Hybrid Collagen-Cellulose-Albumin Biofibers from Skin Waste: A Potential Bioabsorbable Suture Material

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Abstract

Utilization of different biodegradable and environmental friendly biomass wastes to prepare high-value novel materials is gaining importance. Collagen (C) and cellulose are prominent biopolymers from animal and plant kingdom and widely used in bioengineering. Albumin, on the other hand, is the most abundant plasma protein present in blood of mammals. It transports hormone, fatty acids and many drugs and often clinically used to restore blood volume in trauma, burns and surgery patients. In this work, collagen extracted from animal skin waste was blended with hydroxyethylcellulose (HEC) and bovine serum albumin (A) and wet-spun to form hybrid biodegradable C/HEC/A fibers. They were further cross-linked with glutaraldehyde vapors and analyzed for their structural, thermal, mechanical and swelling properties. X-ray diffraction and infra-red spectroscopic studies of the hybrid fiber display the peaks corresponding to collagen, cellulose and albumin. Incorporation of cellulose into the collagen matrix leads to reasonable improvement in mechanical, swelling and thermal properties of hybrid fibers. While the increase in the content of albumin exhibits slight decrease in the thermo-mechanical and swelling properties, a significant improvement in the regularity of fiber surface without altering the porosity is noticed under scanning electron microscope. Hence, the formed hybrid fibers can be potentially used as a suture material as well as for different biomedical applications due to their improved properties.

Keywords: Biowaste, Plant waste, Fiber, Morphology, Trimmings

Introduction

Silk, cotton, Dacron®, nylon, polyester and polypropylene sutures are popular and commonly employed in surgical procedures in different time period. Most of them are nonabsorbable and pose unwanted tissue reaction. Consequently, researchers are currently exploring for self-absorbing bio-based suture materials. Nature provides abundance of structural materials such as protein fibers, which have remarkable properties and performance through evolutionary optimization. Collagen (C), a constituent of skins of domestic animals, is one of the most abundant proteins and made up of polypeptide chains, with the conformation of a left-handed

helix (Thanikaivelan et al. 2012; Anumary et al. 2013). Collagen has a unique structure, size and amino acid sequence and it is considered as an ideal material for biomedical applications with its innate biocompatibility, strength through cross-linking, self-assembly, aggregation and easily modifiable characteristics. Collagen based biomaterials find several applications such as scaffolds, artificial tissue, prosthesis, drug carrier and cosmetics. Collagen has been used as a suture material for many years (e.g. Catgut) due to its controlled biodegradation rate and biocompatibility (Vannas and Larmi 1959; Okada et al. 1992). On the other hand, albumin (A) is the most abundant blood protein constituting about 55% in mammals. It maintains the oncotic pressure required for the distribution of body fluids and also acts as a plasma carrier by non-specifically binding several hydrophobic steroid hormones and as a transport protein for hemin and fatty acids. Albumin is one of the most widely used proteins in research and yet its application in biomedical engineering is limited. Its biodegradability, lack of toxicity and immunogenicity has prompted researchers to coat the surface of a variety of polymeric biomaterials such as polypropylene, polycarbonate, poly(vinyl chloride) and Dacron® vascular grafts (Kamath and Park 1994; Kottke-Marchant et al. 1989). The results suggest that the albumin coating improved the blood compatibility of synthetic polymer based biomaterials. It is also used as a versatile carrier for targeted drug delivery for improving the pharmacokinetic profile of the drugs (Kratz 2008). Although albumin has not been employed as a major component of suture material, albumin based biological glue or sealant for cardiovascular surgery is popular (Zehr 2007; Chao and Torchiana 2003).

Cellulose is another most abundant renewable natural polymer that has widespread applications due to its abundant availability and biodegradability. The estimated yearly biomass production of cellulose is roughly about 1.5 trillion tons, making it an unlimited source of raw material for environmentally friendly and biocompatible products. Cellulose based biomaterials have been used as hemodialysis membranes, coating materials for drugs, and drug releasing scaffolds. Methyl or ethyl hydroxyl cellulose is known to improve tissue compatibility (Miyamoto et al. 1989). Cellulose based suture materials such as cotton, regenerated cellulose yarn, dialdehyde cellulose have been known for long and gained popularity over the years (Ravikumar 2000). Recently, bacterial cellulose derived from *A. Xylinum* has been used as a protective cover for micronerve sutures (Klemm et al. 2001).

Collagen being a natural protein is acquiescent to degradation by microbial attack. One of the approaches to render biostability to collagen is to hybridize with other natural or synthetic polymers (Anumary et al. 2013; Murali et al. 2011). Binary blends of collagen and cellulose derivatives have been probed for making films for tissue engineering, controlling the subcutaneous wound and tissue culture (Anumary et al. 2013). Here, we developed novel hybrid biodegradable fibers using ternary blends of collagen, cellulose and bovine serum albumin. Collagen used in this study is primarily sourced from animal skin waste of leather industry. The formed fibers were characterized for physical, structural, morphological, thermal and biological properties.

Materials and Methods

Materials

The trimmed waste from cow hides were collected from pilot tannery at Central Leather Research Institute (CLRI), Chennai. Hydroxyethyl cellulose (HEC) was purchased from Sigma Aldrich. Bovine serum albumin was procured from Hi Media Laboratories Pvt. Ltd. Glacial acetic acid, potassium chloride and potassium dihydrogen orthophosphate were purchased from Sisco Research Laboratory Pvt. Ltd. Laboratory grade acetone was procured from SD Fine-Chemicals Ltd. Laboratory grade methanol was procured from RFCL Ltd. Analytical grade di-sodium hydrogen phosphate (anhydrous) and sodium chloride were purchased from Merck. Alkaline proteinase from Streptomyces species (deposited in IMTECH, as MTCC 5211) was received as gift sample.

Preparation of Collagen Solution

The hide trimming pieces were soaked, limed, dehaired, relimed, fleshed and delimed completely using conventional procedures. The delimed hide pieces were soaked in 35 and 70% acetone followed by 100% methanol for sufficient duration. This final step was repeated four times in order to completely remove the moisture. Finally, the hide pieces were thoroughly dried in a vacuum drier. The completely dried hide pieces were grounded finely into powder using a Willy mill of mesh size 2 mm. About 0.5 g of hide powder was taken in a beaker and soaked in 45 ml of 0.5 N acetic acid for 2 h. This was thoroughly mixed in a blender for 10 min and filtered using white cloth to get the collagen solution.

Preparation of the Spinning Solution

Different proportions of collagen, HEC and albumin were selected in order to prepare spinning solutions. The proportions such as 100/0/0 wt% C/HEC/A, 100/0/25 wt% C/HEC/A, 100/0/50 wt% C/HEC/A, 100/0/75 wt% C/HEC/A, 100/0/100 wt% C/HEC/A, 100/50/0 wt% C/HEC/A, 100/50/25 wt% C/HEC/A, 100/50/50 wt% C/HEC/A, 100/50/75 wt% C/HEC/A, 100/50/100 wt% C/HEC/A, 100/100/0 wt% C/HEC/A, 100/100/25 wt% C/HEC/A, 100/100/50 wt% C/HEC/A, 100/100/75 wt% C/HEC/A and 100/100/100 wt% C/HEC/A were blended and used as spinning solution in this work.

Fiber Spinning

The spinning solution in the glass beaker was transferred to a 20 ml syringe which was connected to a syringe infusion pump. The spinning solution was pushed through a blunt stainless steel needle (0.5 ± 0.02 mm I.D) at a constant flow rate of 0.5 ml/min into the coagulation bath containing equal ratio of ethanol and acetone (50:50) solution in a Petri plate. The spinning solution coagulated and formed filaments. The filaments were taken out and immersed in the second bath containing ethyl acetate and stretched gently to obtain the fibers and finally dried. Similarly all the proportions of spinning solutions were used to spin the fibers and dried.

Crosslinking with Glutaraldehyde Vapours

Glutaraldehyde solution was prepared at a concentration of 2% and kept in a beaker. Aluminium foil was taken and many small holes were made using pointed needle. Then the foil was stuck inside the beaker containing gluteraldehyde solution. The spun fibres were placed on the foil paper in order to treat with glutaraldehyde vapor. The beaker was sealed tightly using aluminium foil and kept at room temperature for 30 min. The samples were taken out and dried.

Characterization

Cross-sections and surface morphologies of the formed hybrid fibers were observed by a scanning electron microscope (SEM, Hitachi S-3400N) with an applied accelerating voltage of 15kV. Prior to SEM analysis, the fiber samples were gold coated using Hitachi E-1010 Ion sputter. The FTIR spectra were obtained with a Perkin-Elmer RXI FT-IR spectrophotometer in the spectral region of 4000–400 cm^{-1} using the KBr pellet method. X-ray diffraction (XRD) analysis was performed with a General Area Detector Diffraction System (GADDS, Bruker-Axs, Germany) using Cu Kα radiation. Irradiation conditions were 45 kV and 40 mA. Single fiber tenacity or tensile strength and elongation properties were measured using Lenzing Technik Instruments, Lenzing, Austria according to ASTM D-3822. Fibers were cut to 40 mm in length. The ends of the sample were fixed on a sample holder at a gauge length of 20 mm and elongated at a rate of 20 mm/min. All samples were preconditioned at 20°C and 65% relative humidity for 24 h before measurement. Fiber denier was determined by measuring the length and mass of the filament, which is a measure of fiber fineness and necessary to calculate the tenacity.

Results and Discussion

X-ray Diffraction Analysis

X-ray diffraction patterns of collagen, HEC and C/HEC/A hybrid fibers are shown in Fig. 1. It is seen that the diffraction peaks of all the fibers are broad since all of the individual components have macromolecular structure with low crystallinity. Diffraction peaks of HEC and the pure collagen are observed at 20.8° and 22.1°, respectively. This is in agreement with the earlier reports (Thanikaivelan et al. 2012). When the collagen and HEC is blended (C/HEC/A 100/50/0 wt%), there seems to be a little shift in the diffraction peak towards HEC in spite of low HEC content (1:0.5 ratio) indicating possible interaction between them. However, when collagen, HEC and albumin are blended at 100/50/100 and 100/100/100 wt%, the diffraction peaks are at 22.2° and 21.1°, respectively. The hybrid fiber with 100/50/100 wt% composition exhibits diffraction peak close to collagen or albumin due to the fact that protein constituent is much higher than HEC (2:0.5 ratio). Whereas the hybrid fiber with 100/100/100 wt% composition exhibits diffraction peak close to HEC due to the fact that the protein to HEC ratio is 2:1, which is similar to the C/HEC/A 100/50/0 wt% hybrid fiber. The shift in the diffraction peak towards HEC in spite of low HEC content indicates the possible interaction between collagen, albumin and HEC and indirectly shows that the hybrid fibers are homogeneous.

Fourier- Transform Infra Red Spectroscopy

The FTIR spectra corresponding to collagen, HEC, albumin and their hybrid bio-composite fibers are analysed (Figure not shown). The pure collagen fiber exhibits characteristic absorption bands around 1645 cm^{-1} (C=O stretch of amide I), 1547 cm^{-1} (N–H bend, C–N stretch of amide II), 1240 cm^{-1} (C-N stretching and N-H in-plane bending of amide III), 1450 cm^{-1} (aliphatic side chain of various amino acids) and 3423 cm^{-1} (O-H stretch of hydroxyl group). Bovine serum albumin shows characteristic peaks similar to collagen at 1655 cm^{-1} (C=O stretch), 1528 cm^{-1} (N–H bend and C–N stretch), 1242 cm^{-1} (C-N stretch and N-H in-plane bend), 1448 cm^{-1} (aliphatic side chain of various amino acids) and 3294 cm^{-1} (O-H stretch). On the other hand, HEC depicts two major distinctive bands. The band at 2911 cm^{-1} is due to the C-H stretching of ethyl group and the other one at 1060 cm^{-1} is due to the COC stretching of ether.

It is seen from Fig. 2 that the hybrid fibers with binary blend of collagen and HEC (100/100/0 wt.% C/HEC/A) show characteristic bands of both HEC (1065 cm^{-1}) as well as collagen (e.g. 1645 and 1449 cm^{-1}). Whereas the binary blend of collagen and bovine serum albumin (100/0/100 wt.% C/HEC/A) yield hybrid fibers with insignificant difference in their characteristic band assignments as one would expect. The ternary blend of collagen, HEC and albumin (100/100/100 wt.% C/HEC/A) resulted in hybrid fibers having characteristic bands corresponding to proteins (1653, 1532 and 1451 cm^{-1}) and HEC (1058 cm^{-1}). These results indicate the incorporation of HEC and albumin into the pure collagen thereby resulting in the formation of homogeneous fibers. This is in agreement with the XRD results. It is further seen that the amide I, amide II and amide III signature peaks of collagen are seen in all the three types of C/HEC/A hybrid fibers. This confirms that the procedure that we applied to prepare the hybrid composite fibers did not degrade or alter the triple helical structure of collagen molecules.

Fiber Morphology

The surface and cross-sectional scanning electron micrograph (SEM) images of the select hybrid biocomposite fibers are shown in Figs. 3 and 4, respectively. In general, all the wet spun hybrid fibers appeared to be good, however, with varying surface roughness and uniformity. The pure collagen fiber (100/0/0 wt.% C/HEC/A, Fig. 3a) is found to have an uneven and rough surface, which may lead to reduced strength properties. The surface roughness and unevenness of the hybrid fibers are reduced slightly as the content of HEC increases (100/100/0 wt.% C/HEC/A, Fig. 3b). However, the incorporation of bovine serum albumin with collagen/HEC blend (100/100/50 wt.% C/HEC/A, Fig. 3c and 100/100/100 wt.% C/HEC/A, Fig. 3d) seems to reduce the surface roughness and unevenness markedly, eventually leading to the formation of smooth hybrid fibers. It is also noted that both the pure collagen and C/HEC binary blend fibers have a diameter around 50 μm (100/0/0 wt.% and 100/100/0 wt.% C/HEC/A) while the incorporation of bovine serum albumin drastically increases the diameter to around 100 μm (100/100/50 wt.% and 100/100/100 wt.% C/HEC/A).

In general, cross sectional views of the select hybrid fibers exhibit a solid core with the presence of porous network structure (Fig. 4). The pure collagen fiber (100/100/0 wt.% C/HEC/A, Fig. 4a) exhibits a solid core with macro voids. However, as the content of HEC increases (100/100/0 wt.%

C/HEC/A, Fig. 4b, there seems to a significant increase in the porosity in their cross section with the presence of more micro voids. The micro pores in the hybrid fiber seem to decrease gradually (100/100/50 wt.%, Fig. 4c and 100/100/100 wt.% C/HEC/A, Fig. 4d) as the content of the bovine serum albumin increases to a minimal extent. It must be mentioned that the hybrid fibers with more bovine serum albumin content seem to have a non-circular cross section and folding ((Figure not shown). This could be due to the higher coagulation rate of albumin in comparison to collagen or HEC. It is known that a higher coagulation rate results in non-circular forms of solution-spun fibers (Knaul and Creber 1997).

Thermal Properties of the Hybrid Fibers

Thermogravimetric analysis (TGA) provides better understanding of the thermal decomposition behavior and thermal stability of the formed hybrid bio-fibers. The pure collagen, HEC and bovine serum albumin based fibers as well as their hybrid fibers were subjected to TGA analysis and the results such as decomposition temperature and weight loss (%) are shown in Table 1. All the pure as well as hybrid fibers display single stage decomposition between 300 and 340°C. This may be due to the disintegration of the macromolecular structure present in the fibres. It is seen that the pure HEC exhibits higher decomposition temperature (332.61°C) in comparison to pure collagen (317.76°C) as well as bovine serum albumin (307.67°C). The decomposition temperature of hybrid fibers with binary blend of collagen and HEC increases considerably as the content of HEC increases (324.83°C for 100/50/0 wt.% C/HEC/A; 332.90°C for 100/100/0 wt.% C/HEC/A). While the albumin incorporated collagen binary hybrid fiber shows a mixed trend with an initial increase followed by a marginal decrease in their decomposition temperature as the albumin content increases (324.83°C for 100/0/50 wt.% C/HEC/A; 321.80°C for 100/0/100 wt.% C/HEC/A). Hence, it can be hypothesized that the hybrid fibers with higher proportion of HEC would possess higher decomposition temperature. The ternary blend hybrid bio-composite fiber with 100/100/50 wt.% C/HEC/A composition seems to have higher decomposition temperature (~337°C) than that of the hybrid fiber with 100/50/100 wt.% C/HEC/A composition (~322°C). This clearly illustrates the role of HEC in the thermal stability of the formed hybrid fibers. Hybrid fiber with 100/100/100 wt.% C/HEC/A composition exhibits a decomposition temperature around ~326^{o}C, which is due to the high concentration of albumin. The pure collagen, HEC, and bovine serum albumin based fibers as well as their hybrid fibers show a weight loss between 64 and 75%. In general, it can be observed that the incorporation of HEC and bovine serum albumin into the collagen shows significant improvement in the thermal properties of formed hybrid bio-composite fibers.

Mechanical Properties

The tensile strength, elongation and fineness of the single fibers formed from collagen and C/HEC/A blends are presented in Table 2. The pure collagen fiber has tensile strength of about 0.65 g/den, which is increasing significantly as the concentration of HEC increases (0.87 g/den for 100/100/0 wt.% C/HEC/A). On the other hand, the increase in the concentration of bovine serum albumin has little effect on the improvement in strength properties of hybrid fibers. Elongation at break of pure collagen fiber was about 12.60% and it is also increased to 18.50% for 100/100/0 wt.% C/HEC/A as the concentration of HEC increases. The pure collagen fiber

possesses the least fineness about 12.46 den (denier) when compared to that of other C/HEC/A hybrid fibers. The increase in the proportion of HEC showed a marked increase in the fineness of the hybrid fibers. The 100/100/0 wt.% C/HEC/A hybrid fiber has mean fineness value of about 22.51 den as against 18.98 den for the 100/0/100 wt.% C/HEC/A fiber. The 100/100/100 wt.% C/HEC/A hybrid fiber displayed the highest fineness value of 24.53 den. Thus, the addition of both HEC and albumin to collagen exhibited an increase in the fineness of the fibers. Hence, it can be seen that the developed C/HEC/A hybrid fibers show increased mechanical properties as the proportion of HEC increases while bovine serum albumin did not adversely affect them.

Swelling Studies

The extent of swelling of various select hybrid fibers prepared is shown in Fig. 5. It is seen that all the selected hybrid fibers exhibited equilibrium swelling after 240 min. For the binary blend hybrid fiber without the presence of HEC (Fig. 5a), the maximum extent of swelling was about ~ 1800% (100/0/25 wt.% C/HEC/A) in comparison to the pure collagen fiber (1400%). As the concentration of HEC increases in the ternary blend C/HEC/A hybrid fibers, the extent of swelling also increased up to 2000% for 100/50/0 wt.% C/HEC/A and 2730% for 100/100/0 wt.% C/HEC/A compositions (Fig. 5b and 5c). While the increase in the concentration of bovine serum albumin in the ternary blend C/HEC/A hybrid fibers increased the extent of swelling only up to 50% albumin content (2450% for 100/50/50 wt.% C/HEC/A and 3340% for 100/100/50 wt.% C/HEC/A) beyond which the swelling decreased (Fig. 5b and 5c). Hence, it can be inferred that HEC played a vital role in increasing the swelling of C/HEC/A hybrid fibers while bovine serum albumin exhibited a mixed trend. These results are in agreement with the thermal and mechanical property results obtained in this study.

Conclusion

An attempt has been made to fabricate collagen-HEC-bovine serum albumin based hybrid fibers using wet spinning method. Collagen was extracted from trimmed waste of animal skins. The formed hybrid fibers have been analyzed for chemical properties using XRD and FT-IR and the results show evidence for interaction between the components thereby suggesting homogeneous fiber formation. Fibers visualized under SEM show drastic reduction in surface roughness as the albumin content increases. The hybrid fibers display enhanced mechanical and thermal properties as the proportion of HEC increases. A substantial increase in the swelling ability of the hybrid fibers is noticed when the HEC content increased. The results obtained in this work suggest that the C/HEC/A hybrid fibers could be employed as a potential bioabsorbable suture material.

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Table 1. Decomposition temperature and weight loss data of select hybrid fibers from TGA analysis

Collagen (wt.%)	Cellulose (wt.%)	Albumin (wt.%)	Temperature (°C)	Weight loss (%)
100	-	-	317.76	74.57
-	100	-	332.61	64.38
-		100	307.67	73.57
100	50	-	324.83	65.76
100	100	-	332.90	73.71

100		50	324.83	64.46
100	-	100	321.80	68.90
100	50	50	319.11	71.31
100	50	100	321.80	63.03
100	100	50	336.93	67.34
100	100	75	322.81	66.56
100	100	100	325.83	66.61

Table 2. Mechanical properties and fiber fineness of select hybrid fibers

Samples	Tensile strength (g/den)	Elongation (%)	Fineness (denier)
100/0/0 wt.% C/HEC/BA	0.65	12.60	12.46
100/0/50 wt.% C/HEC/BA	0.69	11.40	16.04
100/0/100 wt.% C/HEC/BA	0.72	13.40	18.98
100/50/0 wt.% C/HEC/BA	0.84	17.20	16.55
100/100/0 wt.% C/HEC/BA	0.87	18.50	22.51
100/50/50 wt.% C/HEC/BA	0.73	12.96	20.80
100/50/100 wt.% C/HEC/BA	0.69	13.70	17.29
100/100/50 wt.% C/HEC/BA	0.83	16.23	21.15
100/100/100 wt.% C/HEC/BA	0.84	18.10	24.53

Fig. 1. XRD pattern of collagen, HEC and select hybrid bio-fibers

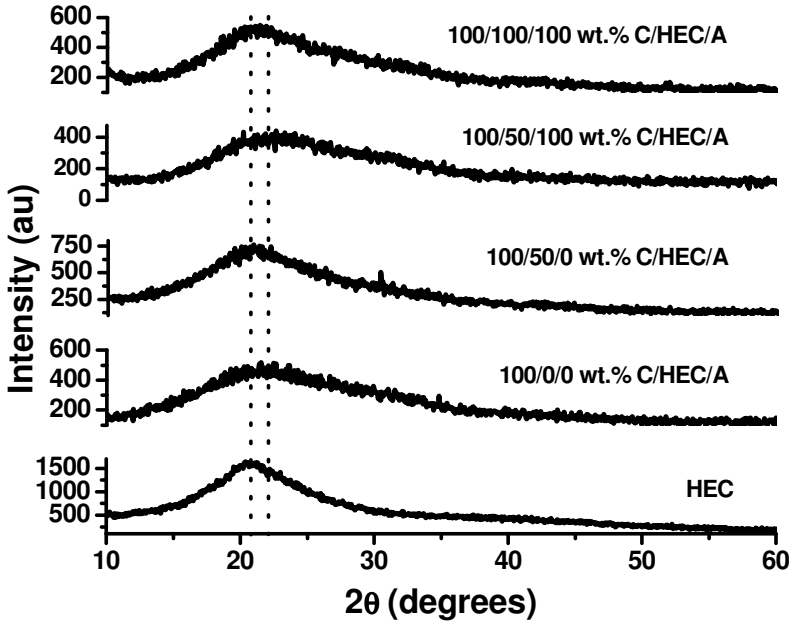

Fig. 2. FT-IR spectra of select hybrid bio-fibers

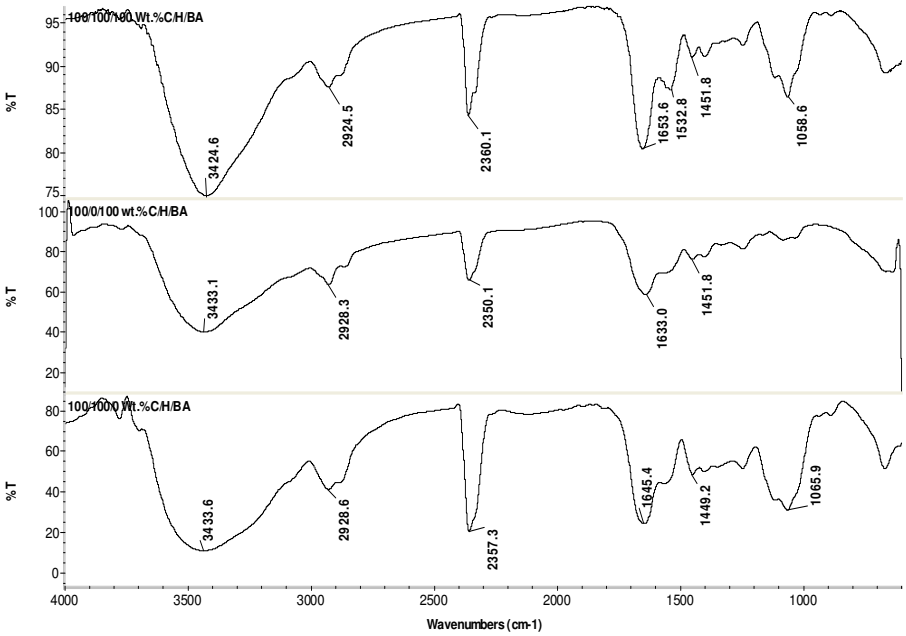

Fig. 3. Scanning electron microscopic images of select hybrid bio-fibers showing the surface; a) 100/0/0 wt.% C/HEC/A; b) 100/100/0 wt.% C/HEC/A; c) 100/100/50 wt.% C/HEC/A; d) 100/100/100 wt.% C/HEC/A

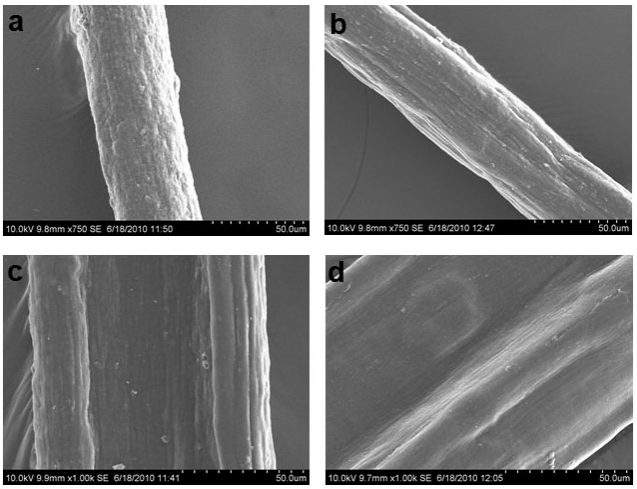

Fig. 4. Scanning electron microscopic images of select hybrid bio-fibers showing the cross section; a) 100/0/0 wt.% C/HEC/A; b) 100/100/0 wt.% C/HEC/A; c) 100/100/50 wt.% C/HEC/A; d) 100/100/100 wt.% C/HEC/A

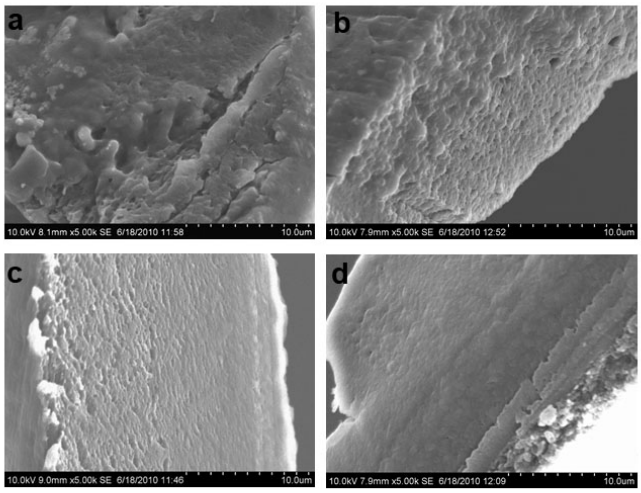

Fig. 5. Swelling property of select hybrid bio-fibers as a function of albumin concentration; a) C/HEC/A hybrid fibers without the presence of HEC; b) C/HEC/A hybrid fibers with 50 wt.% HEC; c) C/HEC/A hybrid fibers with 100 wt.% HEC

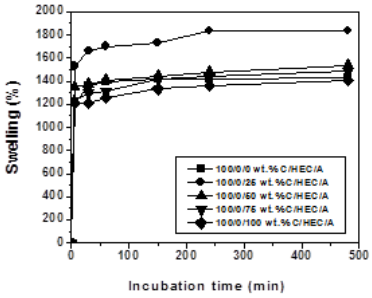

Fig 5a

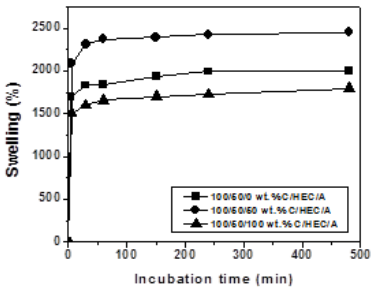

Fig 5b

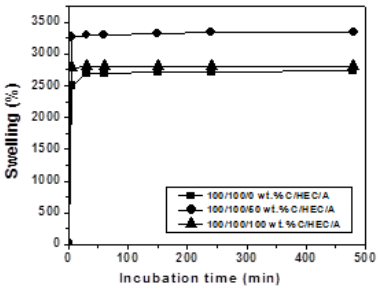

Fig 5c