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Synergistic effects of retinoic acid and graphene oxide on the physicochemical and *in-vitro* properties of electrospun polyurethane scaffolds for bone tissue engineering

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Abstract: Tissue engineering scaffolds simulate extracellular matrixes (ECMs) to promote healing processes of damaged tissues. In this investigation, ECM were simulated by retinoic acid-loaded polyurethane-graphene oxide nanofibers to regenerate bone defects. Scanning electron microscopy (SEM) micrographs, Fourier transform infrared (FTIR) spectrum and X-ray diffraction (XRD) patterns proved the synthesis of graphene oxide (GO) nanosheets. SEM micrographs of nanofibers demonstrated through the formation of homogeneous and bead free fibrous scaffolds that the diameter of fibers were reduced by decreasing the applied voltage in an electrospinning process and the addition of GO. According to the results, the addition of GO to the polyurethane (PU) solution led to an increase in mechanical strength which is the most important parameter in the hard tissue repair. The GO-containing scaffolds showed an increased wetability, swelling, biodegradation and drug release level. Release behavior in nanocomposite scaffolds followed the swelling and biodegradation mechanisms, so osteogenic expression was possible by incorporating retinoic acid (RA) in PU-GO nanofibrous scaffolds. Biological evaluations demonstrated that composite scaffolds are biocompatible and support cellular attachment in which RA-loaded samples represented better cellular spreading. In brief, nanocomposite fibers showed desired that the physicochemical, mechanical and biological properties

and synergic effects of GO and RA in osteogenic activity of MG-63 cells produced favorable constructs for hard tissue engineering applications.

Keywords: electrospinning; osteogenic activity; polyurethane-graphene oxide scaffolds; retinoic acid-loaded nanofibers; tissue engineering.

1 Introduction

Millions of people in the world suffer from skeletal defects. In spite of the fact that many different strategies have been used to reduce or control the problems and improve quality of life, accurate solutions are under discussion. Nonetheless, nowadays tissue engineering studies have replaced different parts of bodies such as bones (1–3), nerves (4), cartilages (5) and wound healing (6) using new approaches. Tissue engineering regulates cellular behavior and regenerates defects by mimicking the extracellular matrix (ECM) (7). In order to mimic an ECM, cellular scaffolds have been made by a variety of techniques such as electrospinning (8–10), freeze casting (11), three dimensional printing (12), etc. Among all the cutting-edge methods, electrospinning has maintained its popularity due to its versatile processing, simplicity, cost effectiveness, high surface area to volume and length to diameter ratios that are essential for various applications (13).

There have been many investigations related to electrospinning technology. Ma et al. (14) characterized poly lactic acid (PLA) nanofibers with the uniform dispersion of hydroxyapatite (HA) and graphene oxide (GO). Results demonstrated that three-dimensional scaffolds enhance cell seeding and growth by mimicking ECMs. Moreover, biocompatibility and cellular differentiation of fibers improved by the addition of HA and GO that produce an appropriate structure for tissue regeneration. Jing et al. (15) fabricated polyurethane (PU)-GO scaffolds by thermally induced phase separation and reported the incorporation of GO into the scaffolds enhanced chemical properties such as roughness and hydrophilicity, and thus promote cellular attachment and growth. Lim et al. (16)

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prepared graphene hydrogels using hydrothermal treatment of large-area GO which were compatible for cells. Guided filopodia of MG-63 on the hydrogel demonstrated compatibility of structure for bio-applications. Nair et al. (17) presented the incorporation of GO into the gelatin-HA freeze dried scaffolds to enhance mechanical strength and osteogenic differentiation. Hence, three-dimensional composite scaffolds that were reinforced by GO represented osteopromotive properties and induced osteogenic differentiation of human adipose-derived mesenchymal stem cells without the need for osteogenic supplements. Another investigation reported that retinoic acid (RA) plays an undeniable role in osteogenic differentiation (18), and Glanz et al. (19) differentiated mouse adipose-derived stromal cells in the presence of high temperature-requirement protease A1 (HtrA1) and RA. They reported that HtrA1 enables molecular action of RA in regulating mouse adipose-derived stromal cells lineage commitment.

In the present study, RA-loaded polyurethane-GO nanofibrous scaffolds were fabricated by a electrospinning technique and the synergic effects of GO and RA on the osteogenic expression in the resultant nanofibers were evaluated. So, the scaffolds were characterized to introduce the suitable composition in terms of physicochemical, biological and drug release level for regeneration of bone defects. As a first step of this study, GO was prepared by the Hummers' method. PU solution, incorporated with a specific amount of GO, was constructed by electrospinning under optimum conditions. Finally, the RA release level which is the important parameter in orthopedic tissue regeneration was investigated. The combinational effect of GO and RA in osteogenic expression is the strength of this study.

2 Materials and methods

2.1 Materials

Polyurethane (PU, Estane 5701) was purchased from the BF Goodrich Chemical. Ltd. (Kallo, Belgium). Tetrahydrofuran (THF), dimethylformamide (DMF) and hydrochloric acid (HCL) were purchased from Merck Co. Ltd.

(Darmstadt, Germany). Graphite flakes (+100 meshes), sulfuric acid (H_2SO_4 , D=1.840 g/ml), potassium permanganate (KMnO_4), hydrogen peroxide solution (H_2O_2) were purchased from Sigma Co. Ltd. (MO, USA). The RA was gift sample from Daroo-Pakhsh Co. Ltd. (Tehran, Iran). All chemicals were used directly without further purification.

2.2 GO synthesis

GO was synthesized by using the Hummers' method (20). Briefly, the oxidation of graphite flakes was followed by H_2SO_4 and KMnO_4 under continuous stirring for 3 days. After the color of the solution changed to dark brown, H_2O_2 solution was added and the color changed to yellow which is the sign of high oxidation level. The washing process was carried out using decantation of the supernatant with a centrifugation technique. So, the products were filtered and washed by 1 M of aqueous HCl solution and deionized water until the pH was 7. Finally, the solid was lyophilized at temperature of -58°C and pressure 0.5 torr for 72 h.

2.3 Preparation of nanocomposite fibers

The polymeric solutions were prepared by dispersion of 1.5 (%.wt) GO in a mixture of DMF:THF (1:1) with 1 h Ultrasonication (WUC-D10H, WISD Co, Dihan, South Korea). PU grains were dissolved in suspension with a concentration of 8 (w/v %) for electrospinning (Full Option Lab ES, Nano Azma Co, Iran). The solutions were magnetically stirred for 12 h at room temperature to obtain a homogeneous composite solution. After that, 0.5 (%.wt) RA was added to the solution with optimum concentration of polymers and no light radiation. Composite solutions were delivered in a plastic syringe which was fitted with a stainless steel needle (25 gauge) and had an injection rate of 0.2 ml/h. The needle tip of the syringe was connected with the high voltage power supply with the applied voltage of 12 and 10 kV. Nanofibers were simply collected on collector in the form of a flat plate which was kept at a distance of 20 cm from the needle tip. All samples were dried overnight under vacuum at room temperature. Table 1 shows the composition and condition of prepared fibers.

Table 1: Composition and condition of prepared fibers.

Codes	Composition	PU (%w/v)	GO (%.wt)	RA (%.wt)	Voltage (kV)
P812	PU-RA	8	1.5	0.5	12
P810	PU-RA	8	1.5	0.5	10
PG810	PU-graphene oxide-RA	8	1.5	0.5	10

2.4 Characterization of GO and composite scaffolds

2.4.1 Morphology observation

The morphological characteristics of the GO- and RA-loaded PU-GO nanofibrous scaffolds were observed by scanning electron microscopy (SEM, Stereoscan S 360, Leica, Cambridge, England) at an accelerating voltage of 20 kV. All samples were coated with a thin layer of gold in double 30 s consecutive cycles at 45 mA to reduce charging and produce a conductive surface.

The size distribution of the fiber was determined by SEM micrographs of polymeric fibers and software image measurement (KONK Image Measurement Light, Edition 11.2.0.0); therefore, five images with same magnification (5000×) from different parts of the samples were prepared, and at least 25 measurements were performed to examine size distribution.

2.4.2 Fourier transform infrared spectroscopy (FTIR)

Chemical characteristics of raw materials and composite scaffolds were evaluated by the Fourier transform infrared spectrophotometer (FTIR, Nicolet Is10, Thermo Fisher Scientific, MA, USA). So, 1 mg of the samples (powder) was completely mixed with 300 mg of KBr and pelletized under vacuum. Then, pellets were analyzed between 400 and 4000 cm^{-1} with a resolution of 4.0 cm^{-1} and eight scans (21).

2.4.3 X-Ray diffraction pattern (XRD)

The phase composition of the GO was determined using an X-ray diffractometer (XRD, Philips, PW3710 with Cu-K α radiation, PANalytical Co., Almelo, Netherlands) at 40 kV and 30 mA. XRD diagrams were constructed from 2 θ data in the range of 0–80°.

2.4.4 Mechanical strength

Mechanical properties of RA-loaded PU-GO nanofibrous scaffolds were evaluated by a tensile strength test system (STM 20, Santam Co., Tehran, Iran) by using a 100N load cell under a crosshead speed of 10 mm/min (22). The scaffold membranes were cut into strips (80 × 20 × 0.2 mm³) for the tensile tests. Each experiment was repeated five times to calculate the average and standard deviations.

2.4.5 Hydrophilicity evaluation

The wettability of the PU, PU-GO nanofibrous scaffolds was characterized by water contact angle analysis, and measured using the sessile drop method at room temperature (DSA 100, KRÜSS GmbH Co., Hamburg, Germany). Five samples were used for each test. The average value was reported with standard deviations.

The phosphate buffered saline (PBS, pH = 7.4) absorption capacity of the nanofibers was calculated after immersing constructs with a diameter of 3 × 3 cm² in 45 ml PBS at 37 ± 0.5°C. So the dry weight (W_0) of samples was determined and dipped in PBS for 2, 4 and 6 h. Then, at each measurement the specimens were weighed in wet conditions (W). The swelling ratio was calculated according to equation 1 (23). Each of the swelling experiments were repeated five times to calculate the average and standard deviations.

$$\text{Swelling ratio (\%)} = [(W - W_0) / W_0] * 100 \quad [1]$$

2.4.6 Hydrolytic biodegradation

The hydrolytic biodegradation rate was determined using the following procedure: the dry weight (W_0) of constructs with a diameter of 3 × 3 cm² was measured before immersing in 45 ml PBS at 37°C with a rotational speed 30 rpm (Thermoshaker, LS-100, Thermo Scientific, USA). So, the samples were placed in PBS for 24, 72, 144, 216 and 288 h to determine the time-dependent degradation behavior of samples. At the end of each time point, the sample was washed with distilled water, freeze-dried (temperature about –58°C and pressure 0.5 torr for 24 h) and weighed (W), while the PBS solution was updated every 2 days. The biodegradation of the samples were calculated by using equation 2 (24). Each of the biodegradation experiments were repeated five times to calculate the average and standard deviations.

$$\text{Biodegradation ratio (\%)} = [(W - W_0) / W_0] * 100 \quad [2]$$

2.4.7 Release behavior

The actual drug content of the RA loaded nanofibers was measured by using a UV-Vis spectrometer at a wavelength of 350 nm (WPA biowave II, Biochrom, Cambridge, UK) after dissolving constructs in DMF:THF (1:1). Ten milligrams of scaffolds with retinoic acid were immersed in 2 ml PBS:ethanol (50:50) at 37°C and rotational speed 30 rpm (25). The concentration of RA in media was evaluated

during 1, 3, 6, 9, 24, 72 and 96 h using a UV-Vis spectrophotometer at 350 nm; in addition, PBS:ethanol was refreshed at the mentioned time points. Blank solution was PBS: ethanol media. PU-GO scaffolds without RA were soaked in this media. This experiment was repeated for five samples to calculate average and standard deviations (26).

2.5 *In-vitro* behavior

2.5.1 Cell culture

To investigate cellular behavior and their interaction with composite samples, 1×10^4 of a human osteosarcoma cell line (MG-63, Pasteur Institute of Iran) were seeded within each sterile sample (1 cm^2) (27) and immersed in Dulbecco's Modified Eagle Medium (DMEM) with phenol red supplemented with 10% (v/v) fetal bovine serum (FBS), 1% penicillin-streptomycin (all from Gibco-BRL, Life Technologies, Grand Island, NY, USA) for 24 h at 37°C , 5% CO_2 and 95% humidity. Cells seeded on cell culture plates without scaffold were also maintained as controls.

2.5.2 Cell attachment

The cell-scaffold interaction was evaluated after 1 day of cell culture. Consequently, cell-loaded samples were washed with PBS twice, fixed with 2.5% glutaraldehyde solution for 2 h at ambient temperature. Scaffolds were dehydrated through ethanol solutions of ascending concentrations (i.e. 30, 50, 70, 90, and 100%) for about 20 min; then, constructs were left to dry in air to be ready for SEM assessment.

2.5.3 MTT assay

The cell viability of the nanofibrous scaffolds were investigated using the MTT assay. So, after cell culture, the scaffolds immersed in culture medium for 24 h and MTT assay followed by extract and MG-63 cells for 3 days. Finally, cell viability in different concentration of extract were determined and compared with the cells cultured in medium without sample that served as control (100% cell viability).

2.5.4 Alkaline phosphatase (ALP) assay

To determine ALP activity, 5×10^4 MG-63 cells per well (28) were seeded on scaffolds as described above. Lysing the cells followed by 0.1% Triton X-100 to the scaffolds and

freeze-thawing at 37°C to evaluate ALP activity at days 1 and 4 according to the MAN company instructions. The lysis cells was incubated with p-nitro phenyl phosphate (PNPP) solution at 37°C for 30 min and stopped with NaOH (1N). ALP activity was determined at 405 nm.

2.6 Statistical analysis

Data were processed using Microsoft Excel 2013 software and the results were presented as mean $\pm$ standard deviation of at least five experiments. Significance between the mean values was calculated using standard software program (SPSS GmbH, Munich, Germany) and $p \leq 0.05$ was considered significant.

3 Results and discussion

3.1 Physicochemical characterization

3.1.1 Morphology observation

GO was produced using the Hummers' method and SEM micrographs (Figure 1C) indicate a formation of nanosheets with a lateral diameter of 30 nm. PU fibers were fabricated using the electrospinning technique (Figure 2C) with different concentrations in range of 6–10 (%wt) and preparing 8 (wt.%) PU solution led to fabrication of a bead free microstructure that was introduced as the optimum concentration. The morphology of PU and PU-GO nanofibers are shown in Figure 1A, B and D. Figure 1A belongs to a higher applied voltage (12 kV) in the electrospinning process compared with 10 kV in Figure 1B and D. SEM micrographs exhibited a randomly oriented, 3D framework and an interconnected porous microstructure that simulated native ECM. According to the SEM images, all the fibers were smooth and relatively uniform. Also, GO nanosheets showed good distribution in the PU solution but due to lower lateral diameter of sheets compared with fibers, they were embedded along the nanofibers. As can be seen in Figure 1E, applying lower voltage (10 Kv), results in the formation of fine fibers ($282 \pm 85 \text{ nm}$), while thick fibers ($799 \pm 86 \text{ nm}$) are dominant in higher voltages of electrospinning. The important role of GO in fabrication of ultra-fine nanofibers with an average diameter of $232 \pm 90 \text{ nm}$ should not be ignored. This phenomenon can be affected by a reduction in the viscosity of the solution.

Figure 1: SEM micrographs (A–D) and average fiber diameter (E) of electrospun scaffolds (a: P812, b: P810, c: GO, d: PG810). Both lower voltage in the electrospinning technique and the addition of GO produced thinner fibers are used. There are no significant differences between diameters of fibers.

3.1.2 FTIR spectroscopy

FTIR spectra of synthesized GO and RA-loaded PU-GO scaffolds and the schematic of chemical molecular structure and electrospinning process are illustrated in Figure 2A–C. For pure GO, an intense OH peak is represented at 3413 cm^{-1} , C=O peak is related to carboxylic acid and carbonyl functional groups are indicated at 1718 cm^{-1} . Hydroxyl and ether groups demonstrated at 1413 and 1220 cm^{-1} , respectively. The peak at 1612 cm^{-1} is assigned to adsorption of water molecules (14). The characteristic absorptions peaks of the PU are observed at 3320 cm^{-1} (N-H stretching frequency), $2835\text{--}2954\text{ cm}^{-1}$ ($\text{-CH}_2\text{-}$ and -CH_3 stretching frequencies), 1731 cm^{-1} (carbonyl urethane stretching), 1526 cm^{-1} (CHN vibration), 1223 cm^{-1} (coupled C-N and C-O stretching), and 1079 cm^{-1} (C-O stretching) (29). Hydrogen bonding between PU and GO shifted the peak around 3320 cm^{-1} to a higher wave number that shows interfacial interactions between PU and GO. As shown in Figure 2A, specific (C-O stretch vibration) absorbance around 1247 cm^{-1} is attributed to

retinoic acid. Also, the carbonyl group of this growth factor is distinguished at 1690 cm^{-1} (30).

3.1.3 XRD pattern

Evaluating the structure of GO is possible by determining the distance between layers. The XRD pattern of synthesized graphene oxide in a range of $5^\circ\text{--}75^\circ$ (Figure 2B) represented the characteristic peak of graphite at 26.58° disappeared as Fu et al. (31) noted. Diffraction peak at 9.98° indicated the distance between graphene oxide layers (32).

3.1.4 Mechanical strength

The tensile strength and modulus of nanofibers indicates the effect of the incorporation of GO into scaffolds on the mechanical behavior of the resultant constructs. Figure 3 shows a significant enhancement in both strength and modulus after the addition of GO owing to the considerable stiffness of GO. This result is consistent with Jing et al.'s achievements in the improvement of the strength by increasing the amount of GO in the thermally induced and electrospinning PU scaffolds (15, 33). The tensile strength were 7.5 ± 1.06 and 23 ± 4.9 MPa and the tensile modulus was 2.5 ± 1.7 and 14 ± 1.06 MPa for PU and PU-GO nanofibrous scaffolds, respectively. So the addition of 1.5 %wt GO created about three times higher tensile strength and five times higher tensile modulus. In fact, the intrinsic properties of GO nanosheets, stability of GO in suspension and interfacial interactions between GO and PU have an effect on the enhancement of the mechanical behavior (15). These results confirmed that incorporation of GO in nanofibers acts as a good reinforcement owing to the interfacial interactions between GO sheets and PU molecules and its influence on stress transfer. Hence, scaffolds are promising in providing a balance between the fiber diameter and their effect on the mechanical strength.

3.1.5 Hydrophilicity evaluation

Wettability of polymeric scaffolds plays a serious role in tissue engineering studies and enhances cellular interactions such as adhesion, proliferation and differentiation. The water drop contact angle values of PU and PU-GO nanofibers measured 129.167° and 101.679° , respectively (Figure 4A). The results reveal that addition of GO to PU solutions decreased the superhydrophobicity nature of PU nanofibers slightly because of the considerable density of hydroxyl and carboxyl functional groups on GO. According

Figure 2: FTIR spectrum of composite scaffolds (A), XRD diffraction pattern of GO (B) and schematic of the molecular structure of materials and the electrospinning process (C).

Figure 3: Tensile strength and modulus of PU and PU-GO fibers. Addition of GO improved both tensile strength and tensile modulus (* $p \geq 0.01$).

to Figure 4A, the swelling ratio of nanocomposite samples follow obtained consequences of contact angle measurements. Not only did absorption of scaffolds increase as a function of time until 6 h, but also the addition of GO enhanced this parameter compared with constructs free of GO. The amount of PBS absorption increased from

160.12 ± 14 to 265.45% ± 23% for PU and PU-GO, respectively, that proved improvement of absorption.

3.1.6 Hydrolytic biodegradation

The hydrolytic biodegradation of PU fibers investigated in this study was used to evaluate time-dependent stability of scaffolds. The mass loss during 24, 72, 144, 216 and 288 h was observed to be 2 ± 1.4, 6.5 ± 2.1, 8.59 ± 0.7, 9.11 ± 1.56 and 9.83% ± 0.9% for PU and 6.44 ± 2.2, 9.01 ± 1.11, 11.76 ± 2.45, 14.73 ± 0.88 and 15.5% ± 1.99% for PU-GO scaffolds, respectively (Figure 4B). By-passing the incubation time, the surface of 3D fibers will become rougher and finally degradation of polymer chains will be followed by breaking apart the fibers. High surface area in electrospinning fibers causes more hydrolysis places to show the positive effect on enhancement of biodegradation rate even though superhydrophobicity of PU tends to reduce this rate. Owing to superhydrophobicity the PU scaffolds exhibited a low biodegradation rate after 12 days. However, the addition of GO improved the mass loss rate that was affected by the improvement of wettability.

Figure 4: PBS absorption ratio of electrospinning fibers after 2, 4 and 6 h and wettability of PU and PU-GO scaffolds (A), hydrolytic biodegradation of fibers during 24, 72, 144, 216 and 288 h (B). Increasing GO enhanced wettability, absorption and biodegradation behavior as a function of time (* $p \geq 0.01$).

3.1.7 Release behavior

RA-loaded samples demonstrated a strong effect on osteogenic induction based on other investigations (17–19). Consequently, it is expected that this growth factor would be useful for hard tissue engineering applications. So the release behavior of RA from biodegradable scaffolds should be discussed. The RA release level and its calibration curve are represented in Figure 5. Results indicate the concentration of RA increased in the release medium as a function of time. Until 96 h the maximum amount of released RA was 24.66 ± 2.8 and $48\% \pm 0.8\%$ for PU and PU-GO randomly-oriented scaffolds, respectively. Therefore, it showed that GO has an undeniable role in increasing the release rate by the time. Based on this analysis, the *in-vitro* release mechanisms of nanofibrous constructs are a combination of diffusion and degradation in PBS:ethanol medium. The incorporation of GO in polymeric scaffolds have a great impact on the RA release level that is indirectly effected by improvement of wettability and a higher swelling ratio. Moreover, increasing the biodegradation rate in PU-GO nanofibers leads to an increased rate of release in comparison with PU samples.

Figure 5: RA release behavior of both PU and PU-GO fibers during 96 h and the calibration curve of RA.

Addition of GO increased the release ratio due to the enhancing swelling and biodegradation rate that are main mechanisms of release in RA-loaded fibers (* $p \geq 0.01$, ** $p < 0.01$).

3.2 In-vitro behavior

3.2.1 Cell-scaffold interaction

The attachment of MG-63 cells to PU-GO scaffolds after a 24-h culturing in the presence and absence of RA is shown by SEM micrographs in Figure 6A and B. The SEM micrographs illustrated that scaffolds support cellular adhesion well and the number of attached cells increased in RA-loaded constructs (Figure 6B) compared with PU-GO fibers (Figure 6A). Results confirmed the positive effects of RA on the improvement cellular density and spreading. Based on the statement of Jing et al. (15), lower concentration of GO may have been helpful for cellular attachment. The addition of GO to the composite solution makes the scaffolds suitable for cellular spreading by supplying the required hydrophilicity (15).

Dehydrogenase activity of mitochondria followed an indirect MTT assay after a 3-day analysis in different concentrations of extract. Figure 6C demonstrates cellular viability of PU-GO and PU-GO:RA scaffolds. According to the results, lower concentrations of extracts had more biocompatibility, illustrated by the increasing number of viable cells. In spite of the fact that RA-loaded scaffolds decreased the number of viable cells slightly especially in higher concentrations, all the samples are biocompatible and support the viability due to survival of more than 85% cells, as in a similar study (34). Cellular viability was found to be highest in cell culture plates and was used as a control group.

MG-63 cells seeded on PU-GO fibrous scaffolds in the presence and absence of RA were analyzed for ALP activity

Figure 6: SEM micrographs of MG-63 cells attachment on PU-GO (A) and RA-loaded PU-GO (B) nanofibrous scaffolds after 24 h, results of the MTT assay (C) after 72-h alkaline phosphatase activity MG-63 cells-scaffolds after 1 and 4 days (D). RA-loaded fibrous scaffolds support cellular attachment and spreading more when compared with free RA constructs. More than 85% of cells are viable in all the groups which demonstrates a biocompatible nature. Expression of ALP indicated that scaffolds are able to induce osteogenic behavior (* $p \geq 0.01$, ** $p < 0.01$).

that is the important marker in osteogenic process. In this study lyzed cells in a cell culture medium that is free of scaffolds were used as the control group. The ALP activity of all the constructs increased up to 4 days; however, ALP activity of RA-loaded fibers with better cellular attachment exhibited an increase in both 1 and 4 days compared with free RA scaffolds and the control group. The results suggest that presence of RA in nanocomposite fibers led to higher osteogenic capability. But, the most important role of GO in osteogenic assistance which should be considered as other studies report this (35).

4 Conclusions

In the present study, RA-loaded PU-GO scaffolds were prepared using the electrospinning technique. Homogeneous and bead free microstructures were created at a concentration of 8 (%wt) and the diameter of fibers decreased by reducing the applied voltage and addition of GO. Moreover, GO enhanced the mechanical strength, wettability, water absorption, biodegradation rate and drug release

level. Biological evaluation of nanofibers demonstrated that RA-loaded constructs decreased the number of viable cells slightly; however, the viability of more than 85% cells compared with control group indicated biocompatibility of all the samples. Moreover, because of positive effects of RA on cellular spreading, the slight decrease in a number of cells can be ignored. Expression of ALP in RA-loaded samples indicated that composite scaffolds induce osteogenic differentiation. Properties of nanocomposite scaffolds indicated that they are a suitable option for *in-vivo* studies and the regeneration of tissues.

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