ELECTRON-BEAM-INDUCED GRAFTING OF CHITOSAN ONTO HDPE/ATZ COMPOSITES FOR BIOMEDICAL APPLICATIONS

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Introduction

Chitosan is the deacetylated polysaccharide from chitin, the natural biopolymer mainly found in shells of crustaceans. It has been widely studied for biomedical, pharmaceutical, surgical, and tissue engineering applications [1]. In bone engineering, both *in vitro* and *in vivo* assays have demonstrated that chitosan-based biocomposite scaffolds may favour tissue regeneration. Chitosan is highly biocompatible and no allergic and inflammatory reactions after topical implantation have been observed. The use of chitosan alone in scaffolds is limited by its reduced bioactivities and poor mechanical properties. Recently, radiation-induced grafting has emerged as a promising tool for improving the mechanical properties of natural polymers through the introduction of organic compounds into a polymeric matrix [2]. In some cases, the process is also able to modify the hydrophobic behaviour of synthetic polymers such as high density polyethylene (HDPE) and improve their compatibility with human tissue. Radiation-induced grafting can be an alternative to produce new HDPE hybrid materials with tunable properties as a function of the specific applications. For applications outside the biomedical field, this process was used for anchoring polymers of acrylic acid and methyl methacrylate, onto the PE surface via radical pathways using γ-ray or electron beam (EB) irradiation, followed by graft-polymerisation.

By considering that chitosan cannot be used per se as tissue analogue replacement, in this study, it was grafted onto an HDPE surface after activation via electron-beam irradiation, with the aim to obtain materials for biomedical purposes with improved biocompatibility properties. In a previous study, alumina-toughened zirconia (ATZ) was used as filler with the purpose to endow HDPE with enhanced mechanical properties [3]. In the present paper, the irradiation and grafting of HDPE/ATZ composites (weight ratio: 99/1, 98/2 and 93/7) were investigated in order to study the influence of this filler on the electron-beam irradiation and chitosan grafting reaction. Mechanical properties, wettability, cells adhesion, and viability were investigated.

Results and discussion

HDPE and its composites were EB-irradiated with a low-energy accelerator operated at 145 kV. Iterative treatments in presence of air were used to achieve the desired cumulated dose of 100 kGy. This dose was chosen because it is able to induce significant surface oxidation [4], with limited impact on PE properties (mechanical and ageing). After irradiation, the grafting process was conducted by soaking the irradiated samples into an aqueous acetic acid solution containing chitosan at 1 wt.%, under argon atmosphere, at 70 °C (pH=6). For neat HDPE, different reaction times (30 min, 1, 2, 3, 5, 7 and 15 h) were used in order to find the best grafting conditions (Figure 1). Based on the results (i.e., the lowest measured contact angle) [4], the grafting reaction for the composites was carried out for 3 h after EB irradiation at the dose of 100 kGy. Mechanical properties of irradiated materials were not remarkably affected by irradiation processing.

The ATR–FTIR spectra in different regions of infrared absorption bands before and after 3 h of grafting reaction of the composites are depicted in Figure 2. After functionalisation, the HDPE/ATZ 99/1 composite (line b) shows increased absorption bands with respect to the ungrafted counterpart (line a): this finding can be ascribed to the presence of chitosan (bands at 3398, 1650–1560, and 1080 cm^{-1}). The same bands in the HDPE/ATZ 98/2 and 93/7 composites do not increase so significantly, hence indicating that, for these composites, the functionalisation with chitosan becomes less evident [4]. Figure 3 shows cell adhesion at 10 min (A) and cell viability after 24 h (B) of untreated and chitosan-functionalised HDPE and HDPE/ATZ composites. All the composites containing ATZ particles sustain a higher number of adherent cells, with the exception of 93/7, and increased cell viability, as compared with unfilled HDPE. ATZ may drive the cell response and the simple attraction toward the surface, suggesting that the introduction of ATZ into a non-polar matrix (HDPE) is sufficient to guarantee an interaction, and thus adhesion and viability.

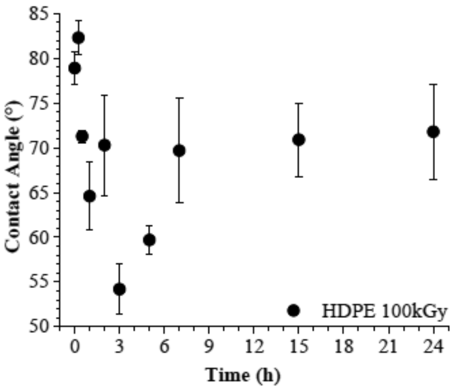

Figure 1: Contact angle values obtained for HDPE surfaces grafted with chitosan at different reaction times after irradiaton at 100kGy.

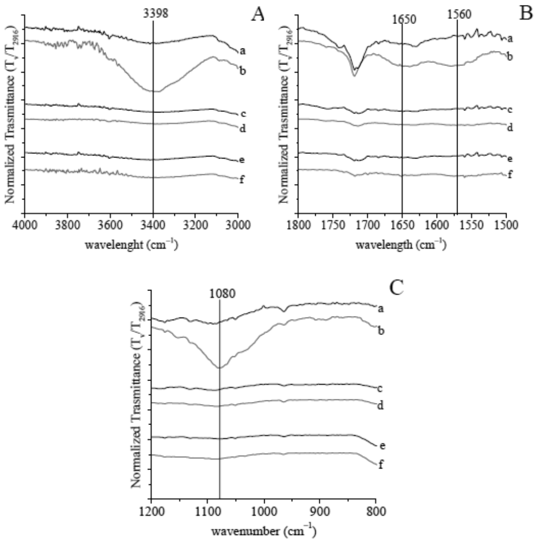

Figure 2: ATR spectra in different regions of infrared absorption bands for (a) HDPE/ATZ 99/1, (c) HDPE/ATZ 98/2, and (e) HDPE/ATZ 93/7 composites before grafting; (b) HDPE/ATZ 99/1, (d) HDPE/ATZ 98/2, and (f) HDPE/ATZ 93/7 composites after 3 h of reaction.

When the ATZ content is 1 wt.% only, the effect on cell interaction is negligible, while it becomes significant when the amount is doubled. For higher ATZ content (7 wt.%) some agglomerates appear, reducing the number of active sites exposed on the surface, then minimising the ATZ effect on the cell interaction. The presence of chitosan-functionalised surfaces further increases cell adhesion. However, cell viability after 24 h significantly decreases. This finding is consistent with the hypothesis of electrostatic interactions occurring between the negative charges of the surface of cell membranes and the $–NH_3^+$ sites on chitosan chains, which first promote interaction.

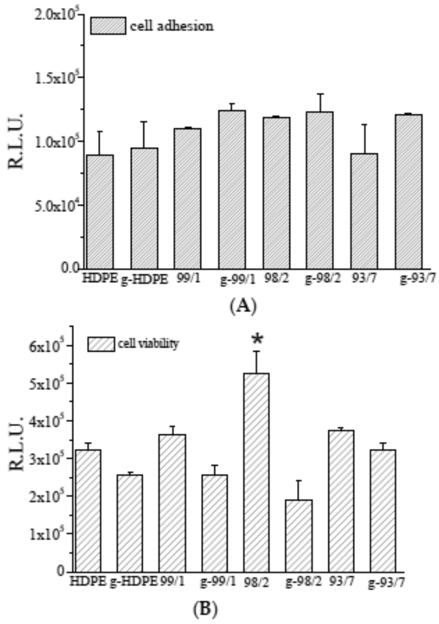

Figure 3: Adherent fibroblast per field at 10 min (A) and the viable cells after 24 h (B) of HDPE/ATZ composites grafted with chitosan.

Conclusions

In this study, chitosan was grafted onto HDPE and HDPE/ATZ composite surfaces after their activation via EB irradiation, aiming at obtaining biomaterials with enhanced biocompatibility. EB treatment was responsible for the modification of wettability, crystallinity, and nano-roughness of HDPE and its composites. The mechanical properties of irradiated materials were not remarkably affected by irradiation processing.

The presence of ATZ, at 2 wt.% or beyond, influenced both the EB irradiation process and the chitosan functionalisation reaction, reducing the oxidation sites and the presence of grafted chitosan on the surface. Biological assays indicated that the electrostatic interactions occurring between the negative charges of the surface of cell membranes and the $–NH_3^+$ sites on chitosan chains were able to promote cell adhesion, whereas the surface oxidation due to the irradiation process could induce some detrimental effects on cell viability.

References

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