In vitro degradation study of novel HEC/PVA/collagen nanofibrous scaffold for skin tissue engineering applications

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A B S T R A C T

The aim of this study was focused on the degradation behavior of electrospun (hydroxyethyl cellulose/poly(vinyl alcohol) HEC/PVA and HEC/PVA/collagen nanofibrous scaffolds, as a potential substrates for skin tissue engineering in two biologically related media: phosphate buffered solution (PBS) and Dulbecco’s modiﬁed Eagle’s medium (DMEM) for 12 weeks incubation period. The scaffolds were characterized at different degradation times by a series of analysis including pH changes of solutions, weight loss, swelling ratio, SEM, ATR-FTIR, DSC, TGA and mechanical properties. The results indicated that HEC/PVA/collagen scaffolds were exhibited slower degradation rate in both medium as compared to HEC/PVA blend nanofibers. All ﬁbers displayed uneven and rough surfaces towards the ﬁnal week of incubation in both PBS and DMEM solution. As degradation time increased, there were little changes in the chemical structure as determined by FTIR spectra while thermal studies revealed that the melting temperatures and crystallinity of scaffolds were slightly shifted to a lower value. Both HEC/PVA and HEC/PVA/collagen ﬁbers showed signiﬁcant decrease in Young’s modulus and tensile stress over 12 weeks degradation. These results show that these nanofibrous scaffold demonstrate degradation behavior that meets the requirement as potential degradable biomaterials for dermal replacement.

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1. Introduction

Biopolymeric scaffolds degradability is one of the main key for designation and fabrication of biomimetic scaffolds in skin tissue engineering applications. Scaffolds should mimic the structure and biological functions of natural extracellular matrix (ECM) as closely as possible to create a conducive living substrate that will induce cells to function naturally. Electrospinning can achieve to mimic the topographic features of ECM due to its unique and facile technique for producing continuous microﬁbres and/or nanofibers. The construction of new scaffolds is not only depends on the selection of material but also the capability of perceiving its potential behavior once implanted and endure recovery time [1]. In tissue engineering, electrospun biodegradable scaffolds must be able to maintain their physical, chemical and mechanical properties to support the growth of new tissue at an injured site till fully regeneration, without any reverse effects on the repair of neighboring tissue and organs [2]. The stability of biopolymers developed as a substrate for tissue engineering with particular prominence of durability during their service life was crucial in tissue engineering field. Indeed, it was important to ensure that the rate of degradation coincide with the rate of new tissue formation. If the rate of degradation is too slow, the new tissue formation will be obstructed, however; if the rate of degradation is too fast, the mechanical stability of the scaffold and developing tissue will be compromised. To achieve this behavior, an in vitro degradation study examining the rate of hydrolysis can be used as an initial estimate of the degradation rate as well as to predict it’s in vivo degradation behavior.

Biodegradable polymers intended for tissue engineering are mostly tailored to decompose via hydrolytic and enzymatic degradeable polymers [3]. There are many factors affect the polymer degradation kinetics such as type of chemical bond, polymer molecular weight, crystallinity and the presence of cross links, fillers, additives and etc. [4]. In order to more accurately mimic the natural ECM, researcher has also investigated the electrospinning of natural materials such as collagen, chitosan, gelatin, alginate and silk fibroin [5,6]. However, these materials often lack the desired
physical properties or difficult to electrospun alone. This unavailability is lead to the development of hybrid materials which consist of a blend of synthetic and natural materials. Niget et al. found that the use of hybrid blend PLLA and chitosan showed decrease in the degradation rate after 8 weeks incubation period compared with pure PLLA scaffolds [2]. Thus, incorporation of natural materials with synthetic polymers can be effectively used to control the degradation rate of polymeric scaffolds used for biomedical application.

Hydroxyethyl cellulose (HEC) is hydrophilic biopolymers with low charge density. HEC is recognized as one of the natural macromolecules polysaccharides that comprised from β-d-glucose rings at the main chain. Strong hydrogen bonding among the hydroxyl groups of HEC makes it available to be employed in extensive utilizations due to its water solution properties. Indeed, the remarkable physical properties associated with cellulose polymers is accumulate from their water and organic solvent solubility, thermal plasticity, thickening and colloid stabilizing abilities. Thereby, the chemical composition of HEC will allow to occupied large amount of relatively easily accessible hydroxyl units that can be attached by a number of functional groups [7–9].

Collagen is one of the most abundant proteins in the ECM with multiple functional characteristics favorable for cells and tissue growth. Collagen presents in skin are mainly synthesized by fibroblast and myofibroblast. In tissue engineering, the blend of collagen with biodegradable synthetic polymers often used as cell culture substrates due to its natural ECM properties and complex signaling capabilities [10,11]. Based on previous reports, the blends of collagen with poly(vinyl) alcohol (PVA) was extensively used in biomedical application owing to its non-toxic, water soluble, biocompatible and biodegradable with excellent mechanical properties [12,13]. Collagen added to the HEC/PVA scaffolds may not only improve the biocompatibility and mechanical properties but also enable manipulate bioactive molecule release from the scaffolds [2].

In particular study, we were focused on the degradation behavior of HEC/PVA incorporated with and without collagen nanofibers in two different biological media. To the best of our knowledge, this is the first report where HEC is blended with PVA and collagen; electrospun to produce nanofibers and investigate their degradation behavior for tissue engineering application. These findings are aimed on the performances of crosslinked HEC/PVA and HEC/PVA/collagen scaffold undergo a series of degradation process and their effect on swelling ratio, weight loss, chemical bonding, mechanical properties, thermal stability and crystallinity. These properties will be helpful to presume the biological features of composite scaffolds in the presence of cells once implanted. The study of the degradation after prolonged periods of immersion (12 weeks) in DMEM and its comparison to the results obtained in phosphate buffered solution (PBS) should be an adequate tool to preview its possible behavior in vivo. The nanofibers were evaluated under physiological conditions of pH 7.4 and body temperature of \( 37 \degree C \).

2. Materials and methods

2.1. Materials

All materials used in this work are commercially available. Hydroxyethyl cellulose was purchased from Merck-Schuchardt, Germany. Poly(vinyl) alcohol (molecular weight 95,000) was purchased from ACROS, New Jersey, USA. Collagen type I, liquid rat tendon excised from tail (0.02 N acetic acid, pH 3.67) was purchased from Merck-Millipore Corporation, Billerica, MA, USA. Analytical reagent grade glutaraldehyde (GA) solution (25%) was purchased from Merck-Schuchardt, Germany. Phosphoric acid was purchased from Merck KGAA-Darmstadt, Germany. Acetone was purchased from R&M Marketing, Essex, UK. Phosphate buffer saline (PBS) was purchased from Gibco Life Technologies, USA. Dulbecco’s Modified Eagle Medium (DMEM) was purchased from Life Technologies, USA. All the chemicals were of highest purity and used without further purification. All the solutions were prepared using Millipore water.

2.2. Solution preparation for electrospinning

The HEC solution with concentration 5 wt% was prepared by dissolving 5 g of HEC powder in 100 ml Millipore water for 2 h at room temperature until a clear solution was obtained with a slight increase in viscosity. PVA solution of 15 wt% was prepared by dissolving 15 g of PVA granules in 100 ml Millipore water with stirring at 80 °C for 2 h. Both solutions were stirred continuously for 12 h at room temperature to ensure a complete mixing and eventually obtain a homogeneous solution. HEC was then blended in PVA solution with the weight ratios of HEC:PVA is 1:1 while collagen solution with concentration 0.38 wt% was added into HEC:PVA solution with the weight ratios of HEC:PVA:collagen is 1:1:1. All solutions were stirred overnight to get homogeneous binary and ternary blend solution respectively.

2.3. Electrospinning of nanofibrous scaffolds

Electrospinning was carried out at room temperature for all the concentrations of HEC/PVA and HEC/PVA/collagen. The polymer solution was filled in a 5 ml syringe fitted with a blunt steel needle of 0.8 mm inner diameter and flow rate of 1 ml/h. The applied voltage was varied from 20 to 25 kV. The electrospun nanofibers were collected using a rotating drum collector wrapped with aluminum foil at the distance of 90–110 cm from tip-to-collector at a rotation speed of 1000–1500 rpm. The humidity of 50% was preserved (Dri-Tech HT-180) in the room. The nanofibrous scaffolds were crosslinked with GA solution in acetic followed by phosphoric acid for 24 h. The nanofibrous mats were rinsed with water 3 times to remove the excess GA. Water resistance of the scaffolds was evaluated by immersing it in distilled water and dried in a vacuum oven for 1 h for further used [14]. The collected electrospun nanofibers were stored in desiccators for further use.

2.4. In vitro degradation study

Electrospun nanofibrous scaffolds (15 mm × 15 mm) were cut into rectangles shape for degradation testing in vitro. In vitro degradation study was carried out in two different media: PBS solution and DMEM media. The nanofibers were sterilized under UV irradiation for 2 h, placed in 24-well plate containing 3 ml of PBS or DMEM solution and incubated at 37 °C and 37 °C containing 5% CO2 and 95% humidity respectively. The incubation medium was replaced by the fresh solution every 4 weeks. Six paralleled scaffold of each degradation period were taken out at different period of time, washed with distilled water and vacuum dried for further characterization.

2.5. pH value, weight loss and swelling ratio

The pH value of PBS and DMEM solution after degradation at each time point were obtained by a pH meter (Mettler Toledo FE20 FiveEasy™ pH meter). Each value was averaged from six samples. All degraded scaffolds were taken out from the media and directly eliminate the excess water at the surface. The scaffolds were immediately weighted by an electrical balance with a resolution of 0.1 mg to obtain the weights of the sample at the swollen states, \( W_s \). Scaffold were then dried in a vacuum desiccators for 24 h. The dried
mass was exemplified as $W_d$. Swelling ratio of nanofibrous scaffolds were calculated according to Eq. (1):

$$\text{Swelling ratio} (%) = \left( \frac{W_t - W_d}{W_d} \right) \times 100\%$$  \hspace{1cm} (1)

Weight loss percentages were calculated from the dried weight obtained before and after degradation using gravimetric method [1]. The percentage of weight loss was determined after drying the samples in vacuum by comparing dry weight, $W_d$ at a specific time with the initial weight, $W_0$ according to Eq. (2):

$$\text{Weight loss} (%) = \left( \frac{W_0 - W_d}{W_0} \right) \times 100\%$$  \hspace{1cm} (2)

2.6. Scanning electron microscope (SEM) study

To investigate the surface morphology of dried network samples, SEM pictures of degraded electrospun HEC/PVA and HEC/PVA/collagen nanofibers scaffold were taken using Scanning Electron Microscopy (SEM) (ZEISS EVO 50) at an accelerating voltage of 15 kV. The electrospun nanofibers were sputter coated with a thin layer of platinum in double 30 s consecutive cycles at 45 mA to reduce charging and produce conductive surfaces (BALTEC SCD 005 Sputter Coater – BALTEC).

2.7. Attenuated total reflectance – Fourier transform infrared (ATR-FTIR) study

ATR-FTIR spectroscopic analysis of electrospun nanofibrous scaffolds was performed on Spectrum One (Perkin–Elmer, USA) spectrophotometer over a range of 500–4000 cm$^{-1}$ at a resolution of 2 cm$^{-1}$ with 100 scans per sample.

2.8. Differential scanning calorimetry (DSC) study

The thermal behavior of the electrospinning fibers were studied with a DSC technique. DSC was performed with a Q500 (TA instruments, New Castle, USA) under atmosphere. About 5 mg of sample was heat-treated from 50 to 250 °C at a heating rate of 10 °C/min. The degree of relative crystallinity, $X_c$, was expected from the endothermic peak by the following equation:

$$X_c = \frac{\Delta H_f}{\Delta H_f^c}$$  \hspace{1cm} (3)

where $\Delta H_f$ = measured enthalpy of fusion from DSC thermograms and $\Delta H_f^c$ = enthalpy of fusion for 100% crystalline PVA which is 138.6 J/g [15].

2.9. Mechanical study

The mechanical properties of the electrospun nanofibers were measured using a universal testing machine (UTM) (AG-500, Shimadzu, Japan); under a cross-head speed of 10 mm/min. Rectangular specimens with dimension 10 mm × 20 mm were used for testing. The room conditions were controlled at 25 °C and 34% humidity. The tensile stress and strain at break are calculated based on the obtained tensile stress–strain curve.

3. Results and discussions

3.1. Morphological changes of electrospun nanofibers scaffold

Figs. 1 and 2 shows morphology changes of the HEC/PVA and HEC/PVA/collagen nanofibers scaffold after degradation in different biological media at various time points. PBS solutions have osmolality and ion concentrations that match with those of human body. DMEM is used in mammalian cell culture to increase media stability, minimize toxic ammonia build up and maximize cell performance. DMEM is a basal medium for supporting the growth of many different mammalian cells including primary fibroblasts, neurons, glial cells and smooth muscle cells. After degradation for 2 weeks, all electrospun fibers exhibited no difference with their original physical structure except for slight swelling properties. After 4 weeks degradation, HEC/PVA and HEC/PVA/collagen blended nanofibers immersed in DMEM and PBS media presented distinct swelling and the fibers start to connect with each other while still no morphology changes observed on the surface of HEC/PVA/collagen nanofibers incubates in PBS solution. In addition, some fibers start to appear breakage and fracture with the increased of degradation period. After degradation for 8 weeks, almost all pores of all fibers surfaces were covered due to swelling effect. The internal nanofibers were furthered visible and ‘fused’ together after degradation for 12 weeks presented uneven and rough surfaces of the fibers. The highly porous three-dimensionally interconnected fiber structure was collapsed and adhered together during degradation. It is found that the nanofibers scaffold were likely to degenerate faster in DMEM media than PBS buffer solution. This dissimilarity was due to the difference chemical reactions of the scaffold with the medium that affect the polymer backbone in a different manner. The nanofibrous scaffold show better hydrolysis rates in DMEM that comprised of 110.3 mM NaCl inorganic salts which is less than NaCl composition in PBS of 137 mM. Although PBS contain higher salt concentration, the hydrolysis activity seems to present an increment in DMEM due to concentration of salt that fell within the scaffolds hydrolytic functional threshold, thus affect the hydrolysis reaction to be increased. Apart of that, all blended nanofibers appeared to be melted during the degradation process. Same results also reported by K. Zhang et al. where the electrospun silk fibroin/P(LLA-CL) and P(LLA-CL) nanofibers emerged to be melted instead of fiber breaking during degradation study [16]. This degradation behavior may caused by the gradual decreases in the molecular weight of blended polymers associated with the increase in plasticization effect of the fibers [17]. Furthermore, the rigid and immobilized polymer chains in the crystalline region, that affect the breakage of fibers along the fiber axis during ‘weak’ point of degradation, imply that the broken end were more vulnerable to hydrolytic effect due to higher exposure of degradation medium during degradation [18]. A longer degradation time study would be preferable to identifying the critical time point for the nanofibers scaffold become weak in terms of polymer chain, soluble and subsequently guaranteed their potential in tissue growth. In skin tissue-engineering, the onset of the maturation phase may vary extensively, depending on the size and type of the wound ranging from approximately 3 days to 3 weeks or may last for a year or longer. The unmatched degradable rate may deteriorate wound healing progress and leaves inappropriate activity at the wound sites.

3.2. Weight loss, swelling ratio and pH value analysis

The efficiency of HEC/PVA and HEC/PVA/collagen crosslinked nanofibers was evaluated by measuring the weight change of the fibers incubated in PBS and DMEM solution for up to 12 weeks as illustrated in Fig. 3. The HEC/PVA/collagen nanofibers scaffold was exhibited slower degradation rate in both medium as compared to HEC/PVA nanofibers. The hydrolysis of protein will mainly resulted in the fracture of peptide bond. Thus, the slower rate of collagen incorporated HEC/PVA in PBS buffer solution might be due to the difficulty of hydrolyzing the peptide bond (−NH$_2$) in neutral
medium and inhibited the degradation rate of HEC/PVA/collagen. Meanwhile, the slow rate of HEC/PVA/collagen fibers degraded in DMEM medium was probably caused by the presence of various chemical such as amino acids, vitamins, glucose and inorganic salts, which might have limited amount of water contact with polymer link and decrease the breakage of backbone. In the meantime, the HEC/PVA nanofibers exhibited minor weight loss at the earlier stage (~4%) might be caused by reaction between aldehyde group and (–OH) during crosslinking process which retarded the penetration of water molecule into polymer chain segments of the scaffolds during initial degradation process. During this reaction, the polymer chains are retained with the matrix until the chains reach a critical molecular weight and become water insoluble. Subsequently, when the chains exit the matrix the weight will slowly decrease by time. In contrast, HEC/PVA/collagen nanofibers exhibited high weight loss at primary weeks might be due to high surface area of nanofibers scaffold resulted in faster diffusion rate and a corresponding builds up of protein degradation product to the medium.

Fig. 4 showed the swelling ratio of HEC/PVA and HEC/PVA/collagen immersed in PBS and DMEM up to 12 weeks incubation. The biomolecular reaction in which water and the functional group possessing the bond involved was increased the degradation rates of this hydrophilic polymer. Swelling ratio property play a main role due to the fact that, when the scaffolds are capable of swelling they allow their pore size to increase in diameter in order to swell thus facilitating the cells not just attach but also allow them to penetrate inside the interpolymer network to grow in a three-dimensional fashion, during in vitro culture studies [19]. The cells will then avail the maximum internal surface area of the scaffold. The scaffolds water uptake in PBS increased from 0 to (140–238%) after 2 weeks and slowly increased until maximum 270% and 251% for HEC/PVA and HEC/PVA/collagen nanofibers respectively. The water uptake of the scaffolds exposed to DMEM media varied from 0 to 150% and 110% after 2 weeks of degradation and increase up to 259% and 356% for HEC/PVA and HEC/PVA/collagen respectively towards the end of degradation study. The swelling ratio was expected to be high initially since the first mechanism occurred during degradation is the absorption of water. After some time point, the chains of the polymer would start to shorten due to cleavage of polymer backbone. Once the chain became small enough to exit the matrix, the scaffold would start disintegrating thus lead to drop in water uptake. The graph illustrated the total water absorption of HEC/PVA/collagen immersed in PBS solution after 12 weeks was at the highest percentage (~400%). This can be explained in terms of density of polymer network as the HEC group
([CH2CH2O]4H) of HEC polymer is bigger than the amino group (−NH2) of collagen. The addition of collagen in HEC/PVA substrate will decrease the free volume for liquid absorption. However, further weight loss of HEC/PVA/collagen was due to large number of ether groups that have strong hydrogen bonding with water molecule resulted in an increased of the amount of water uptake. As mentioned previously, if the scaffolds are capable of swelling they allow their pore size to increase in diameter. Since the scaffold developed from HEC/PVA/collagen showed maximum swelling, it was considered to have high surface area/volume ratio and thus increase the potential of cells to attach and grow in a three-dimensional fashion. Since they show maximum percentage than another scaffold it is obvious that they can avail nutrients of the culture media more effectively, during in vitro cell culture studies. The longer degradation period would be required to indicate the saturated points in which the water absorption of the scaffolds was stabilized.

In this study, the nanofibers were hydrolyzed in PBS and DMEM solution at pH 7.4 and 7.2 respectively, incubate at 37 ± 0.1 °C and the pH changes of both medium solutions at prolongation time up to 12 weeks after submerged with HEC/PVA and HEC/PVA/collagen nanofibers scaffold were illustrated in Fig. 5 (a and b) correspondingly. It was depicted that fiber immersed in DMEM remained pH ~7.2 during 4 weeks degradation as compared to PBS buffered solution as the pH decreased to 6.74 from its original pH which is 7.4. HEC/PVA nanofibers scaffold incubated in both PBS and DMEM solution showed higher degradation rate than HEC/PVA/collagen scaffold.
medium exhibited low pH value differences due to weak hydrogen forces between these polymer chains in aqueous medium that facilitate penetration of water molecule into the polymer. The formation of interpenetrating polymer network from HEC/PVA with and without collagen was believed to involve electrostatic interaction, hydrophilic association and hydrogen bonding [20]. The cationic collagen and HEC in the presence of GA, remains the stability of polymers in the dissolution media, thus limit the release of acidic product and slower the degradation rate of the scaffolds.

3.3. ATR-FTIR spectra analysis

The attenuated total reflection-Fourier transform reflection (ATR-FTIR) spectra of HEC/PVA and HEC/PVA/collagen electrospun nanofibers in different degradation media before and after degradation for 12 weeks were shown in Fig. 6(a, b, c and d). FTIR spectra was carried out to elucidate the presence of HEC, PVA and collagen in the blended nanocomposite and to analyze the interaction (hydrogen bonding) between them.

![Fig. 5](image)

**Fig. 5.** pH value of HEC/PVA and HEC/PVA/collagen nanofibers scaffold after degradation for up to 12 months in (a) DMEM and (b) PBS solution.

The broad peak at 3326 to 3398 cm\(^{-1}\) indicates stretching vibrations of the hydroxyl groups due to the intramolecular and intermolecular hydrogen bands of the OH groups of PVA and HEC. The absorption peak were found to be increased and decreased for both type of fibers after 12 weeks degradation in PBS and DMEM medium respectively. The intensity of absorption indicate a stronger hydrogen bond appeared within molecular chain of HEC/PVA blend nanofibers contributes to slower degradation rate in DMEM solution. The intensity of the bands around 2927–2942 cm\(^{-1}\) corresponded to CH\(_2\) stretching vibrations were almost diminishes after degradation in PBS medium. This result suggests the hydrolysis of HEC/PVA segments chain to soluble oligomers and diffused into degradation medium. In addition, the cellulose ether macromolecules undergo chain scission and grafting to the polymer backbone. The interaction between collagen and HEC/PVA nanofibers scaffold showed no obvious difference before and after degradation. Collagen is a homo-oligomers protein which composed from the identical protein chains. This protein had the characterization absorption band at 1642 cm\(^{-1}\) to 1647 cm\(^{-1}\) (amide I, for C=O stretching), 1558 cm\(^{-1}\) (amide II, for –NH deformation) and 1246 cm\(^{-1}\) (amide III, for N–C–N stretching) attributed to the collagen with the amide I, II and III band regions of the spectrum directly related to the polypeptide conformation [21,22]. The amide I band became week in PBS medium due to reduced stretching vibrations of peptide carbonyl groups (C=O bond) along the polypeptide backbone. The N–H peak that contributed to amide II showed no severe changes in DMEM medium. On the other hand, this peak was almost diminishes after degradation in PBS medium due to weak intramolecular hydrogen bonding within collagen molecule. In addition, it is likely due to the breaking of N–H–O=C interchain hydrogen bonds accompanied by the release of the water bound in collagen [23]. For amide III band, there are bands assigned at 1246, 1248 and 1251 cm\(^{-1}\). This band was arising from C–N stretching and N–H bending with minor changes after 12 weeks degradation period. The relative strong hydrogen bond would effectively lengthen the C=O double bond and thus reduce the energy required to displace the oxygen atom from the carbon [24]. This in turn requires a photon of lower energy, which translates to an absorption peak at lower frequency in the infrared spectrum of the scaffolds. The presence of two peaks at 1720 to 1723 cm\(^{-1}\) indicated stretching of C=O and at 1655 to 1640 cm\(^{-1}\) indicated presence of the residual vinyl acetate groups in the PVA chains (C=C) and the traces of water molecule (bending vibration) [25]. The highly reduced of the absorption peaks in PBS medium showed the occurrence of hydrolysis of PVA led to hydrolytic cleavage of polymer backbone.

3.4. DSC study

In this sub-section, DSC measurements were conducted to further describe the degradation behavior of electrospun HEC/PVA and HEC/PVA/collagen nanofibrous scaffolds. In Table 1, the crystallinity, \(\chi_c\) of HEC/PVA incorporated with and without collagen of nanofibers scaffold were calculated based on the melting enthalpy, \(\Delta H_m\) from DSC thermograms. The \(T_g\) values of both samples immersed in DMEM media showed an increment after 12 weeks incubation indicated slow degradation process were occurred on the samples. In contrast, both electrospun nanofibers scaffold immersed in PBS exhibited a reduction in \(T_g\) values could be attributed to the decreased in molecular weight of the samples. The decreased of \(T_g\) values were associated with the increased in the mobility of the polymer chains as a consequence of hydrolytic process [26]. When introducing these samples to erosion media, the lowered value of \(T_g\) is due to the uptake of water which leads to the recrystallization of the polymer [27]. Thermal studies by DSC as
illustrated in Fig. 7(a, b, c, and d) showed a decrease in melting temperature during in vitro degradation. It can be seen that $\chi_c$ demonstrated relatively lower value after 4 weeks degradation time which is 1.89, 9.42, 6.39 and 5.34 for HEC/PVA (in DMEM), HEC/PVA/collagen (in DMEM), HEC/PVA (in PBS) and HEC/PVA/collagen (in PBS) respectively. However, the degree of crystallinity of these nanofibrous shows augmented significance for up to 8 weeks degradation in range of 6.16–17.71% and seem to be reduced after 12 weeks of hydrolyze process which is from 3.07 to 5.75 % except for HEC/PVA/collagen (in media) that demonstrated a decrease crystallinity through prolongation time of degradation.

This degradation patterned could be elucidated by the fact that initial stage of degradation happened in the amorphous region where the molecular chains are less dense and characteristically disordered in nature [28]. This amorphous nature may enhance the degradation due to the irregularity in the subchain arrangement contributed to the diffusion of water molecule into the polymer network [29]. The cleavage of side chains as well as chain-stripping of PVA will then promoted higher molecular chain mobility [30]. The arrangement of polymer chains will facilitate the crystallization process resulting from selective hydrolysis and removal of the chains in case of the amorphous state occurred first through degradation [31]. Due to the faster erosion of amorphous compared to crystalline polymer regions, the overall crystallinity of samples increases. After 12 weeks degradation, the less value of crystallinity explained the fact that the perfection of the crystallinities in HEC/PVA and HEC/PVA/collagen fibrous scaffolds could deteriorate by degradation. In addition, it can be seen that the $T_m$ of all fibers were

### Table 1
DSC data and the characteristics observed for the electrospun HEC/PVA and HEC/PVA/collagen nanofibers before and after in vitro degradation.

<table>
<thead>
<tr>
<th>Weeks</th>
<th>$T_g$ (°C)</th>
<th>$T_m$ (°C)</th>
<th>$\Delta H_m$ (J/g)</th>
<th>$\chi_c$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEC/PVA (in DMEM)</td>
<td>0</td>
<td>67.38</td>
<td>194.35</td>
<td>19.10</td>
</tr>
<tr>
<td>4</td>
<td>85.73</td>
<td>200.88</td>
<td>2.62</td>
<td>1.89</td>
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<tr>
<td>8</td>
<td>103.55</td>
<td>179.35</td>
<td>17.71</td>
<td>12.78</td>
</tr>
<tr>
<td>12</td>
<td>133.10</td>
<td>178.88</td>
<td>7.26</td>
<td>5.24</td>
</tr>
<tr>
<td>HEC/PVA/collagen (in DMEM)</td>
<td>0</td>
<td>72.02</td>
<td>185.82</td>
<td>8.09</td>
</tr>
<tr>
<td>4</td>
<td>99.21</td>
<td>201.85</td>
<td>9.42</td>
<td>6.80</td>
</tr>
<tr>
<td>8</td>
<td>103.34</td>
<td>200.37</td>
<td>1.75</td>
<td>1.26</td>
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<td>12</td>
<td>105.67</td>
<td>187.28</td>
<td>4.98</td>
<td>3.59</td>
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<tr>
<td>HEC/PVA (in PBS)</td>
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<td>67.38</td>
<td>194.35</td>
<td>19.10</td>
</tr>
<tr>
<td>4</td>
<td>110.03</td>
<td>213.48</td>
<td>6.39</td>
<td>4.61</td>
</tr>
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<td>8</td>
<td>106.70</td>
<td>202.60</td>
<td>11.59</td>
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<tr>
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<td>202.01</td>
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<td>201.86</td>
<td>6.16</td>
<td>4.44</td>
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<tr>
<td>12</td>
<td>101.74</td>
<td>194.34</td>
<td>4.25</td>
<td>3.07</td>
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</table>

### Table 2
Mechanical data of HEC/PVA and HEC/PVA/collagen nanofibers before and after degradation for up to 12 weeks.

<table>
<thead>
<tr>
<th>Time</th>
<th>Sample</th>
<th>Young’s modulus (MPa)</th>
<th>Ultimate tensile stress (MPa)</th>
<th>Ultimate tensile strain (%)</th>
</tr>
</thead>
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<tr>
<td>Before degradation</td>
<td>HEC/PVA</td>
<td>97.8</td>
<td>2.31</td>
<td>12.43</td>
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<tr>
<td></td>
<td>HEC/PVA/collagen</td>
<td>106.1</td>
<td>2.95</td>
<td>20.35</td>
</tr>
<tr>
<td>After degradation</td>
<td>HEC/PVA/collagen (DMEM)</td>
<td>28.7</td>
<td>0.95</td>
<td>43.24</td>
</tr>
<tr>
<td>for up to</td>
<td>HEC/PVA (DMEM)</td>
<td>24.76</td>
<td>0.99</td>
<td>58.45</td>
</tr>
<tr>
<td>12 weeks</td>
<td>HEC/PVA (PBS)</td>
<td>36.35</td>
<td>1.27</td>
<td>28.58</td>
</tr>
</tbody>
</table>
decreased after 12 weeks degradation further prove that the damage of crystalline structure was achieved during the degradation process (Table 1).

3.5. Mechanical characterization

The stress–strain curves of HEC/PVA and HEC/PVA/collagen nanofibrous scaffolds before and after degradation are shown in Fig. 8 and the data are summarized in Table 2. The Young’s modulus of the fibrous exhibited significant decrease after degradation time from 97.8 to 106.1 MPa at week 0 to 11.04–36.35 MPa at weeks 12. After 12 weeks incubation, significant drop of tensile stress from 2.95 MPa to the lowest value of 0.60 MPa clearly displayed that the degraded scaffolds lost their structural properties over prolongation incubation time [28]. In addition, this decrement might be caused by the reduction of molecular weight with degradation time resulted in rapid reduction of modulus and tensile stress [32]. The tensile stress (MPa) was found to be less than Young’s modulus showed that the degraded scaffolds could still bear pressure instead of bear load. In contrast, the tensile strain of fibrous scaffolds demonstrated increased in value from 12.43 to 20.35 % at weeks 0 to 28.58–58.45% at weeks 12. The decreased in Young’s modulus (MPa) and increased in tensile strain (%) revealed the plasticizing effect was obtained after degradation period. The HEC/PVA/collagen immersed in PBS solution depicted lowest value of modulus and tensile stress might be due to high cleavage of polymer backbone linkages between the polymer repeating units. However, the incorporation of collagen into HEC/PVA immersed in DMEM presented higher tensile stress contributed to the strong adhesion between the materials caused by the presence of various chemical such as amino acids, vitamins, glucose and inorganic salts which increase the rigidity of the resultant degraded scaffolds.

4. Conclusions

In this study, the degradation behavior of electrospun HEC/PVA and HEC/PVA/collagen nanofibrous scaffolds was characterized in two different biological media which is DMEM and PBS for 12 weeks. The fibers exhibited uneven and rough surfaces towards the final week of incubation in both medium. Much faster degradation behavior is observed in PBS when compared to that determined in DMEM. This effect is more pronounced in HEC/PVA samples. The addition of collagen in the HEC/PVA matrices reduced the degradation rate of the blended nanofibrous scaffolds. Reductions in weight of the scaffolds were more obvious in PBS than those in DMEM. Water absorption rate of the degrade scaffolds were found to be higher at the initial stage and then got lower after 12 weeks prolongation time. Along with degradation, less value of crystallinity was obtained explained the fact that the perfection of the crystallinitis in HEC/PVA and HEC/PVA/collagen fibrous scaffolds could deteriorate by degradation. The blended nanofibrous scaffolds lost their structural properties over relatively 12 weeks in vitro degradation, as reflected by the decrease in its Young’s modulus and tensile stress over time. These results could provide fundamental experimental references for the degradation behavior of biodegradable polymeric scaffolds used in skin tissue engineering for in vivo.

Fig. 7. DSC thermogram before and after degradation for incubation up to 12 weeks. (a) HEC/PVA/collagen nanofibers immersed in DMEM solution; (b) HEC/PVA nanofibers immersed in DMEM solution; (c) HEC/PVA/collagen nanofibers immersed in PBS solution; (d) HEC/PVA nanofibers immersed in PBS solution.
Fig. 8. Stress–strain curves (i) before and (ii) after degradation for incubation up to 12 weeks. (a) HEC/PVA/collagen nanofibers immersed in DMEM solution; (b) HEC/PVA nanofibers immersed in DMEM solution; (c) HEC/PVA/collagen nanofibers immersed in PBS solution; (d) HEC/PVA nanofibers immersed in PBS solution.

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