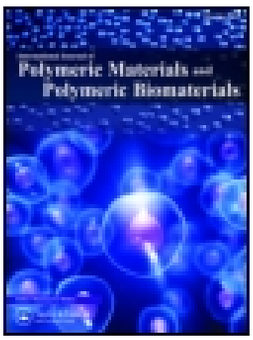

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Surface modification of poly (ethylene terephthalate) fabric by soy protein isolate hydrogel for wound dressing application

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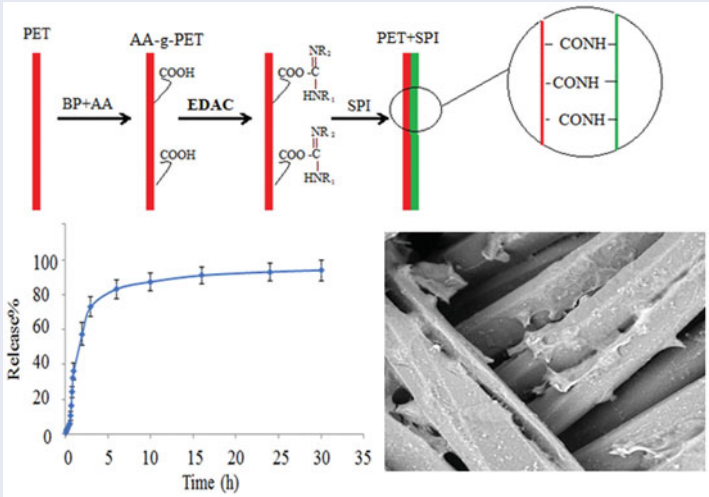
ABSTRACT

In this study, a composite of poly (ethylene terephthalate) (PET) fabric and soy protein isolate (SPI) hydrogel loaded with gabapentin was developed. For covalent attachment of SPI on the surface of PET fabric, graft polymerization of acrylic acid (AA) on the surface of PET fabric was performed and then carboxyl groups available in the structure of AA were activated using EDAC and then SPI was coated on the surface of PET fabric. The results revealed appropriate connection between hydrogel and modified fabric. The hydrogel was characterized by swelling test and the drug release behavior was investigated. It was found that the casting temperature affects the swelling ratio of the hydrogel and an appropriate release profile of the drug was observed. The surface of fabric was characterized by contact angle measurement, electron microscopy, and infrared spectroscopy. *In vitro* cell culture study was performed using NIH 3T3 mouse fibroblasts to investigate the biocompatibility of final composite and MTS results along with morphology of cells on the surface of PET fabric coated with SPI revealed the biocompatibility of final product and no cell cytotoxicity was observed in modified PET fabric.

GRAPHICAL ABSTRACT



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KEYWORDS
Gabapentin; poly (ethylene terephthalate) fabric; soy protein isolate (spi); surface modification; wound dressing

1. Introduction

In recent years a lot of studies have been focused on the wound and its healing process[1–5]. Every year many people from all over the world suffer from different types of wounds. Although traditional wound dressings have occupied a large part of wound dressing market, researchs on the modern wound dressing such as hydrogels, hydrocolloids, foams etc. have been expanded as an ideal wound dressing can accelerate wound healing process[2,6–8]. It has been demonstrated that wound healing can be accelerated in a moist environment. Meanwhile, it should be noted that excessive exudates can disturb wound rehabilitation as well. Consequently, utilizing a dressing with the ability to absorb

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wound exudates along with providing a moist environment for the wound is desirable[2]. Hydrogels are cross-linked polymeric networks with high capacity of liquids absorption and have been used in different medical applications such as tissue engineering, drug delivery, and wound dressing[2,9]. Particular advantages of hydrogels such as providing a moist environment along with exudate absorption, pain relief of patient and oxygen permeability make hydrogels as a suitable substrate for wound dressing[1,3,7,10]. However poor mechanical properties of hydrogels is a challenging issue[7]. Balakrishnan et al. fabricated alginate/gelatin hydrogel for wound dressing application and their results showed the ability of the hydrogel to provide a moist environment and prevent accumulation of exudates. Moreover, complete improvement of full-thickness wounds in a rat model upon using alginate/gelatin hydrogel was observed[9]. Recently, plant-derived biomaterials have been more attractive compared to animal derivatives due to their biocompatibility and less immunogenicity responses[11–14]. Moreover, non-animal resource products, don't have the risk of disease transmission that occurs in human and animal resource products[15].

Soy protein isolate (SPI), is a plant-derived protein obtained from soybean[12,13,16] and its application in biomedical engineering is growing in recent years owing to its low price, non-animal resource, easy handling etc.[16]. SPI can stimulate collagen deposition and integrates into blood clots and therefore helps the wound healing process[16]. Peles and Zilberman fabricated SPI film loaded with gentamicin as an antibiotic for wound dressing application and their results showed that the SPI film has good potential to be utilized as wound dressings substrate due to its suitable properties and drug release profile to protect against bacterial infection[16].

Regarding poor mechanical properties of hydrogels, this study was aimed to coat SPI hydrogel on poly (ethylene terephthalate) (PET) fabric as substrate and the protective layer.

PET is an important polymer with a wide range of applications in different shapes of film, fiber, and fabric because of its affordable prices, good mechanical properties and available resources[17–19]. Another advantage of using hydrogels is the ease of loading drugs/bioactive agents into them. In this study, our focus is on healing diabetic relevant wounds. Neuropathic pain is one of the pain syndromes that occur in diabetic patients. There has been little concern about the influence of diabetes on the different parts of nervous system leading to high injury and death. Opioid analgesics and non-steroidal drugs are may be considered in selected clinical circumstances. Other medications such as gabapentin (1-(amino methyl) cyclohexane acetic acid) is an anti-epileptic agent but now it is also recommended as first line drug in neuropathic pain, particularly in diabetic neuropathy[20–23].

SPI hydrogel loaded with gabapentin was coated on the surface of PET fabric to fabricate a dressing for highly exudate wounds with neuropathic pain. Drug release profile of gabapentin was evaluated and biocompatibility of modified PET fabrics was also investigated regarding different chemicals used in surface modification procedure.

2. Materials and methods

2.1. Materials

Soy protein isolated (SPI) was purchased from Matin Co. (Iran). Acrylic acid (AA), benzoyl peroxide (BP), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) were obtained from Sigma-Aldrich (USA), acetone and toluene were purchased from Merck (Germany). PET fabric was received from Hijab Co. (Iran). Phosphate Buffer Saline (PBS) was purchased from Yasa Teb Co. (Iran) and Gabapentin (GB) was received as a gift from Sobhan Daru Co. (Iran). Dulbecco's modified Eagle medium (DMEM) was purchased from Gibco. Fetal bovine serum, penicillin, and streptomycin were obtained from Sigma-Aldrich (Denmark). Cell Titer 96 AQueous Non-Radioactive Cell Proliferation Assay 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) was purchased from Promega, Madison, WI.

2.2. Methods

2.2.1. Preparation of SPI hydrogel

SPI solution was prepared with the concentration of 9.5% (w/v) in distilled water and was stirred on a magnetic stirrer for 1 h at room temperature to obtain a homogeneous solution. The solution was adjusted to PH 7.0, heated at 105 °C (the unfolding temperature) for 30 min in an oil bath and then cooled at room temperature[12,13,24]. The preheated solution was cast at different temperatures of 25, 40, 60 and 80 °C for 24 h.

2.3. Swelling test

The water uptake of synthesized hydrogel was investigated in PBS solution for 24 h. Hydrogel samples were weighed, immersed in PBS, removed out periodically, wiped with a piece of filter paper and re-weighed. The water uptake percentage was calculated by equation (1) whereas SW% is swelling percentage, W_1 is the initial hydrogel weight (before immersion in PBS) and W_2 is the weight of hydrogel after immersion in PBS[12,25].

$$SW\% = [(W_2 - W_1)/W_1] * 100 \quad (1)$$

2.4. Incorporation of gabapentin (GB) in SPI

In order to study the drug release profile of GB from SPI hydrogel, GB was incorporated into SPI solution (5% w/w) and cast on the cover glass at 25 °C. GB loaded SPI hydrogel submerged in 5 mL of PBS (PH =7.4) at 37 °C and the drug release was measured using UVmini-1240 spectrophotometer (Shimadzu, Japan) at different intervals and wavelength of 210 nm. The released amount of GB in PBS was calculated from standard curve according to Equation (2) whereas %R is release percentage, C is the released GB and C_0 is the total drug that loaded in SPI hydrogel[12,26].

$$R\% = (C/C_0) * 100 \quad (2)$$

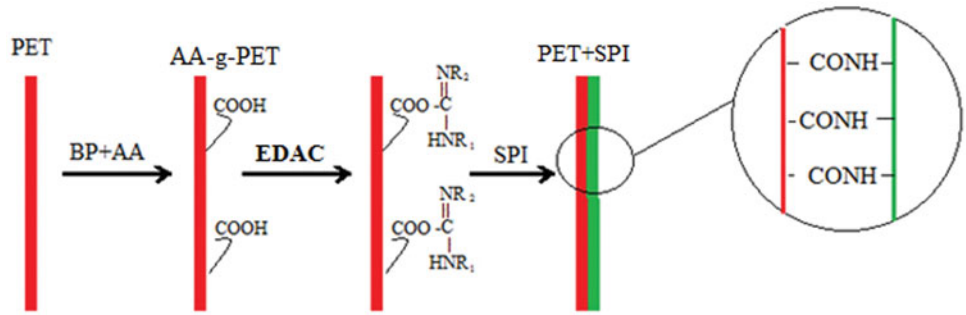

Figure 1. Schematic illustration of the chemical reaction occurred in surface modification of PET fabric by SPI.

2.5. Functionalization of PET fabric by SPI

Radical graft polymerization of AA on the surface of PET fabric was carried out in a test tube. The PET fabrics were placed into a test tube containing 20% (v/v) of AA. BP (as initiator) was dissolved in toluene in desired concentration and added to the reaction tube. The final mixture was then placed in a water bath at 75 °C for 12 h. The fabrics were taken out after the reaction process, washed in boiling water for 5 h to remove any homopolymer adhering to the surface and finally dried at room temperature[17,27]. The graft yield (G%) was obtained using the equation (3) where W_0 and W_g are the weights of PET fabrics before and after the graft reaction, respectively[28].

$$G\% = \left[(W_g - W_0)/W_0\right] * 100 \quad (3)$$

The AA grafted PET fabric (AA-g-PET) was immersed in EDAC/PBS (10 mg/mL) for 24 h at 2 °C to activate the carboxyl groups[29,30]. Then the prepared SPI solution was coated on the surface of activated AA-g-PET at 25 °C for 24 h (Figure 1).

2.5. Characterization of PET fabric

2.5.1. Thermal gravimetric analysis (TGA)

TGA investigation on the thermal behavior of AA-g-PET and PET samples were carried out using Thermal Analyzer (Rheometric Scientific, UK) under argon atmosphere between ambient temperature and 700 °C at a rate of 10 °C/min. The initial decomposition temperature (IDT) and T_{50} (the temperature related to 50% weight loss) were found from the thermogram[31].

2.5.2. Static water contact angle (WCA) measurement

The static WCA was measured using the photographic method by USB digital microscope (Micro View, Japan). A distilled water droplet (10 µL) was applied to the AA-g-PET and PET fabrics and contact angle measurement was carried out 20 s after the water drop was placed on the surface of specimens. The contact angle was obtained from the photographs by Digimizer software at the point of fabric/water intersection[32,33].

2.5.3. Scanning electron microscopy analysis

The surface of the AA-g-PET and PET fabrics were studied by scanning electron microscopy (SEM) (Philips, Netherlands) at 30 kV acceleration voltage. All the samples were sputter coated with a 30 nm layer of gold in advance.

2.5.4. Attenuated total reflectance infrared spectroscopy (ATR-FTIR)

Surface changes of the fabric samples were investigated by BOMEM ATR-FTIR (Hartmann & Braun, Canada) analysis. The spectra of samples were obtained in the range of 400-4000 cm^{-1} with a wavenumber resolution of 8 cm^{-1} using a Ge crystal.

2.5.5. Mechanical properties measurement

Mechanical properties of PET fabric with and without SPI hydrogel were determined using a uniaxial tensile testing machine (Zwich 1446-60, Germany) by applying a 20 kN load cell at room temperature and at the constant crosshead speed of 60 mm/min. For mechanical test, samples were cut into rectangular shape with dimensions of 2.5×15 cm^2 and mechanical properties were measured. At least three samples were tasted for each type of fabric and result were reported as mean ± SD.

2.6. Cell studies

2.6.1. Cell culture of 3T3 fibroblasts

NIH 3T3 mouse fibroblasts (ATCC, U.K.) were cultured in DMEM supplemented with 10% fetal bovine serum, 50 U/mL penicillin and 50 U/mL streptomycin. During the cell culture, the culture medium was replaced every 3 days and cultures were incubated at 37 °C with 5% CO_2. Cells were detached by 0.05% trypsin/0.05% EDTA after reaching about 80% confluence. Cell seeding onto AA-g-PET fabric was carried out at a density of 5,000 cells/cm^2 and incubated at 37 °C with 5% CO_2.

2.6.2. Metabolic activity and proliferation of 3T3 fibroblasts

MTS cytotoxicity assay was used to measure cell viability and metabolic activity on the samples, according to the manufacturer's directions. Cell seeding at a density of

5,000 cells/cm^2 was done and activity of cells was evaluated during a 7-days period. On days 1, 4, and 7, the cell-seeded specimens were punched in diameter of 10 mm, transferred to 48 well plates, and washed with PBS. Respectively 200 µL of culture medium and 50 µL of MTS were added to each well. Cells were maintained for an additional 4 h in an incubator with a humidified atmosphere at 37 °C and 5% CO_2. Finally, 100 µL of the solution in each well was pipetted out and transferred to 96 well plate, and the absorbance at 490 nm was recorded using a spectrophotometer Microplate Reader (Wallace VICTOR3 1420 multilable counter, PerkinElmer). Cell metabolic activity was obtained from absorbance on each substrate at different time points.

2.6.3. Cell morphology

SEM was used to study the morphology of cells on PET modified substrates after 1 and 7 days of cells seeding. Samples were fixed in 2.5% glutaraldehyde in cacodylate buffer for 12 h and washed with cacodylate buffer. Then samples were dehydrated with adding ethanol gradient and treated with hexamethyldisilazane to more water extraction. The dehydrated constructs were dried in desiccators under vacuum for 12 h and sputter coated with a thin Platinum layer to obtain SEM images of 3T3 fibroblasts grown on samples after 1 and 7 days of cell culture.

2.7. Statistical analysis

The experimental data were analyzed using analysis of variance (ANOVA) at $\alpha = 0.05$ to determine the significance of differences between results. Data are reported as mean ± standard deviation.

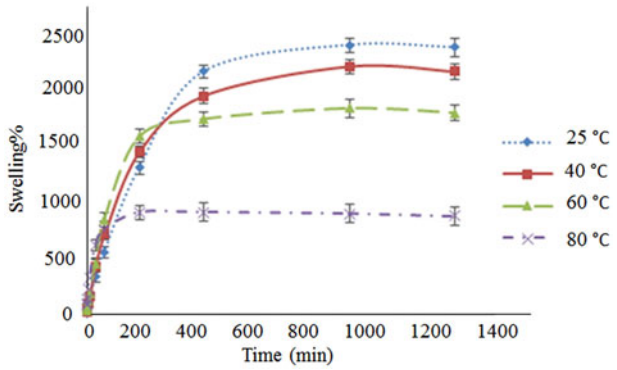

Figure 2. Swelling percent of SPI hydrogels were cast at different temperature ($n = 3$, $p \leq 0.05$).

3. Results and discussion

3.1. Fabrication of SPI hydrogel

In this study, the SPI hydrogel was preheated at 104 °C for 30 min according to reports from previous researches[12,13,24], and then SPI solution was cast at different temperatures of 25, 40, 60 and 80 °C for 24 h. Swelling ratio measured as an important factor indicating the ability of wound dressing to absorb wound exudates and body fluids as well as affecting drug release profile. As shown in Figure 2, the casting temperature has significant effect ($p < 0.05$) on swelling of SPI hydrogel whereas maximum equilibrium swelling (over 2000%) was observed at casting temperature of 25 °C and swelling was found to decrease at higher temperatures. In agreement with Flory-Rhener theory, when a biopolymer network gets in contact with an aqueous medium, the network starts to swell due to the thermodynamic compatibility of polymer chains and water[25]. Soy protein is a globular protein and its preheating at 105 °C leads to protein unfolding. Maltais et al. studied the structure of SPI cold-set hydrogel and reported that preheating process leads to protein denaturation and exposition of hydrophobic groups that enhances the degree of aggregation[12,24]. It seems that structural units of denatured SPI with hydrophobic outside embed water molecules upon immersing in the water (Figure 3). Casting at high temperatures after preheating process lead to reverse the structure of SPI hydrogel to the first configuration and less swelling[26,34]. Since the hydrogel was cast at 25 °C has shown the maximum swelling ratio, further experiments have been done using this case.

3.2. Release profile of gabapentin

Since hydrogels are known as a good carrier for drugs and also to investigate the drug release ability of fabricated SPI hydrogel, GB was incorporated into SPI solution and the resultant solution was cast at 25 °C and the release profile of GB measured up to 30 h (Figure 4). An initial quick burst release was observed whereas GB release from SPI hydrogel reached 80% after 5 h in PBS solution. It may be correlated to high solubility of GB in water and significant swelling of

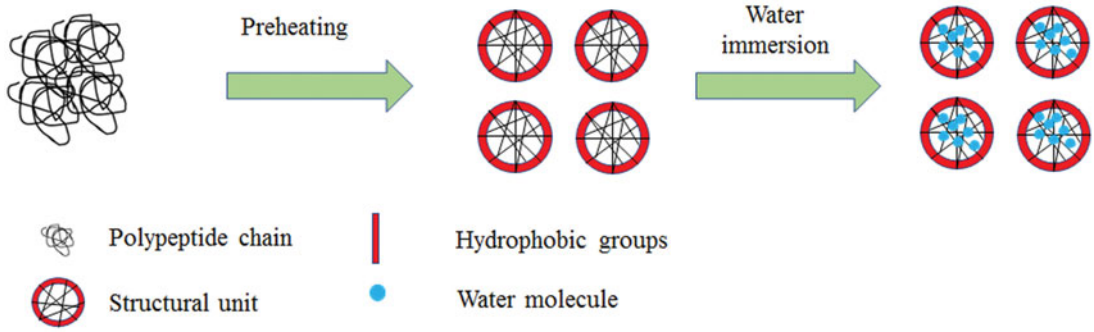

Figure 3. Schematic illustration of the hydrophobic groups exposition and embedding of water molecules in the structural units of SPI hydrogel caused by preheating.

SPI hydrogel. As the hydrogel network was exposed to aqueous media, polypeptide chain interaction decreased, the water molecules penetrated into the network and osmotic pressure led to drug diffusion outward[12]. After this initial quick burst release, the drug release profile followed a slower rate and about 20% of GB was released over 25 h which would be practical for wound dressing application in painful diabetic ulcer[16].

According to the GB chemical structure (Figure 5), existing of carboxylic acid and amine groups provide good water solubility (about 10 mg/mL) for GB[23,35]. Moreover, lack of chemical crosslinking between polypeptide chains in SPI hydrogel is another reason for this initial quick release. Similar result was obtained by Chien et al. whereas they reported an initial burst release (about 60% of whole drug in less than 10 h) and a steady release afterward till 7 days [26]. Peles and Zilberman in their study on gentamicin incorporated SPI film for wound dressing application, investigated the release of the drug in an eight-week period. They divided the release period to three steps consisting of first 6 h (step 1), first week (step 2) and during weeks 2-8 (step 3). The results of their experiment demonstrated 66% and 94% drug release for the step 1 and step 2 respectively. They discussed this burst release profile during the first stage is correlated to hydrophilic nature of drug and SPI initial rapid water intake[16].

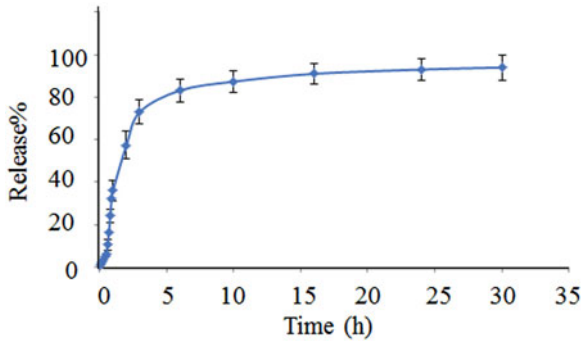

Figure 4. Release profile of GB incorporated in SPI hydrogel ($n = 3$, $p \leq 0.05$).

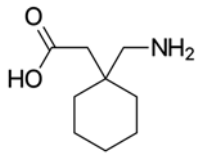

Figure 5. Chemical structure of GB.

3.3. Modification of PET fabric by acrylic acid grafting

In grafting procedure, the PET fabric was entered into AA grafting solution and BPO as the initiator was added to the mixture. The AA grafting procedure was started with the generation of radicals on the PET backbone by BPO. In the following these radicals initiated the graft polymerization of AA to organize graft brush layer onto the PET fabric surface. Afterward, primitive PAA micelles were formed with the extension of graft polymer chains restricted in these reaction sites on the PET fabric surface. With the chain propagation, the adjacent particles ultimately joined into one another, forming a continuous graft coating layer. This regular behavior has been stated in similar systems previously[17,28,31]. Figure 6 shows the morphology of the pure PET and AA-g-PET fabrics which revealing the presence of AA layer on the surface of the PET fabric. It is clearly shown that the surface of the AA-g-PET fabric has been almost covered by AA (spherical particles at submicron scale), and the surface roughness seems to be increased.

In order to study the surface change of the PET fabric after AA graft polymerization, FTIR spectra of the PET and AA-g-PET were recorded (Figure 7). Characteristic peaks of PET were observed at 1716 cm^{-1} which is related to C = O vibration, and 1100 cm^{-1} due to presence of C-O-C in both PET fabrics with and without AA. Comparing with the PET spectrum, the C = O vibration signal at 1716 cm^{-1} is broadened and new peak at 1689.3 cm^{-1} is observed in the FTIR spectra of PET fabric grafted with AA which can be attributed to C = O vibration band in the structure of AA. Moreover, a strong bond observed at 1303.8 cm^{-1} is assigned to the vibration of C-O in the grafted AA[17,36,37].

The grafting of AA onto the PET fabric surface was accomplished in order to promote a surface that provided a high density of carboxyl groups. It was observed that the amount of PAA graft was considerably affected by the BPO concentration. Figure 8 shows the effect of initiator concentration on the grafting efficiency of AA on the surface of the PET fabric. As can be seen from this figure, increasing initiator concentration up to 0.006 g/mL significantly enhanced

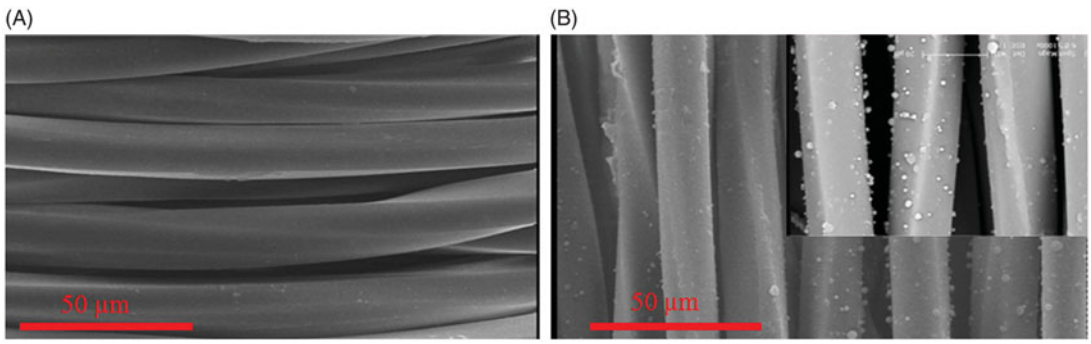

Figure 6. SEM photographs of (A) pure PET and (B) AA-g-PET (The magnification is 500 for A and B).

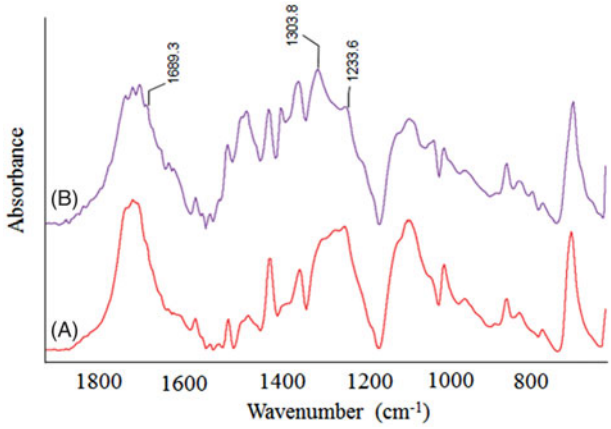

Figure 7. FTIR spectra of the PET (A) and AA-g-PET (B).

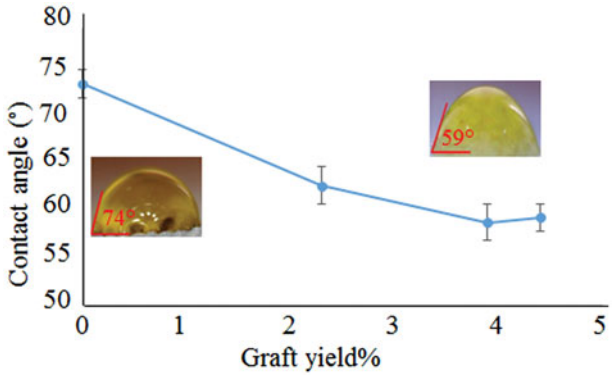

Figure 8. Influence of the initiator concentration on graft yield ($n = 3$, $p \leq 0.05$).

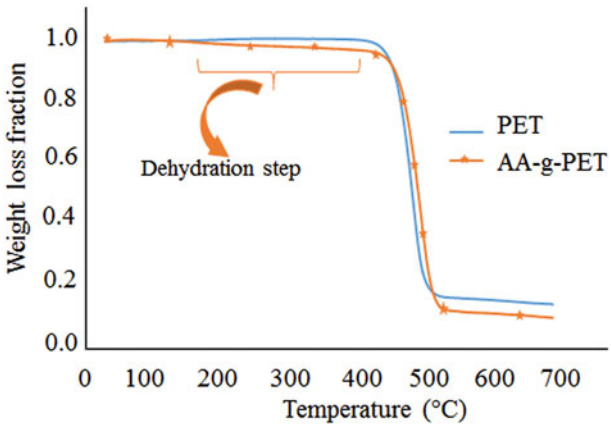

Figure 9. Variation of static WCA with the degree of AA grafting on AA-g-PET fabric ($n = 3$, $p \leq 0.05$).

the graft yield, while more amount of initiator does not have any noticeable effect on graft yield. These observations were quite similar to Ongun et al. for radical graft polymerization of methacrylamide onto the PET fibers. These authors also found that increasing of BPO concentration up to 0.009 mol/L enhanced the graft ratio of methacrylamide while a significant decrease in graft ratio was observed beyond 0.009 mol/L of BPO concentration[27].

The primary growth in the graft yield with the increase in BPO concentration was caused by increasing the number of active sites involved in the reaction and the unlimited monomer accessibility to the primary radicals, while increasing the initiator concentration more than 0.006 g/mL led to homopolymerization and increasing of reaction medium viscosity. Extensive homopolymerization reduces effective monomer concentration in the AA solution and increases solution viscosity which hinders the diffusion of the remaining monomer to propagation sites[38].

Figure 9 illustrates the effect of acrylic acid grafting on hydrophilicity of PET fabric. It is clearly shown that the WCA decreases gradually from 74° for pure PET fabric to about 59° for AA-g-PET which indicates the hydrophilicity of AA-g-PET fabrics has enhanced due to the hydrophilic nature of acrylic acid. This is in line with the graft yield results whereas higher grafting efficacy leads to higher wettability. Our outcomes are consistent with other studies showing the improvement of PET hydrophilicity by AA graft polymerization[28,33]. It would be worth commenting that grafting yield more than 3.9% did not influence on contact angle significantly ($p \geq 0.05$) which is likely due to saturation of surface of the PET fabrics with PAA at grafting yields more than 3.9%.

The thermograms of the PET and AA-g-PET fabrics are shown in Figure 10 indicating that the grafting of AA onto the PET fabric affect the thermal characteristic of PET. The thermal stability of PET fabric enhances by AA grafting. The IDT and T_{50} of the PET fabric were found to be 418 and 490 °C, while these parameters increased to 430 and 499 °C for the AA-g-PET, respectively indicating the enhancement in thermal stability of PET fabrics after grafting. This can be attributed to formation of stable anhydride due to PAA chains cyclization (Figure 11) resulting from dehydration of PAA chains at high temperatures on the surface of PET fabrics[30]. Our results are in accordance of previous studies whereas they also showed the increasing of thermal stability of PET after grafting with PAA[30,38].

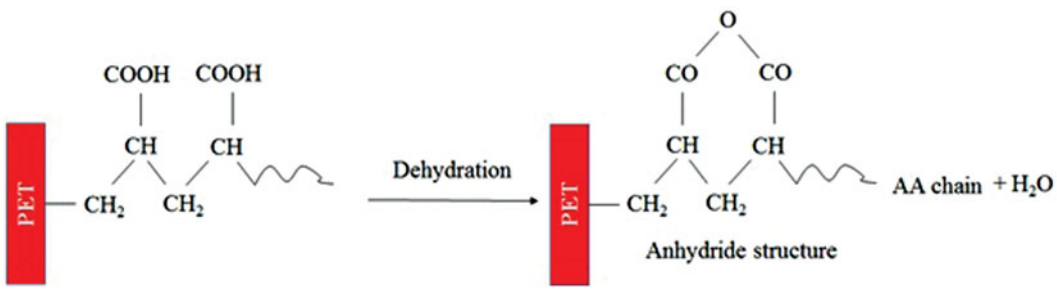

Figure 10. TGA thermograms of pure PET and AA-g-PET.

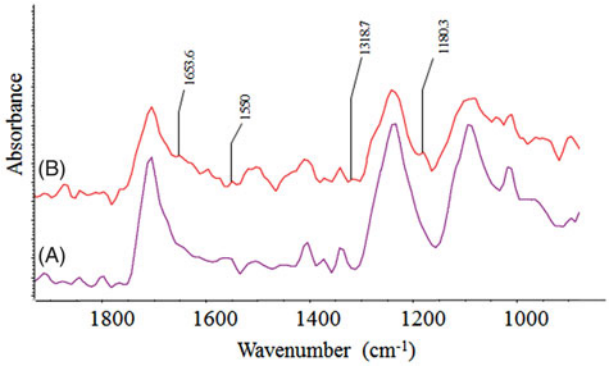

Figure 11. Formation of stable anhydride caused by chains cyclization at high temperatures in TGA test.

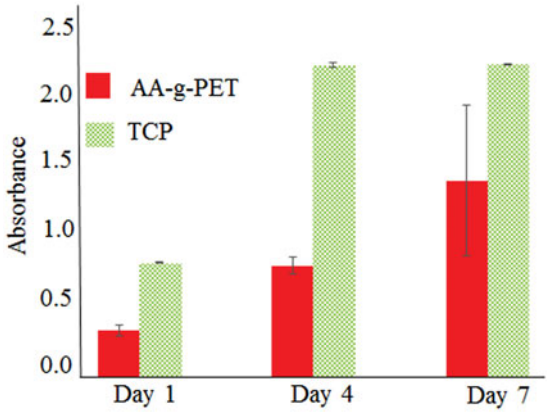

Figure 12. FTIR spectra of (A) pure PET and (B) SPI-PET.

Table 1. Mechanical properties of PET and SPI-PET samples.

Mechanical properties	PET	SPI-PET
Tensile strength (MPa)	134.14 ± 5.02	137.95 ± 4.72
Strain at break (%)	24.12 ± 0.44	22.29 ± 1.33

3.4. Covalent attachment of SPI on the surface of AA-g-PET fabric

With respect to weak mechanical properties of SPI hydrogel, this study was intended to coat SPI hydrogel on the PET fabric. For this purpose, the AA-g-PET fabric was immersed in EDAC/PBS solution (10 mg/mL) for 24 h at 2 °C to activate the carboxyl groups and afterward prepared SPI solution was coated on the surface of PET fabric at 25 °C (Figure 1)[29,30].

In order to investigate the covalent bond formation between SPI and activated AA-g-PET fabric after coating, FTIR spectra of the AA-g-PET and SPI coated AA-g-PET (SPI-PET) were recorded. Figure 12 shows the FTIR spectra of AA-g-PET and SPI-PET. According to these spectra, PET shows some strong characteristic signals at 1716, 1100 and 1250 cm^{-1} that are attributed to stretching vibration bonds of C = O and C-O-C, respectively and are visible in both spectra[17,36]. Comparing with the AA-g-PET spectrum, some additional peaks at 1180, 1318, 1550 and 1653 cm^{-1} appeared in SPI-PET spectrum. The absorption peaks at 1180 and 1318 cm^{-1} are attributed to stretching vibration of C-N in the amine groups. Also, the weak signal at 1550 cm^{-1} is assigned to the amid groups (N-H vibration) appeared in FTIR spectrum of the grafted PET fabric. The carbonyl peak at 1653 cm^{-1} from SPI masked by the inherent broad C = O band absorption in the PET fabric[27,30].

3.5. Mechanical properties

Table 1 shows the mechanical properties of PET and SPI-PET samples. No noticeable difference was observed between mechanical properties of PET fabric with and without SPI.

3.6. In vitro cell culture study

In order to study the biocompatibility of the modified PET fabric with regard to different chemicals used in surface

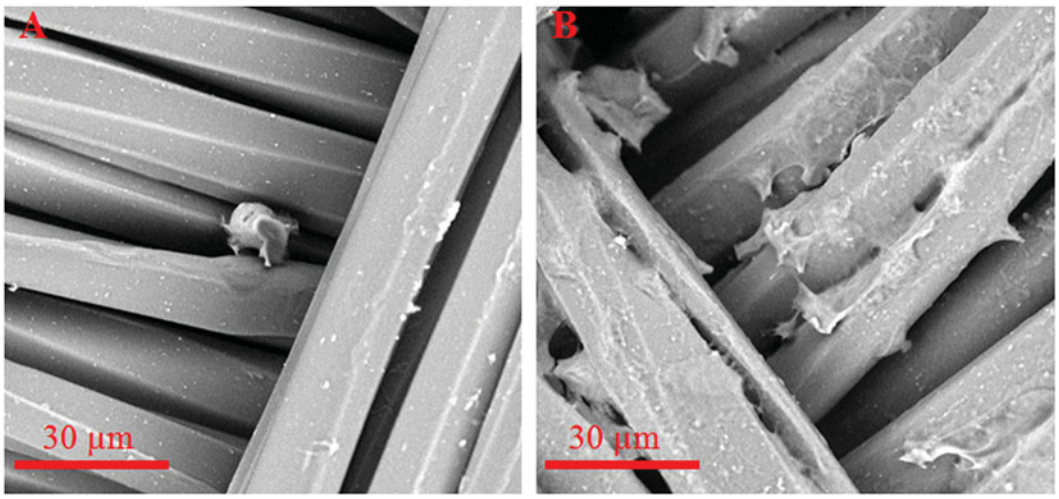

Figure 13. NIH 3T3 mouse fibroblasts' proliferation on AA-g-PET fabric after 1 and 7 days of cell seeding ($n = 3$, $p \leq 0.05$).

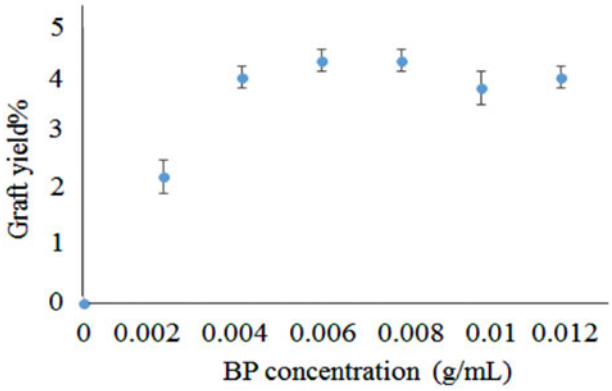

Figure 14. Morphology of NIH 3T3 mouse fibroblasts on AA-g-PET fabric (A) after 1 day and (B) after 7 days of cell culture (The magnification is 2500 for A and B).

modification procedure, MTS cytotoxicity assay was used to measure cell viability and their metabolic activity on the samples. The MTS result of cells seeded on the AA-g-PET fabric and TCP (as control) are presented in Figure 13. As can be seen from this figure, significant proliferation took place through the one-week culture indicating the biocompatibility of modified PET fabric.

Figure 14 shows SEM images of cells seeded on the AA-g-PET fabric after 1 and 7 days of cell seeding revealing cell proliferation and spreading on the fabric and confirming the biocompatibility of modified fabric.

4. Conclusion

Thermally induced and GB incorporated SPI hydrogel was fabricated at different casting temperatures and the temperature of 25 °C was chosen as desirable casting temperature due to the maximum swelling ratio. With regard to hydrogels' poor mechanical properties, PET fabric was used as substrate and protective layer for SPI hydrogel. The surface of the PET fabric was modified by AA graft polymerization in order to improve the fabric-hydrogel connection. FTIR result proved covalent bonding between SPI and modified surface of the PET fabric. MTS cytotoxicity assay and SEM illustrations were used to investigate the biocompatibility of modified PET fabric. Our overall results showed that the fabricated substrate during this study can be considered as an appropriate candidate for wound dressing applications particularly in diabetic ulcer due to presence of gabapentin.

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