this study, keratin/gelatin blend films were prepared and characterized their morphology, secondary structures and thermal properties.

MATERIALS AND METHODS

This study was done for 3 months from November 10, 2010 to February 10, 2011. The experiment was performed at Department of Chemistry, Faculty of Sciences, Mahasarakham University, Thailand.

Materials: Human hairs were collected from local hair salon in Maha Sarakham Province, Thailand. The hairs were warmed at 40°C, washed with distilled water for 2 times. They were removed external lipid by immersion in n-hexane for 12 h, then air-dried before use. Other chemical reagents used were analytical grade.

Methods

Keratin extraction: Human keratin was extracted using modification Shindai method (Nakamura *et al*., 2002).

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Gelatin solution: The 1% of gelatin solution was prepared by weighing 1 g of gelatin powder and then added 100 mL of distilled water. The mixture was then magnetic stirred until gelatin dissolves absolutely.

Keratin/gelatin blend films preparation: The keratin/gelatin blend films were prepared by mixing 1% wt. of keratin and 1% wt. gelatin at different ratios; 1:1, 1:2 and 2:1. The 20 mL of mixture was poured on the polystyrene plates, then taken to an oven at 40°C for 3 days to obtain films. In addition, the native Keratin (K) and Gelatin (G) films were also prepared to use as control.

Characterization of films: All films were cut and observed their morphology under the Scanning Electron Microscope (SEM) (JEOL, JSM-6460LV, Tokyo, Japan). The samples were sputter coated with gold by double side of carbon for enhancing surface conductivity. The secondary structures of the films were analyzed using FTIR spectroscopy (Perkin Elmer-Spectrum Gx, USA) in the spectral region of ~4000-400 cm^{-1} at 4 cm^{-1} spectral resolution and 32 scans. Thermal properties were also measured using Thermogravimetric Analyzer (TGA), TA instruments, SDT Q600 (Luken's drive, New Castle, DE). The films weight of 8-10 mg were prepared and loaded in a platinum crucible. The samples were non-isothermal heated from 50 to 800°C at a heating rate of 20°C min^{-1}. The TGA was carried out in nitrogen with the flow rate of 100 mL min^{-1}. The TG and heat flow were recorded with TA Instrument's Q series explorer software. The analyses of the data were done using TA Instrument's Universal Analysis 2000 software (version 3.3B).

RESULTS

Morphology: The morphology of the films was observed under SEM as shown in Fig. 1. Both native gelatin and

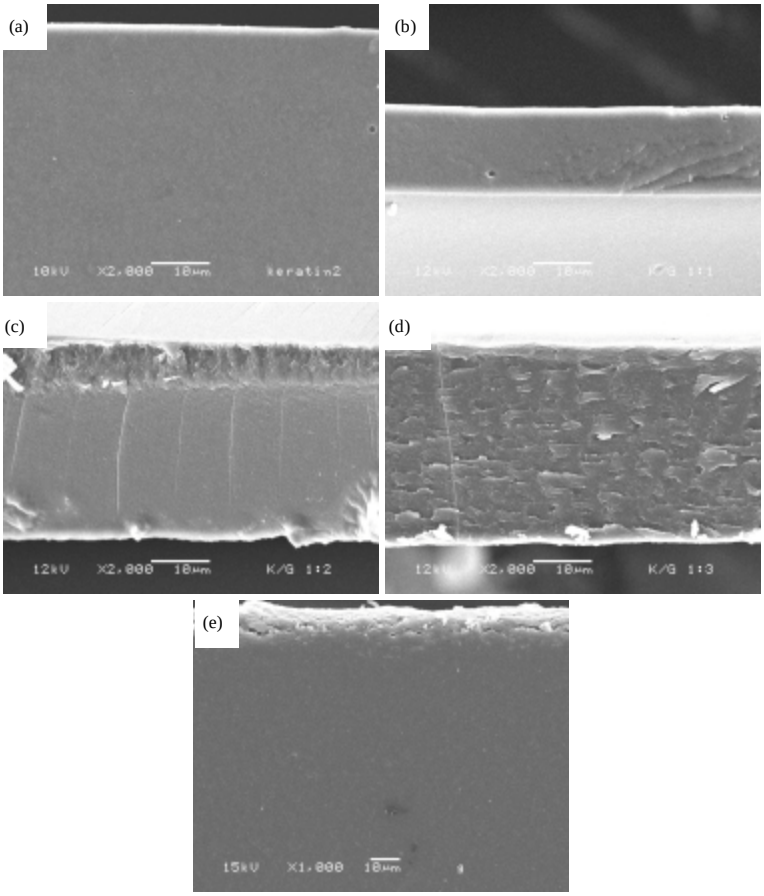

Fig. 1 (a-e): (a) SEM micrographs of native keratin, (b) Keratin/gelatin blended at 1:1, (c) 1:2, (d) 2:1 and (e) Native gelatin. Scale bars = 10 μm

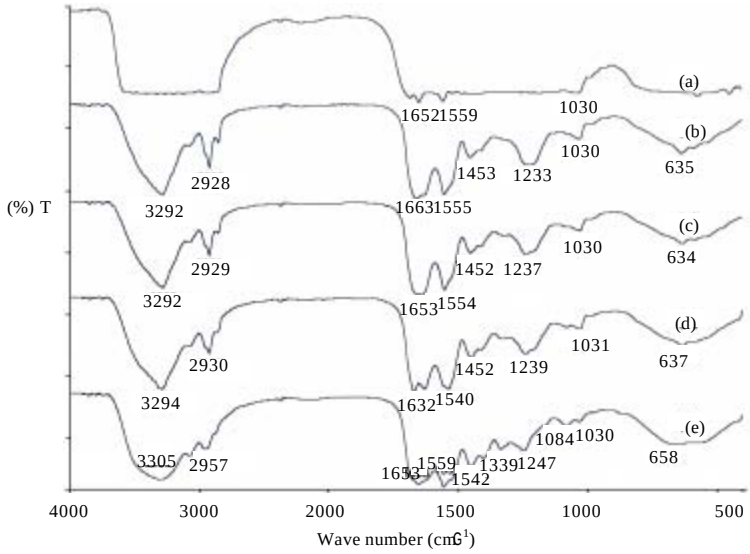

Fig. 2: FTIR spectra of native keratin (a), keratin/gelatin blended at 2:1 (b), 1:1 (c), 1:2 (d) and native gelatin (e)

keratin showed smooth surfaces through out the film area. The native gelatin slightly smoother than the native keratin since the last film surfaces composed the small bead like particles cover the keratin texture. All of blend ratios have smooth surfaces as same as the native. Considering to the different ratios of the blend films, cross section images showed different characteristics. The miscible texture of keratin and gelatin was found at the ratio of 1/1. The rough of film was observed when increased the content of both native types. The highest rough of film from cross section was occurred when blended ratio was keratin (2)/gelatin (1). However, all of blend films were homogeneously mixed and did not separate in their texture.

Secondary structures: As shown in Fig. 2, IR spectrum of native keratin showed different profiles comparison to native gelatin as well as the blend films. Strong absorption peaks of keratin film indicated at 3205, 1684, 1559 and 1030 cm^{-1}. Native gelatin film showed strong absorption peaks at 3305, 1653, 1559, 1452, 1247 and 1084 cm^{-1}. The blend films showed similar absorption peaks in all of blend ratios but some different points were also observed. The blend ratio of keratin (1)/gelatin (1), IR spectrum showed the absorption peaks mixed characteristics both native types at 3292, 2929, 1653, 1554, 1452, 1237 and 1030 cm^{-1}. When increased the gelatin content (keratin (1)/gelatin (2)), IR spectrum was similar to the IR spectrum of native gelatin.

Thermal behaviors: Thermal properties of the films were analyzed from Thermogravimetric (TG),

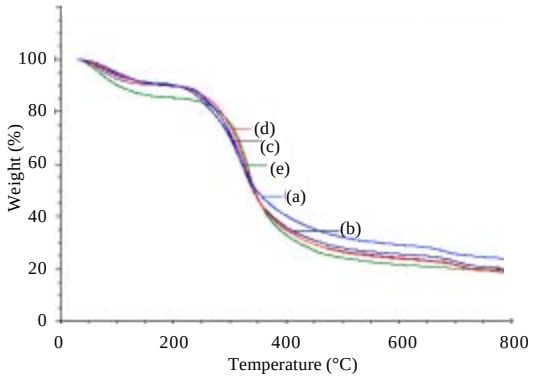

Fig. 3: TG curves of different films: native keratin (a), keratin/gelatin blended at 2:1 (b), 1:1 (c), 1:2 (d) and native gelatin (e)

differential TG (DTG) and heat flow results. As shown in Fig. 3, all of films did not completely decompose even at 800°C. At the last temperature, native keratin remained its weight in the highest percentage while native gelatin was the lowest. However, native gelatin has the highest of temperature of maximum decomposition ($T_{d, max}$). The results were clearly indicated by DTG curves (Fig. 4). From the results, native keratin has the lowest of $T_{d, max}$. The $T_{d, max}$ of keratin film was increased when blended with gelatin. With the heat flow curves showed exo/endo-thermic peaks of the films which summarized in Table 1. The highest of exo-thermic peaks of native keratin and gelatin were 533 and 527°C, while endo-thermic peaks were 669 and 492°C, respectively. The blend films

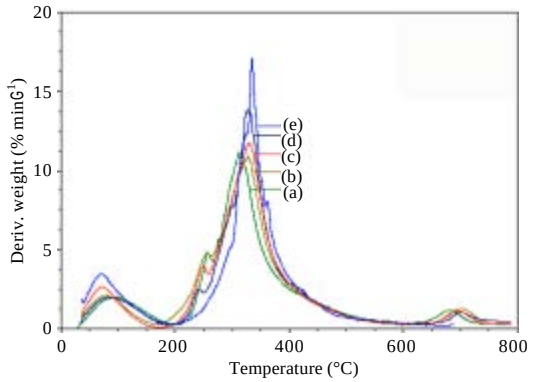

Fig. 4: DTG curves of different films: native keratin (a), keratin/gelatin blended at 2:1 (b), 1:1 (c), 1:2 (d) and native gelatin (e)

Table 1: Exo/endo-thermic peaks of different films

Film types	Exothermic peaks (°C)	Endothermic peaks (°C)
Keratin	220 273 409 533	104 259 311 474 669
Keratin/gelatin (2:1)	196 271 423 554 765	91 255 333 522 765
Keratin/gelatin (1:1)	202 267 317 410 539 757	83 250 302 339 511 716
Keratin/gelatin (1:2)	205 257 376 735	95 242 330 692
Gelatin	205 251 355 407 527	81 226 332 363 492

showed higher exo/endo-thermic peaks than that of native films, especially high content of keratin.

DISCUSSION

Recently, protein based-materials such as collagen (Sumita *et al*., 2006), gelatin (Xing *et al*., 2010), silk fibroin (Guziewicz *et al*., 2011) or keratin (Reichl, 2009) have been applied in various fields. Of these, keratin-based materials have attracted for development due to their natural abundance (Rouse and Van-Dyke, 2010). Among keratin sources, hair keratins contain a much higher content of cysteine residues and form tougher and more durable structures (Moll *et al*., 2008). Like many materials, keratin could be prepared in several forms. Keratin film has been used for a long history. Generally, pure keratin film was too fragile, poor strength and flexibility (Yamauchi *et al*., 1996; Fujii *et al*., 2004). To solve this limitation, addition of both natural (Tanabe *et al*., 2002; Yamauchi *et al*., 2003) and synthetic (Tonin *et al*., 2007) polymers to keratin was developed.

Blending keratin with gelatin found that all of films have homogeneous texture with smooth surfaces. This result suggested that miscibility between native keratin and gelatin was improved. On the other hand, gelatin has smoother surface than keratin. This can be described by the difference of their internal composition. Keratin composed of both hydrophobic amino acids such as glycine, alanine and hydrophilic amino acids such as glutamic acid, serine and cysteic acid (Ki *et al*., 2007; Cardamone, 2008, 2010). The blend films between keratin and gelatin showed smooth texture without separation phase. This suggested that the interaction between keratin and gelatin could be formed via hydrophilic amino acids.

FTIR was powerful equipment for analysis the secondary structure of protein (Tasukada *et al*., 1995; Kweon *et al*., 2000). The sensitive regions at amide I (1700-1600 cm^{-1}), amide II (1600-1400 cm^{-1}) and amide III (1400-1200 cm^{-1}) were analyzed. Native keratin showed strong peaks at 1684 and 1652 cm^{-1} represent β-turn and α-helix structures, respectively. In addition, the absorption peak at 1559 cm^{-1} assignments to tryptophan (Church *et al*., 2010) while peak at about 1030 cm^{-1} was sulfitolysis cleavage of the cysteine disulfide bond (Cardamone, 2008). For native gelatin, almost absorption peaks as well as conformation were similar to native keratin, except peak at 1246 cm^{-1} (β-sheet) (Church *et al*., 2010). The blend films showed strong absorption peaks of mixture keratin and gelatin characteristics. It could be observed that keratin blended with gelatin enhancement the β-sheet formation of the films. The result confirmed that interaction between the native types was occurred.

The TG and DTG curves of films indicated that native keratin has the highest of $T_{d, max}$ comparison to other films. Gelatin film showed strong one peak at 336°C while native gelatin exhibits major peak at 312°C with small two peaks at 257 and 681°C. The blend films indicated strong peaks at about 330°C with two shoulder peaks like keratin. It was noted that keratin blended with gelatin at 1:1 ratio has higher $T_{d, max}$ than other ratios. This result might be suggested that this ratio was suitable blending to give the homogeneous texture of the film. Heat flow curves showed endothermic peaks about 80-100°C which was associated with the evaporation of water (Du *et al*., 2009; Mo *et al*., 2009). Moreover, different exo/endo-thermic peaks of all films are present at temperatures higher than 250°C, responsibility to denaturation of crystalline parts of proteins (Zoccola *et al*., 2009). In addition, thermal behavior of one component is more strongly influenced by blending with other component. This result confirms the FTIR spectra that keratin and gelatin are able to form intermolecular hydrogen bonds (Vasconcelos *et al*., 2008).

CONCLUSION

SEM images indicated that native gelatin and keratin have smooth surfaces contrast with the blend films. The keratin blended with gelatin at 1:1 ratio appeared the highest miscible texture while at 2:1 ratio was the highest rough surfaces. From IR results, the keratin/gelatin blend films have the characteristics of both native types which can be suggested that intermolecular interactions between keratin and gelatin were formed. With TG and DTG

curves, native gelatin has the highest of temperature of maximum decomposition. Moreover, keratin blended with gelatin showed higher exo/endo-thermic peaks than that of native films as shown by heat flow curves.

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REFERENCES

Aboushwareb, T., D. Eberli, C. Ward, C. Broda, J. Holcomb, A. Atala and M. Van-Dyke, 2009. A keratin biomaterial gel hemostat derived from human hair: Evaluation in a rabbit model of lethal liver injury. J. Biomed. Mater. Res., 90: 45-54.

Aluigi, A., A. Varesano, A. Montarsolo, C. Vineis, F. Ferrero, G. Mazzuchetti and C. Tonin, 2007. Electrospining of keratin/poly(ethylene oxide) blend nanofibers. J. Applied Polym. Sci., 104: 863-870.

Aluigi, A., C. Vineis, A. Varesano, G. Mazzuchetti, F. Ferrero and C. Tonin, 2008. Structure and properties of keratin/PEO blend nanofibers. Eur. Polym. J., 44: 2465-2475.

Apel, P.J., J.P. Garrett, P. Sierpinski, J. Ma and A. Atala *et al*., 2008. Peripheral nerve regeneration using a keratin-based scaffold: Long-term functional and histological outcomes in a mouse model. J. Hand Surg. Am., 33: 1541-1547.

Cardamone, J.M., 2008. Keratin transamidation. Int. J. Biol. Macromol., 42: 413-419.

Cardamone, J.M., 2010. Investigating the microstructure of keratin extracted from wool: Peptide sequence (MALDI-TOF/TOF) and protein conformation (FTIR). J. Mol. Struct., 969: 97-105.

Church, J.S., A.J. Poole and A.L. Woodhead, 2010. The raman analysis of films cast from dissolved feather keratin. Vib. Spectrosc., 53: 107-111.

Du, C., J. Jin, Y. Li, X. Kong, K. Wei and J. Yao, 2009. Novel silk fibroin/hydroxyapatite composite films: Structure and properties. Mater. Sci. Eng. C, 29: 62-68.

Fujii, T., D. Ogiwara and M. Arimoto, 2004. Convenient procedures for human hair protein films and properties of alkaline phosphatase incorporated in the films. Biol. Pharm. Bull., 27: 89-93.

Guziewicz, N., A. Best, B. Perez-Ramirez and D.L. Kaplan, 2011. Lyophilized silk fibroin hydrogels for the sustained local delivery of therapeutic monoclonal antibodies. Biomaterials, 32: 2642-2650.

Joshi, S.G., M.M. Tejashwini, N. Revati, R. Sridevi and D. Roma, 2007. Isolation, identification and characterization of a feather degrading bacterium. Int. J. Poult. Sci., 6: 689-693.

Katoh, K., T. Tanabe and K. Yamauchi, 2004. Novel approach to fabricate keratin sponge scaffolds with controlled pore size and porosity. Biomaterials, 25: 4255-4262.

Ki, C.S., E.H. Gang, I.C. Um and Y.H. Park, 2007. Nanofibrous membrane of wool keratose/silk fibroin blend for heavy metal ion adsorption. J. Mem. Sci., 302: 20-26.

Kurimoto, A., T. Tanabe, A. Tachibana and K. Yamauchi, 2003. Keratin sponge: Immobilization of lysozyme. J. Biosci. Bioeng., 96: 307-309.

Kweon, H.Y., I.C. Um and Y.H. Park, 2000. Thermal behavior of regenerated *Antheraea pernyi* silk fibroin film treated with aqueous methanol. Polymer, 41: 7361-7367.

Lee, K.Y. and W.S. Ha, 1999. DSC studies on bound water in silk fibroin/S-carboxymethyl keratein blend films. Polymer, 40: 4131-4134.

Lee, K.Y., 2001. Characterization of silk/fibroin/S-carboxymethyl kerateine surfaces: Evaluation of biocompatibility by contact angle measurements. Fibers Polym., 2: 71-74.

Lee, K.Y., S.J. Kong, W.H. Park, W.S. Ha and I.C. Kwon, 1998. Effect of surface properties on the antithrombogenicity of silk fibroin/S-carboxymethyl kerateine blend films. J. Biomater. Sci. Polym. Ed., 9: 905-914.

Meyers, M.A., P.Y. Chen, A.Y.M. Lin and Y. Seki, 2008. Biological materials: Structure and mechanical properties. Prog. Mater. Sci., 53: 1-206.

Mo, C., P. Wu, X. Chen and Z. Shao, 2009. The effect of water on the conformation transition of *Bombyx mori* silk fibroin. Vib. Spectrosc., 51: 105-109.

Moll, R., M. Divo and L. Langbein, 2008. The human keratins: Biology and pathology. Histochem. Cell Biol., 129: 705-733.

Nakamura, A., M. Arimoto, K. Takeuchi and T. Fujii, 2002. A rapid extraction procedure of human hair proteins and identification of phosphorylated species. Biol. Pharm. Bull., 25: 569-572.

Peplow, P.V. and G.J. Dias, 2004. A study of the relationship between mass and physical strength of keratin bars *in vivo*. J. Mater. Sci. Mater. Med., 15: 1217-1220.

Prasong, S., 2010. Characterization of tetracycline-loaded thai silk fibroin/gelatin blend films. J. Applied Sci., 10: 2893-2898.

Reichl, S., 2009. Films based on human hair keratin as substrates for cell culture and tissue engineering. Biomaterials, 30: 6854-6866.

Rouse, J.G. and M.E. Van-Dyke, 2010. A review of keratin-based biomaterials for biomedical applications. Materials, 3: 999-1010.

Sierpinski, P., J. Garrett, J. Ma, P. Apel and D. Klorig *et al.*, 2008. The use of keratin biomaterials derived from human hair for the promotion of rapid regeneration of peripheral nerves. Biomaterials, 29: 118-128.

Sumita, Y., M.J. Honda, T. Ohara, S. Tsuchiya, H. Sagara, H. Kagami and M. Ueda, 2006. Performance of collagen sponge as a 3-D scaffold for tooth-tissue engineering. Biomaterials, 27: 3238-3248.

Tachibana, A., S. Kaneko, T. Tanabe and K. Yamauchi, 2005. Rapid fabrication of keratin-hydroxyapatite hybrid sponges toward osteoblast cultivation and differentiation. Biomaterials, 26: 297-302.

Tamilmani, P., A. Umamaheswari, A. Vinayagam and B. Prakash, 2008. Production of an extra cellular feather degrading enzyme by *Bacillus licheniformis* isolated from poultry farm soil in Namakkal district (Tamilnadu). Int. J. Poult. Sci., 7: 184-188.

Tanabe, T., N. Okistu, A. Tachibana and K. Yamauchi, 2002. Preparation and characterization of keratin-chitosan composite film. Biomaterials, 23: 817-825.

Tasukada, M., G. Freddi, P. Monti, A. Bertoluzza and N. Kasai, 1995. Structure and molecular conformation of tussah silk fibroin films: Effect of methanol. J. Polymer Sci. Part B Polymer Phys., 33: 1995-2001.

Tonin, C., A. Aluigi, C. Vineis, A. Varesano, A. Montarsolo and F. Ferrero, 2007. Thermal and structural characterization of poly (ethylene-oxide)/keratin blend films. J. Therm. Anal. Calorim., 89: 601-608.

Varesano, A., A. Aluigi, C. Vineis and C. Tonin, 2008. Study on the shear viscosity behavior of keratin/PEO blends for nanofibre electrospinning. J. Polym. Sci., 46: 1193-1201.

Vasconcelos, A., G. Fredi and A. Cavaco-Paulo, 2008. Biodegradable materials based on silk fibroin and keratin. Biomacromolecules, 9: 1299-1305.

Watcharin, O., S. Yaowalak, S. Wilaiwan and S. Prasong, 2009. Morphology, secondary structure and thermal properties of silk fibroin/gelatin blend film. Pak. J. Biol. Sci., 12: 1526-1530.

Wilaiwan, S., S. Yaowalak, B. Yodthong and S. Prasong, 2010. Silk fibroin/gelatin hybrid films for medical applications: Study on chlorhexidine diacetate. J. Biol. Sci., 10: 455-459.

Xing, Q., F. Zhao, S. Chen, J. McNamara, M.A. DeCoster and Y.M. Lvov, 2010. Porous biocompatible three-dimensional scaffolds of cellulose microfiber/gelatin composites for cell culture. Acta Biomater., 6: 2132-2139.

Yamauchi, K., A. Yamauchi, T. Kusunoki, A. Kohda and Y. Konishi, 1996. Preparation of stable aqueous solution of keratins and physiochemical and biodegradational properties of films. J. Biomed. Mater. Res., 31: 439-444.

Yamauchi, K., H. Hojo, Y. Yamamoto and T. Tanabe, 2003. Enhanced cell adhesion on RGDS-carrying keratin film. Mater. Sci. Eng., 33: 467-472.

Yamauchi, K., M. Mamiwa and T. Mori, 1998. Cultivation of fibroblast cells on keratin-coated substrata. J. Biomater. Sci. Polym., 9: 259-270.

Zoccola, M., A. Aluigi and C. Tonin, 2009. Characterisation of keratin biomass from butchery and wool industry wastes. J. Mol. Struct., 938: 35-40.