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Physico-Chemical and Biocompatibility of Poly(vinyl alcohol): Polyethylene Glycol/κ-Carragreenan Membrane by Freeze-Gelation Method

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The aim of this study was to fabricate membrane based on poly(vinyl alcohol) (PVA):polyethylene glycol (PEG)/κ-carragreenan (KC) with different weight ratio (w/v) by freeze gelation method. The prepared membrane was characterized by using SEM, FT-IR, TGA, AFM and XRD techniques. The results revealed that FTIR and SEM showed good molecular interaction among the PVA/PEG with κ-carragreenan. The rate of thermal degradation stabilized with increased κ-carragreenan ratio and also increased the percent of crystallinity. Further water uptake also performed under phosphate buffer saline (PBS) media and results showed increased rate of swelling with increased κ-carragreenan concentration, which enhanced the stability of water uptake. Moreover cell viability performed using MTT method. The highest compatibility showed for lower concentration κ-carragreenan (PVA/PEG/KC10%). Overall results exhibited moderate swelling and degradation, good mechanical properties with strong molecular interaction between PVA/PEG with κ-carragreenan these all characters might have advantage in the field of biomedical applications.

Keywords: Poly(vinyl alcohol), Polyethylene glycol, κ-Carragreenan, Morphology, Biocompatibility.

INTRODUCTION

Extensive research has been undertaken to construct biopolymer based biomimetic polymer materials as substitute materials for biomedical applications such as drug delivery, tissue regeneration. Recently, tissue engineering has become most popular disciplines of research, which include both biological and engineering principles [1]. Tissue engineering is a technique to construct new tissue from cultured cells, considered as a potential alternative to organ or tissue transplantation [2]. Polymer materials act as transplant vehicles for cultured cells and also as a template to guide tissue growth play a significant role in modifying the cultured cells to a new tissue [3-5]. In this context many reaserch investigation efforts have been performed to control and modify the material′s chemistry for enhancement of highly specific binding interactions between material and cells which involve improvement of the material surface to provide for cell adhesion with extracellular matrix components [6,7]. The varieties of biodegradable polymers are used as biomaterials in variety of forms like films, hydrogels, fibres, membranes and 3D scaffolds. There are several methods for preparation of biomaterials and new processes are emerging for different applications [8]. Currently, freeze gelation technique is attracting much attention in the preparation of polymer based biomaterials and these involves the process of freezing followed by gelation of the polymer in the presence of solvents below its freezing temperature [9,10]. Various synthetic and natural polymers like gelatine, chitosan, PVA and PEG have been used for preparation of freeze gelled membrane in recent past [11-13] and hybrid based biomaterials have been increasing due to their compatibility, flexibility and strength. In this scenario, expanding library of natural and synthetic polymers available for biomedical applications, block copolymers of poly(vinyl alcohol) (PVA), polyethylene glycol (PEG) and κ-carragreenan have emerged as a promising biodegradable material due to their highly controllable physical and chemical properties.

Poly(vinyl alcohol) (PVA) is a water-soluble, biocompatible and hydrophilic polymers used widely in the area such

as food chemistry, pharmaceuticals and medicines. It is a polymer of intense interest for biomedical applications [14,15]. Poly-(vinyl alcohol) presents a high tendency to form hydrogen association and easily forming gels. The hydrogen bonding plays an important role in the properties of PVA, such as its wide crystallinity range, high crystal modulus and water solubility [16]. Costa-Júnior *et al*. [17] reported the PVA/chitosan crosslinked film for biomedical applications. PVA with other polymers opened a window of research for tailoring the properties of interest. On the other hand, to increase properties of matrix, polyethylene glycol (PEG) is considered due to its biodegradable, non-toxic, biocompatible and non-immunogenic nature and holds the inert surface that reduces inflammation side effect after biomaterial implantation. PEG has various biomedical applications such as in drug delivery systems, tissue engineering applications like neural, cartilage, cardiac, liver and skin regeneration [18-21]. However synthetic polymers are limits their bioactivity for some biomedical applications hence, it is great advantage to increase bioactivity of matrix by combination of synthetic polymer with natural such as κ-carragreenan, it has been explored for biomedical field due to advantage of properties *viz*. non-toxic, easily gel forming properties and easily miscible with other polymers. In this article research focussed on fabrication of biopolymer based membrane by using poly (vinyl alcohol), poly (ethylene glycol and κ-carragreenan for biomedical applications such as tissue regeneration..

EXPERIMENTAL

Poly(vinyl alcohol) (*m.w.* 170,000) was obtained from Spectrum Reagents and Chemical Pvt. Ltd. Edayar Cochin, India and polyethylene glycol was procured from Spectrochem Pvt. Ltd. Mumbai, India and κ-carragreenan was obtained from TCI Chemicals Pvt. Ltd. Tokyo, Japan. Phosphate buffer saline (PBS) and lysozyme enzyme (ex. white egg) were purchased from Sisco Research Laboratory Pvt. Ltd., India. The double distilled water and alcohol was used as a solvent.

Preparation of PVA/PEG/KC membrane: A solution of PVA and PEG was prepared by dissolving equal weight percent (2% w/v) in distilled water and stirred under magnetic stirrer. Similarly, κ-carragreenan (KC) solution was prepared by dissolving different weight percent (10 & 20% w/v) in distilled water and stirred for 24 h until homogenous clear viscous solution was obtained. Later mixed all three solutions and stirred under magnetic stirrer for overnight. After ensuring complete homogeneous nature of PVA/PEG/KC solution, blend solution was sonicated to remove the air bubbles then poured on the dried petri-plates and freezed in deep freezer at -18 ºC and then the frozen petri-plate dipped in mixture of water/ethyl alcohol (1:3). After complete gelation, petri-plated were dried in room temperature then the formed gel membrane were dried under hot air oven at 40 ºC for 12 h to remove the solvent from petri dish. Then membranes were removed from the petri-plates, later set aside in polythene bags and stored in vacuum desiccator until use. Membranes were named as PVA, PVA/PEG, PVA/PEG/KC1(10%) and PVA/PEG/KC2 (20%).

SEM analysis: The morphology of films was assessed by scanning electron microscopy (JSM-6360, JEOL/Germany). The images were obtained using an accelerating voltage of 10 kV. All the specimens were sputter-coated with a conductive layer of gold to avoid charging under high electron beam. The film specimens were mounted on metal stubs using a double-sided sticky carbon tapes.

FTIR analysis: The interaction among the blend components was examined with an attenuated total reflection (ATR) method of IR spectrometer FTIR spectroscopy (attenuated total reflection, Prestige 21, Shimadzu, Japan). The FTIR spectra were obtained in the wavenumber range from 4000 to 650 cm^{-1} at 4 cm^{-1} resolution. The FTIR spectra were normalized and major vibration bands were identified associated with the main chemical groups.

Thermal analysis: Thermogravimetric analysis (TGA) was carried out on Perkin-Elmer STA 6000 instrument. The samples of 5-10 mg were taken and warmed up until 700 ºC at a heating rate of 10 ºC/min under N_2 atmosphere (flow rate 100 mL/min).

XRD analysis: X-ray diffraction patterns of the pure and blend polymers membrane formulations were obtained by using Panalytical X'PERT PRO, Netherland. The system was operated during the experiment by using Lynx-Eye as a detector at a voltage of 40 kV and at a constant current of 40 mA. The scanning was conducted over the 2θ range from 35º to 146º using Nickel-filtered Cu*K*α radiation (λ = 1.5418 Å) with a scan speed of 4º/min. The pure samples and blend polymers were poured into the cavity of sample tray and film specimens of approximately 0.5 mm thick were cut to fit the square tiles of sample holder before running the test.

AFM analysis: Atomic force microscopy (AFM) (Gwyddion 2.25) was used to collect the surface topography structure of the membranes at ambient temperature. Topographic images were observed by contact angle mode using aluminum coated cantilever.

Swelling studies: The swelling property of blend membrane was determined in aqueous media for different interval of time. The wet weight (W_s, swollen weight) and dry weight (W_d, dried at 40 ºC overnight) were measured. Then, the swelling index was calculated using following equation:

$$S(\%) = \frac{W_s - W_d}{W_d} \times 100$$

MTT assay: The study of cell viability was investigated by using standard MTT assay protocol. Briefly, NIH 3T3 fibroblast cells were cultured in T-25 flask, trypsinized and aspirated into a 5 mL centrifuge tube. The cell pellet was obtained by centrifugation at 300 × g. The cell count was adjusted, using DMEM HG medium such that 200 µL of suspension contained approximately 1×10^4 cells. The test samples were sterilized with 70% ethanol dried and coated with 0.1% gelatin. The wells of the 96 well plates were also coated with gelatin and dried for 2 h. The samples were placed into the wells in triplicates. To each well (96 well microtitre plate), 200 µL of cell suspension was added and the plate was incubated at 37 ºC and 5% CO_2

atmosphere for 24 h. After 48 h, the spent medium was aspirated and 200 µL of medium containing 10% MTT reagent was then added to each well to get a final concentration of 0.5 mg/mL and the plate was incubated at 37 ºC and 5% CO_2 atmosphere for 3 h. The culture medium was removed completely without disturbing the crystals formed. Then in 100 µL of solubilization solution, DMSO was added and the plate was gently shaken in a gyratory shaker to solubilize the formed formazan. The samples were then removed from the wells and the absorbance was measured using a microplate reader at a wavelength of 570 nm and the percentage viability was calculated.

RESULTS AND DISCUSSION

SEM analysis: The morphology structure of PVA, PVA/PEG and PVA/PEG/KC1 and 2 membranes are shown in Fig. 1. A pure PVA presented a continuous heterogeneous and uniform shape structure and ice particle structure embedded within the PVA were aggregated on the surface of PVA. Similarly, addition of PEG changed the surface structure and PEG merged within the PVA clearly visible on the membrane, further incorporation of κ-carragreenan (KC) drastically changed the morphology, which consist of small micrometric ridges appeared on the surface in smaller concentration of KC10% and in higher volume KC20% surface exhibited large ridges due to interaction sites of external stimuli with the hydrophilic groups (-OH) of κ-carragreenan in PVA/PEG/KC membrane. Though the membrane surface exhibited heterogenous with rough surface after blending with κ-carragreenan. This may be due to the strong intermolecular hydrogen bonding interaction between PVA/PEG and κ-carragreenan to form a tight structure with stable network. Similar results were found by Afnan *et al.* [22] by using PVA/PEG blends. Overall results suggested that PVA and PVA/PEG films alone showed an absence of ridges, which was appeared after incorporation κ-carragreenan indicated a good miscibility among three of the biopolymers.

FTIR analysis: The FTIR spectra of pure PVA and its blends PVA/PEG, PVA/PEG/KC at different loadings of κ-carragreenan in the wavenumber range of 4000-400 cm^{-1} are shown in Fig. 2. All three polymers had good solubility in water. This was because of the formation of strong H-bonds between

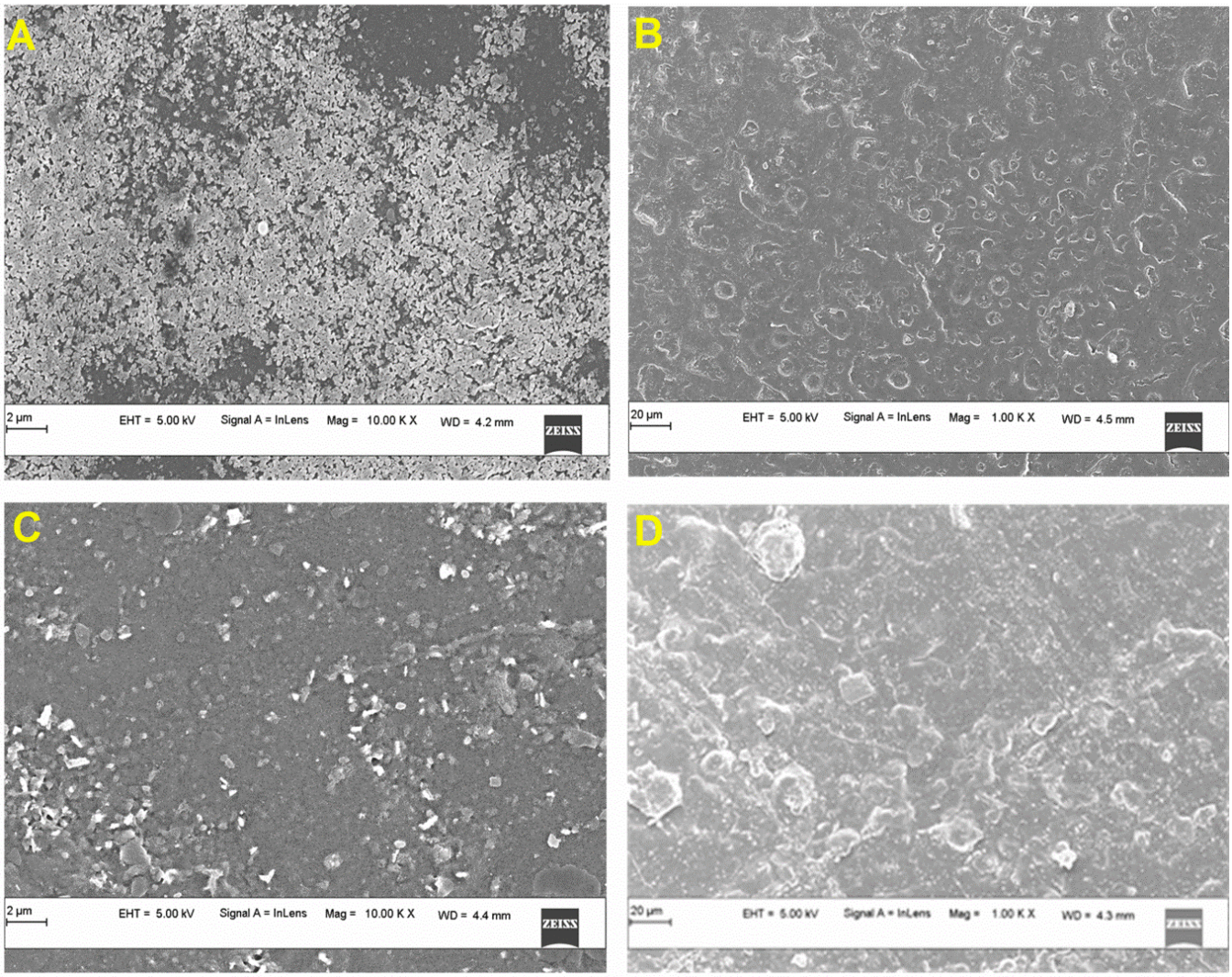

Fig. 1. FESEM images of film pure (A) PVA binary (B) PVA/PEG and blend (C) & (D) PVA/PEG/KC1 & 2, respectively in the range scale bar of 2-20 µm

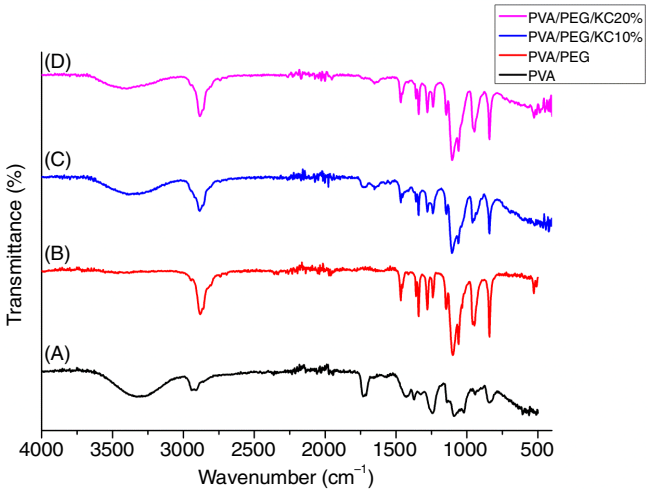

Fig. 2. FTIR spectra of (A) Pure PVA (B) PVA/PEG and blend (C) PVA/PEG/KC1, (D) PVA/PEG/KC2

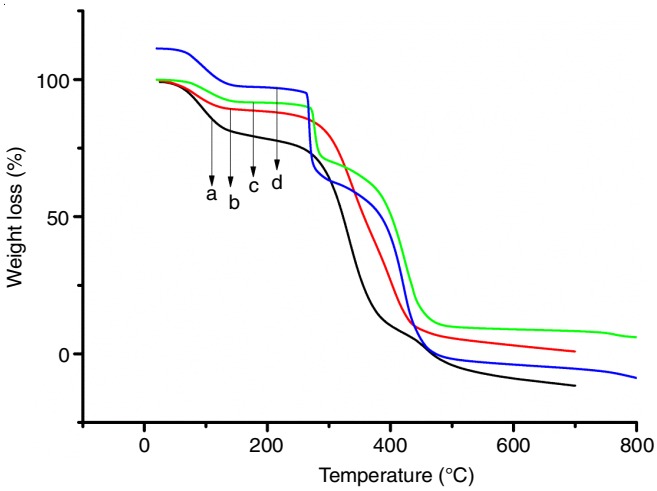

Fig. 3. TGA curve of (a) PVA (b) PVA/PEG (c) PVA/PEG/KC1 and (d) PVA/PEG/KC2

the functional group of polymers and water molecules (hydrophilic property). It was clear from the spectra of all PVA/PEG/KC blends that extensive H-bonding existed in the range 3500-3000 cm^{-1} due to stretching vibration of -OH groups which results from strong H-bonds that form during blending for pure PVA, which exhibited all the main typical hydroxyl group peaks and acetate group [23]. More specifically, the broad band observed between 3450 and 3200 cm^{-1} were associated with the stretching O-H from the intermolecular and intramolecular hydrogen bonds and the bands for C-H observed at 2920 and 2880 cm^{-1}. The band at 1083 cm^{-1} corresponds to O-H bending. The bending and wagging of CH_2 vibrations and C-H wagging were observed at 1428 and 1378 cm^{-1}, respectively [24,25]. Another sharp band observed at 1652 cm^{-1}corresponds to the C-O stretching from acetate group of PVA [26] and the peaks between 1735-1720 cm^{-1} were associated with stretching C=O and C-O from acetate group of PVA (saponification reaction of polyvinyl acetate) [27]. The characteristic absorption bands of PVA/PEG blends were observed at 3300, 1421, 1330, 1100, and 851 cm^{-1} correspond respectively to -OH, C_6H_6, C-O-C, C-C and C-H [28]. A strong absorption peak was also observed at 2900 cm^{-1} and linked to the stretching mode of the CH_2 group. The absorption band of -OH groups observed in the range 3550-3000 cm^{-1} got wider with higher intensity in the spectra of PVA/PEG/KC1 indicating occurrence of H-bond interactions between the -OH groups present in the blend PVA/PEG and the -OH groups of KC. This may be attributed to a good miscibility of PVA/PEG with κ-carragreenan, which causes a strong intermolecular connection in H-bond interactions. However, there was slightly shift of peaks in PVA/PEG/KC towards a higher wavenumber.

Thermal analysis: The TGA thermograms of obtained membranes of PVA, blend PVA/PEG and mixture of PVA/PEG/KC at different κ-carragreenan loadings are shown in Fig. 3. Fig. 3 clearly shows the dependency of the thermal behaviour on κ-carragreenan loading. It was observed that most of the κ-carragreenan blends showed a better thermal stability as compared to pure PVA and PVA/PEG, which resulted a shift of the degradation temperatures to higher values. By incorporating κ-carragreenan, thermal stability of PVA/PEG film could be improved remarkably. It is cleared that weight of all the membrane samples investigated were decreased gradually as the temperature increased. There was a remarkable difference in the thermal degradation behaviour of pure PVA and PVA/PEG compared to that PVA/PEG/KC membrane. Thermogram of all samples exhibited a three-step degradation process. The first step degradation for PVA and PVA/PEG occurred at 180 ºC and 200 ºC, respectively, due to evaporation moisture content compared to PVA/PEG/KC1 & 2 occured at 210 ºC and 200 ºC, respectively. The second phase occurred at 430, 450, 300 and 350 ºC for PVA, PVA/PEG, PVA/PEG/KC1 & 2, respectively. Further final phase degradation occurred for PVA and PVA PEG at 640 ºC and 670 ºC as compared to PVA/PEG/KC1 & 2 at 740 ºC and 790 ºC due to byproduct of biopolymer. Fahad *et al.* [29] found the similar results for PVA/PEG films with graphene composites. Overall results suggested after adding κ-carragreenan, a stability of matrix increased in higher level (Fig. 3d) and in lower concentration of κ-carragreenan, thermal stability decreased as shown in Fig. 3c. The changes in thermal behaviour suggested that a good miscibility among PVA/PEG and κ-carragreenan concentration.

XRD analysis: The X-ray patterns of pure and blend membranes are displayed in Fig. 4. The XRD patterns of PVA membrane showed a sharp diffraction peak at 2θ = 11.5º, 19.57º, 25.2º, 40.19º, which correspond to the semi-crystalline nature of PVA film. The results of PVA were similar to that of the other researcher [30,31]. The PVA/PEG membrane showed the most prominent peak occurred at 11.4º, 19.4º, 23.5º, 39.9º which was attributed to the crystalline zones. The X-ray pattern on blend PVA/PEG/KC1 membrane shown a peak at 2θ = 9.6º, 19.4º, 23.1º, 39.8º the diffraction pattern of membrane intense peak shifted to a lower value in the presence κ-carragreenan, which suggested that there was a transformation from semi-crystalline form to crystalline nature. It is noticeable that blend with low content of κ-carragreenan was more compatible, due to an increased degree of crystallinity (88% PVA/PEG/KC1) compared to the other membranes PVA-57% PVA/PEG-35% and PVA/PEG/KC2-78%. Overall results suggested that the

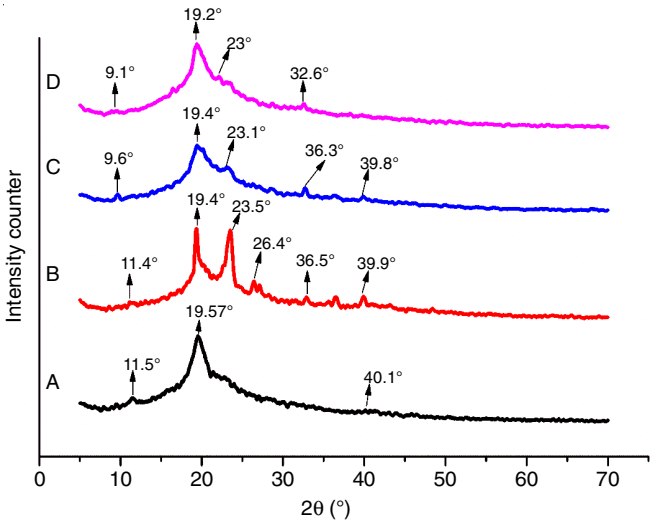

Fig. 4. XRD of membrane pure PVA binary PVA/PEG and blend membrane PVA/PEG/KC1 (10%), PVA/PEG/KC2 (20%)

prominent effect of KC10% in lower level showed an increased crystalline, which might be due to the good miscibility and interaction between -OH group of PVA/PEG and κ-carragreenan.

AFM analysis: The AFM images of membrane samples prepared with different blend ratios are shown in Fig. 5. The AFM images of blend of κ-carragreenan with PVA/PEG showed a increased roughness and surface roughness was higher than pure PVA and PVA/PEG alone. As the concentration of κ-carragreenan (20% PVA/PEG/KC2) increases, the roughness Ra value from 6.6 nm to 0.391 μm for PVA and PVA/PEG/KC2, respectively. This suggests that the κ-carragreenan was mixed well at the molecular level when present in low concentration (KC 10%, 23.7 nm). At higher concentration, dispersion and aggregation of κ-carragreenan observed in the membrane leads to the phase separation and rougher surface. AFM results were consistent with the results of SEM analysis.

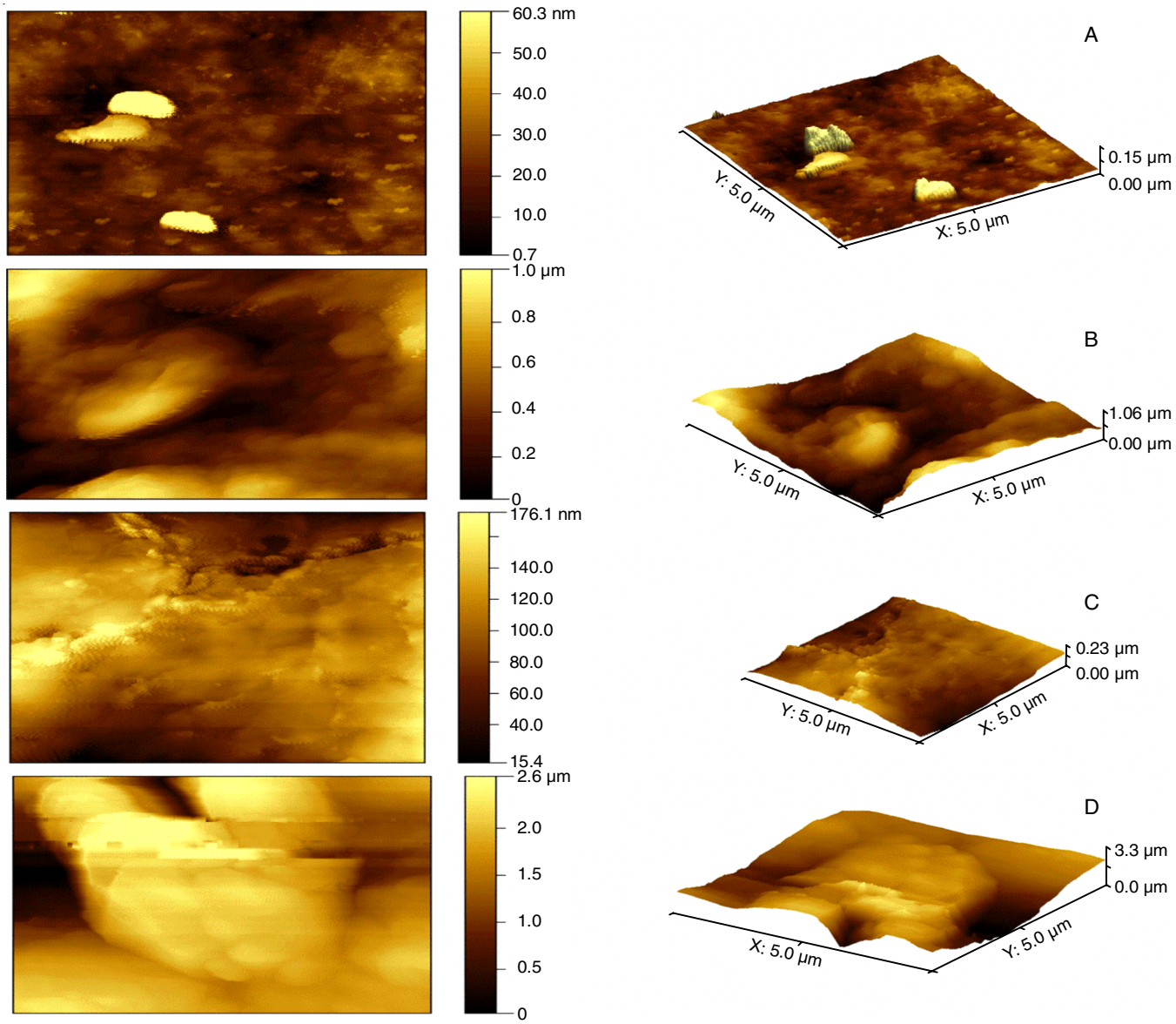

Fig. 5. AFM images of the membrane pure PVA, binary PVA/PEG and blend membrane PVA/PEG/KC1 (10%), PVA/PEG/KC2 (20%)

Swelling study: Swelling phenomenon happened to each cured membrane after dipped in PBS at different interval of time (30, 60, 90, 120 min) at room temperature. The membrane PVA and PVA/PEG alone exhibited higher water uptake unto 85% and 105%, respectively, after adding κ-carragreenan, the swelling rate increased to 130% (Fig. 6d). This indicated a strong intermolecular bonding between PVA/PEG and κ-carragreenan due to strong hydrogen bonding of κ-carragreenan and OH group of PVA/PEG and overall results indicated blend membrane (PVA/PEG/KC2) maintained their water uptake behaviour upto 90 min. The swelling ratio percent of the hydrogel film samples were measured [32] using the following equation:

$$\text{Swelling } (\%) = \frac{W_s - W_d}{W_d} \times 100$$

where, W_d and W_s represents the dried and swollen weight of film before and after immersed in PBS media, respectively.

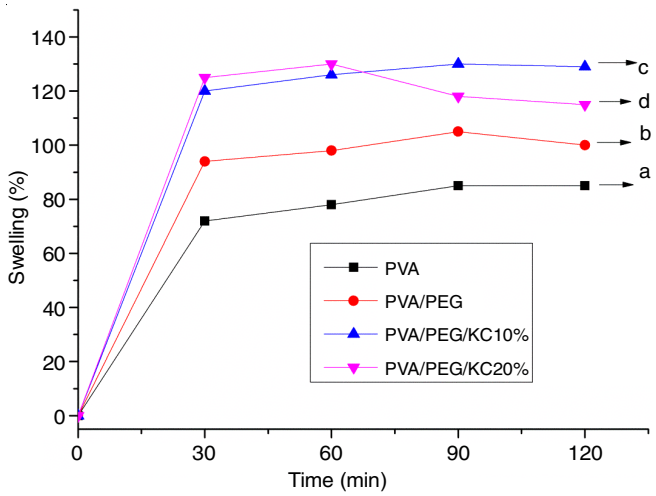

Fig. 6. Swelling behaviour of the membrane pure PVA, binary PVA/PEG and blend membrane PVA/PEG/KC1 (10%), PVA/PEG/KC2 (20%)

MTT assay: Biocompatibility or cell viability test was measured by using MTT assay after 24 and 72 h cell seeding by using NIH3T3 fibroblast cell line. Figs. 7 and 8 show the cell viability percent and optical images of cell attachment and proliferation. These prepared membrane exhibited comparable good biocompatibility. Cells seeded on a tissue culture plate without sample was taken as a control. The study resulted numbers of cell growth varying from 94 to 107% and 89 to 94% for PVA/PEG/KC1 & 2 compared to control as 100% for both 24 and 72 h, respectively. Results suggested that both membranes were biocompatible and non-toxic, consequently PVA showed 106% and PVA/PEG 83% for 24 h and after 72 h cell viability decreased to 91 and 69%. Hence overall results indicated that blend membrane with PVA/PEG/KC1 showed a relatively highest compatible compared to other membranes. It was believed that higher attachment of cells over the test sample due to sufficient chemical cues to the cell for their attachment and proliferation over the sample. These results were associated with the proliferation of cells on a polymer template that begins with attachment of cells on matrix, spread, proliferate and differentiate [33]. An overall result suggested that NIH3T3

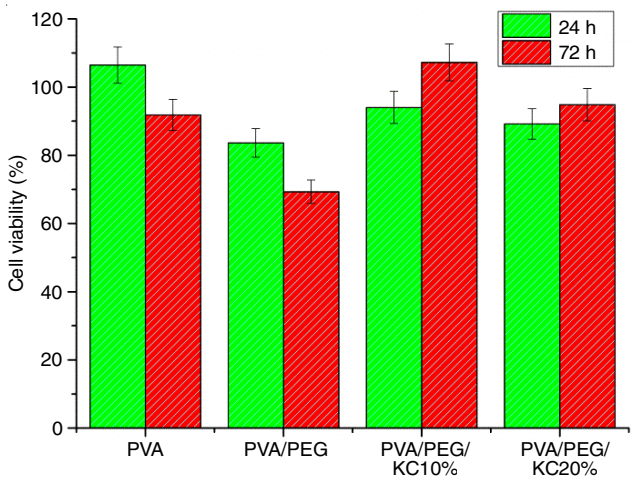

Fig. 7. Cell viability percent of the membrane pure PVA, binary PVA/PEG and blend membrane PVA/PEG/KC1 (10%), PVA/PEG/KC2 (20%)

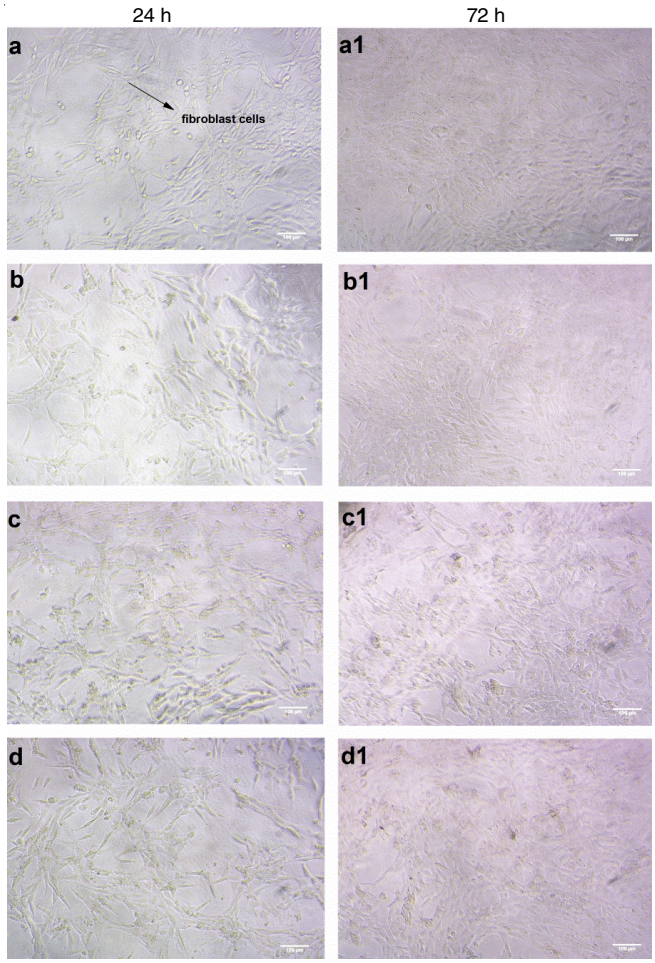

Fig. 8. Optical images of cell viability of the membrane pure PVA (a & a1), binary PVA/PEG (b & b1) and blend membrane PVA/PEG/KC1 (10%) (c & c1) and PVA/PEG/KC2 (20%) (d & d1) after 24 and 72hr NIH 3T3 fibroblast cell seeding

fibroblast cells were more biocompatible after adding κ-carragreenan compared to PVA and PVA/PEG alone. According to the ISO guidelines [34], if viability is reduced to < 70 % of control, then the test material has cytotoxic potential within the matrix.

Conclusion

In the present study, PVA/PEG based membranes with different amounts of κ-carragreenan incorporated in PVA/PEG were prepared by freeze-gelation method and the physico-chemical parameters were investigated by using SEM, FT-IR, TGA, AFM and XRD techniques. Further, swelling behaviour with biocompatibility study were also analyzed using NIH3T3 fibroblast cells by MTT method. The prepared membrane PVA/PEG/KC1 (10%) showed a good compatibility among other membrane PVA, PVA/PEG and PVA/PEG/KC2. Addition of κ-carragreenan in PVA/PEG increased the swelling rate onto 60 min and also decreased the thermal degradation rate compared to PVA and PVA/PEG membranes. Moreover, a good biocompatibility was showed at lower concentration of κ-carragreenan which revealed the highest viability of cells. Overall results obtained from this study suggested that the potential efficiency of PVA/PEG/KC membrane may have advantage to act as suitable scaffold for applications in skin tissue engineering.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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