Chapter

Development, Characterization and Properties of Silk Fibre and Grafted Silk Fibre Reinforced Polymer Composite Films

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Abstract

The use of natural fibres over synthetic fibres is gaining widespread importance due to its availability; renewability, low density and satisfactory mechanical properties making them an ecological alternative to synthetic fibres. The innumerable properties of silk fibre have made it superior to be used by researchers both in the plastic and biomedical sector. Silk fibre reinforced PVA (polyvinylalcohol) and PVA/PVP (polyvinyl pyrrolidone) films were prepared via solution casting technique. The effect of silk fibre concentration, on the structural, thermal, mechanical, bio-degradable and the morphological properties of the composite films was assessed. The results indicated that the addition of silk fibres improved the thermal, morphological, mechanical and biodegradable properties of the films. The extensive use of silk fibroin in the biomedical field, due to its robust properties has made it a promising material, suitable in tissue engineering applications. Keeping this in view, the current study also focuses on re-tailoring the properties of silk fibres by grafting a natural polysaccharide like chitosan and thereby fabricate composite films of PVA reinforced with this grafted fibre. The films were tested for their potential applications in tissue engineering, by subjecting them to *in vitro* biocompatibility tests. The films were also tested for their antibacterial properties. The results thus obtained indicated that the films were non-toxic in all concentrations and were found to be suitable for biomaterial applications.

Keywords: silk fibre, chitosan, grafting, tissue-engineering, biodegradation

1. Introduction

With the advent of polymer technology and large scale production of synthetic fibre reinforced composites, natural fibres as reinforcements have gained greater insights due to ecological concerns, accessibility, relatively low cost and biodegradability. This thrust for green products has empowered the mankind to consider these natural fibres, to be an alternative over conventional glass and carbon fibres [1]. Natural fibres are however considered far more superior over synthetic fibres as they are known to possess good relative mechanical properties, flexibility during processing, biodegradability and minimum health hazards [2].

Among the natural fibres, silk, a natural animal fibre is widely used due to its enormous applications. Silk is a strong and filamentous fibre produced by the larva of silkworm, during metamorphosis [3]. Of the many varieties of silk fibres, the best known is the *Bombyx mori*, which is essentially recognized for its strength and lustre [4].

It is structurally made up of a protein called fibroin which is composed of amino acids like glycine, alanine and serine, with a crystallinity in the order of 70–75 and 25–30% amorphous in nature. Besides, polypeptide chains in silk lie close enough to each other, to form a network of hydrogen bonds. As a result, bond formation is enabled with other hydrogen atoms of the polymer matrix resulting in higher thermal stability [3, 5]. The vast properties of silk fibres are mainly due to its high ultimate tensile strength which is about 208.45 MPa, elongation at break: 19.55%, modulus of 6.10 GPa and density of 1.33 g/cm^3 [6]. Furthermore, silk also exhibits high softening temperature and decomposes at a higher temperature [3]. Moreover, it is a potential candidate in the field of medical, pharmaceutical and agricultural areas and is also known to possess properties, like microbial resistance, oxygen permeability, biocompatibility, water absorbability [7–9]. Due to these properties, the emphasis lies on the study of silk based fibre reinforced polymer composites.

The surface modification of biomaterials using biomolecules is known to improve blood compatibility [10] or to enhance cell attachment and proliferation [11]. Over the centuries, grafting of silk fibres using vinyl monomers was performed to improve properties of the fibre to make it equally competent over man-made fibres [12, 13]. Although, grafting of vinyl monomers improves the properties of fibres, it however has a major limitation, due to the damaging products released by slow degradation [14]. Therefore, environmental surface modification of fibres was a technique introduced in an attempt to retain the properties of silk by grafting natural polysaccharides such as chitin and chitosan. Chitosan, is a derivative of chitin and is prepared through the deacetylation of chitin. It is a major component present in marine invertebrates such as crustacean shells (shrimps, crabs etc.), exoskeleton of insects and a few fungi [15]. Chitosan and silk fibroin are natural biopolymers that are applied in tissue engineering and biomedical fields. Chitosan has been proposed as a biomaterial for biomedical applications mainly due to its biocompatibility [16, 17]. The properties of chitosan including easy availability, biodegradability, bioactivity and nontoxicity, as well as bio-adhesion and antimicrobial properties are the major reasons for its applications widely considered by researchers [18].

Glycosaminoglycans (GAGs), which are native components of the extracellular matrix (ECM), are known to structurally resemble chitosan, making it one of the promising biomaterials, for cartilage repair [19, 20]. Furthermore, it accelerates wound healing [21, 22] and amends the immune system by macrophage activation [23] to generate cytokines thus, inhibiting infections [24]. In view of this, the grafting of silk fibres using chitosan was adopted for the current study.

The best route to successfully graft chitosan over silk is via acylation as it provides an enhanced surface area for grafting. Chitosan grafting over silk and via acylation has been successfully performed by researchers to enhance the properties of silk in the textile industry [25, 26]. However, the application of grafted silk fibres in the field of tissue engineering remains unexplored. Therefore, the current chapter focuses on the preparation and properties of silk fibre reinforced PVA and PVA/PVP composite films and thereafter study the effect of surface modification of silk fibre by grafting a natural polysaccharide like chitosan and thus explore the potential of these fibre reinforced composites for packaging and biomedical applications respectively.

2. Silk fibre reinforced PVA and PVA/PVP composite films

2.1 Preparation

Silk fibre reinforced PVA (SF-PVA) and PVA/PVP (SF-PVA/PVP) films were prepared as follows. The degummed silk fibres were rinsed with distilled water to remove any impurities/solid dust particles sticking to the fibres. Further, the fibres were dried completely and then incised into small particles and powdered. This powdered silk was used for the preparation of films. Using the solution-casting technique, SF-PVA and SF-PVA/PVP films were prepared by mixing different weights of silk fibre and the polymer. For the blend composites, the weight of PVP was kept constant and the weight of silk fibres and PVA was varied. The solvent used for the preparation of films was double distilled water. The SF-PVA and SF-PVA/PVP solutions were mixed, for about 8 hours at 80°C. Further, to avoid agglomeration of fibres and to enhance its dispersion in the polymer matrix, the solutions were ultra-sonicated. The solutions were finally poured to a petri-dish and subjected to evaporation and final drying in an oven at 50°C [27, 28].

2.2 Characterization of the composite films

2.2.1 Morphological properties

The morphology of the films was observed using field emission scanning electron microscopy (FESEM) [27, 28]. The surface morphology and cross sections depicting thickness of a few selected films is as depicted in **Figure 1**. The thickness of the film corresponds to 27.53 μm. PVA film, without the fibre shows a homogenous and continuous matrix throughout without cracks. Similarly, in case of blend film, the compatibility of both PVA and PVP was clearly observed due to the smooth and homogenous surface resulting from the interaction between the two polymers. In both the cases, when the film is reinforced with 9 wt% silk, the smoothness of the surface is however lost and appears rough. The images depict that the fibres are randomly distributed and embedded well in the matrix indicating proper mixing of fibres. This could possibly be due to the interaction of the polar functional groups of the fibre with that of the matrix. When the fibre concentration is further increased to 15 wt%, the fibres seem to be less adhered to the matrix phase and tend to agglomerate indicating phase discontinuity [29] resulting in loss of film homogeneity.

2.2.2 Thermal properties

Thermogravimetric analysis (TGA) was performed for both SF-PVA and SF-PVA/PVP films as depicted in **Figure 2**. For SF-PVA films, the pristine PVA film exhibited three decomposition steps. The initial decomposition, due to the loss of water from the sample occurred at 80–150°C with a mass loss of 7.6%. The maximum degradation occurred from 250–400°C accompanied by a major weight loss of about 62.1%. This was due to the structural degradation followed by scissions of polymer chains in PVA. Further decomposition occurred from 450°C. This was due to the breakdown of C-C bonds in the polymer backbone. The addition of silk fibre (3–15 wt%) reduced the percentage mass loss (59.7–45.5%) [27]. This decrease in mass loss and the resultant increase in thermal stability is attributed to the higher thermal stability of silk fibres, which on interaction with the polymer matrix act as barriers for better heat insulation and lower the rate of degradation of the polymer [30].

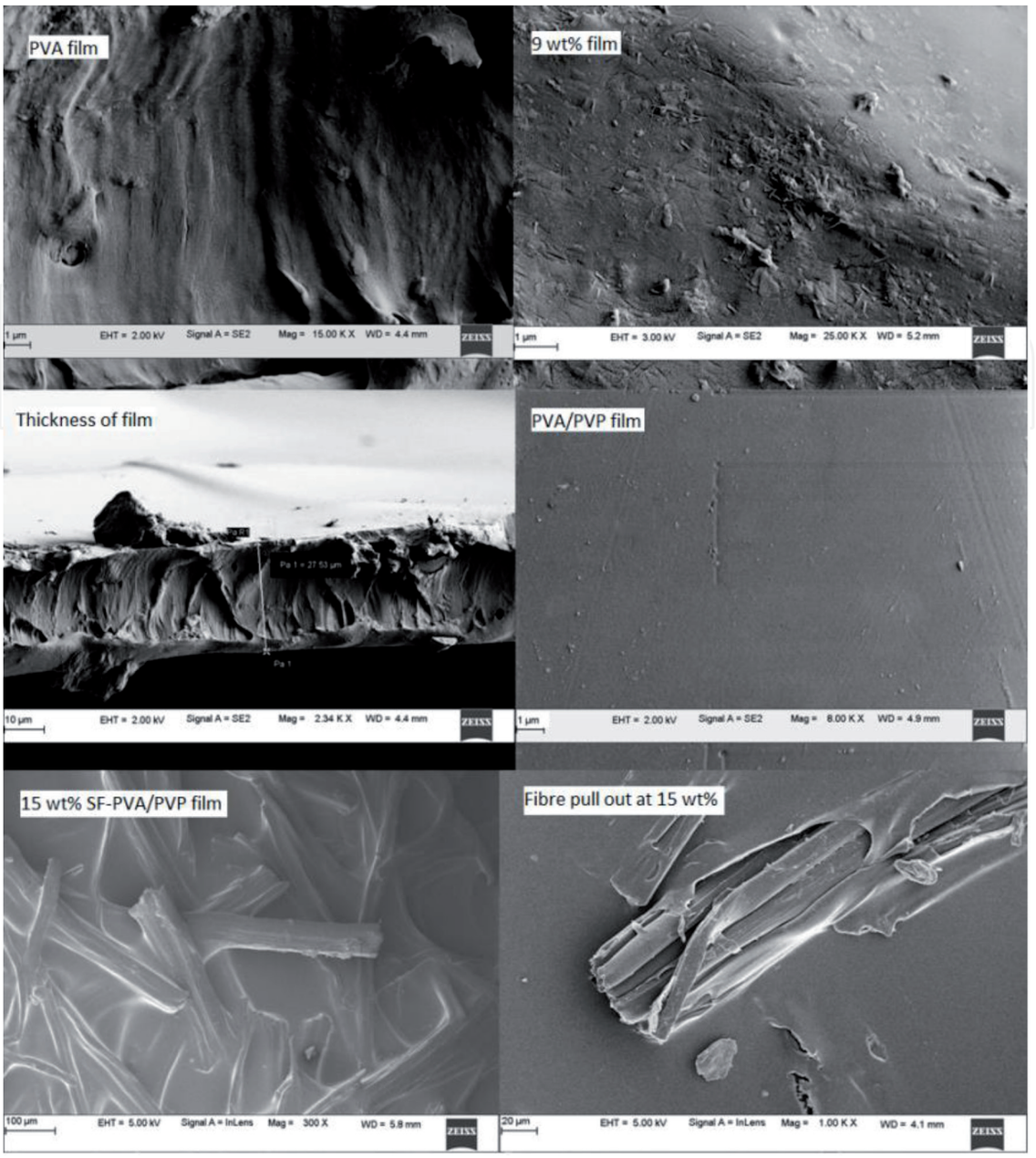

Figure 1.
FESEM images of SF-PVA and SF-PVA/PVP films.

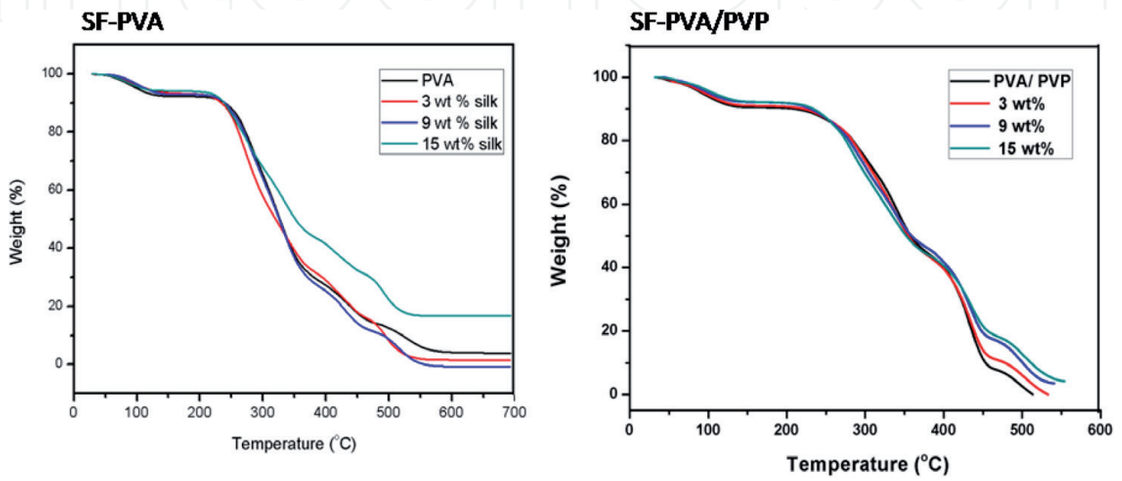

Figure 2.
TGA curves for SF-PVA and SF-PVA/PVP films.

The TGA of PVA/PVP film (**Figure 2**) and the film composites showed three decomposition steps. For the pure blend, the initial decomposition, with a mass loss of 7.4%, occurred at 70–140°C which was due to the loss of bound water molecules and acetic acid in the polymer [31]. Major degradation, due to the melting and breakdown of the blend segments, occurred between 200 and 365°C followed by a major weight loss of about 42%. Further decomposition and degradation of the sample resulted in a mass loss of 36% which occurred from 370 to 460°C. This was mainly due to the condensation and cyclization of the polyaromatic PVP [32]. With the addition of silk fibres, the percentage weight loss was slightly reduced to 40%, followed by an additional decrease to 36% (15 wt%) with a maximum residual content. It is quite obvious that due to the presence of amide groups, silk is known to possess a higher thermal stability as indicated by its high melting temperature, than PVA. The existence of a strong intermolecular hydrogen bonding interaction between hydroxyl groups of PVA and amide groups of silk in the composites resulted in an improved thermal stability and mechanical strength.

To further explain, after a thermal analysis, the final residual mass of a composite (char) is a measure of its flame resistance. This residual char, when exposed to a higher temperature can thermally insulate the undecomposed polymer from degrading [28]. The high varying nitrogen content in silk fibres (about 15–18%), has provided the fibre with a self-extinguishing property and hence a higher flame resistance. Therefore, it can thus be concluded that the decrease in mass loss with increasing fibre concentration, is mainly due to the thermal stability and flame resistance properties of silk fibres in the composites [33, 34].

From differential scanning calorimetry (DSC), the melting temperature, glass transition (T_g) and enthalpy values (ΔH_m) were assessed and summarized in **Table 1** and depicted in **Figure 3**. The melting temperature values for PVA and SF-PVA films were to a certain extent close to each other. The T_g for pure PVA film observed was 85.89°C [27]. Substantial increase in the T_g and ΔH_m values was observed with the addition of silk fibres, as compared to the PVA film. The addition of silk reinforcements hinders the chain mobility, thus shifting the T_g [30].

For SF-PVA/PVP films, a single glass transition temperature was observed. The occurrence of a single glass transition temperature (T_g) indicates the miscibility between the two polymers [35]. For the blend films, this endothermic transition appeared at a temperature of 93°C. Glass transition temperature (T_g) was further increased to higher temperatures (97, 101, and 105°C for 3, 9 and 15 wt%

Silk fibre content (wt%)	T_g (°C)	T_m (°C)	ΔH_m (J/g)	Mass loss (%)
SF-PVA films				
0	85.9	222.7	21.9	62.1
3	101.9	223.04	23.5	59.7
9	106.7	221.9	28.7	58.7
15	93.9	221	29.8	45.5
SF-PVA/PVP films				
0	93	215	27.4	42
3	97	217	21.6	41.4
9	101	219	23.3	40
15	105	220	28.6	36

Table 1.
Thermal properties of SF-PVA and SF-PVA/PVP films.

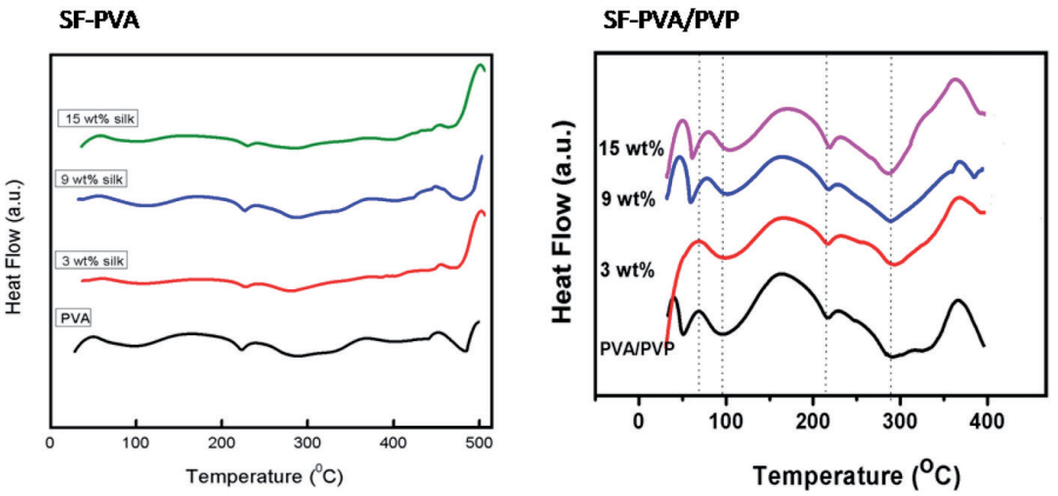

Figure 3.
DSC curves for SF-PVA and SF-PVA/PVP films.

respectively) with the increased amounts of silk fibre [28]. This increase in T_g along with the increase in the concentration of silk is due to the interaction of the silk fibre with the PVA/PVP matrix, thus, hindering chain mobility as previously discussed [30].

2.2.3 Mechanical properties

Tensile strength, Young's modulus and percentage elongation at break of the SF/PVA films was assessed and illustrated in **Figure 4**. For the SF-PVA film, the tensile strength and Young's modulus values effectively increased up to a fibre concentration of 12 wt% and thereafter decreased. The maximum values recorded for tensile strength and Young's modulus were 41.87 ± 2.08 and 202.08 ± 2.53 MPa respectively [27]. This was probably due to the hydrogen bonding interactions between the PVA and silk fibre that resulted in an increased mechanical property. When the fibre concentration was relatively low (3 wt%), the matrix was not restrained by adequate fibres and thus a remarkably high strain occurred which was confined to a small area in the matrix. This can cause rupturing in the bonds between the matrix and the fibre ensuing in an insufficient mechanical strength [36]. When the fibre concentration was increased, the load was shared between the fibres and the matrix, wherein the maximum load was taken by the fibres by the stress transfer mechanism. At a high fibre concentration, the $–NH_2$ and CO– groups of silk have a tendency to entangle and agglomerate among each other thus failing to interact with PVA [37] and hence a resulting decrease in the mechanical strength. The interfacial adhesion of the fibre and matrix plays a pivotal role in understanding the strength of a composite [36]. Highest percentage elongation at break was seen in PVA film, while the films reinforced with silk fibre, showed lower values due to the decrease in the concentration of PVA. This resulted in a fragile character and less ductile nature of the films.

For the SF-PVA/PVP films, the tensile strength and Young's modulus values increased up to 9 wt%, and subsequently decreased at a fibre concentration of 12 wt%. The maximum values for 9 wt% film, recorded a tensile strength of 30.3 ± 1.58 MPa and Young's modulus of 276.5 ± 4.05 MPa [28].

It is obvious that when a polymer matrix is reinforced with short fibres such as silk, the mechanical keying effects between the polymer chains and the fibre primarily restricts the segmental motion of the polymer matrix. Further, as previously

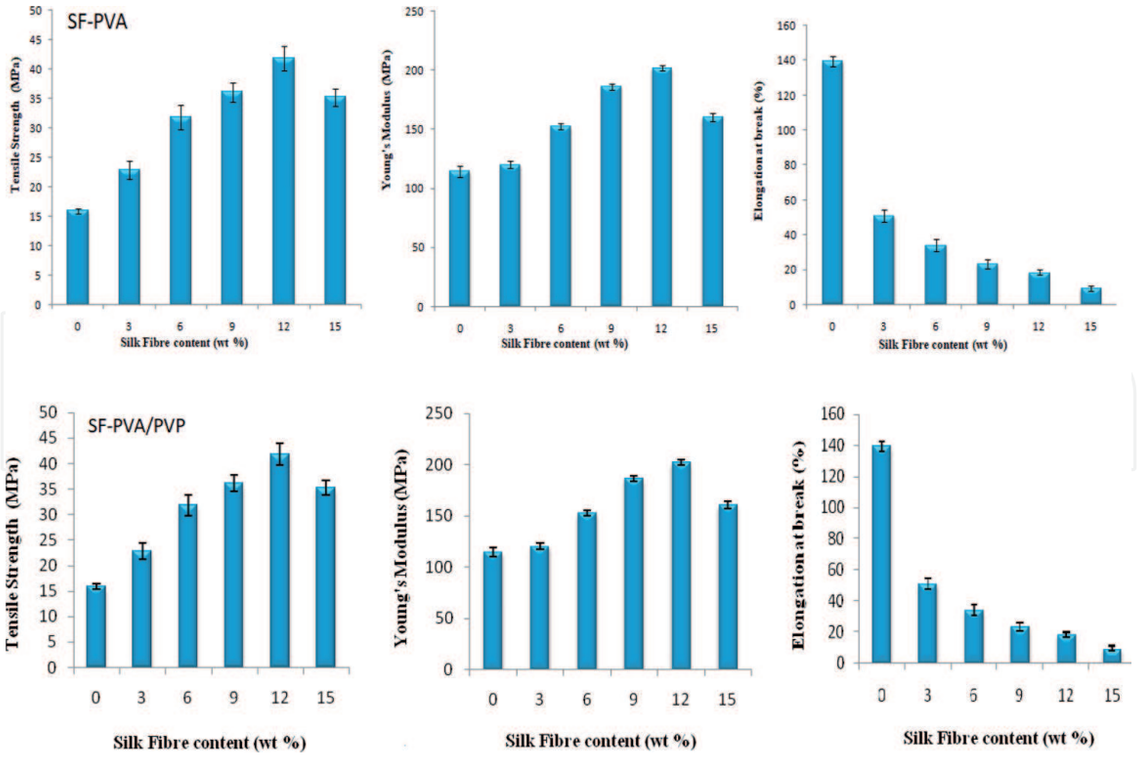

Figure 4.
Mechanical properties of the composite films.

discussed, the chemical interactions such as hydrogen bonding between the matrix and fibre, improves the strength of the composite.

In short fibre reinforced composites; the matrix plays a dynamic role as it provides a cushioning effect to the embedded fibres, although the load is shared by both fibre and matrix. When the concentration of the matrix is decreased, followed by a relatively increase in the concentration of the fibres, the increased load results in more strain resulting in breaking, thus leading to de-bonding between the fibre and matrix [38]. In addition to this, as evident from FESEM analysis (**Figure 1**) the silk fibres were less adhered to the matrix at the surface of the film (15 wt% fibre concentration) [28].

The trend observed in the percentage elongation at break was similar to that observed in the case of SF-PVA films. This decrease was again ascribed to the associated decrease in the concentration of the matrix [28].

2.2.4 Biodegradation by soil burial tests

The biodegradability of the film samples was evaluated using the weight loss method [39] and depicted in **Figure 5**. For a period of 8 days, the average weight decrease was 5% and the weight of the films further gradually decreased as the time increased. For SF-PVA/PVP films, for a period of 8 days, the average weight decrease was found to be 12% and as time increased, the weight of the films also gradually decreased. After 64 days the average weight decrease was approximately 36 and 32% for SF-PVA and SF-PVA/PVP respectively. It was observed that, as the percentage of silk fibre increased there is enhancement in the rate of bio-degradation of the films when compared to the pure PVA and blend film without the fibre. As the soil microbes attack, structural deformations in the film composites occurred resulting in the brittleness of the films. Therefore the films became hard and fragile in nature. When this protein based silk fibre, is buried in soil, it tends to undergo degradation

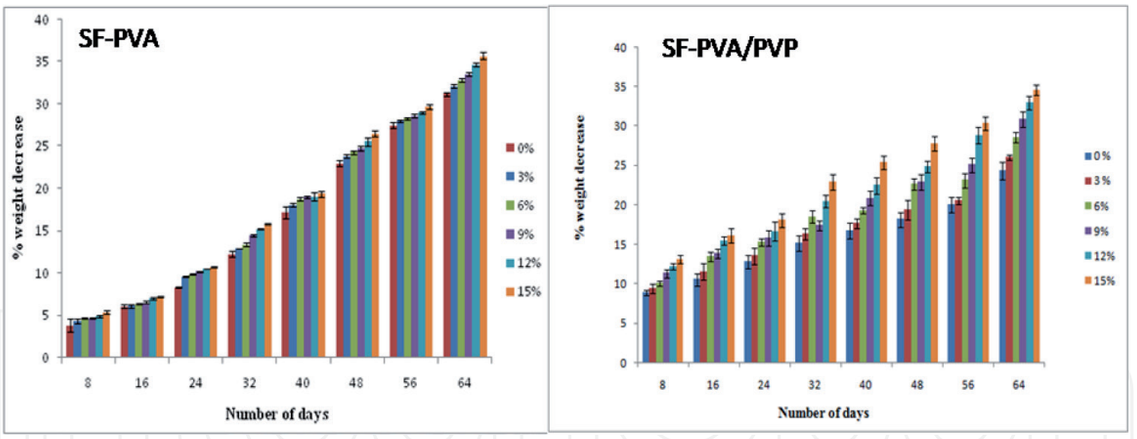

Figure 5.
Graphs depicting soil burial degradation tests for composite films.

which could be attributed to the penetration of water from the cut edges of the composite films, resulting in loosening of the complex structure and consequently results in the weight loss of the films [40].

3. Surface modification of silk by grafting chitosan

The grafting of chitosan over silk was performed using the optimized procedure with slight modifications, reported by Davarpanah et al. [26, 41]. Briefly, degumming of silk was performed using sodium dodecyl sulphate and sodium carbonate. The material mass to liquor ratio maintained was 1:25. The fibres were further washed with warm and cold distilled water and dried. Acylation of the degummed fibres was performed using succinic anhydride in N,N-dimethyl formamide (DMF). The fibre samples were washed with DMF followed by acetone, to remove unreacted anhydrides. Grafting of chitosan was carried out in acetic acid media followed by drying. In **Figure 6**, the images of the fibres before and after surface modification are shown. Total percentage fibre weight gain was calculated by the difference in weight gain of the acylated and the grafted silk fibre and was found to be 17% [42].

3.1 Preparation of grafted silk fibre reinforced PVA composite films

Post grafting, the fibres were dried completely and incised into small particles and finely powdered. Fibre reinforced PVA films were prepared by mixing different weight percentages of grafted silk and PVA, followed by the solution-casting technique [42] as described previously under Section 2.1.

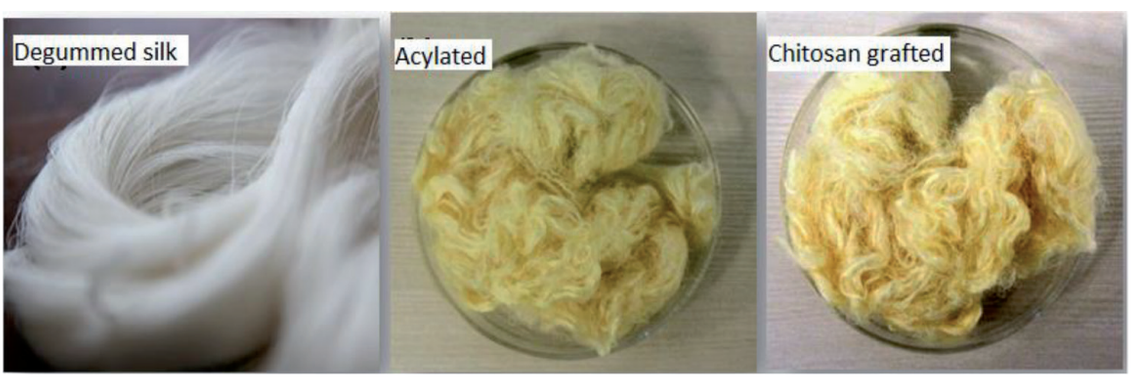

Figure 6.
Image depicting degummed, acylated and chitosan grafted silk fibres.

3.2 Characterization and *in vitro* biocompatibility studies

3.2.1 Morphological properties

From scanning electron microscopy the chemically treated and grafted fibres were examined. **Figure 7** depicts the surface of degummed silk fibre and thus appears smooth. The acylated fibres were characterized by a uniform surface with few foreign particles and the roughness of the fibres further increased due to grafting of chitosan, which clearly shows the presence of particles firmly attached on the fibre.

The surface topography and roughness of the pristine film as well as the fibre reinforced composites was studied using atomic force microscopy (AFM). Atomic force microscopy (AFM) images for PVA and composite film samples are depicted in **Figure 8**. The AFM of PVA film appeared smooth and the surface roughness (RMS) value obtained for PVA film was 86.32 ± 58 nm. For the composite films, AFM revealed the presence of grafted silk fibres randomly scattered along the matrix. The roughness values substantially increased with the addition of the fibres and the films appeared corrugated and irregular. It is apparent that, a fibre, when added to a polymer matrix and later casted to form a film, a repulsive interaction is known to exist between the two phases (matrix + fibre), which eventually results in changes in surface roughness. This is mainly due to the microphase separation and changes in the alignment of the polymer chains [43] that results in changes in the topography.

3.2.2 Thermal properties

TGA curves showed three decomposition steps for PVA film as depicted in **Figure 9**. The trend in decomposition observed for PVA was similar to that observed previously. The initial decomposition for PVA occurred at 80–150°C. The

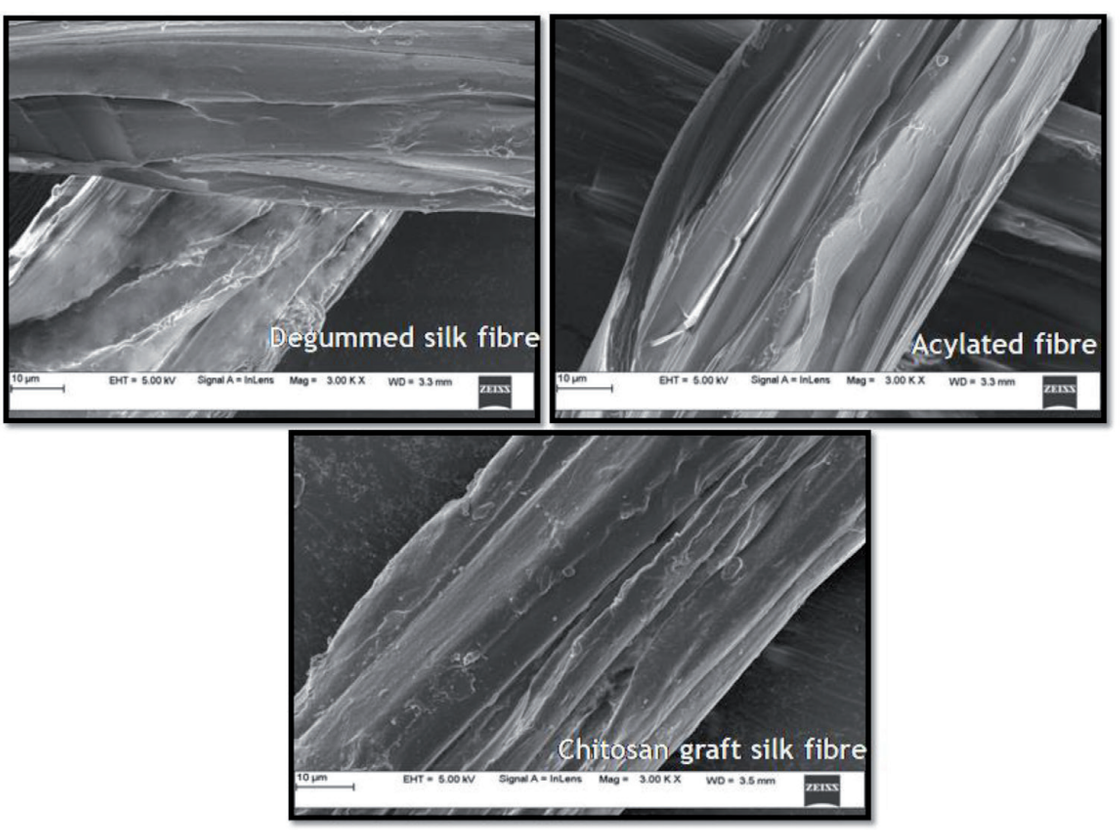

Figure 7.
FESEM images of degummed, acylated and chitosan grafted silk fibres.

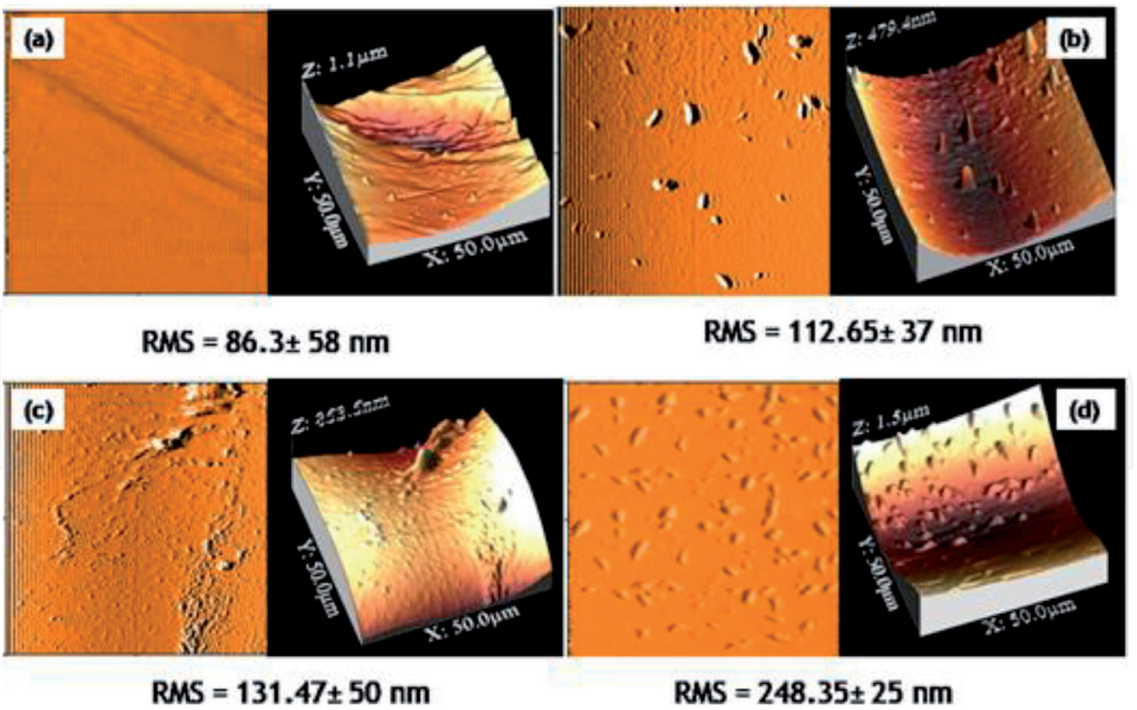

Figure 8.
AFM images of (a) PVA film (b) 3 wt% film (c) 9 wt% film (d) 15 wt% film.

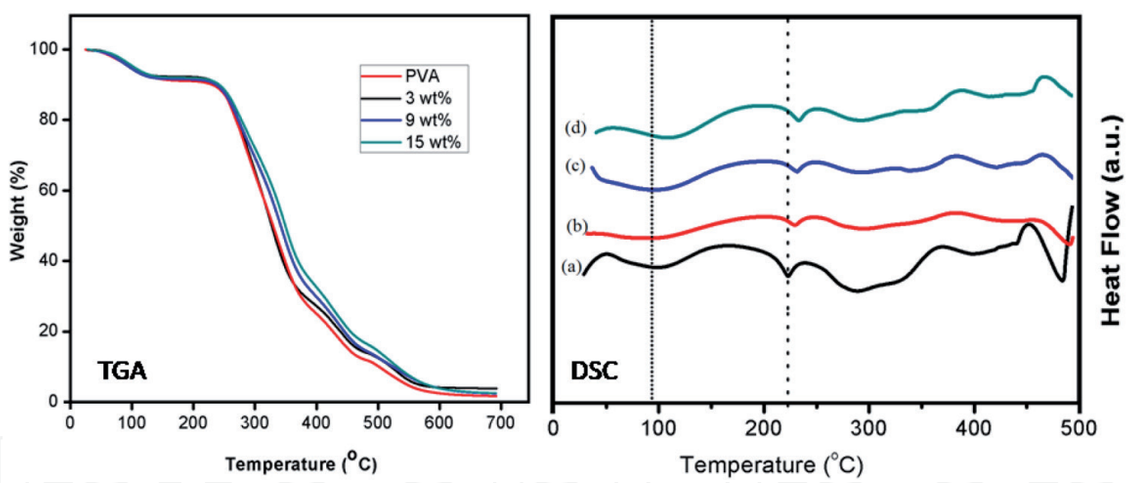

Figure 9.
TGA and DSC curves for grafted fibre reinforced composite films.

weight loss of about 8% was observed which was due to the loss of bound water from the sample. The maximum degradation occurred from 250 to 400°C accompanied by a major weight loss of about 59.4%. As previously discussed, this was due to the structural degradation along with the chain scissions in PVA [42]. Further decomposition followed by the decrease in the weight of sample was observed from 450°C. This was due to the breakdown of C–C bonds in the polymer backbone [44].

Incorporation of chitosan onto the silk fibres did not affect its thermal properties. As a result, the higher thermal stability of silk fibre was retained. The higher thermal stability of silk fibres reduced the percentage weight loss to about 58%, followed by further reduction in weight to about 54.9 and 50% for 3, 9 and 15 wt% incorporated grafted fibres respectively [42]. Thus, the hydrogen bonding interaction of PVA, with the functional groups of silk fibre was still observed.

DSC analysis showed a substantial increase in T_g and a slight increase in melting temperatures indicating better thermal stability as depicted in **Table 2** and **Figure 9**.

Silk fibre content (wt%)	T_g (°C)	T_m (°C)	ΔH_m (J/g)	Mass loss (%)
0	98.8	222.6	22.8	59.4
3	101.9	222.3	23.5	58
9	106.7	224.6	26.62	54.9
15	109.9	224.8	28.9	50

Table 2.
Thermal properties of grafted silk fibre composites.

The T_g for the PVA film observed was 98.8°C. The grafted silk when added to the matrix, further increased the T_g, and ΔH_m values. Thus, the mobility of polymer chains was hindered by further addition of silk; thereafter resulting in a substantial increase in T_g values [30, 42].

3.2.3 Biodegradation by soil burial tests

The prepared film composites turned brittle and lost their flexibility when exposed to soil, after a certain period of time. For a period of 8 days the weight decrease was found to be approximately 8% and as the time increased the percentage decrease in the weight of the samples also increased. After 64 days the weight decrease was approximately 40%. Compared to our previous results as discussed, the trend observed in this study as depicted in **Figure 10**, was that the weight decrease slightly increased due to the incorporation of chitosan grafted silk fibres. Although chitosan is known to be antibacterial in nature, it is however known to be an efficient soil amendment material and shows degradation in soil. This is due to the fact that certain microbes are known to possess an enzyme chitosanase that promotes degradation of chitosan. Berkeley [45] reported that chitosan-hydrolyzing enzymes (chitosanases) are produced by the genera *Arthrobacter*, *Bacillus*, *Streptomyces*, *Aspergillus*, and *Penicillium*. Besides, the increased surface roughness with the addition of silk fibres is also known to enhance the rate of biodegradation.

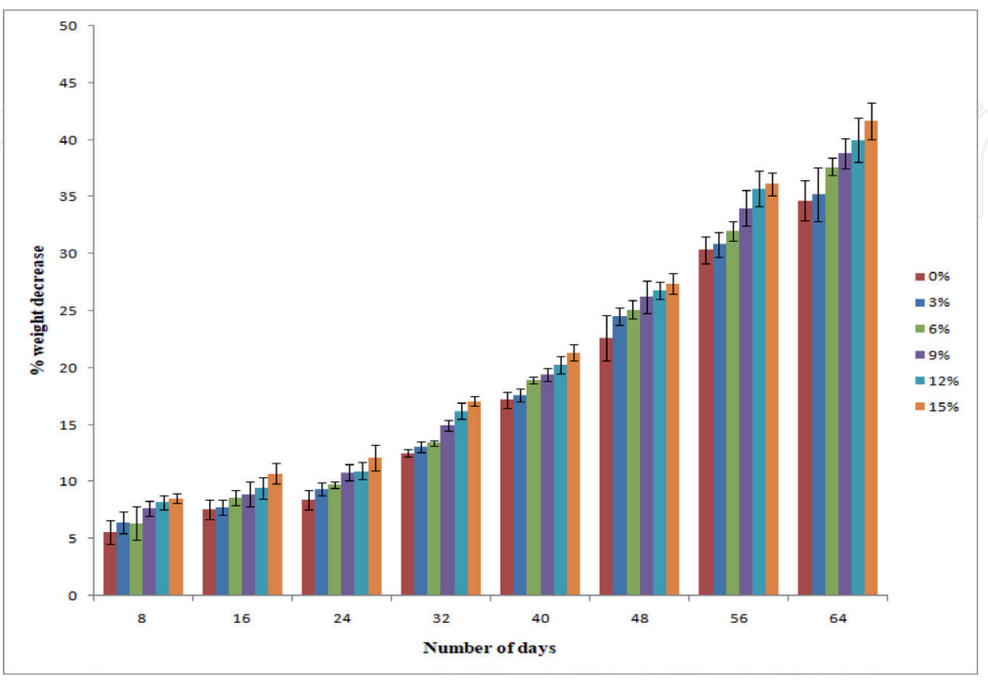

Figure 10.
Graph depicting soil burial degradation test.

Higher surface roughness increases biodegradation by providing more sites for bacterial colonies to settle and proliferate [46].

3.2.4 Antibacterial tests

In vitro antibacterial studies were performed for 2 g negative and 2 g positive bacterial strains. PVA film did not show any activity on all four bacterial strains. The positive control streptomycin showed highest antibacterial activity. **Table 3** shows the zone of inhibition calculated for the films and the activity against the bacterial strains. Due to the lower concentrations of the chitosan grafted silk fibre (3 and 6 wt%), the films showed little or no activity. However as the fibre concentration was increased, the films showed good activity and consequently at 15 wt% concentration, due to a relatively high concentration of chitosan, significant activity was seen against *S. aureus* and *E. coli* the reason being the bacteriostatic as well as bactericidal activity of chitosan towards pathogenic strains of bacteria. It is believed that the polycationic nature of chitosan, due to the positively charged $-NH_3^+$ groups of glucosamine, might be a crucial factor contributing to its interaction with the negatively charged surface components of many fungi and bacteria. Consequently, this leads to extensive cell surface alterations, leakage of intracellular substances, and impairment of vital bacterial activities [47–50].

3.2.5 In vitro hemocompatibility

Hemolysis is a phenomenon that occurs, when the cells swell to the critical bulk and results in the lysis of cell membranes. The resulting broken red blood cells can release adenosine diphosphate, which escalates the assembly of blood platelets, thereby accelerating the formation of clotting and thrombus. It is an additional setback associated with the biocompatibility of material [51]. It is quite obvious that when red blood cells (RBC's) come in contact with water, they tend to hemolyse and when an incompatible material comes in contact with these cells, this problem intensifies. The membrane stability of the RBC's was studied for PVA and film composites, in relation with the standard drug diclofenac sodium [52]. Percentage inhibition of hemolysis for test samples was studied and calculated as follows

$$\text{Percentage inhibition of haemolysis (\%)} = \frac{\text{Control} - \text{Test sample}}{\text{Control}} \times 100 \quad (1)$$

	Zone of inhibition (mm)			
Sample films	***S. aureus***	***Bacillus subtilis***	***E. coli***	***K. pneumoniae***
Control	—	—	—	—
3 wt%	8 ± 1	—	—	—
6 wt%	8 ± 1	—	11 ± 0.00	—
9 wt%	9.00 ± 0.00	9 ± 0.00	13.66 ± 0.57	9 ± 0.00
12 wt%	12 ± 0.00	12.66 ± 0.57	14.66 ± 1.15	10 ± 0.00
15 wt%	17.33 ± 1.15	13.33 ± 0.57	15.33 ± 1.15	12.66 ± 0.57
Chitosan	22 ± 0.57	19. 33 ± 1.15	21 ± 0.57	22 ± 0.57
Streptomycin	25 ± 1	22. ± 1	28.66 ± 0.57	19.33 ± 0.57

Table 3.
Antibacterial activity of composite films reinforced with grafted fibre.

A higher percentage inhibition was observed for all composite films in comparison with the pristine PVA film as depicted in **Figure 11**. The highest percentage value obtained was for 15 wt% films (79.0 ± 1.8), which were almost similar to the standard diclofenac (82.5 ± 1.2) [52]. Previous reports suggest that chitosan promoted surface induced hemolysis, which can be ascribed in part to the electrostatic interactions [53, 54]. When the concentration of chitosan was increased to about 100 mg/ml, traces of haemolytic activity was observed and the largest haemolytic activity observed was less than 10%, signifying a wide safety margin suitable for blood contact applications [55]. Thus, on comparing the low hemolytic activity with the high erythrocyte agglutination, it can be seen that, chitosan only induces the surface adhesion of erythrocytes and does not critically damage the cell membrane [56, 57]. The results thus obtained for the films, did not rupture the cell membrane, subsequently making them hemocompatible.

3.2.6 Cell proliferation studies by trypan blue assay

The principle underlying the trypan blue dye exclusion assay is that a dead or a dying cell possesses a membrane that happens to be permeable to the dye trypan blue and thus will stain blue. On the contrary, the viability of the cells can be determined as the viable cells are capable of repelling the dye and hence do not stain [58]. **Figure 12** denotes the cultured mouse fibroblast cells growing on PVA and composite films. The cells displayed spindle shaped morphology, rapidly proliferated and grew into small colonies by strongly attaching on the film substrates as compared to the control (Dubecco's Modified Eagle's Medium) and PVA film. It was observed that the composite films of all concentrations supported cell growth and were thus non-toxic. These results when further corroborated with AFM investigations, demonstrated that the enhanced surface roughness of the films provided a larger surface area for the cells to adhere. As a result, cell growth was facilitated when compared to the comparatively smooth pristine film, signifying that surface roughness of the substrate played a huge role in regulating cell adhesion and growth [52].

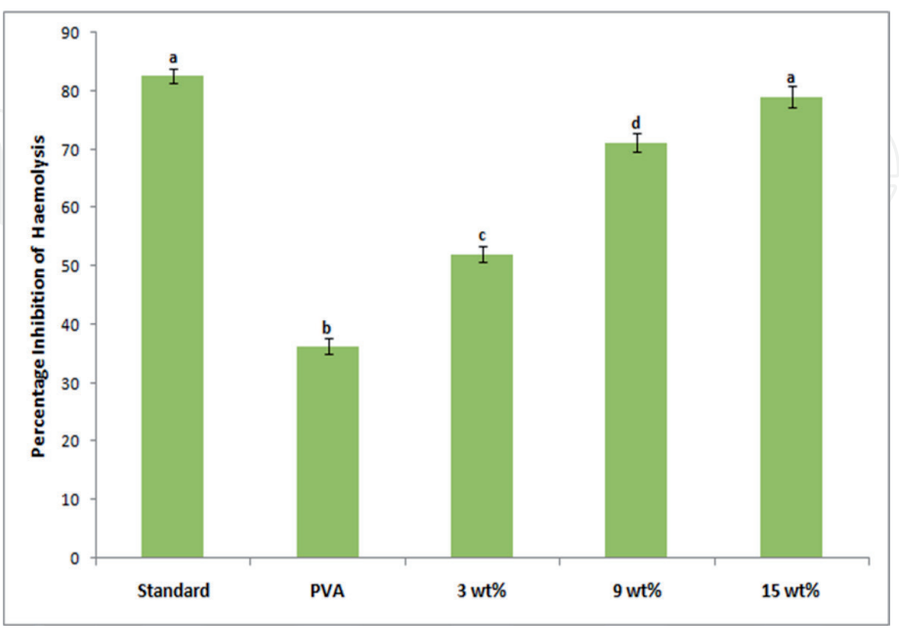

Figure 11.
Graph depicting percentage inhibition of haemolysis. Values expressed as Means ± SD of triplicate measurements (n = 3). Means with different letters in each column is significantly different (p <0.05, ANOVA, Bonferroni).

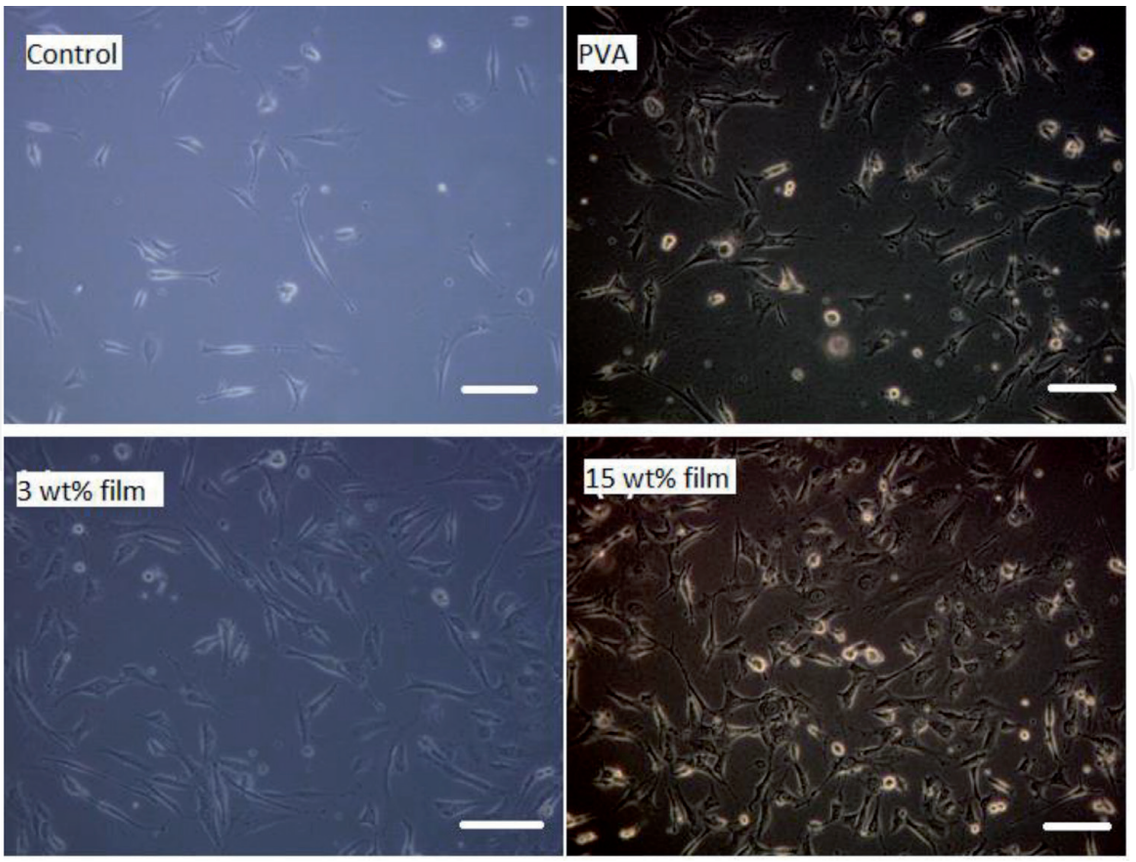

Figure 12.
Pictomicrographs of attachment of fibroblast cells (Scale bar-500 μm).

3.2.7 MTT assay

The mitochondrial activity of cells is measured by a colorimetric assay (MTT assay) that can reduce MTT to a purple coloured product called formazan. The number of viable cells is related to the amount of colour produced. Yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) is reduced to purple coloured product called formazan in viable cells [59]. This insoluble purple formazan is dissolved using a solvent such as dimethyl sulphoxide (DMSO), so as to form a coloured solution [60]. Solubilization of the cells results in liberation of the purple product, which can be detected using a colorimetric measurement. The resulting purple solution is spectrophotometrically measured [61]. The increase in the absorbance is related to the amount of formazan formed resulting due to rapid proliferation of cells, thus indicating the mitochondrial activity of cells.

The cellular activity on the substrate was studied using cultured mouse astrocytes. The results demonstrated cell viability values higher than 80% for all film samples (**Figure 13**). This enhanced viability is ascribed to the initial time taken by the cells to adapt to the nature of the substrate [62]. Although there was no huge difference in the cell activity of all test samples, highest activity observed was for 3 wt% films (103.25 ± 13.23). The films were thus nontoxic and supported cell growth [52].

3.2.8 Micronucleus cytome assay

The *in vitro* cytokinesis-blocked micronucleus cytome (CBMN-cytome) assay is a modified CBMN assay which is based on the assessment of micronucleus (MN)

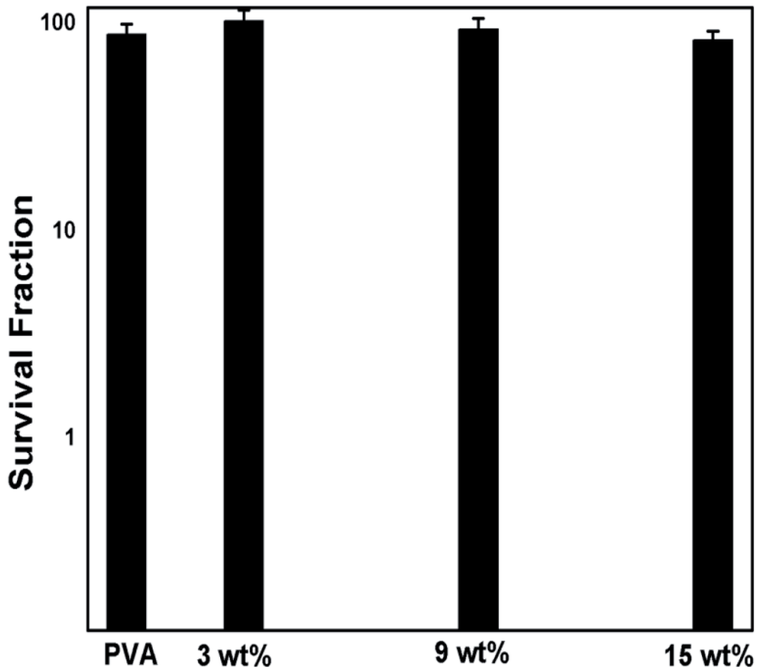

Figure 13.
Cell viability by MTT assay. Results are expressed in percentage survival fraction.

in nucleated cells that have completed only one nuclear division. The MN assay is a simple and rapid method that is perfectly suitable to elicit the biocompatibility of the samples and thus study the genotoxicity of the polymer composite samples. The CBMN cytome assay is not only restricted to micronucleus measurement, but also assists in the assessment of relevant markers like nucleoplasmic bridges (NPBs), nuclear buds (NBUDs), apoptotic and necrotic cells [63]. **Figure 14** reveals the presence of micronucleus, NPBs, NBUDs, and necrotic cells. The MN refer to small nuclei formed from acentric fragments or whole chromosomes, which lag behind and does not get included in either of the daughter nuclei, formed during cell division [64]. Hence, MN provides an indication of both chromosome breakage and chromosome loss.

MN cytome assay as described by Fenech [63], with slight modifications was used in the study. The cells used for the study were cultured normal lung cells

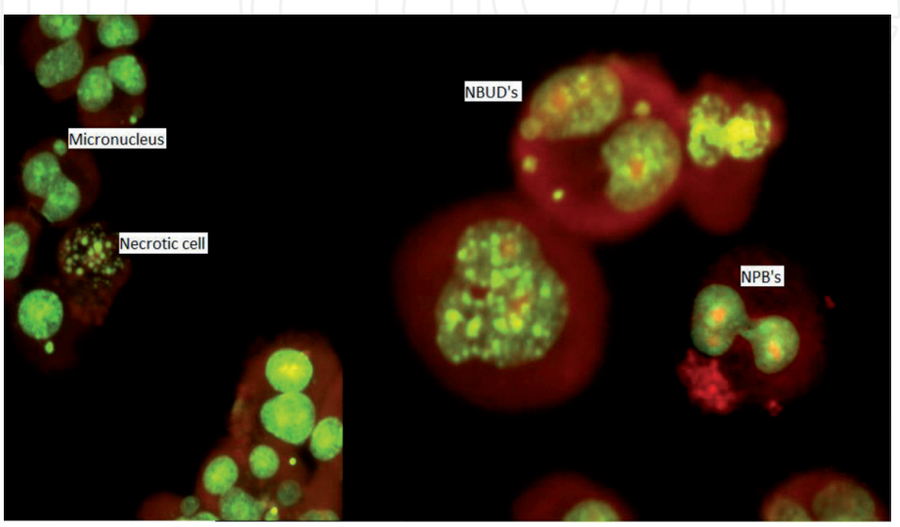

Figure 14.
Image depicting typical L132 cells.

(L132) cells. Only binucleated cells (BNC) with intact cytoplasm were considered for scoring micronuclei (MNi) by ignoring cells with broken cytoplasm and cells with fused nuclei [65]. No significant difference in the MN yield of micronuclei was observed between the cultures treated with PVA, 3, 9 and 15 wt% silk fibre reinforced films when compared to the negative control, revealing the lack of genotoxic effect. In relation to nuclear division index values, no significant differences were observed between the different composite films and negative control, demonstrating the absence of cytotoxicity.

4. Conclusions

The surging interest towards silk fibres due to its innumerable properties was the motivation of this study. A comprehensive study on the use of silk fibres as reinforcements, for the development of biocompatible and biodegradable composites was conducted. The composite films were developed by solution casting and the effect of silk fibre concentration on the properties of the composite films was assessed.

Biocomposite films of PVA and PVA/PVP; reinforced with degummed short silk fibres were prepared and showed enhanced properties. The results showed that increasing the fibre concentration, diminished the mechanical properties and the optimum concentration was found to be 12 and 9 wt% respectively. The added short silk fibres, improved the thermal properties of the composite films as confirmed by DSC/TGA. Morphological properties of the film composites indicated a poor fibre matrix adhesion (FESEM) at the fibre concentration of 12 and 15 wt% respectively. The prepared films were found to biodegradable and the biodegradation was aided by the presence of soil microbes.

The properties of silk fibres were re-tailored by grafting a natural polysaccharide like chitosan, thereby fabricating films of PVA, suitable for biomedical applications. Films of PVA reinforced with grafted silk showed improved properties as a result of fibre-matrix interactions and hence prove to be effective to be used as scaffolds for tissue engineering applications. Besides, the composite films also showed enhanced biodegradable characteristics with increasing concentrations of grafted silk fibres. The efficacy of the films to exhibit antimicrobial activity was undoubtedly due to the presence of chitosan on silk. The films were further evaluated for their blood compatibility, cytotoxicity and genotoxicity studies and proved to be nontoxic in almost all concentrations.

Acknowledgements

The authors are grateful to DST PURSE, Mangalore University for providing the necessary facilities for the characterization of the samples.

Conflict of interest

The authors of this manuscript declare that they do not hold any conflicts of interest that might have any bearing on research reported in their submitted manuscript.

DOI: http://dx.doi.org/10.5772/intechopen.85022

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