Polyglycerol dendrimers immobilized on radiation grafted poly-HEMA hydrogels: Surface chemistry characterization and cell adhesion

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HIGHLIGHTS

- Radiation-grafted PHEMA hydrogels have been obtained by simultaneous gamma-irradiation of LDPE and HEMA monomer.
- PGLD dendrimer was immobilized onto PHEMA for application in tissue engineering.
- The microstructural characterization of LDPE-g-PHEMA-i-PGLD by RMN, XPS, AFM and MALDI-TOF are made.
- Measurements of water uptake and contact angle of LDPE-g-PHEMA are compared to those of LDPE-g-PHEMA-i-PGLD.
- The MC3T-E1 osteoblast cell adhesion and growth on LDPE-g-PHEMA-i-PGLD were studied.

ARTICLE INFO

ABSTRACT

Radiation induced grafting of poly(2-hydroxyethylmethacrylate) (PHEMA) on low density polyethylene (LDPE) films and subsequent immobilization of poly(glycerol) dendrimer (PGLD) has been performed with the aim to improve cell adhesion and proliferation on the surface of the polymer, in order to enhance their properties for bone tissue engineering scaffolding applications. Radiation grafting of PHEMA onto LDPE was promoted by γ-ray radiation. The covalent immobilization of PGLD on LDPE-g-PHEMA surface was performed by using a dicyclohexyl carbodiimide (DCC)/N,N-dimethylaminopyridine (DMAP) method. The occurrence of grafting polymerization of PHEMA and further immobilization of PGLD was quantitatively confirmed by photoelectron spectroscopy (XPS) and fluorescence, respectively. The LDPE-g-PHEMA surface topography after PGLD coupling was studied by atomic force microscopy (AFM). The hydrophilicity of the LDPE-g-PHEMA film was remarkably improved compared to that of the ungrafted LDPE. The core level XPS ESCA spectrum of PHEMA-grafted LDPE showed two strong peaks at 286.6 eV (from hydroxyl groups and ester groups) and 289.1 eV (from ester groups) due to PHEMA brushes grafted onto LDPE surfaces. The results from the cell adhesion studies show that MCT3-E1 cells tended to spread more slowly on the LDPE-g-PHEMA than on the LDPE-g-PHEMA-i-PGLD.

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1. Introduction

The 20th century was a watershed for the development of life saving surgical procedures and techniques that also vastly improved the quality of human life. The clinical surgeries were informed by the extraordinary explosion in knowledge of human anatomy, physiology, and biology. All the knowledge was converged to a complex array of pre-existing lines of work from the interdisciplinary between the clinical medicine, engineering, and science. Actually, an intense research has been devoted to the development of scaffolds in the area of medicine that has come to known as tissue engineering.

The success of polymeric scaffolds is determined by the response it elicits from the surrounding biological environment. This response is governed, to a large extent, by the surface properties of the scaffold. Multiple approaches have been developed to provide micrometer to nanometer scale alterations in polymer surface to enable improved protein and cell interactions. Chemical modification of polymeric scaffold surfaces by radiation grafting is one of the upcoming approaches that have been employed successfully to provide cell adhesion and proliferation on polymeric scaffolds (Shariatpanahi et al., 2006; Zhu et al., 2009).

The achievements regarding cell culture on hydrogels surfaces prepared by radiation-grafting can be described as promising techniques to prepare scaffolds to tissue engineering due to their

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biocompatibility and a vast literature has been published in this field (Hoffman, 2002; Grøndahl et al., 2005; Luk et al., 2013). However, the cellular response to radiation-grafted polymer surfaces including adhesion and growth of cells cannot be controlled or modified through changes in the graft-polymer structure. Graft copolymers do not possess functional groups, other than end groups, that permit chemical modification to change their properties, thereby limiting the applications of these materials in tissue engineering.

There is a need for the development of polymer materials which include a sufficient concentration of derivatizable groups to permit the chemical modification of the polymer for different biomedical applications. This strategy is important to provide a better control of the process of cell adhesion and growth on synthetic surfaces.

Macromolecules with dendritic structure appear to be promising alternatives because of their high periphery functionality. Polymers with a dendritic (multi-level) branched architecture have been investigated extensively over the past 20 years. These fascinating macromolecules have tree-like structures, with "branches" consisting in repeating units divergently emerging from a central core (Gillies and Frechet, 2005; Svenson and Tomalia, 2005). The functional groups on dendrimer periphery make the covalent or ionic attachment of a biological molecule possible to the graft copolymer. The dendrimer-functionalized graft copolymers can be used in a wide range of biomedical applications including tissue engineering and drug delivery.

Due to their bionertia and excellent bulk properties, polyethylene (PE) are used in a wide range of orthopedic applications (García-Rey et al., 2013). However, the nonpolar nature confers to the PE surface hydrophobicity and the absence of wettability thus limiting their biomedical applications (Santavirta et al., 1993).

The improvement of the PE wettability continues to be an area of active biomedical concern and intensive research effort on the grafting of hydrophilic monomers onto polyethylene, including the grafting of poly(hydroxyethylmethacrylate) (PHEMA) hydrogels (Abdel-Bary, et al., 1995; Yue-E et al., 2003; Bhattacharya and Misra, 2004).

Over the past three decades, various research groups have focused remarkably on radiation grafting of PHEMA onto PE for preparing biomaterials with suitable biocompatibility and good mechanical properties (Cohn et al., 1984, Ferreira et al., 2012). The graft copolymerization of PHEMA will result in the incorporation of hydroxyl groups into PE polymer. Due to the high dipole and high polarizability of the O–H bond, a significant enhancement in the hydrophilic properties of PE will be anticipated. This approach follows the experimental observation that PHEMA hydrogels structures permit a water content similar to that in living tissues (Wicherle and Lim, 1960).

The present work was dedicated to the design and development of new biomaterials suitable for hard tissue implants based on poly(glycerol) dendrimers (PGLD). The study aims to the synthesis of poly(2-hydroxyethyl methacrylate) hydrogels (PHEMA) grafted onto low density polyethylene (LDPE-g-PHEMA) based polymer matrix functionalized with PGLD.

The high compatible properties of PGLD with blood tissue are an advantage in constructing scaffolds for tissue engineering (Fernandes and De Queiroz, 2009). Additionally, the spheroidal structure of PGLD which contains large amounts of peripheral groups and interior cavities are potential vectors for growth factor and ligands for adhesion receptors on cells.

The chemical strategy of this research was structured in two parts: i) the synthesis of the radiation graft copolymer LDPE-g-PHEMA and ii) the immobilization of the PGLD onto the graft copolymer for obtaining the LDPE-g-PHEMA-i-PGLD. Both surfaces, LDPE-g-PHEMA and LDPE-g-PHEMA-i-PGLD were characterized by

X-ray photoelectron spectroscopy (XPS), contact angle and atomic force microscopy (AFM). In addition, swelling degree and bioactivity tests are presented and compared in terms of the surface composition. Finally, preliminary cell adhesion and proliferations tests were performed by means of seeding MC3T3-E1 osteoblast cell on the LDPE-g-PHEMA and LDPE-g-PHEMA-i-PGLD surfaces.

2. Materials and methods

2.1. Radiation grafting of PHEMA onto LDPE films and characterization

The 2-hydroxyethylmethacrylate (HEMA) monomer was purchased by Sigma-Aldrich Co and was distilled in vacuum before using. Commercial LDPE films of 50 µm thickness supplied by Union Carbide were used as a substrate for the radiation graft polymerization. The LDPE films were cut into 2.0×2.0 cm^2 pieces, and ultrasonically cleaned twice in methanol for 5 min, and dried in a vacuum oven at 50 °C for 24 h. All other chemicals were reagent grades. The graft polymerization reaction was carried out in 100 mL glass ampoule containing a methanol (MeOH) solution of HEMA (30% w/w). HEMA grafted onto LDPE (LDPE-g-PHEMA) was synthesized by placing a LDPE film in a glass ampoule in a solution of HEMA/MeOH (30% w/w). The ampoule was degassed by bubbling nitrogen (N$_2$) for 15 min., sealed and then irradiated with a ^{60}Co γ-source at an irradiation dose of 0.5–2.0 kGy and a dose rate of 0.061 kGy.h^{-1} to give graft yields on the interval of 5–50% (w/w). The PHEMA homopolymer was removed by washing the LDPE-g-PHEMA films with 400 mL water:MeOH mixture (30:70) several times (for 1 h each) on the magnetic stirrer at room temperature (25 °C). The LDPE-g-PHEMA films were then extracted with water:MeOH (30:70) in a Soxhlet for 8 h to remove the undesirable residual monomer and PHEMA homopolymer, and finally dried under reduced pressure (10^{-3} mbar) at room temperature (25 °C). The grafting yield was determined by the percentage increase in weight of the LDPE films and the grafting process was confirmed by photoelectron spectroscopy (XPS). The grafting yield was calculated by Eq. (1).

$$Grafting(\%) = \frac{(W_f - W_i)}{W_i} 100 \tag{1}$$

where W_f and W_i are the weights of the samples before and after grafting.

The chemical compositions of the nongrafted and functionalized LDPE surfaces were determined by XPS. The XPS measurements were performed on a ESCA-36 McPherson spectrometer with a monochromatic Al Kα X-ray source (1486.71 eV photons) at a constant dwelling time of 100 ms and a pass energy of 40 eV. The samples were mounted on standard sample studied by means of double-sided adhesive tape. The core-level signals were obtained at a photoelectron takeoff angle (measured with respect to the sample surface) of 90°. The X-ray source was run at a reduced power of 225 W (15 kV and 15 mA). The pressure in the analysis chamber was kept at 10^{-7}–10^{-8} Torr during each measurement. All binding energies (BEs) were referenced to the C1s hydrocarbon peak at 284.8 eV. Surface elemental stoichiometries were determined from the spectral area ratios, after correction with the experimentally determined sensitivity factors, and were reliable to within ± 10%. The elemental sensitivity factors were calibrated with stable binary compounds of well-established stoichiometries.

For determination of equilibrium water absorbency, the LDPE-g-PHEMA graft copolymers were immersed into phosphate buffered saline solution (PBS), pH 7.0 at 37 °C for different periods of time. It was found that the equilibrium water absorbency is

achieved after 2.0 h. In order to determine the equilibrium water absorbency gravimetrically, the surface of the swollen LDPE-g-PHEMA film was wiped with filter paper in order to remove the free water and then the samples were weighed. Swelling percentage was determined by Eq. (2).

$$Swelling(\%) = \left[\left(\frac{W_s - W_d}{W_d} \right) \right] 100 \qquad (2)$$

where W_s and W_d are weights of the swollen and initial films, respectively. The swelling measurements were repeated for a total of three samples. The accuracy of the measurements was $\pm$ 3%.

The hydrophilicity/hydrophobicity of LDPE-g-PHEMA surfaces was measured by contact angles with deionized water. The contact angles were measured in air by a sessile-drop method using a home-made optical contact angle goniometer. The LDPE and LDPE-g-PHEMA films were conditioned at the equilibrium humidity of the instrument. The water contact angle was measured by putting a sessile drop (3 μL) of bi-distilled and deionized water on five different sites of air-side surface of the polymer surfaces (1×1 cm^2). Three angles were measured on each sample. This was repeated for a total of three samples.

To investigate the surface topography of LDPE modified surfaces, atomic force microscopy was used. Atomic force microscopy was performed on a commercial SPM-9500 J3, Shimadzu using optical beam deflection to monitor the displacement of a micro-fabricated silicon cantilever having a spring constant of 60 N/m. AFM was performed with silicon nitride probes mounted on cantilevers in a tapping mode to avoid surface damage. The force of interaction was approximately 10^{-9} N and the AFM images were obtained under ambient laboratory conditions (1 atm, 25 ºC).

2.2. PGLD synthesis and characterization

PGLD with generation 3 (G-3) was synthesized by step-by-step allylation and dihydroxylation reactions according to Haag et al., 2002 . The PGLD dendrimer with a number average molecular weight of 1.7 kDa and with an average of 26 hydroxyls per molecule was synthesized in a step-growth process denominated divergent synthesis. The successful synthesis of PGLD G-3 (88% yield) was confirmed by ^{1}H- NMR and ^{13}C- NMR spectroscopy. ^{1}H- NMR: (300 MHz, CD$_3$OD). Shift (δ, ppm): 4.8 (OH), 4.04 (OCH_2–CH–CH$_2$), 3.95–3.40 (CH$_2$–O–CH$_2$). ^{13}C- NMR (75 MHz, CD$_3$OD). Shift (δ, ppm): 62.9 (CH$_2$–OH, terminal unity), 82.0–81.5, 80.6–79.8, 74.7–73.9, 73.8–72.2, 71.8–70.7, 64.7, 63.1 (CH$_2$–O–CH$_2$, polyether backbone).

A Bruker MALDI-MS with time-of-flight (TOF) mass analyzer and microchannel plate detector (MCP) was used for the determination of large molecular weight PGLD dendrimer molecules. The energy source was a nitrogen laser operating at a wavelength of 337 nm and pulse width of 3 ns. The matrix used was indoleacrylic acid which was prepared by forming a saturated solution in acetone. Then a target plate was spotted with 5 μL of matrix and followed by 5 μL of dilute sample. The sample was allowed to dry completely before use. Standards were used to calibrate the instrument. The real mass of the PGLD G-3 determined from MALDI mass spectra were found to be 1592 (Na$^+$) (Fig. 1). This value was consistent with the theoretical molecular weight of PGLD G-3 (1689) reported by literature (Haag et al., 2002).

2.3. PGLD immobilization onto PHEMA hydrogels

PGLD was coupled to LDPE-g-PHEMA hydrogels using dicyclohexyl carbodiimide (DCC)/N,N-dimethylaminopyridine (DMAP) as coupling esterification methodology (Neises and Steglich, 1978). The LDPE-g-PHEMA was immersed in a 1:4 mixture of pyridine and CH$_2$Cl$_2$ containing 30 mol% of DMAP and PGLD at a concentration of

Fig. 1. Maldi-Tof mass spectra of polyglycerol G3.0 dendrimer.

0.5 M. After stirring at room temperature (25 °C) for 6 h, the LDPE-g-PHEMA containing the coupled PGLD (LDPE-g-PHEMA-i-PGLD) was transferred into a solution of water/pyridine (1:1 v/v) and maintained for 24 h. The LDPE-g-PHEMA-i-PGLD was washed with NaHSO$_4$ (1 M), then Na$_2$CO$_3$ (10% w/w) and finally, with saturated NaCl solution to remove pyridine and DMAP. The esterification reaction between LDPE-g-PHEMA and PGLD was accomplished by fluorescence measurements (Varian Cary Elipse). Previously to the immobilization onto LDPE-g-PHEMA chains, PGLD was labeled with rhodamine B by reacting the PGLD G-3.0 with 8 equiv of rhodamine B isothiocyanate (6.0×10^{-5} mol) (Márquez and Sabater, 2005). The labeled PGLD was purified by dialysis until no traces of free dye were detected by HPLC. Fig. 2 illustrates the reaction scheme of the PHEMA radiation grafting onto LDPE and PGLD immobilization onto LDPE-g-PHEMA surface.

2.4. Cell adhesion

MC3T-E1 osteoblast cell adhesion on LDPE-g-PHEMA and LDPE-g-PHEMA-i-PGLD was analyzed by neutral red assay after 2, 4, 6 and 24 h. MC3T3-E1 cells, an osteoblastic cell line established from normal mouse calvaria, were grown in a α-modified minimum essential medium (α–MEM; 1.8 mM Ca^{2+}, 0.81 mM Mg^{2+}, 1.0 mM H$_2$PO$_4$, 50 μg/mL ascorbic acid) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin (GIBCO-BRL) in 5% CO$_2$ at 37 °C. The medium was changed twice weekly. After 7 day culture, the MC3T3-E1 cells were removed from the culture dish using a trypsin solution (0.25% trypsin, 0.05% EDTA, 0.1% glucose in PBS), centrifuged and resuspended in DMEM medium to adjust the cell density to 2.0×10^5 cells/mL. A volume of 100 μL of the cell suspensions was placed on LDPE-g-PHEMA and LDPE-g-PHEMA-i-PGLD. Tissue culture polystyrene commercial 24-well cell culture plates were used as control. To access the number of viable adherent cells on LDPE-g-PHEMA and LDPE-g-PHEMA-i-PGLD substrates neutral red assay (NR) was performed. At the end of cell incubation time at 37 °C the medium was removed and replaced with 200 μL/well of NR solution (0.4% w/v in water). After the reaction time, the medium was discarded and the cultures were washed twice with PBS. The cells were lysed with 1% (w/v) acetic acid in 50% ethanol solution for 10 min to
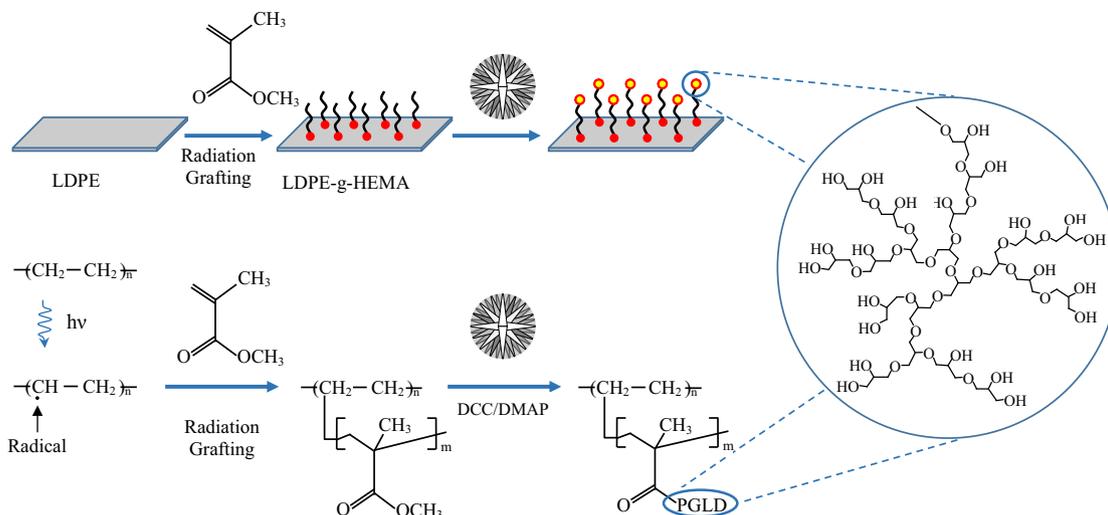
Fig. 2. Illustration of the radiation grafting of the LDPE-g-PHEMA-i-PGLD synthesis.
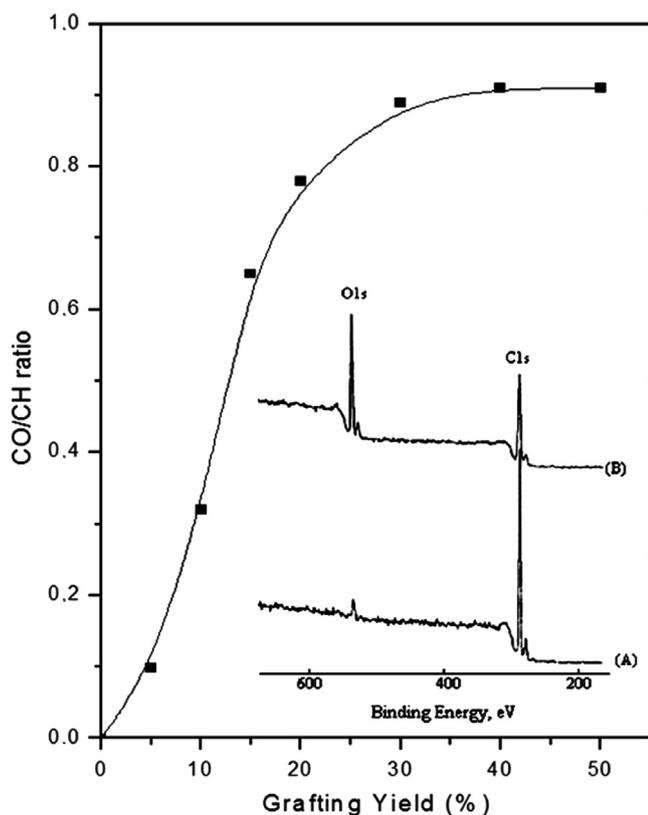

Fig. 3. CO/CH ratio for LDPE-g-PHEMA obtained from XPS spectra (inside) of LDPE (A) and LDPE-g-PHEMA (B). Grafting yield: 50% (w/w).
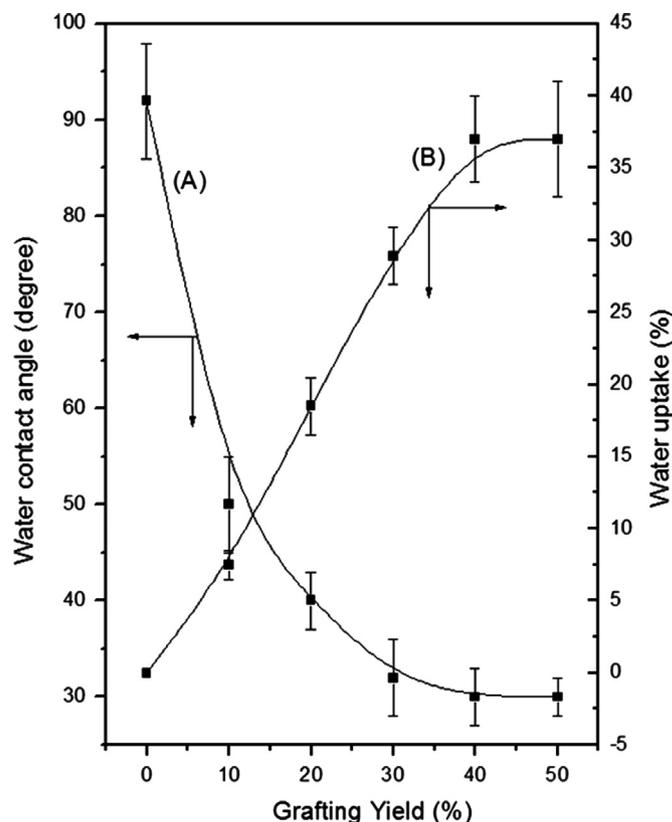

Fig. 4. Water contact angle measurement (A) and water uptake (B) of the grafted LDPE films as a function of grafting yield (%).

release the dye. The lysates were collected and the optical density was measured (540 nm).

3. Results and discussion

In order to verify the presence of PHEMA on LDPE surfaces, the XPS analysis was carried out. LDPE and LDPE-g-PHEMA surfaces were characterized by XPS (Fig. 3). The high resolution C_{1s} and O_{1s} core levels were taken on LDPE-g-PHEMA surface. After the PHEMAgrafting on LDPE, the oxygen atomic percent of LDPE-g-PHEMA increased at the expense of a decrease in the carbon atomic percent. Using the peak fit software available with the XPS instrument; these two peaks can be analyzed. As can be seen in Fig. 3 the values of the ratios CO/CH peaks of different grafting yields were calculated and plotted against the degree of grafting. It can be seen that ratios of the CO/CH peaks increased with the grafting yield and level off at a 50% (w/w) grafting yield. This means that the distribution of grafted chain in the surface increased with increasing grafting yield. At saturated level of CO/CH ratio, the distribution of PHEMA grafted chain may be interpreted as homogeneous on the surface of the polymeric matrices.
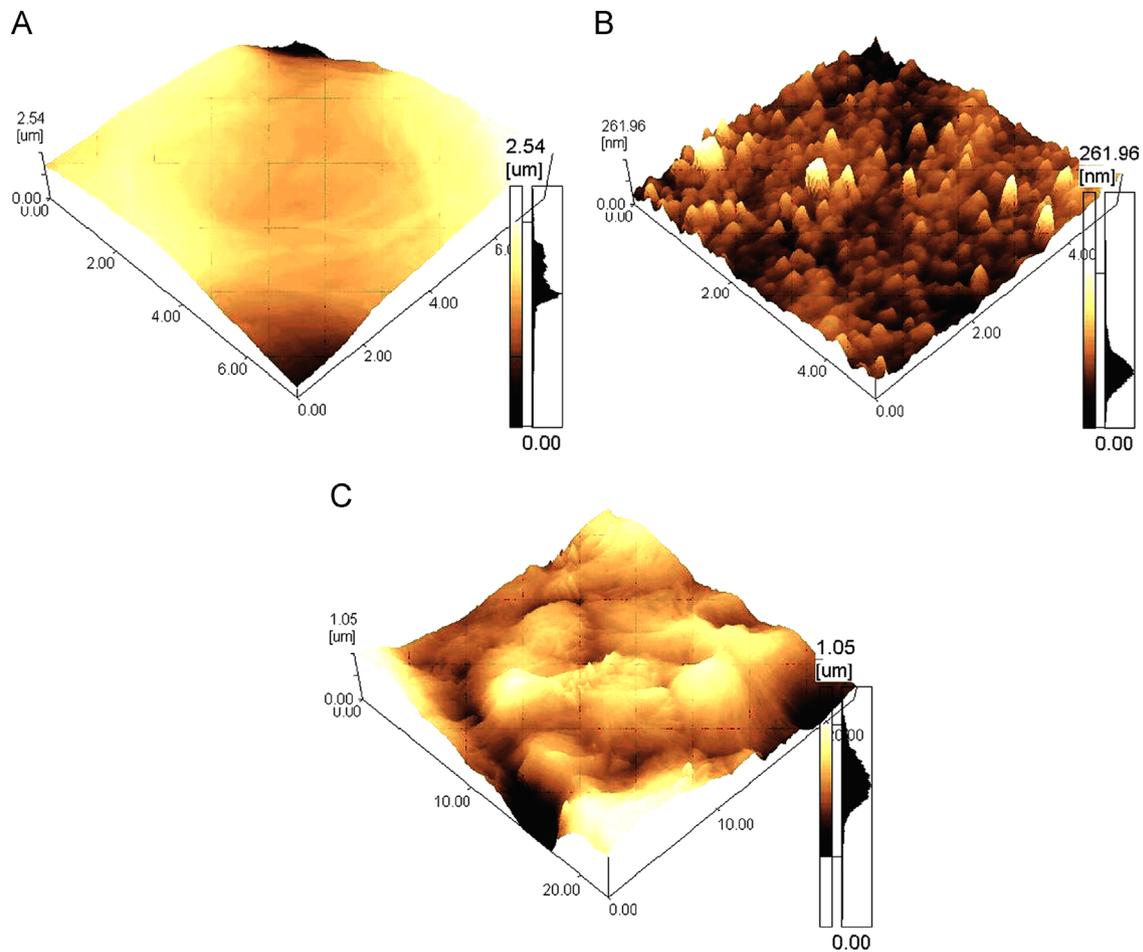
Fig. 5. AFM images of LDPE (A), LDPE-g-PHEMA (B) and LDPE-g-PHEMA-i-PGLD (C) surfaces.

Fig. 4 shows the influence of grafting yield on the water contact angle and water uptake of the final grafted LDPE. The water contact angle decreased sharply from 92° to 42° at grafting yield of 30% (w/w) and then decreased slowly to a constant value of 32° at grafting yield of 50% (w/w). The water uptake increased correspondingly. The reduction of water contact angle is certainly caused by the introduction of PHEMA. Therefore, it can relatively reveal the amount of PHEMA on LDPE surface. Surfaces with contact angles in the range of 20–60° are generally considered to be hydrophilic and show more resistance to protein adsorption (Andrade and Haldy, 1986).

Dendrimer macromolecules are open and soft material rather than rigid and due to this reason they tend to deform on surfaces. The electrostatic interactions between the brushes of grafted PHEMA on LDPE surfaces and the surface groups of PGLD may produce deformations on immobilized dendritic macromolecule (PGLD).

Fig. 5 shows the AFM images of control LDPE, LDPE-g-PHEMA and LDPE-g-PHEMA-i-PGLD, respectively. For control LDPE film (Fig. 5-A) the surface is relatively smooth, whereas the LDPE-g-PHEMA exhibits the micro scale holes and values (Fig. 5-B). The globular structures (Fig. 5-C) could be attributed to the individual separated grafted PHEMA chains changing the LDPE surface morphology suggesting the existence of specific interactions occurring between the grafted PHEMA brushes and the adjacent PGLD molecules. These interactions could exhibit a wide variety of conformations, including collapsed and expanded PHEMA brushes. After immobilization of PGLD, the film surfaces demonstrate the large-scale globular structures (Fig. 5-C) caused from the pattern PGLD, which is in the regular chemical structure (Li et al., 2000).

The results from the MC3T3-E1 osteoblast cell adhesion tests are shown in Fig. 6. After cultivation on LDPE-g-PHEMA and LDPE-g-PHEMA-i-PGLD for 1 h, only 20% of MCT3T3-E1 cells were judged as spread on the polymer surfaces. During the next few hours, however, the percentage of spread cells increased rapidly from 20% to 80%, and all cells finished spreading within 24 h (Fig. 6). The LDPE-g-PHEMA films were found to be resistant to cell adhesion as compared to LDPE-g-PHEMA-i-PGLD films (Fig. 6). A further interesting possibility for the PGLD-g-PHEMA-i-PGLD would be to immobilize on dendrimer surface certain growth factors (osteogenic protein-1, BMP-2 or IGF-1/TGF-b1), which are known to stimulate bone formation could be tied to the biomaterial.

4. Conclusions

The results of this study clearly show that osteoblasts are able to grow onto LDPE-g-PHEMA-i-PGLD. The favorable cell attachment properties seems promising for the use of LDPE-g-PHEMA-i-PGLD in bone tissue engineering. Since LDPE-g-PHEMA-i-PGLD possesses multiple hydroxyl groups that are easily functionalized, it can be tailored for specific purposes. This newly developed LDPE-g-PHEMA-i-PGLD might reduce the need for autologous bone and is therefore an interesting alternative for biomaterials field. The next step will be to test the LDPE-g-PHEMA-i-PGLD in *in vivo* animal experimentation. The results of this *in vitro* study are quite promising; therefore there is a high chance that the animal experiments will also be successful.
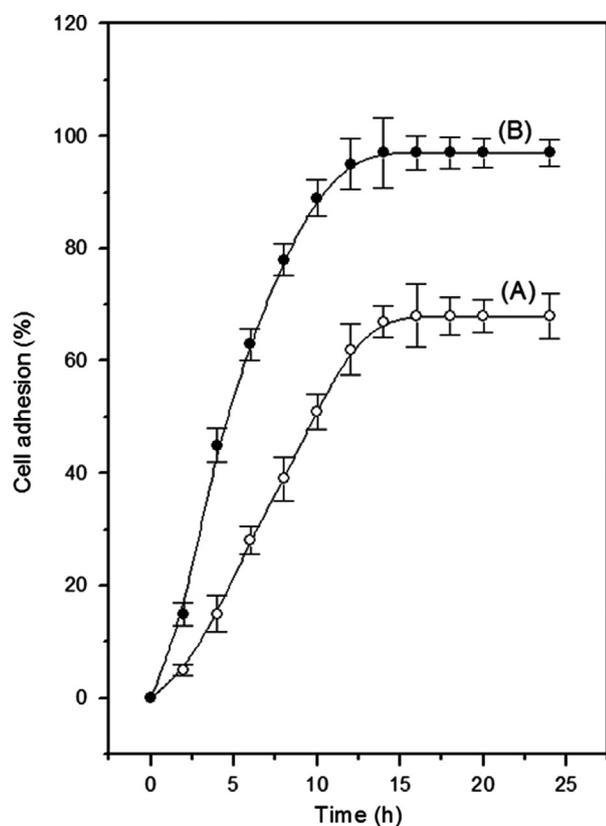
Fig. 6. MC3T3 osteoblast cell adhesion assays on LDPE-g-PHEMA (A) and LDPE-g-PHEMA-i-PGLD (B).

Acknowledgments

References

Abdel-Bary, E.M., Dessouki, A.M., El Mesr, E.M., El Miligy, A.A., 1995. Radiation-induced grafting of binary monomer (acrylic acid/2-hydroxyethyl methacrylate) onto LDPE and PP films. Polym. Plast. Technol. Eng. 34, 383–403.

Andrade, J.D., Haldy, V., 1986. Protein adsorption and materials biocompatibility. Adv. Polym. Sci. 73, 1–63.

Bhattacharya, A., Misra, B.N., 2004. Grafting: a versatile means to modify polymers: techniques, factors and applications. Prog Polym. Sci. 29, 767–814.

Cohn, D., Hoffman, A.S., Ratner, B.D., 1984. Radiation-grafted polymers for bioma-terial applications. I. 2-hydroxyethyl methacrylate: ethyl methacrylate grafting onto low density polyethylene films. J. Appl. Polym. Sci. 29, 2645–2663.

Fernandes, E.G.R., De Queiroz, A.A.A., 2009. A bioconjugated polyglycerol dendri-mer with glucose sensing properties. J. Mater. Sci. Mater. Med. 20, 473–479.

Ferreira, L.M., Leal, J.P., Rodrigues, P.A., Alves, L.C., Falcão, A.N., Gil, M.H., 2012. Characterization of PE-g-HEMA films prepared by gamma irradiation through nuclear microprobe techniques. Radiat. Phys. Chem. 81, 1319–1323.

García-Rey, E., García-Cimbrelo, E., Cruz-Pardos, A., 2013. New polyethylenes in total hip replacement: a ten- to 12-year follow-up study. Bone Jt. J. 95-B, 326–332.

Gillies, E.R., Frechet, J.M.J., 2005. Dendrimers and dendritic polymers in drug delivery. Drug Discov. Today 10, 35–43.

Grøndahl, L., Chandler-Temple, A., Trau, M., 2005. Polymeric grafting of acrylic acid onto poly(3-hydroxybutyrate-co-3-hydroxyvalerate): surface functionalization for tissue engineering applications. Biomacromol. 6, 2197–2203.

Haag, R., Sunder, A., Hebel, A., Roller, S., 2002. Dendritic aliphatic polyethers as high-loading soluble supports for carbonyl compounds and parallel membrane separation techniques. J. Comb. Chem. 4, 112–119.

Hoffman, A.S., 2002. Hydrogels for biomedical applications. Adv. Drug Deliv. Rev. 54, 3–12.

Li, J., Piehler, L.T., Qin, D., Baker, J.R., Tomalia, D.A., 2000. Visualization and characterization of poly(amidoamine) dendrimers by atomic force microscopy. Langmuir 16, 5613–5616.

Luk, J.Z., Cooper-White, J., Rintoul, L., Taran, E., Grøndahl, L., 2013. Functionalised polycaprolactone films and 3D scaffolds via gamma irradiation-induced graft-ing. J. Mater. Chem. B 1, 4171–4181.

Márquez, F., Sabater, M.J., 2005. Emission frequency modulation by confinement effect: rhodamine-B incorporated within a dendritic structure. Phys. Scrip. T118, 190–192.

Neises, B., Steglich, W., 1978. Simple method for the esterification of carboxylic acids. Angew. Chem. Int. Ed. Engl. 17, 522–524.

Santavirta, S., Nordstrom, D., Metsarinne, K., Konttinen, Y.T., 1993. Biocompatibility of polyethylene and host response to loosening of cementless total hip replacement. Clin. Orthop. Relat. Res. 297, 100–110.

Shariatpanahi, R.E., Orang, F., Emami, S.H., Naimi, T., 2006. Cell growth on tissue-engineering scaffolds prepared by gamma irradiation grafting of N-vinyl-2-pyrrolidone onto polyvinyl alcohol. J. Biomater.Sci. Polym. Ed. 17, 659–667.

Svenson, S., Tomalia, D.A., 2005. Dendrimers in biomedical application reflection on the field. Adv. Polym. Drug Deliv. Rev. 57, 2106–2129.

Yue-E, F., Tianyi, S., Wenfeng, W., Fang, S., 2003. Kinetics of radiation-induced graft copolymerization of 2-hydroxyethyl methacrylate onto polyethylene mem-branes. J. Appl. Polym. Sci. 38, 821–828.

Wicherle, O., Lim, D., 1960. Hydrophilic gels for biological use. Nature 185, 117–118.

Zhu, J., Tang, C., Kottke-Marchant, K., Marchant, R.E., 2009. Design and synthesis of biomimetic hydrogel scaffolds with controlled organization of cyclic RGD peptides. Bioconjug. Chem. 20, 333–339.