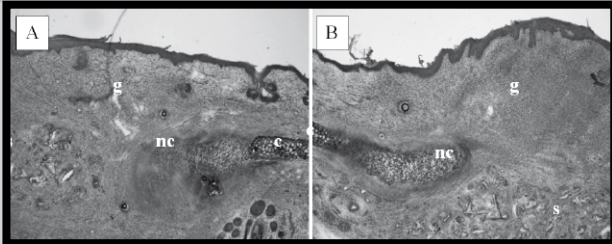


**FIG.3.** Histochemical pictures of PLG scaffolds (A) and PLG-Hyal (B) scaffolds 1 week after implantation; acid phosphatase (AcP) staining, optical microscope Olympus BH2, objective 10x; s – scaffold, arrows indicate macrophages stained in red.



**FIG.4.** Histological pictures of PLG scaffolds (A) and PLG-Hyal (B) scaffolds 4 weeks after implantation; MGG staining, optical microscope Olympus BH2, objective 4x; s – scaffold, g – granulation tissue, c – cartilage, nc – neo-cartilage regenerating towards scaffolds.

All animals survived the surgery. No wound healing complications were observed after the surgery and during the whole experiment.

For histological evaluation tissue slices containing implanted scaffolds were stained with May-Grünwald-Giemsa (MGG), an acid-sensitive histological stain enabling visualization of inflammatory cells and evaluation of degradation process of aliphatic polyesters [9]. Moreover, histochemical staining for the activity of acid phosphatase was made in order to evaluate the number of inflammatory cells and the extent of inflammation in tissues around the implants.

1 week after implantation both scaffolds were infiltrated mainly by neutrophils, e.g. cells predominant in acute inflammation. Inflammatory exudate in the implant site was observed. It was stronger in the case of PLG than in PLG-Hyal scaffolds. Macroscopically, bigger swelling was also seen around PLG implants. The activity of AcP was slightly higher in PLG-Hyal implants due to faster macrophage influx (FIG.3). It suggests, that more intense inflammation helped in tissue transformation, because it was clearly seen that PLG-Hyal scaffolds were better fixed in newly forming granulation tissue compared to PLG scaffolds. Both scaffolds were transparent and their microstructure was not changed.

4 weeks after implantation the exudate was not present and the scaffolds were well settled in granulation tissue (FIG.4). Inflammatory cells such as macrophages, mast cells, eosinophils, and numerous multinucleated foreign body giant cells (FBGC) were observed close to the implants. Slices stained for AcP showed similar activity of the enzyme in tissues with both implanted materials. Macroscopically, the ears were much thicker in the case of PLG than in the case of PLG-Hyal implants. The scaffolds changed colour to brown indicating early degradation process of PLG polymer. New cartilage tissue regenerating towards the scaffolds was clearly visible.

In conclusion, both materials developed in this study seem to be good scaffolding materials promoting regeneration of auricular cartilage, although the quickest tissue regeneration was found after implantation of PLG-Hyal.

## Acknowledgements

The authors thank Dr. P. Dobrzyński (PAN, Zabrze) for providing polymer samples, Dr. W. Ścierański (Silesian Medical University, Department of Otolaryngology) for help in implantation, M. Żolnierek (Coll Medicum UJ, Kraków) for help in biological studies, Dr. C. Paluszkiwicz (AGH-UST, Krakow) for help in FTIR measurements and B. Trybalska, (AGH-UST, Krakow) for SEM evaluation. This study was supported by AGH-UST (grant number: 10.10.160.253).9

## References

- [1] Ciorba A, Martini A, Tissue engineering and cartilage regeneration for auricular reconstruction, *Int. J. Pediatric Otorhinolaryngology* 2006;70:1507-1515.
- [2] Romo T, Fozao M, Sclafani A, Microtia reconstruction using a porous polyethylene framework, *Facial Plast. Surg.* 2000;126:538-547.
- [3] Vunjak-Novakovic G, Martin I, Obradovic B, Treppo S, Grodzinsky AJ, Langer R, Bioreactor cultivation conditions modulate the composition and mechanical properties of tissue-engineered cartilage. *J Orthop Res* 1999;17(1):130-8.
- [4] Lee C-T, Lee Y-D, Preparation of porous biodegradable poly(lactide-co-glycolide)/hyaluronic acid blend scaffolds: Characterization, in vitro cells culture and degradation behaviors *J Mater Sci: Mater Med* 2006;17:1411-1420.
- [5] Dobrzyński P, Kasperczyk J, Janeczek H, Bero M, Synthesis of biodegradable copolymers with the use of low toxic zirconium compounds. 1. Copolymerization of glycolide with L-lactide initiated by  $Zr(Acac)_4$ . *Macromolecules* 2001;34: 5090-5103.
- [6] Goldberg AF, Barka T, Acid phosphatase activity in human blood cells", *Nature* 1962;195:297.
- [7] Pamuła E, Błażewicz M, Paluszkiwicz C, Dobrzyński P, FTIR study of degradation products of aliphatic polyesters-carbon fibres composites, *J Mol Struct* 2001;596:69-75.
- [8] Wang W, A novel hydrogel crosslinked hyaluronan with glycol chitosan, *J Mater Sci: Mater Med* 2006;17:1259-1265.
- [9] Schwach G, Vert M. In vitro and in vivo degradation of lactic acid-based interference screws used in cruciate ligament reconstruction. *Int J Biol Macromol* 1999;25:283-291.

## INJECTABLE CELL IMMOBILIZATION SYSTEMS FOR BONE REGENERATION

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[*Engineering of Biomaterials*, 69-72, (2007), 5-6]

### Abstract

Cell immobilization or encapsulation has been extensively investigated with the purpose of providing immunisolation but few attempts have been made to use this strategy for tissue regeneration.

Our research efforts are currently focused on the functionalization of natural polymers, namely polysaccharides, to promote their interaction with biological systems. Osteoprogenitor and stem cells were immobilized within alginate microspheres conjugated with oligopeptides including the Arg-Gly-Asp (RGD) sequence. Polymers were further modified to improve their biodegradability. After immobilization and under dynamic cell culture conditions, immobilized cells were viable, proliferated and differentiated. Immobilized cells further synthesized an extracellular matrix and expressed bone phenotypic markers, which indicates the capability of this approach to promote the regeneration of bone tissue. Current efforts are focused on promoting vascularization of bone tissue by using angiogenic factors as well as endothelial cells.

## Acknowledgements

The authors are grateful to FCT - Fundação para a Ciência e a Tecnologia and to FLAD - Luso-American Foundation, for financial support.

## CHARACTERIZATION OF FERROMAGNETIC COMPOSITE MATERIALS FOR BIOMEDICAL APPLICATIONS

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## Abstract

The great technological development in materials engineering together with quantum mechanical effect called giant magnetoresistance (GMR) observed in thin film structures composed of alternating ferromagnetic and nonmagnetic metal layers have evolved to the point where their synergistic combination have culminated in a new field of multidisciplinary research and in new applications.

Paper shows results of investigations of nanocomposites consisting of magnetic metallic (alloy) nanoparticles (MMNPs) embedded in a dielectric matrix (like SiO<sub>2</sub> or Al<sub>2</sub>O<sub>3</sub>). The studied (Fe<sub>0.45</sub>Co<sub>0.45</sub>Zr<sub>0.10</sub>)<sub>x</sub>(Al<sub>2</sub>O<sub>3</sub>)<sub>100-x</sub> (17<x<65at.%) films with thickness *d* of 3 to 5μm were manufactured using ion-beam sputtering of the compound target with argon onto the motionless water-cooled substrate. The films were deposited onto glass-ceramic substrates for electrical measurements and on thin aluminium foils. The composites have been investigated by means of Mössbauer spectroscopy, AC/DC measurements and magnetometry.

Incorporation of oxygen into the nanocomposite structure allows formation of soft ferromagnetic core (alloy)–shell (oxide) structure that is opening wide opportunities for tailoring of magnetic, electric and magnetotransport properties of nanocomposites.

[*Engineering of Biomaterials*, 69-72, (2007), 6-7]

## Introduction

The quick advances in material sciences have provided a broad framework for implementing multifunctionality in materials. Multifunctional materials embedded in an adaptive composite system have presented an exceptional possibility in engineering consideration problems. It is believed that broadly discussed in literature [1-9] the ferromagnetic nanomaterials possessing giant magnetoresistivity (GM) and spin-dependent tunnelling (SDT) effects are very promising for applications in biomedical and bioengineering fields as they allow the elaboration and production of magnetic nanosensors. The elementary concept of sensors in biomedical application is to prepare multifunctionally highly integrated composite provided data on the structural environment to a processing and control computerised system which in turn signals can modify the structural properties. Particularly, core-shell nanocomposites contained soft magnetic Fe-based oxides have captured a dominant position due to their superior chemical and thermal stability, hardness, non-toxicity and biocompatibility [10].

In order to exploit the full potential of such materials a detailed understanding of nanostructure property correlation is needed. Present paper is aimed on the investigation of nanocomposites consisting of magnetic metallic (alloy) nanoparticles (MMNPs) embedded in a dielectric matrix (like SiO<sub>2</sub> or Al<sub>2</sub>O<sub>3</sub>). Incorporation of oxygen into the nanocomposite structure allows formation of soft ferromagnetic core (alloy)–shell (oxide) structure that is opening wide opportunities for tailoring of magnetic, electric and magnetotransport properties of nanocomposites.

## Materials and methods

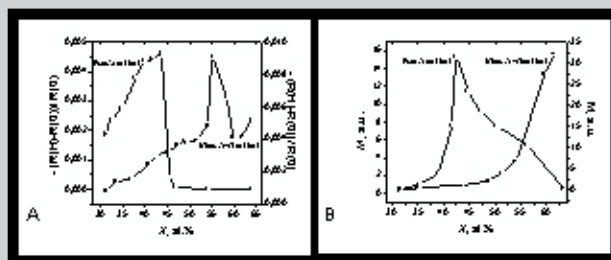


FIG.1. DC magnetoresistance (A) and magnetization *M* at *B*=400mT (B) of the composite films of series 1 (pure Ar ambient) and 2 (mixed Ar+O ambient) vs composition *X*.

The studied (Fe<sub>0.45</sub>Co<sub>0.45</sub>Zr<sub>0.10</sub>)<sub>x</sub>(Al<sub>2</sub>O<sub>3</sub>)<sub>100-x</sub> (17<*x*<65 at.%) films with thickness *d* of 3 to 5μm were manufactured using ion-beam sputtering of the compound target with argon onto the motionless water-cooled substrate. The films were deposited onto glass-ceramic substrates for electrical measurements and on thin aluminium foils for Mössbauer investigations. Films were deposited either in pure argon (Ar) at a pressure of 8.0×10<sup>-4</sup>Pa (samples of series 1) or in argon-oxygen (Ar+O) gas mixture at the total pressure of 9.6×10<sup>-2</sup> Pa and partial pressure of oxygen of about 4.4×10<sup>-2</sup> Pa (samples of series 2).

The composites have been investigated by means of Mössbauer spectroscopy, AC/DC measurements and magnetometry. Mössbauer spectra have been recorded at room temperature in transmission geometry using <sup>57</sup>Co/Rh source (40mCi) and fitted MOSMOD program. The AC/DC conductance was measured in the temperature range of 77-350K using flow cryostat system. For DC conductivity  $\sigma$  measurements the two probe method was used. The real *Z'* and imaginary *Z''* parts of impedance in the frequencies *f*=102-106Hz were measured by a precise AC bridge HP-