Radiation Grafting of Acrylic Acid/*N*-Vinyl Pyrrolidone Binary Mixture Onto Poly(ethylene terephthalate) Fabric and Growth of Human Mesenchymal Stem Cell

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ABSTRACT: Radiation grafting of acrylic acid (AA)/*N*-vinyl pyrrolidone (NVP) binary mixture onto poly(ethylene terephthalate) (PET) knittings was investigated by pre-irradiation technique. The influence of the grafting conditions, such as monomer composition, reaction temperature, and the effect of storage time with temperature after irradiation on the degree of grafting was determined. ATR-FTIR spectroscopy analysis of the grafted knittings confirmed the existence of amide group of NVP in the knittings. The concentration of peroxides and effect of storage time on peroxide concentration were also determined by 2,2-Diphenyl-1-picrylhydrazyl at different temperatures. There was an increase in surface roughness of grafted PET in comparison to virgin PET as determined by atomic force microscopy and scanning electron microscopy. The grafted knittings were subsequently immobilized with collagen Type I which was further apt for the study of growth and morphology of human mesenchymal stem cell (hMSC). The immobilization of collagen on PET knittings has provided an excellent surface for the growth of hMSCs. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 115: 116–126, 2010

Key words: PET knitting; acrylic acid (AA); *N*-vinyl pyrrolidone (NVP); radiation; grafting

INTRODUCTION

Surface modification by tethering polymer chains to solid substrates is a useful method for the development of materials possessing specific surface properties which are useful in achieving better host-implant reactions.[1–3] The polymer chains chemically tethered to a substrate polymer are being successfully achieved by graft polymerization. The grafting process offers an interesting route as the surface active sites are available for the interaction with various monomers.[4–7] A large number of polymer-monomer combinations are available where selective functionalities are created by radiation grafting. The hydrophilic polymers derived from *N*-vinylpyrrolidone (NVP) are useful materials which promotes endothelial cell growth without protein precoating.[8–10] NVP is a hydrophilic and nonionic monomer which undergoes polymerization through radicals, thermal or photochemical irradiation in the presence of a crosslinker. Uncrosslinked PVP has had a long history of use in the medical and pharmaceutical fields and has often been found to be nontoxic and nonthrombogenic. The expected low antigenicity of PVP gels make them suitable for many biomedical applications.[11,12] The gels find their use as hemodialysis membranes,[13] wound dressings[14,15] and implantable drug delivery systems.[14,16] Razzak et al.[17] prepared PVP and poly(vinyl alcohol) (PVA) blended hydrogel for wound dressing. The *in vitro* assessment studies showed it as a useful material with accessing healing rate and manageable efficiency requirements. Today one tries to promote the replacement of an injured tissue or organ by a new but identical tissue. For this application the weak textiles like nonwovens, wovens, knitted structures, braided structures etc, perform best by providing just space. Textile materials offer interesting features, such as porosity and compliance which are often not exerted by other polymeric materials. Surgical implantation of these materials is encounted with both thrombosis and inflammation at the site of injury.[18,19] These processes are related and both contribute to the healing of tissue into and around the material. Poly(ethylene terephthalate) (PET) in different shapes and forms is used worldwide clinically in cardiovascular devices.[20] However, PET is reputed to be both thrombogenic and moderately inflammatory in nature, so it is necessary to modify PET to make it

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bioreceptive for its use as a biomaterial. PET has excellent mechanical strength, good stability in the presence of body fluids and high radiation resistance, which makes it suitable for sterilization but its surface needs precise modification for the immobilization of biomolecules.[7] The key requirement for modification is to acquire specific functional groups for immobilization of biomolecules. The modification of PET has been carried out by different methods involving chemical hydrolysis, UV, plasma, ozone, and graft polymerization to introduce various functional groups such as carboxylic acid, sulphonic acid, amide, amine, acrylate, pyrrolidone, and glycol.[6,7,21–28]Radiation-induced grafting of vinyl monomers onto PET has been frequently used for various biomedical applications.[5,29–33] To improve the biomedical applicability, polymeric materials are often immobilized with biologically active components onto the surface.[34–36]

Gamma radiation-induced graft polymerization is a promising way of combining two or more highly incompatible polymers, so that materials with desirable properties are achieved.[37] Chapiro et al.[38] grafted NVP on to silicone tubes and observed significant improvement of blood-compatibility. Jabbari et al.[39] grafted PVA with NVP or acrylic acid (AA) monomer using gamma radiation and showed that the use of NVP for grafting PVA improve cell viability. Taher et al.[40] prepared the membranes by direct radiation grafting of NVP/acrylamide (AAm) on to low density polyethylene films. The optimum comonomer composition at which highest grafting yield was obtained was found to be 20/80 wt % of AAm/NVP. These membranes were used in removal of heavy metals from waste water. The copolymerization of NVP with AA and methacrylic acid (MAA) has also been reported earlier by Chapiro and Trung.[41] It has been observed that the rate of polymerization slowly decreases from pure AA to pure NVP. The copolymers formed in DMF contain a somewhat larger fraction of AA. This effect could be due to the formation of a complex between AA and dimethyl formamide which suppresses the effect of molecular complex arising from mixtures of AA and NVP on the copolymerization. The reactivity ratios showed that MAA is more reactive towards NVP than AA and the reaction leads to copolymers with high content of MAA over a wide range of monomer feed. In our earlier studies, PET knitting was modified by radiation-induced graft polymerization of MAA and NVP mixture.[5] The grafted surfaces were evaluated for collagen immobilization to make them bioreceptive for tissue engineering. The reaction parameters of grafting of AA onto PET fabric has been reported earlier.[42] In the present study, functional designing of the PET knitting under various conditions using preirradiation grafting of binary mixture of AA/NVP was investigated. The effect of grafting conditions, such as the comonomer concentration, monomer concentration, reaction temperature, and the effect of storage time after irradiation of preirradiated PET knittings on the degree of grafting was investigated. The concentration of peroxides and effect of storage time on peroxide concentration were also determined. The change in surface topography and morphology by grafting were also determined by AFM and scanning electron microscopy (SEM) respectively. The grafted knittings were immobilized with collagen Type I and subsequently, the growth of human msenchymal stem cell (hMSC) was studied.

EXPERIMENTAL

Materials

Weft knitted textured PET fabric of denier 80/34 (mass of 9000 m/number of filaments in yarn) used in this study was of textured yarn supplied by Reliance Industries (Mumbai, India). AA (used after vacuum distillation), Tetrahydrofuran (THF) and toluene (both used as received) were obtained from Merck, India. *N*-vinyl pyrrolidone (NVP) (used after vacuum distillation), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) were received by Fluka. Collagen Type I (from Rat Tail, Sigma, US) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC, Sigma-Aldrich, US) were used as received. Deionised water was used for all experiments.

Knitting

Single end weft knitting was carried out on Krenzl, Switzerland, weft knitting machine of diameter, 3.5 in. Gauge was 14 needles/in.

Heat setting

For dimensional stability, heat setting was carried out at 200°C in free shrink condition on EARNST BENZAG, Switzerland heat setting machine.

Extraction of spin finish

Heat set knitted fabric was soxhlet extracted in methanol for 10 h for the removal of spin finish. Then it was removed and boiled in distilled water for 1 h followed by drying overnight at 60°C.

Irradiation

Knitted PET fabrics were exposed to γ-rays from a ^{60}Co source (900 Curies) in the presence of air. The dose rate of radiation was 0.16 kGy/h.

Graft polymerization

Grafting was carried out in glass ampoules of 2 × 10 cm^2 size with B-24 joints. A weighed amount (~500 mg) of fabric was placed in gamma chamber for irradiation. After the irradiation of 40 kGy, the fabric was immediately placed into ampoules containing monomer and the solvent (THF/water). Nitrogen was purged into the ampoule to remove air trapped inside the reaction mixture. The ampoule was subsequently placed in a water bath maintained at required temperature. After a desired period, the ampoule was removed and the sample was washed with boiling distilled water for 10 h to remove any surface adhered homopoylmer. The samples were then dried in an oven at 60°C under vacuum and the degree of grafting was determined using the following expression.[42]

$$\text{Degree of grafting } (\%) = \frac{W_g - W_i}{W_i} \times 100 \qquad (1)$$

Where, W_i and W_g are the weight of the ungrafted and grafted fabrics, respectively.

Functional group analysis by titration method

In this method, PET samples were taken in 0.01 *M* sodium hydroxide solution in a measuring flask and keept it at 40°C for 24 h. The grafted carboxylic acid groups of AA were neutralized by 0.01 *M* sodium hydroxide and the concentration of remaining sodium hydroxide was determined by titrating against 0.01 *M* HCl using phenolphthalein as an indicator. A blank experiment was also carried out.

Peroxide determination

The amount of peroxide formed on and near the PET fabric after preirradiation in air was quantified with DPPH.[43,44] The samples were placed in a 1 × $10^{-3}M$ toluene solution of DPPH in a glass ampoule of 2 × 10 cm^2 size. Nitrogen was purged into the ampoule to remove air trapped inside the reaction mixture and the ampoule was kept at 70°C for 5 h to decompose the peroxides. The DPPH molecules consumed were measured from the difference in absorbance at 510 nm between the control and peroxidized sample spectrophotometerically. The concentration of peroxides is measured by calibration plot using different concentration of DPPH solutions in toluene.

Attenuated total reflectance (ATR-FTIR spectroscopy)

ATR-FTIR spectra were recorded on Perkin–Elmer spectrum **one** spectrophotometer.

Atomic force microscopy

Topographical studies of the fiber surface were carried out in air using AFM, Nanoscope IIIa (Digital Instruments, Veego Metrology Group). It was operated in the contact mode using an etched silicon tip attached to the end of a cantilever (115–135 μm in length). The AFM measurements were carried out at a cantilever resonant frequency of around 277.5 kHz and a scan rate of 0.5 Hz. The spring constant of the cantilever was in the range of 20–80 N/m.

Scanning electron microscopy

The surface characteristics of virgin and grafted PET knittings were studied using STEREO-SCAN 360 (Cambridge Scientific Industries), scanning electron microscope, after coating them with silver.

Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) analyzes of samples were carried out on Perkin–Elmer–Pyris system in the temperature range of 50–300°C. The heating rate was 10°C/min and the thermograms were run under constant flow rate (20 mL/min) of nitrogen. The heat of fusion (ΔH_f) was obtained from the area under the melting thermogram. The crystallinity in sample was obtained by the following expression:

$$\%\ \text{Crystallinity} = \frac{\Delta H_f}{\Delta H_f\ (\text{crys})} \times 100 \qquad (2)$$

where, ΔH_f is the heat of fusion of the sample and ΔH_f(crys) is the heat of fusion of 100% crystalline PET and was taken as 28.1 cal/g.[45]

Collagen immobilization

Original or PAA-grafted PET fabrics 1 × 1 cm^2 were washed in boiling distilled water for 2 h. The surfaces were placed in EDAC (26 m*M*) solution for 24 h to activate the carboxyl groups.[25] After activation, the samples were washed with distilled water and subsequently dipped in collagen Type I solution at pH 4.6 (in acetate buffer) for 24 h at 4°C. Then the samples were then washed in distilled water for 1 min to remove the unbound collagen and dried in vacuum at 60°C. The collagen immobilized samples were further used for the growth of human mesenchymal stem cells (hMSCs).

Cell culture

Human Bone Marrow (1–2 mL) in T25 culture flask (BD) along with 3 mL of culture media was

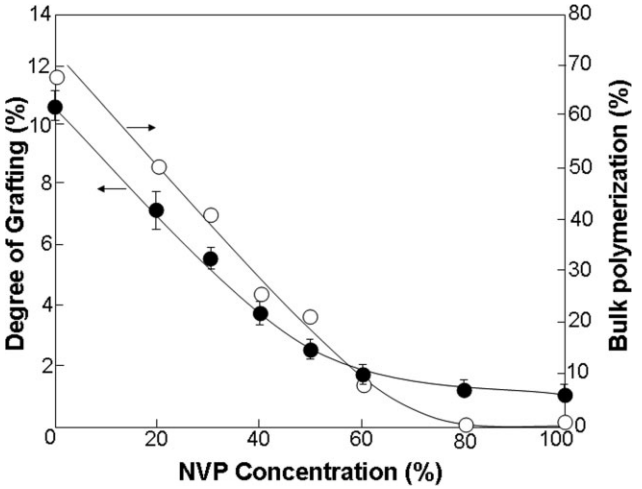

Figure 1 Influence of NVP concentration in feed on the degree of grafting and bulk polymerization. Reaction conditions: solvent (THF/water, 60/40) 60%, monomer concentration (AA/NVP) 40%, temperature 65°C, dose 40 kGy, time 10 h.

incubated at 37°C in a humidified atmosphere containing 95% air and 5% CO_2. Media consisted of Dulbecco Modified Essential Medium with low glucose (DMEM-LG, GIBCO) supplemented with 10% FBS (HYCLON), 1% antibiotic (Penstrep, GIBCO), and 2 m*M* L-Glutamine (GIBCO). The cultures were incubated for 24 h which allowed adherent cells to stick to the plastic surface. The medium was changed after the first 24 h to remove nonadherent cells. Subsequently, the medium was removed three times a week. At day 4 and 5, spindle shape cells were observed adhering to the plastic culture flask. When culture flask became near-confluent, cells were detached with 0.25% trypsin containing 1 m*M* EDTA for 3 min at 37°C, and subsequently replated at 5×10^3 cells/cm^2 for continued passaging.

Cell growth on fabrics

The sterilized (by gamma radiation) collagen immobilized PET-*g*-AA/NVP fabrics were cut into small pieces (0.5× 0.5 cm^2). The virgin and modified PET fabrics were placed on the bottom of the 12-well tissue culture plates containing media for overnight. Culture-expanded hMSCs were seeded at a concentration of 2×10^6 cells/mL on the fabrics and cultured for 4 days in DMEM-LG with supplements. Virgin PET fabric was used as control. After 4 days, the cell-seeded fabrics were harvested and the cell morphology and its attachment on PET fabrics were observed by SEM. For SEM, cell-seeded PET fabrics were washed thrice with PBS and subsequently fixed in 1% (v/v) glutaraldehyde and 4% (v/v) formaldehyde. This was followed by serial washing with 30, 40, and 50% (v/v) ethanol and then freeze drying.

RESULTS AND DISCUSSION

The grafting of AA in conjunction with NVP was carried out to create hybrid network on the fabric surface. The introduction of carbonyl functionality made the surface bio-interactive and also now biocompatible features due to the presence of NVP for the cell seeding and grafts. The influence of comonomer concentration on the degree of grafting is shown in Figure 1. As the concentration of NVP increases in the comonomer, the degree of grafting decreases. In pure NVP, very little grafting takes place and on the other hand, the AA leads to high level of grafting along with substantial homopolymerization. As NVP concentration increases in the monomer mixture, bulk polymerization in grafting solution (copolymer formed by bulk polymerization of AA and NVP) also decreases. This may be due to the deactivation of some of the radicals thereby resulting in decrease in the grafting. The composition of AA on PET-*g*-AA/NVP with respect to feed composition is presented in Figure 2. The trend of experimental values is similar to the calculated values (from their reactivity ratios, r_1= 0.27 and r_2= 0.041 for AA and NVP respectively).[46] The similar trend has been obtained by Chapiro and Trung in bulk polymerization as well.[41]

The variation of the degree of grafting with the monomer concentration is presented in Figure 3. Initially, the degree of grafting increases and reaches maximum at 60% monomer concentration and then tends to decrease. The initial increase in the degree of grafting with the increase in the monomer concentration is due to the unhindered accessibility of the

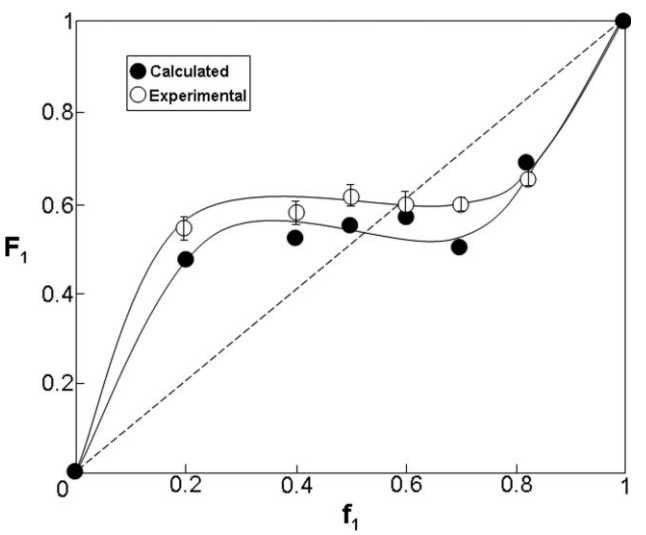

Figure 2 Influence of mole fraction of AA in copolymer on the feed composition. See Figure 1 for the reaction conditions.

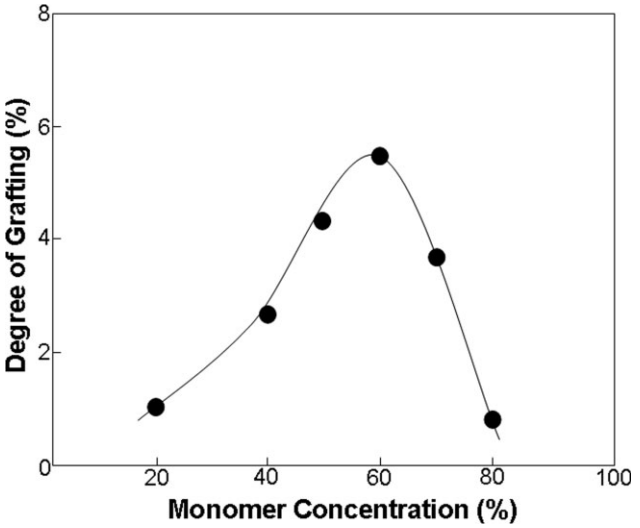

Figure 3 Influence of monomer concentration on the degree of grafting. Reaction conditions: monomer AA: NVP (50 : 50), solvent THF/water (60/40), time 10 h, temperature 65°C, dose 40 kGy.

monomer to the primary radicals, P·/PO· resulting in a smooth initiation step and propagation step [eqs. (3) and (4)]:

$$\mathrm{P\cdot/PO\cdot} + \mathrm{M} \xrightarrow{k_i} \text{initiation} \quad (3)$$

$$\mathrm{PCHX\cdot} + \mathrm{M} \xrightarrow{k_p} \text{propagation} \quad (4)$$

Beyond 60%, the effective concentration of monomer is reduced by extensive bulk polymerization. Due to which the rate of propagation (k_p) decreases considerably, and chain transfer (k_{tr}) to another species (Q), such as copolymer in solution dominates over the chain propagation. The degree of grafting, as a result, shows a decreasing trend.[7,42] Whereas in our previous investigation, the grafting maxima was observed at 40% of monomer concentration in case of NVP/MAA (20 : 80 in feed) on PET.[5] This is because in current system we are using 50 : 50 ratio of NVP/AA in feed. At 20 : 80 ratio of NVP/AA, almost half of the monomer undergoes bulk polymerization (Fig. 1) which is a very high content in comparison to NVP/MAA system because AA has tendency for faster homopolymerization than MAA leading to the quick gel formation. Therefore, as we increase the monomer concentration in current system, the relative fraction of AA increases which enhances the bulk polymerization of AA and NVP, thereby leading to increase in viscosity of reaction medium.

The influence of the reaction temperature on degree of grafting is shown in Figure 4. The grafting was carried out from 50 to 80°C at 60% monomer concentration. The degree of grafting increased up to 65°C and thereafter tended to decrease. Similar results were obtained in our previous studies on radiation-induced grafting of AA onto PET.[42] It may be understated that with the increase in temperature, the concentration of propagating chains is increased because of a higher peroxide decomposition rate which ensures fast initiation and propagation. However, beyond 65°C, the termination of two growing chains by mutual recombination becomes a major factor due to extensive bulk polymerization which favors more chain transfer in the system. It is possible that the chain transfer steps given by eqs. (5) and (6) (at temperature higher than 65°C) are dominated to such an extent that the limiting degree of grafting is reduced. It may also be possible that some of the primary radicals (P·/PO·) became deactivated in the reaction medium [eq. (7)], contributing to the reduced degree of grafting at higher temperatures.[7,42]

$$\mathrm{PCHX\cdot} + \mathrm{PCHX\cdot} \xrightarrow{k_c} \text{dead polymer} \quad (5)$$

$$\mathrm{PCHX\cdot} + \mathrm{Q} \xrightarrow{k_{tr}} \text{chain transfer} \quad (6)$$

$$\mathrm{P\cdot \text{ and } PO\cdot} + \mathrm{PCHX\cdot} \longrightarrow \text{deactivation} \quad (7)$$

Ionizing radiation can induce excitations or radical formations in polyesters that can evolve in charge transfer, chain break, disproponation, β-scission and other ordinary ion and radical reactions. Increasing formation of radicals with the dose (time) can allow intermolecular radicalic reactions effective for an increase in the molecular weight. Oxygen stored in the polymer is a well known radical scavenger; it

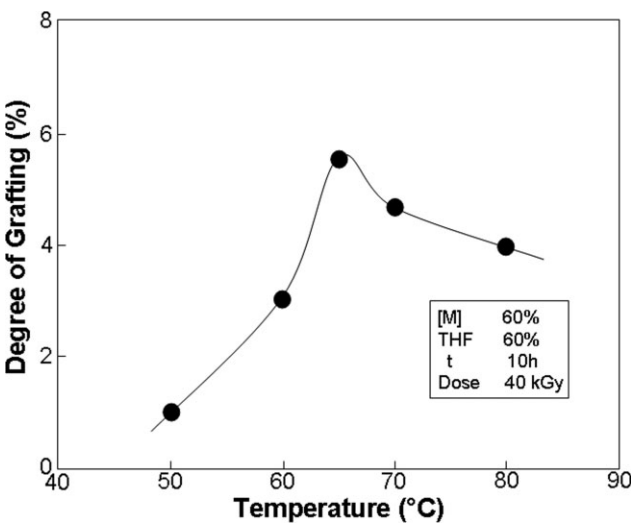

Figure 4 Influence of reaction temperature on the degree of grafting. Reaction conditions: solvent (THF/water, 60/40) 40%, monomer concentration (AA: NVP = 50 : 50) 60%, dose 40 kGy, time 10 h.

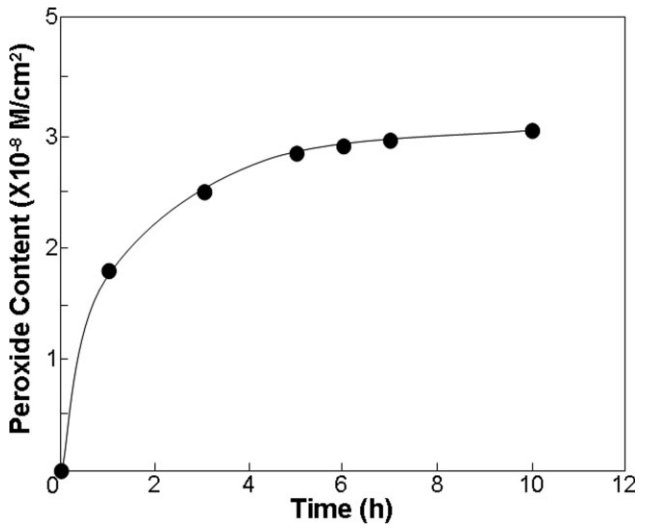

Figure 5 Influence of reaction time on the peroxide content at 70°C formed on PET during preirradiation at 40 kGy.

quickly reacts with formed radicals producing peroxides (hydroperoxides and diperoxides) [eqs. (8)–(13)] that can evolve into stable products like alcohols, aldehydes, ketones, carboxylic acids and esters.[47] When the hydroperoxides formed on and near the polymer substrate are heated at high temperatures, the dissociation of the peroxide leads to an equal number of PO· and OH· radicals. This means that the reactive sites in the backbone polymer are generated by the decomposition of diperoxides [eq. (14)] or hydroperoxides [eq. (15)] at high temperatures. The DPPH technique was utilized to evaluate the concentration of peroxide formed in the irradiated PET fabric by counting the quantity of DPPH consumed from the reaction of peroxide radicals with DPPH. Figure 5 shows the decomposed peroxide concentration calculated from the DPPH consumption as a function of the reaction time at 70°C for the PET fabric irradiated by γ-rays at a dose of 40 kGy. The decomposition of peroxides continues for up to 5 h and then levels off. The similar trend was observed by Kwon et al. on ultra high-molecular weight polyethylene.[43] Hu et al.[48] also measured the peroxide density on PET at 10 kGy with dose rate 7.8 kGy/h which was 0.85 nmol/cm^2.

$$\mathrm{PH} \xrightarrow{\text{Y-rays}} \mathrm{P}\cdot + \mathrm{H}\cdot \quad (8)$$

$$\mathrm{P}\cdot \xrightarrow{\text{Air } (O_2)} \mathrm{POO}\cdot \quad (9)$$

$$\mathrm{POO}\cdot + \mathrm{PH} \longrightarrow \mathrm{POOH} + \mathrm{P}\cdot \quad (10)$$

$$\mathrm{POO}\cdot + \mathrm{P}\cdot \longrightarrow \mathrm{POOP} \quad (11)$$

$$\mathrm{POO}\cdot + \mathrm{POO}\cdot \longrightarrow \mathrm{POOP} + \mathrm{O_2} \quad (12)$$

$$\mathrm{P}\cdot + \mathrm{P}\cdot \longrightarrow \mathrm{P-P} \quad (13)$$

$$\mathrm{POOP} \longrightarrow 2\mathrm{PO}\cdot \quad (14)$$

$$\mathrm{POOH} \longrightarrow \mathrm{PO}\cdot + \mathrm{OH}\cdot \quad (15)$$

The effect of the storage temperature and time on the formation of peroxides on PET which were irradiated to a total dose of 40 kGy is shown in Figure 6. The peroxides on PET at room temperature increase rapidly than at −20°C (in freezer) with storage time up to 20 days. Irradiation of PET fabric leads to the formation of trapped radicals and peroxides which then initiates the grafting. These trapped

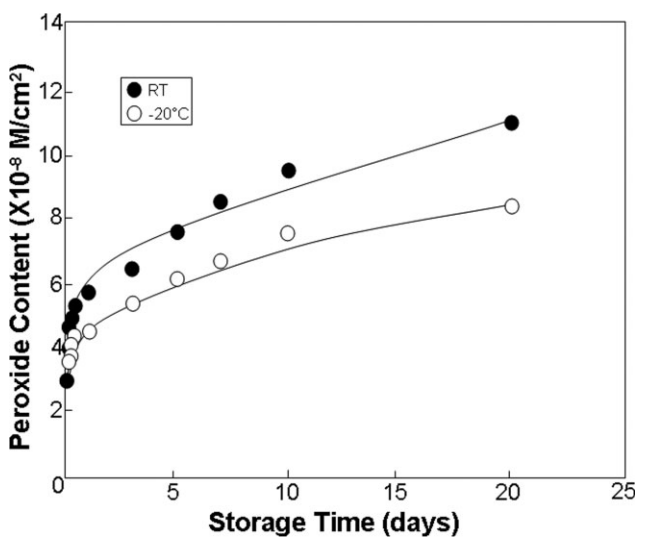

Figure 6 Influence of storage temperature and time on the peroxide content formed on PET during storage. Reaction conditions: temperature 70°C, time 5 h.

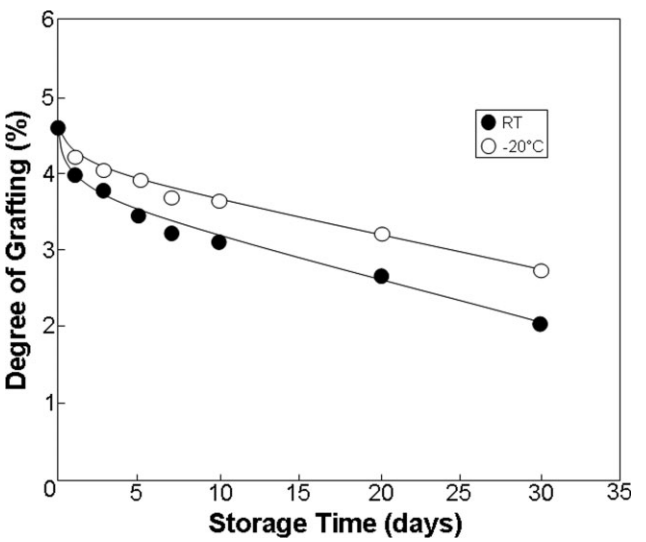

Figure 7 Influence of storage temperature and time on the degree of grafting. Reaction conditions: solvent (THF/water, 60/40) 40%, monomer concentration (AA: NVP = 50 : 50) 60%, dose 40 kGy, temperature 70°C, time 10 h.

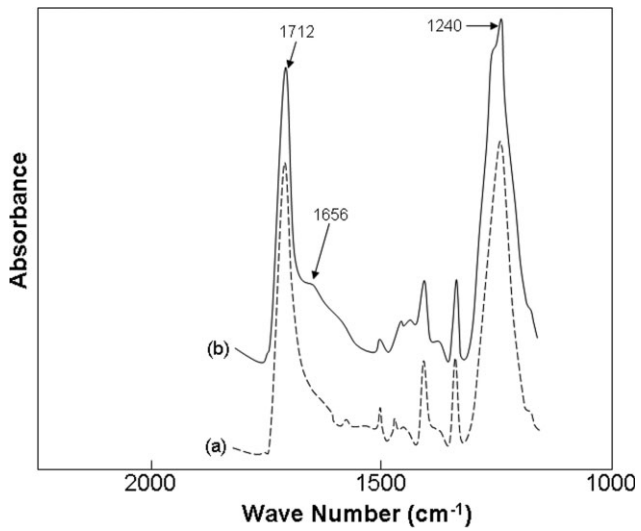

Figure 8 ATR-FTIR spectra of PET (a) virgin and (b) 5.5% grafting (F_1= 0.63).

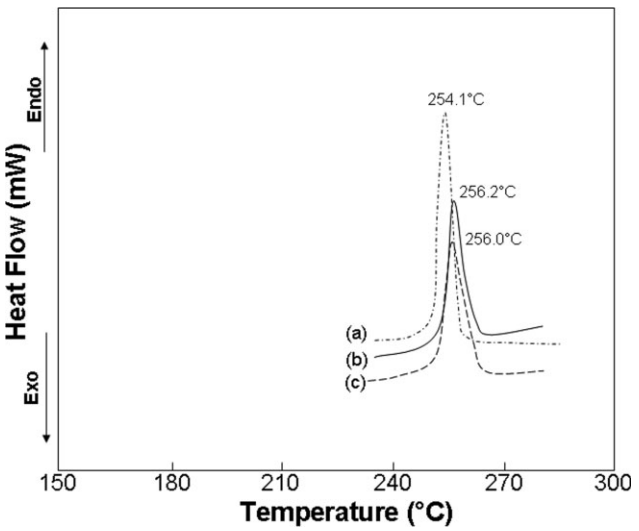

Figure 9 DSC thermograms of PET fabrics (a) virgin, (b) 2.5% grafted (F_1= 0.63), and (c) 5.5% grafted (F_1= 0.63).

radicals in the presence of air are easily oxidized to peroxides but the diffusion of oxygen is temperature dependent.[43,44,49] So, as the storage temperature decreases, the diffusion of oxygen is also decreased which may be the reason of lesser peroxide content at −20°C than room temperature.

Figure 7 shows the effect of storage time on the degree of grafting of AA/NVP (50 : 50) onto the preirradiated PET fabrics. When the samples are stored in freezer at −20°C, the decrease of the degree of grafting is small in comparison to room temperature storage. The grafting is initiated by the trapped radicals and peroxides which were formed during irradiation but during storage, some of the trapped radicals are converted into peroxides and some may be deactivated by mutual combination [eq. (13)]. The decrease in the degree of grafting at room temperature storage and −20°C may be due to the deactivation of trapped radicals during storage by mutual recombination. But this deactivation may be less when storage temperature is −20°C than at room temperature. The similar behavior was obtained by Chen et al. when they carried out the electron beam preirradiated grafting of acrylamide onto PP films during storage.[49]

The ATR-FTIR of ungrafted and grafted PET samples is presented in Figure 8. The appearance of band at 1656 cm^{-1} which is characteristic peak of —C=O of amide bond, shows the presence of NVP on the grafted sample. The peak at 1712 cm^{-1} is also observed which corresponds to the —C=O of ester group of polyester.[42] The DSC results indicate that the thermal stability of PET is increased after grafting of NVP/AA (Fig. 9). The melting of PET fabric is shifted from 254 to 256°C (Fig. 9) for grafted samples but there is no increase in melting temperature from 2.5% grafting to 5.5% grafting. The observed increase in melting point indicates the effect of grafting on the crystalline structure of the fibers whereas the amount of grafting does not affect the crystalline structure. The increase in melting point of PP on grafting of MAA was also observed by Atia et al.[50] It has been observed that slight decrease in crystallinity occurs with increase in the degree of grafting (Table I). The decrease in crystallinity measured by DSC is very close to theoretical calculated value of fraction of crystalline PET present in respective grafted samples. These results therefore suggest that the grafting proceeds solely within the noncrystalline region of PET matrix and the inherent crystallites remain unaffected. The similar results were obtained in our previous publication of grafting of AA onto PP films.[51]

The surface topography of PET fiber, as observed by AFM, underwent significant changes as a result of the gamma treatment and the subsequent grafting process (Fig. 10). The surface roughness is increased from 9.4 nm for virgin PET to 10.7 and 126.8 nm for gamma treated and 5.5% grafted PET respectively. The roughness is increased on gamma treatment is due to the chain break, disproportionation and

TABLE I
Changes in Heat of Fusion (ΔH_f) and % Crystallinity of Graft Copolymers (PET-*g*-AA/NVP) with Different Degree of Grafting

		% Crystallinity	
Degree of grafting	ΔH_f (J/g)	DSC	Calculated
Virgin PET	58.85	50.1	–
2.5% (F_1 = 0.63)	55.62	47.4	48.9
5.5% (F_1 = 0.63)	56.82	48.3	47.5

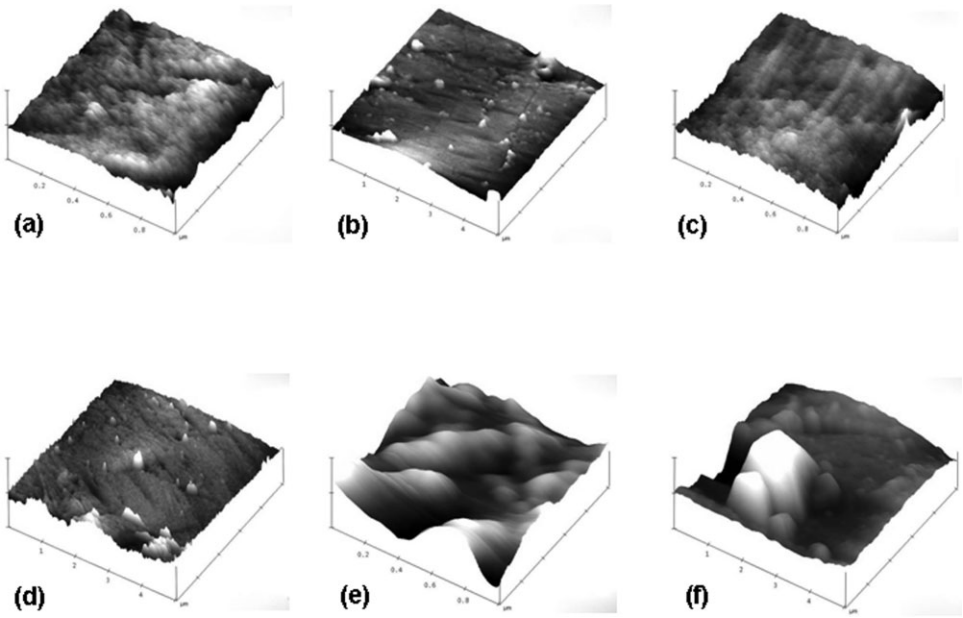

Figure 10 AFM of PET fiber surfaces (a) and (b) virgin, (c) and (d) gamma irradiated, and (e) and (f) 5.5% grafted (F_1= 0.63).

β-scission.[52] Grafting led to a substantial increase in the surface roughness, which may be due to the grafted chains form their own domains and morphologies at the surface. These results indicate that the surface morphology of the PET fiber is significantly affected by the grafting which is also confirmed by the SEM (Fig. 11). PAA/PNVP grafted chains are hydrophilic in nature and hence experience incompatibility with PET matrix, leading to the phase separation.

The grafted knittings are immobilized with collagen Type I and growth of hMSC have been carried out (Figs. 12 and 13). SEM observation demonstrated that the MSCs are attached to the surrounding fibers after day 1 (Fig. 12). The attachment of cells are better on collagen immobilized grafted fabrics in comparison to virgin fabric and MSCs have fully developed into their original morphology on day 4 (Fig. 13). Moreover, MSCs have presented a better cell morphology, as shown by light microscopy [Fig. 13(a)] of cells, on collagen immobilized grafted surface in comparison to virgin surface. This may be due to the hydrophobicity of virgin PET surface and absence of protein on the surface, cells have not adhered properly, so could not attain their original morphology. The surface immobilized proteins can interact with integrins of the cellular membrane represents an interesting way to achieve cell adhesion and activation. Therefore, the immobilization of collagen on grafted surfaces improves the

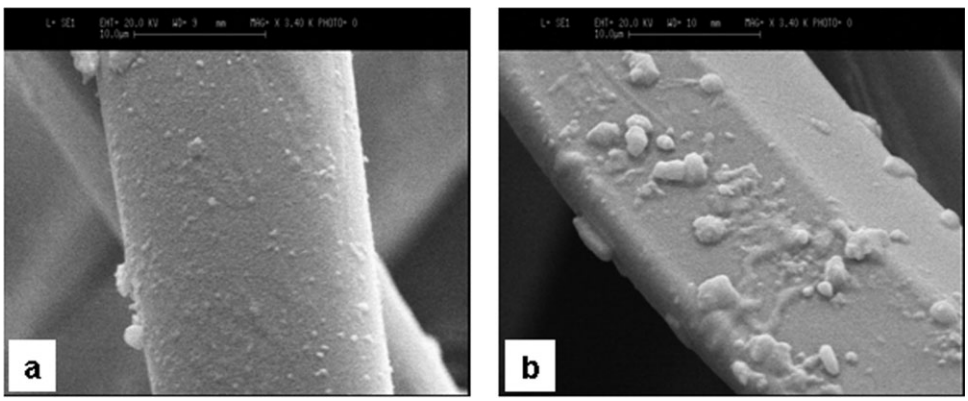

Figure 11 SEM of PET fabrics (a) virgin and (b) 5.5% grafted (F_1= 0.63).

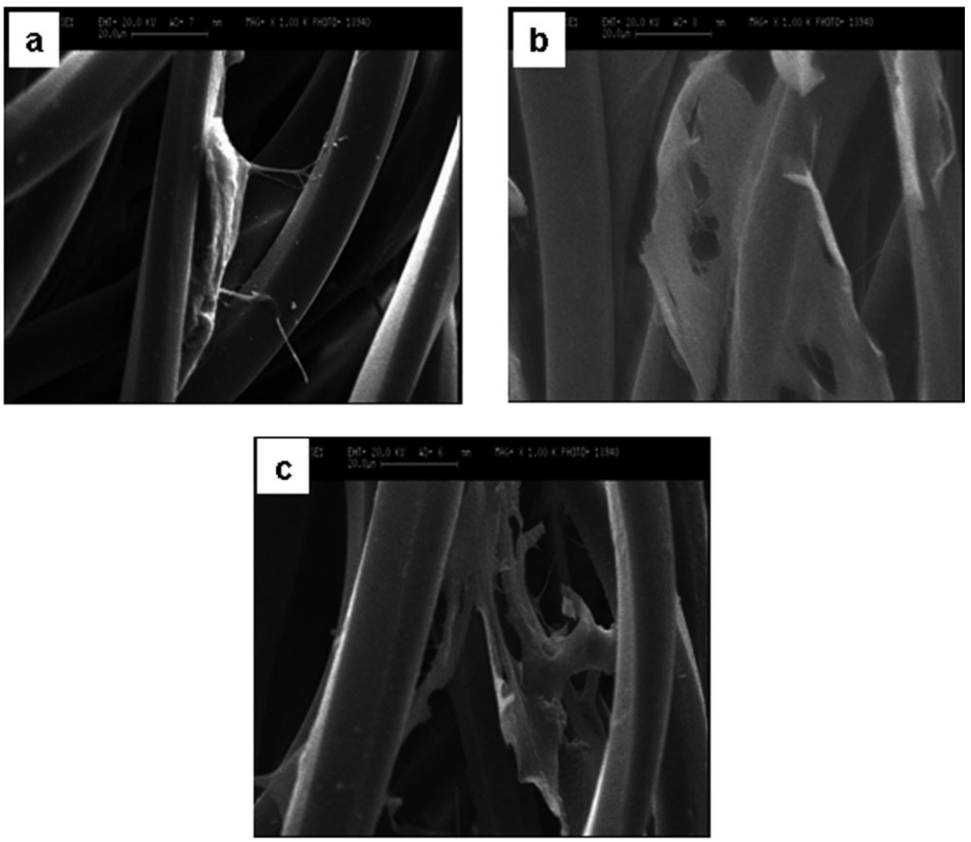

Figure 12 SEM of hMSCs (1 day cultured) in virgin PET (a), 2% grafting (b), and 5.5% grafting (c).

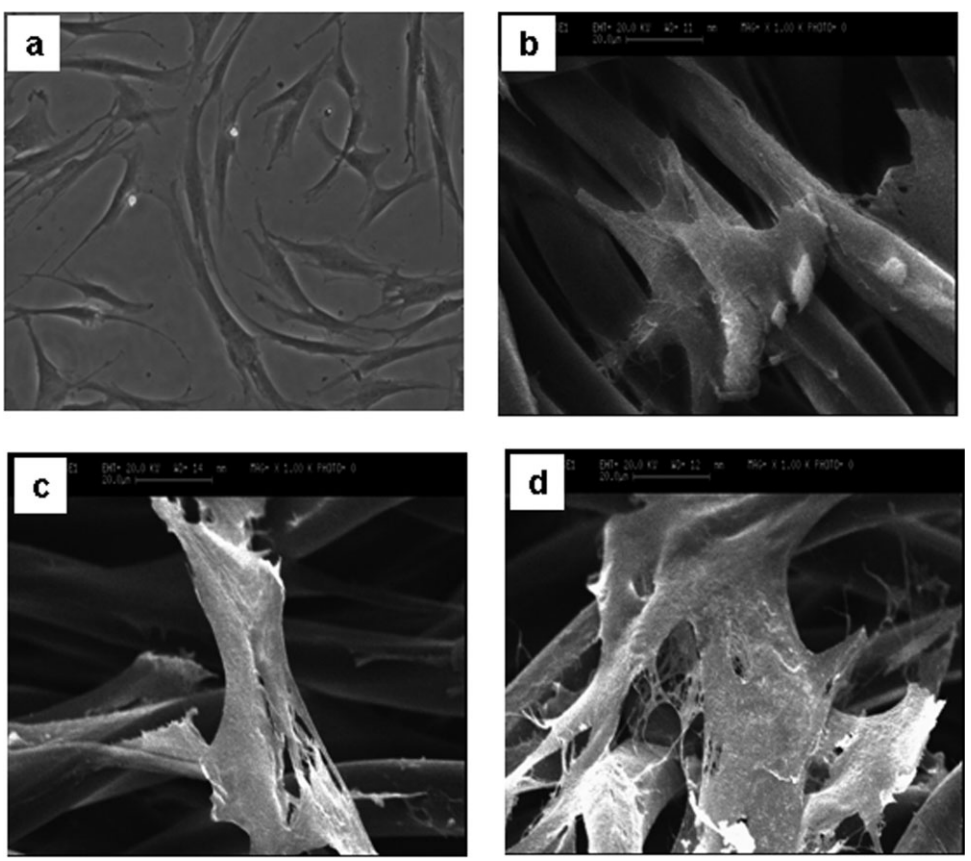

Figure 13 Light microscopy image of hMSCs (a), SEM of hMSCs (4 days cultured) in virgin PET (b), 2% grafting (c), and 5.5% grafting (d).

hydrophilicity; bioreceptivity as well as cytocompatibility of the surfaces for adherence and growth of hMSCs.[53] These observations clearly indicate that the collagen immobilized grafted fabrics are excellent substrates for adherence and growth of hMSCs. The detailed study on cell viability of hMSCs on these collagen immobilized bioreceptive surfaces is in under progress and will be published subsequently.

CONCLUSIONS

The composition of AA in copolymer with respect to feed composition was found to be following similar trend as calculated values from their reactivity ratios (r_1= 0.27 and r_2= 0.041 for AA and NVP respectively). The grafting maximum has been observed at 60% of monomer concentration beyond which the graft level has decreased due to extensive bulk polymerization. The reaction temperature influenced the grafting significantly. Abrupt fall in the equilibrium degree of grafting was observed beyond 65°C. The peroxide content on PET at room temperature has increased rapidly than at −20°C (in freezer) with storage time up to 20 days. This was due to the decrease in diffusion of oxygen as the storage temperature decreases. The degree of grafting was decreased when the samples were stored at room temperature and −20°C which may be due to the deactivation of trapped radicals during storage by mutual recombination. The presence of characteristic band of carbonyl group of amide at 1656 cm^{-1} confirms the existence of NVP on grafted PET fabric. The crystallinity of grafted samples decreased since grafting proceeds solely within the noncrystalline region of PET matrix. The surface roughness was increased with grafting as observed by AFM and SEM which was due to incompatibility of hydrophilic PAA/PVP system with PET matrix. The collagen immobilization on these surfaces was shown to have excellent surface for adherence and growth of hMSCs.

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