Proceedings of the 50th Zakopane School of Physics, Zakopane, Poland, May 18–23, 2015

Effect of Magnetite Composite on the Amount of Double Strand Breaks Induced with X-Rays

A. Wiecheć[a,*], K. Nowicka[b], M. Błażewicz[b] and W.M. Kwiatek[a]

[a]Institute of Nuclear Physics, Polish Academy of Sciences, PL–31342 Kraków, Poland

[b]AGH University of Science and Technology, Faculty of Material Science and Ceramic, Department of Biomaterials, Kraków, Poland

The aim of this study was to find out if polylactide (PLA) modified with magnetite might affect the amount of DNA double strand breaks induced with X-rays. The human osteosarcoma cells (MG63) were seeded on the polystyrene cell culture dishes (PS), PLA and PLA modified with magnetite substrates. The double strand breaks were analyzed after X-ray irradiation (dose rate 2 Gy/min), in the first day of culturing. The number of double strand breaks increased in the PLA modified with magnetite, for example after 1 Gy of X-rays irradiation, double strand breaks/cell equaled: 24.5 vs. 17.5 and 17.3, for PLA modified with magnetite vs. PLA and PS, $p < 0.0003$. We conclude that PLA modified with magnetite changed the number of double strand breaks induced with X-rays. However, more research is needed to confirm that such composite might be considered as radiosensitizer in radiotherapy.

DOI: 10.12693/APhysPolA.129.174

PACS: 87.50.–a, 87.53.-j, 81.05.Mh, 81.05.Ni, 81.05.Pj, 81.05.Qk, 75.50.Bb

1. Introduction

Magnetite, due to the magnetic properties, can be considered as radiosensitizer in radiotherapy [1]. However, despite its non-toxic parameters for cells, it may be cumulated in some human organs, not necessarily in those that require radiotherapy [2]. Thus, the application of magnetite in composites would allow limiting particles to local destinations and also using low concentration of iron, which seems to be advantageous from the standpoint of medical applications. The aim of this study was to find out if bioresorbable polylactide (PLA) modified with magnetite might affect the radiosensitivity measured as the amount of DNA double strand breaks (DSBs) induced with X-rays in osteosarcoma cells.

2. Materials and methods

2.1. Polylactide composite modified with magnetite

The samples were produced using PLA (Ingeo 3051D) purchased from NatureWorks. The polymer was dissolved in dichloromethane (POCH) at 10 wt%. The composite material was produced by a casting method using 1 wt% of the magnetite (Sigma Aldrich). The pure polylactide was reference material. The surface state of polylactide modified with 1% magnetite was characterized by the dynamic method of wetting angle determination (static drop method) (DSA10Mk2, Kruss, Germany). The roughness was measured using a static profilometer with ceramic head manufactured from Hommel Werke Company. Magnetic measurements of composite were performed on a Vibrating Sample Magnetometer (VSM — LakeShore's 7300 model). Magnetic properties of composite and pure magnetite were compared.

*corresponding author; e-mail: Anna.Wiechec@ifj.edu.pl

2.2. DSBs detection in γ-H2AX foci assay

The human osteosarcoma cancer cells (cell line MG63, European Collection of Cell Cultures, Salisbury, UK) were seeded on: (a) polystyrene cell culture dishes (PS) (Sarstedt, Germany), (b) PLA, (c) the composite PLA with 1% magnetite. The day after seeding, MG63 cells were irradiated with X-rays (doses of: 1, 2, and 4 Gy, dose rate 2.1 Gy/min) from the X-Ray Tube MG325 (250 kV, 10 mA). Then, they were incubated for 1 h and subjected to the procedure of γ-H2AX foci immunostaining (according to [3]). The number of γ-H2AX foci per cell was counted. At least 100 cells per sample was analyzed. The experiment was repeated twice. The numbers of γ-H2AX foci were expressed as Means ± SD. The one way analysis of variance (ANOVA) was performed. The p values equal to or less than 0.05 were considered significant.

3. Results and discussion

The surface characteristics parameters confirmed biocompatibility of composite under study. The value of the contact angle for the composite was slightly higher than the value for the pure polymer ($\theta = 77.3°$ vs. $\theta = 78.7°$). This means that the composite material possessed more hydrophilic surface than pure PLA. Magnetite in the polymer slightly affected the surface roughness. The addition of magnetite to the polylactide caused changes in properties of the surface.

The roughness profile (on the base of Ra parameter — arithmetic mean deviation of the assessed profile) for the pure polymer was about 1 μm, and the composite value increased to approximately 1.5 μm (Fig. 1). Results obtained for Rz parameter indicated the increase of maximum height of the composite profile. Rt parameter (the distance between the highest and the lowest points on the surface) confirmed the highest roughness of composite in comparison to pure polylactide. The best cellular alignment was found on the 1 μm-wide polylactide substrates [4]. However, an increase of the composite roughness enhanced osteoblast's adhesion, which was observed by Stodolak et al. [5]. Pure polylactide has no magnetic properties, while the addition of magnetite gives magnetic properties of the entire composite.

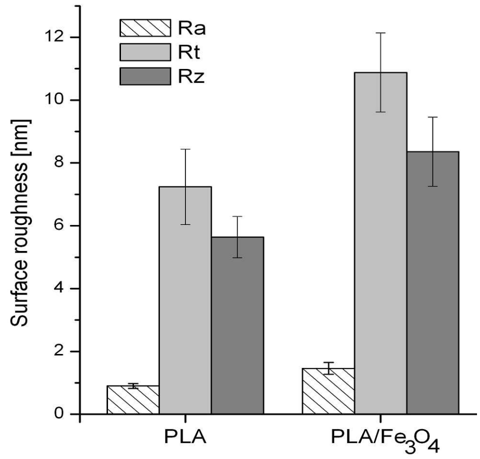
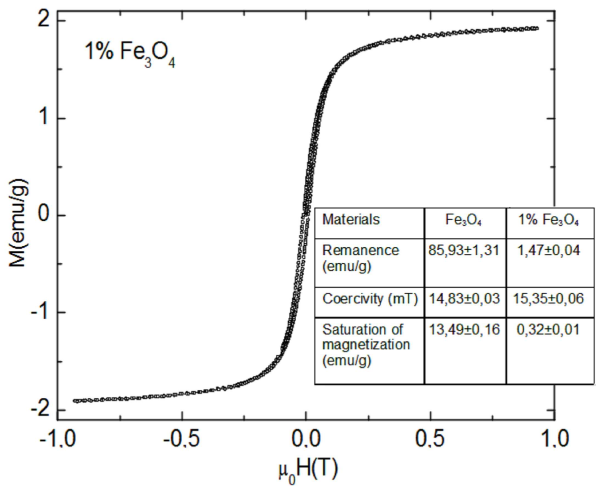


Fig. 1. Surface roughness of materials. *Ra* — arithmetic mean deviation of the assessed profile, *Rt* — total height of the profile: height distance between the deepest valley and the highest peak on the evaluation length, *Rz* — maximum height of the profile.



Materials	Fe_3O_4	1% Fe_3O_4
Remanence (emu/g)	85,93±1,31	1,47±0,04
Coercivity (mT)	14,83±0,03	15,35±0,06
Saturation of magnetization (emu/g)	13,49±0,16	0,32±0,01

Fig. 2. Hysteresis loop for composite PLA modified with 1% magnetite. Inset table shows comparison of magnetic properties of the composite vs. the pure magnetite.

Magnetic properties of applied composite and pure magnetite was compared (Fig. 2). The remanence for pure modifier stands at 85.93 emu/g while for the composite 1.47 emu/g. The coercivity was almost constant (14.83 mT for the pure magnetite and 15.35 mT for the composite). This means the external magnetic field with the same value for both the composite as well as for the pure magnetite is needed to reduce remanence to zero value. The smaller value of saturation magnetization for composite in comparison to pure magnetite indicated the magnetization at a maximum value of external magnetic field. This value is proportional to the amount of magnetite in the sample.

There was no influence of substrates on presence of DSBs in non-irradiated cells (Fig. 3). At about 3–4 γ-H2AX foci per cell were observed, meaning that applied materials were not genotoxic. However, magnetic particles enhanced number of DSBs induced by X-ray (Fig. 3). For all applied doses (1, 2, 4 Gy), the composite with magnetite statistically significantly increased the number of DSBs induced by X-rays.

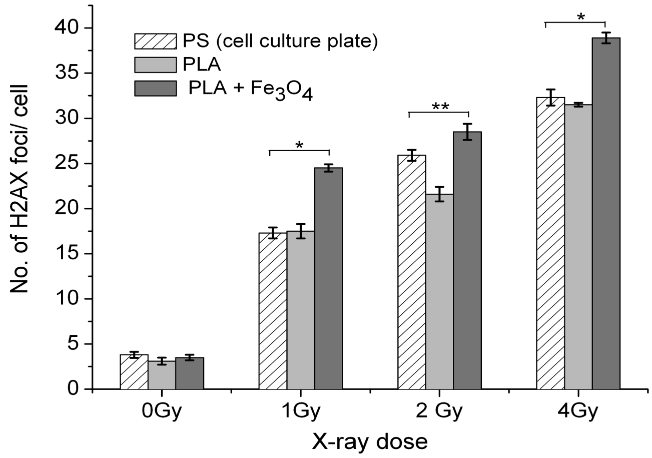


Fig. 3. Number of gamma-H2AX foci (marker of DNA double strand breaks) in MG63 cells after X-ray irradiation. Statistical signal $*p < 0.0003$, $**p < 0.02$.

We conclude that PLA modified with magnetite changed the number of DSBs induced with X-rays. However, further research is needed to confirm that such composite might be considered as radiosensitizer in radiotherapy.

Acknowledgments

This work was partially financed from statutory works of AGH University of Science and Technology (No. 11.11.160.616).

References

[1] D. Kwatra, A. Venugopal, S. Anant, *Transl. Cancer Res.* **2**, 330 (2013).

[2] A. Ruiz, Y. Hernandez, C. Cabal, E. Gonzalez, S. Veintemillas-Verdaguer, E. Martınez, M.P. Morales, *Nanoscale* **5**, 11400 (2013).

[3] J.E. Cleaver, *Photochem. Photobiol.* **87**, 1230 (2011).

[4] X.F. Walboomers, H.J.E. Croes, L.A. Ginsel, J.A. Jansen, *J. Biomed. Mater. Res.* **47**, 204 (1999).

[5] E. Stodolak, C. Paluszkiewicz, M. Błażewicz, I. Kotela, *J. Mol. Struct.* **924–926**, 562 (2009).