RSC Advances

# Cytotoxicity control of SiC nanoparticles introduced into polyelectrolyte multilayer films


| | |
|---|---|
| Journal: | *RSC Advances* |
| Manuscript ID: | RA-ART-04-2014-003914.R1 |
| Article Type: | Paper |
| Date Submitted by the Author: | 26-Jun-2014 |



| | |
|---|---|
| Complete List of Authors: | Mzyk, Aldona; Institute of Metallurgy and Materials Science; Polish Academy of Sciences, Reymonta St.25,<br>Major, Roman; Institute of Metallurgy and Materials Science; Polish Academy of Sciences, Reymonta St.25,<br>Lackner, Juergen; Joanneum Research Forschungsges mbH, Institute of Surface Technologies and Photonics, Functional Surfaces, Leobner Strasse 94, A-8712,<br>Bruckert, Franz; Laboratoire des Matériaux et du Génie Physique, Grenoble Institute of Technology- Minatec, 3, Parvis Louis Néel, BP 257, 38016,<br>Major, Boguslaw; Institute of Metallurgy and Materials Science; Polish Academy of Sciences, Reymonta St.25, |

Journal Name

RSCPublishing

ARTICLE

# Cytotoxicity control of SiC nanoparticles introduced into polyelectrolyte multilayer films

Cite this: DOI: 10.1039/x0xx00000x

A. Mzyk[a], R. Major[a], J. M. Lackner[b], F. Bruckert[c], B. Major[a]

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

Nowadays, biosensors technology development is directed toward improvement of sensing devices biocompatibility. Polyelectrolyte multilayer film (PEMs) consisting of natural polymers seems to be appropriate for electrode coverage and semiconducting nanoparticles localization in order to create controllable polymer – nanoparticles tridimensional network. However, control of nanoparticles release from films and in a consequence their cytotoxicity is still a challenge. In this study we demonstrated that cytotoxic effect of silicon carbide (SiC) nanoparticles introduced by the plasma activated chemical vapor deposition (PACVD) method into poly-L-lysine/hyaluronic acid (PLL/HA) or poly-L-lysine/ alginate acid (PLL/ALG) films could be controlled by chemical cross-linking of the polyelectrolyte film. Herein, we tested two types of cross-linkers N-hydrosulfosuccinimide/1-ethyl-3-(3-dimethylamino-propyl)carbodiimide (NHS/EDC) and genipin reagent. Analysis of nanoparticles distribution by scanning electron microscopy (SEM) has shown conglomerates formation in each film type. High resolution transmission electron microscopy (HRTEM) images indicated cubic structure of SiC and nanoparticles localization in the polymer coating. Among tested cross-linkers, either NHS/EDC or genipin seems to be suitable for nanocomposite properties control due to the low cytotoxicity and an effective stabilization of polymer – nanoparticle interaction, thus lower nanoparticles release rate from films.

## 1. Introduction

Recent years have reported increasing importance of polyelectrolyte multilayer films (PEMs) for biomedical applications as thin coatings on artificial prosthesis or to cover electrodes in biosensors.[1-3] PEMs constitute a versatile system to build architectures whose properties can be tuned in terms of thickness, film internal structure and degree of ionization of the polyelectrolytes.[4-6] These structures can be obtained from conductive biopolymers which provide the ability to accommodate nanoparticles (NPs) in controlled experimental conditions. Modified PEMs bring together the properties of tridimensional networks offered by polymers and the intrinsic functionalities of nanoparticles[7]. This opens the possibility to explore the application of such nanocomposites in remotely controlled triggered drug release or biological processes detection *in vivo*, and simultaneously provides prosthesis or sensor biocompatibility.[8,9] Among different kinds of nanomaterials, such as metals, metal oxide, carbon based materials and others, silicon carbide (SiC) which is a wide band gap semiconductor, has become more and more popular. It has a range of physical, chemical, mechanical and electronic properties, which make it suitable for designing and improving sensing devices.[10] So far SiC nanoparticles have been used as a part of biosensor for electrocatalytic and flow injection analysis of insulin, determination of purine and pyrimidine DNA bases and electrochemical detection of nitric oxide.[11-13] It has been shown that the distribution of NPs within the film, their size and the total loaded amount can be tuned by controlling the film architecture.[14-16] However, controlling of nanoparticles release from films and in a consequence their cytotoxicity is still a challenge. Polymers chosen for experiments have natural origin. The advantage of these biopolyelectrolytes concerns their ability to specificially interact with living cells, their bioavailability and possible biodegradability, as specific enzymes are present in tissue and biological fluids.[17] Hyaluronan (HA) which is one of the glycosaminoglycans and poly-L-lysine (PLL) consists of repeated sequence of well recognized amino acids, present in the extracellular matrix of human tissues, were used as coating components sensitive for surrounding cells response.[18] The PLL/HA multilayer type is well described in the literature for their physico-chemical characteristics, biocompatibility and cellular reactions.[19-20] Alginate (ALG) as a polysaccharide derived from algae which is biocompatible and not recognize by human enzymatic degradation system was chosen as potential time dependent stabilizer of the polymer film. In order to control swelling behavior, two chemical cross-linkers were suggested due to their variable mechanism of action. In contrary to the most popular glutaraldehyde cross-linking reagent, either NHS/EDC or genipin is non cytotoxic *in vitro*, and its non-reacted derivatives could be washed out easily due to water solubility. However, when washing procedure is impossible to be carried out like a during *in vivo* cross-linking process, then only a genipin could be applied.[21,22]

In this study we demonstrated for the first time to our knowledge, that cytotoxic effect of SiC nanoparticles introduced by PACVD method into PEMs could be controlled by chemical cross-linking of the PEM coating. We described the relation between nanoparticles and various polymer types, as well as the kind and concentration of different cross-linkers in order to obtain bases information about nanoparticles - biopolymer interaction for designing biocompatible sensors in a conscious way.

## 2. Materials and methods

The polyelectrolyte coatings were made from the cationic poly- L-lysine purchased from Sigma-Aldrich, the anionic hialuronic acid bought from LifeCore'sOnCore$^{T}$ and sodium alginate purchased from Sigma-Aldrich, hereafter referred as PLL, HA and ALG respectively. The average molecular weight of the PLL was 15-30 kDa and HA was 176-350 kDa. Medium molecular weight sodium alginate was applied. Chemicals for cross-linking i.e. 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide (EDC), N-hydrosulfosuccinimide (NHS) were supplied from Sigma-Aldrich and genipin from Challenge Bioproducts Co. Ltd. Cytotoxicity was assessed by staining with propidium iodide purchased from Sigma Aldrich.

### 2.1. Polyelectrolyte multilayer film manufacturing

Polyelectrolyte multilayered films (PEMs) were deposited with a "layer-by-layer" technique onto 1.5 cm x 1.0 cm substrate material. Substrate was activated by 10 M NaOH and washed with pure Milli-Q water. PLL, HA and ALG were dissolved in 400 mM HEPES/0.15 M NaCl solution with concentration of 0.5 mg/ml, 1 mg/ml and 1 mg/ml, respectively. The pH of solutions was set at 7.4 by adding 0.5 M NaOH. Films were manufactured with an automatic dipping machine by an alternately immersing substrate in solutions of PLL and HA or PLL and ALG for 8 min each. After each deposition step, the substrate was rinsed in 0.15 M NaCl solution buffered at pH 7.4 to remove excess polyelectrolyte. The process was repeated until the desired number of 12 bilayers was obtained. Multilayer films were further processed for a cross-linking or were left in a non-cross-linked state. Afterwards, samples were subjected to final rinsing steps and stored at 4$^{o}$C in 400 mM HEPES/0.15 M NaCl solution buffered at pH 7.4 until further proceedings.

### 2.2. Chemical cross-linking

The chemical stability of multilayer polyelectrolyte films was caused by the chemical cross-linking process. Process was performed in two different ways. In the first one 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysulphosuccinimide (NHS) was applied according to the protocol described elsewhere. Reagents were prepared in 0.15 M NaCl solution buffered at pH 5.5 and mixed together in 1:1 volume ratio immediately before application. Three different EDC concentrations (260 mM, 400 mM, 800 mM) were tested. In all reactions 100 mM NHS was used. Polyelectrolyte films were incubated in EDC/NHS for 18 hours at 4$^{o}$C. Then the cross-linker solution was removed, and films were rinsed several times with 400 mM HEPES/0.15 M NaCl solution buffered at pH 7.4 to wash out non-reacted amounts of reagents. Washing procedure which is very important for exact cytotoxicity investigations was performed in two steps. At first coatings have been rinsing two times for an one hour under slow shaking at room temperature each time. Then in the second step coatings were conducted to three short (10 minutes each one) washes.
The second cross-linking protocol concerned application of genipin. Reagent was prepared in three different concentrations (1 mM, 10 mM, 50 mM) by preliminary dissolution in ethanol and finally in 0.15 M NaCl slolution buffered at pH 5.5. Polyelectrolyte films were incubated in genipin for 18 hours at RT and then rinsed with distilled water.

### 2.3. Nanoparticles introduction

Nanoparticles were introduced to cross-linked and non-cross-linked films by the plasma activated chemical vapor deposition (PACVD), in 5 to 10s lasting processes[23]. Before deposition, the substrates were dried and introduced into the vacuum chamber. Substrates were mounted parallel to the target surface in a ~120 mm distance. The chamber was pumped to reach the vacuum down to at 5 Pa. SiC coatings were grown in hexamethyldisiloxane (HMDSO, Sigma Aldrich) atmosphere of 20 Pa pressure. The nanoparticles were achieved at 32°C with less than 2°C heating during deposition in plasma. Due to process specificity it is impossible to determined exact quantity of the introduced nanoparticles. Therefore average number of single SiC nanoparticles per 100 nm$^2$ surface was calculated based on high resolution transmission electron microscopy (HRTEM) images.

### 2.4. Topography and microstructure analysis

Topography observations were done using Scanning Electron Microscopy technique (SEM) on Quanta 200 3D. Microstructure characterization was performed using Transmission Electron Microscopy technique (TEM), on cross-section. The Tecnai G$^2$ F20 (200kV) FEG was used for analysis[24]. Thin foils for TEM observations were prepared directly from the place of interest using Focused Ion Beam technique (equipped with in-situ micromanipulator). The Quanta 200 3D DualBeam was used for FIB preparation. Observations were performed in a bright field mode.

### 2.5. Cytotoxicity

Potential cytotoxic effect was determined according to the ISO 10993-5:2009 "Biological evaluation of medical device – Part 5: Tests for in vitro cytotoxicity "standards. Samples in size of 1.5 cm$^2$ were placed in confluent mouse fibroblast (L929; ATCC) cultures (about 5 x 10$^5$ cells) and have been incubated for 48 hours at 37$^{o}$C. Then cells were stained by propidium iodide (PI). Cultures incubated either with cross-linked, non-cross-linked and samples without nanoparticles were analyzed in comparison with control cultures. Images were taken with the Axio Observerver Z1 inverted microscope equipped with a camera and quantified using AxioVision 4.6 software (Carl Zeiss MicroImaging). A statistical analysis (two-way ANOVA and Tukey post hoc test, P value smaller than 0.05 was considered as significant – Statistica 10.0 PL) was performed on three replicates from each treatment.

## 3. Results

### 3.1. Topography and microstructure analysis

The top view analysis was performed by scanning electron microscopy technique. Controlling the spatial organization of monodisperse and non-aggregated nanoparticles in PEMs remains a challenge. Analysis of nanoparticles distribution has shown aggregate-like structure formation in each type of investigated polyelectrolyte films (Fig. 1). No significant differences between samples, except variable homogeneity in aggregates distribution related to PACVD protocol specificity and not connected with a polymer coating type were observed. TEM analysis exhibited micro-scale inhomogeneity caused by the nanoparticles penetration through the coating assembly. It was found that nanoparticles have run through polymer coating and formed a phase directly on the substrate (Fig. 2). Only a small number of nanoparticles were anchored in the polymer multilayer. Nanoparticles phase analysis

was performed using selected area electron diffraction pattern (SAED) and a high resolution transmission electron microscopy technique (HRTEM). The analysis revealed silicon carbide (SiC) phase (Fig. 2b and Fig. 3). Images indicated on cubic 3C-SiC polytype of nanoparticles with size in range of 1-5 nm and mean value of the 13±2 single particles per 100 $nm^2$ of coating surface. Nanoparticles did not form a separate layer within polyelectrolyte multilayer film. The clear interface between SiC nanoparticles and polymer matrix has been indicated. The thickness of the mixed polymer with SiC layer was on the level of 150 nm. The HMDSO thin film which appeared due to PACVD process conditions was the outermost layer of composite coatings with thickness up to 20 nm.

### 3.2. Cytotoxicity

The biological verification considered cytotoxicity of coatings modified by SiC nanoparticles has shown changes dependent on type of applied polyelectrolytes, cross-linker type and cross-linking rate. Both type of PEMs (PLL/HA, PLL/ALG) indicated cytotoxicity when compared with control culture. PLL/HA films in all cases affected cells viability much more than coatings consisted of PLL/ALG polymers. Either PLL/HA or PLL/ALG multilayers without introduced nanoparticles and cross-linked by NHS/EDC were less cytotoxic than the same non-cross-linked films (Fig. 4, Fig.5). There was no significant difference between the samples treated with various EDC concentrations in case of PLL/HA film. The genipin application resulted in the similar effect as an EDC cross-linking. There was no significant difference between the results obtained for various cross-linked samples without nanoparticles and similar non-cross-linked PLL/HA films (Fig. 6), whereas PLL/ALG multilayers without nanoparticles and cross-linked were less cytotoxic than the same non-cross-linked films (Fig. 7). The coatings with SiC indicated higher cytotoxicity than those without nanoparticles. It was found that cross-linking of PLL/HA films with nanopartices by NHS/EDC decreased the cytotoxicity (Fig. 4). Herein, the highest influence of nanoparticles on cell viability was observed for non-cross-linked multilayers. The cytotoxicity decreased with application of higher cross-linker concentration. The contribution of dead cells in case of films, which were cross-linked by 800 mM EDC, was on the same level as in control cultures.

The PLL/HA films with nanoparticles after cross-linking by the highest genipin concentration were slightly more cytotoxic than, that cross-linked by the 800 mM NHS/EDC. However there was no significant difference between non-cross-linked and cross-linked samples with 1 mM genipin (Fig. 6). A decrease of cytotoxicity was observed after application of 10 mM reagent. Cytotoxic effect of PLL/ALG coatings, which were modified by nanoparticles, was lower than in case of such PLL/HA films. Changes in the contribution of dead cells for PLL/ALG multilayers after cross-linking by NHS/EDC were similar to observed for corresponding PLL/HA films (Fig. 5). The highest influence of nanoparticles on cell viability was observed for non-cross-linked multilayers. The cytotoxicity was decreasing with an application of higher cross-linker concentration. There was no significant difference between cytotoxic influence of non-cross-linked and cross-linked by 1 mM genipin samples (Fig. 7). Decrease of cytotoxicity was observed after application of 10 mM reagent at least. Contribution of dead cells after culture incubation with samples cross-linked both of 10 mM and 50 mM genipin was equal with control culture. These studies have shown SiC nanoparticles could cause cytotoxic effect, however cross-linking either by NHS/EDC or genipin could reduce cells dead rate to the level of control culture.

## 4. Discussion

### 4.1. Microstructure

In this work nanoparticles were introduced by the PACVD on the level of several atomic layers. The application of such parameters in the traditional coating/substrate set up should result in the two dimensional model of thin film nucleation.[25,26] In the studies nanoparticles were deposited on a porous polymer coating. However, aggregates were formed in the film structure and bonds within the PEM coating were broken. Li. et al. described such interaction between water molecules and cubic SiC surface leads to dissociation of water molecules accompanied by changes in both its structural and electrochemical properties. The -OH and -H groups bond mainly on nanocrystals.[27] We supposed that such charged molecules interact with polymer films in varying degrees depend on multilayer cross-linking state. The high surface energy makes inorganic nanoparticles generally extremely unstable, liable to undergo chemical reactions with the environment and also to self-aggregate. Properties of nanoparticles are size- and shape- dependent — a statement that nowadays can be regarded as axiomatic. These features of inorganic nanoparticles, in part determined by the conditions of synthesis, create enormous difficulties in their fabrication and application.[28] The effect of cross-linkers on nanoparticles binding/location in the film, which will be useful to understand mechanism of their release dependence on different type and concentration reagents will be an objective of the Attenuated Total Reflection Fourier Transform Infrared spectroscopy (ATR-FTIR) further investigations.

**Cytotoxicity**

Analyzed PLL/HA and PLL/ALG films indicated low cytotoxicity. It was noticed that PLL/HA affected cells viability much more than PLL/ALG polyelectrolytes. It has placed probably due to higher rate of swelling and release of polymer chains from PLL/HA. Boeckel et al. performed in vitro evaluation of cytotoxicity of hyaluronic acid as an extracellular matrix on osteoblastic cells by the MTT assay. Results of their research suggested a decrease in cell viability in the presence of HA.[29] Whereas Fischer et al. have tested in vitro cytotoxic influence of polycations application on fibroblast culture. They found that poly-L-lysine induced necrotic cell reaction.[30] Multilayers cross-linked either by NHS/EDC or genipin were the same or less cytotoxic than the similar non-cross-linked films. Cytotoxicity decrease after cross-linking was tightly connected with coatings structure stabilization and swelling limitation. Investigated cross-linkers were chosen for the nanoparticles release rate control due to their widely described in recent years influence on PEMs biocompatibility and physico-chemical characteristics.[31] Each type of cross-linking in adequate concentration results irreversible linkage between layers. The cross-linking saturation point, where tested films had the same cytotoxicity level as control samples, was found for 50 mM genipin and 800 mM NHS/EDC. Since the final results of both cross-linking methods was the same it seems to be reasonable to consider them as an equally effective. Other parameters like stiffness, surface morphology or coating adhesion to the substrate was changing in response to apply different reagent concentration.[32] Therefore, in our opinion method effectiveness should be considered simultaneously from the point of view of several parameters crucial for composite application. Authors' recent investigations have shown that genipin could be less effective due to coating delamination risk from the substrate after cross-linking at the saturation point. Obviously this is the result of specificity and differences in cross-linking action mechanism of applied reagent. The most important observation comes from this studies is that SiC nanoparticles could cause cytotoxic effect. Contrary, in literature

silicon carbide was considered as a highly biocompatible material.[33] However, only a few toxicological data are available for these NPs. Barillet et al. presented global toxicological profile of SiC nanoparticles on A549 lung epithelial cells, using a battery of classical in vitro assays. They used five type of SiC-NPs, with varying diameters and Si/C ratios. Authors have shown that SiC-NPs were internalized in cells where they cause a significant, though limited, cytotoxic effect. Cell redox status was deeply disturbed: SiC-NP exposure caused reactive oxygen species production, glutathione depletion and inactivation of some antioxidant enzymes: glutathione reductase, superoxide dismutase, but not catalase. Finally, the alkaline comet assay shown that SiC-NPs were genotoxic.[34] Taken together these data proved that SiC-NPs biocompatibility should be revisited. Allen et al. have investigated the effects of two forms of silicon carbide (alpha-SiC and beta-SiC) on macrophages, fibroblasts and bone cells in vitro. Both alpha- and beta-SiC were well-tolerated by cells at concentrations of up to 0.1 mg/ml but caused severe cytotoxicity at a concentration of 1 mg/ml.[35] Pourchez et al. described non cytotoxic effect of SiC nanoparticles on macrophages culture. Simultaneously remarkable pro-oxidative reactions and inflammatory response were recorded, whose intensity appears related to the physico-chemical features of nano-sized SiC particles. *In vitro* data clearly showed an impact of the extent of nanoparticle surface area and the nature of crystalline phases (α-SiC vs. β-SiC) on the TNF-α production, a role of surface iron on free radical release, and of the oxidation state of the surface on cellular $H_2O_2$ production.[33] In this study we have shown that silicon carbide nanoparticles induced a slight but significant cell death. We assumed that difference in cytotoxicity was mainly connected with nanoparticles release rate due to coating cross-linking range. The cross-linking stage is responsible for the coating stiffness, character of the internal structure and the surface morphology what was shown in our previous work.[6] Herein, we present that as a consequence changes in these parameters affected adhesion strength of the outermost HMDSO thin layer co-deposited during PACVD process on the surface of composite coating. We have observed better adhesive properties for the rougher and stiffer films, cross-linked with a higher reagents concentration. The HMDSO was reported in our previous investigations as a non-cytotoxic, therefore could be considered as a protecting coating. However, in some cases where nanoparticles are used for triggering drug/growth factors release this PACVD side effect coating could be not desired. Moreover, biomechanical stimulation control of various cellular reactions in response to the contact with a composite could be achieved only by its cross-linking. The HMDSO film delamination could increase cytotoxicity due to uncover of SiC aggregates and expose cells for an interaction with nanoparticles. It seems to be reasonable to recognize mechanism governs dynamic of nanoparticles release/binding state of cross/linked films. Obviously, nanoparticles modes of action need to be investigated in order to have a clearer view on phenomena of observed cytotoxicity.

## 5. Conclusions

Results indicate the cytotoxic effect on the cellular viability of the silicon carbide cubic nanoparticles formed by PACVD method. Therefore, it is appropriate that biosensors technology using this kind of semiconductors should seek to find solution for biocompatibility improvement. Herein, we proposed nanoparticles introduction into polyelectrolyte multilayer films and their release control by chemical cross-linking of coatings. The highest necrotic effect of SiC introduction into non-cross-linked films was observed probably due to less stable and more relaxed structure, higher swellability and in a consequence higher particles release rate. Among tested cross-linkers, either NHS/EDC or genipin seems to be suitable for nanocomposite properties control due to the low cytotoxicity and an effective stabilization of polymer – nanoparticle interaction, so decrease in nanoparticles release rate from films.

We also found that polymer selection plays a key role in stable structure formation. The PLL/ALG films were better choice probably due to lower swelling rate. However, this needs confirmation studies. Future investigations should be also concentrated on identifying nanoparticles cytotoxicity modes of action. Finally, biosensors technology according to suggested here development path needs selection of appropriate conductive polymers and PACVD method improvement in order to obtain mono-dispersive, non-aggregated nanoparticles distribution in biocompatible films.

## Acknowledgements

The research was financially supported by the European Union under the European Social Found within Project No. POKL.04.01.00-00-004/10 and Project No. 2011/03/D/ST8/04103 "Self-assembling, biomimetic porous scaffolds in terms of inhibiting the activation of the coagulation system" of the Polish National Center of Science**.** The experiments were executed with the support of Laboratoire des Matériaux et du Génie Physique Grenoble Institute of Technology - Minatec.

## Notes and references

[a]aldonamzyk@googlemail.com
[a]r.major@imim.pl
[b]juergen.lackner@joanneum.at
[c]Franz.Bruckert@phelma.grenoble-inp.fr
[a]b.major@imim.pl

1 H.W. Liao, C.L. Nehl, J.H. Hafner, *Nanomedicine* 2006, **1**, 201–208.
2 B. Sepulveda, P.C. Angelome, L.M. Lechuga, L.M. Liz-Marzan, *Nano Today*, 2009, **4**, 244–251.
3 R. Major, *Journal of Material Science Materials in Medicine,* 2013, **24**, 725-733.
4 G. Decher, *Science*, 1997, **277**, 1232–1237.
5 D. Yoo, S.S. Shiratori, M.F. Rubner, *Macromolecules*, 1998, **31**, 4309–4318.
6 A. Mzyk, R. Major, M. Kot, J. Gostek, P. Wilczek, B. Major, *Archives of Civil and Mechanical Engineering,* 2014, **14(2)**, 262-268.
7 L. Shen, L. Rapenne, P. Chaudouet, J. Ji, C. Picart, *Journal of Colloid and Interface Science*, 2012, **388**, 56–66.
8 A.L. Daniel-da-Silva, A. M. Salgueiro, T. Trindade, Gold Bull, 2013,
9 G. Lind, C. E. Linsmeier, J. Thelin, J. Schouenborg, *Journal of Neural Engineering*, 2010, **7**, 046005.
10 H. Zhao, L. Shi, Z. Li, C. Tang, *Physica E*, 2009, **41**, 753.
11 A. Salimi, L. Mohamadi, R. Hallaj, S. Soltanian, *Electrochemistry Communications*, 2009, **11**, 1116–1119.
12 R. Ghavamia, A. Salimi, A. Navaee, *Biosensors and Bioelectronics*, 2011, **26**, 3864–3869.
13 S. Miserere, S. Ledru, N. Ruille, S. Griveau, M. Boujtita, F. Bedioui, *Electrochemistry Communications,* 2006, **8**, 238–244.
14 S. Joly, R. Kane, L. Radzilowski, T. Wang, A. Wu, R.E. Cohen, E.L. Thomas, Langmuir, 2000, **16,**1354–1359.
15 W.Y. Yuan, J.H. Fu, K. Su, J. Ji, Colloids Surf. B, 2010, **76**, 549–555.
16 A. Agarwal, T.L. Weis, M.J. Schurr, N.G. Faith, C.J. Czuprynski, J.F. McAnulty, C.J. Murphy, N.L. Abbott, Biomaterials, 2010, **31**, 680–690.

17 V. Gribova, R. Auzely-Velty, C. Picart, *Chem. Mater.,* 2012, **24**, 854-869.
18 L. Richert, F. Boulmedais, P. Lavalle, J. Mutterer, E. Ferreux, G. Decher, P. Schaaf, JC. Voegel, C. Picart*, Biomacromolecules,* 2004, **5**, 284-294.
19 C.J. Detzel, A.L. Larkin, P. Rajagopalan, *Tissue Eng. B*, 2011, **17(2)**, 101-113.
20 C. Picart, P. Lavalle, P. Hubert, F.J.G. Cuisinier, G. Decher, P. Schaaf, *Langmuir*, 2001, **17**, 7414.
21 Y. Hao, P. Xu, C. He, X. Yang, M. Huang, J. Xing, J. Chen, *Nanotechnology,* 2011, **22,** 285103, 1-9.
22 K. Ulubayram, E. Aksu, S.I. Delilogluguruhan, K. Serbetci, N. Hasirci, *J. Biomater. Sci. Polymer Edn*, 2002, **13**(11), 1203–1219.
23 J. M. Lackner, W. Waldhauser, R. Major, L. Major, P. Hartmann, *Surface & Coatings Technology*, 2013, **215**, 192-198.
24 L. Major, W. Tirry, G. Van Tendeloo, Surface & Coatings Technology, 2008, **202**, 6075-6080.
25 J. A. Thornton, *Ann. Res. Mat. Sci.*, 1977, 239.
26 V. Valvoda, *Surface and Coating Technology*, 1996, **30**, 61-65.
27 Y. Li, C. Chen, J. T. Li, Y. Yang, Z.M. Lin, *Nanoscale Research Letters*, 2011, **6**, 454.
28 B.A. Rozenberg, R. Tenne, *Prog. Polym. Sci*, 2008, **33**, 40-112.
29 D.G. Boeckel, R.S. Shinkai, M.L. Grossi, E.R. Teixeira, *Oral Surg Oral Med Oral Pathol Oral Radiol.,* 2012, **S2212-4403(12)**, 01016-4.
30 D. Fischer,Y. Li, B. Ahlemeyer, J. Krieglstein, T. Kissel, Biomaterials, 2003, **24(7)**, 1121-31.
31 C.P. Va´zquez, T. Boudou, V. Dulong, C. Nicolas, C. Picart, K. Glinel, *Langmuir*, 2009, **25**, 3556-3563.
32 A.L. Daniel-da-Silva, A.M. Salgueiro, T. Trindade*, Gold Bull*, 2013, **46**, 25–33.
33 J. Pourchez, V. Forest, N. Boumahdi, D. Boudard, M. Tomatis, B. Fubini, N. Herlin-Boime, Y. Leconte, B. Guilhot, M. Cottier, P. Grosseau, *Journal of Nanoparticle Research*, 2012, **14(10)**, 1143.
34 S. Barillet, M.L. Jugan, M. Laye, Y. Leconte, N. Herlin-Boime, C. Reynaud, M. Carrière, *Toxicol Lett.*, 2010, **198 (3)**, 324-30.
35 M. Allen, R. Butter, L. Chandra, A. Lettington, N. Rushton, *Biomed Mater Eng.*, 1995, **5(3)**, 151-9.

## Figure captions

Fig. 1. Example of SEM images of nanoparticles distribution in PEM – smaller frame indicates area used for FIB thin foil preparation.

Fig. 2. Cross section TEM analysis of the silicon carbide nanoparticles introduction into porous polymer coatings: a) bright field image; b) selected area electron diffraction patterns. The red arrow indicates a border between polymer porous layers.

Fig. 3. HRTEM analysis of the silicon carbide nanoparticles introduction into porous polymer coatings.

Fig. 4. Cytotoxicity analysis of PLL/HA films with or without incorporated SiC nanoparticles and cross-linked by variousconcentration of NHS/EDC. Data represent mean ±SD; n=3; *P< 0.05 vs. sample non-cross-linked without NPs, #P< 0.05 vs. sample non-cross-linked with NPs.

Fig. 5. Cytotoxicity analysis of PLL/ALG films with or without incorporated SiC nanoparticles and cross-linked by various concentration of NHS/EDC. Data represent mean ±SD; n=3; *P< 0.05 vs. sample non-cross-linked without NPs, #P< 0.05 vs. sample non-cross-linked with NPs.

Fig. 6. Cytotoxicity analysis of PLL/HA films with or without incorporated SiC nanoparticles and cross-linked by various concentration of genipin. Data represent mean ±SD; n=3; *P< 0.05 vs. sample non-cross-linked without NPs, #P< 0.05 vs. sample non-cross-linked with NPs.

Fig.7. Cytotoxicity analysis of PLL/ALG films with or without incorporated SiC nanoparticles and cross-linked by various concentration of genipin. Data represent mean ±SD; n=3; *P< 0.05 vs. sample non-cross-linked without NPs, #P< 0.05 vs. sample non-cross-linked with NPs.

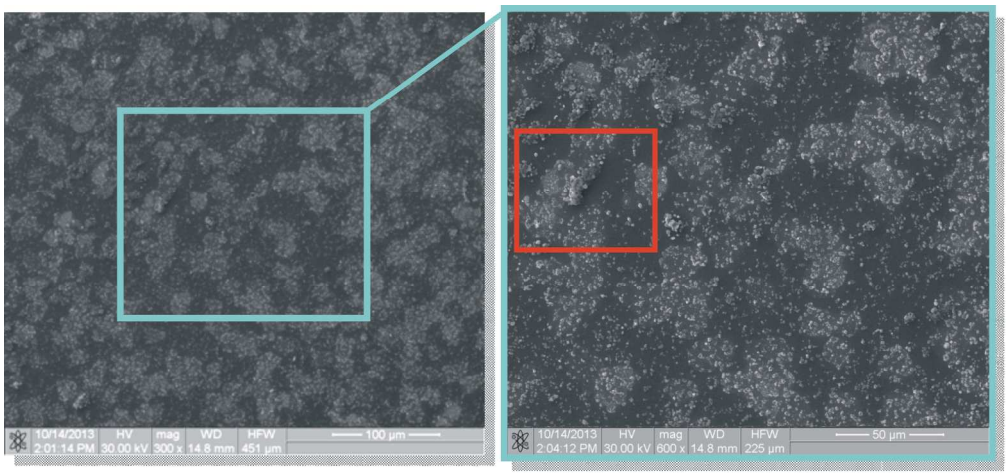



Fig. 1. Example of SEM images of nanoparticles distribution in PEM – smaller frame indicates area used for FIB thin foil preparation.

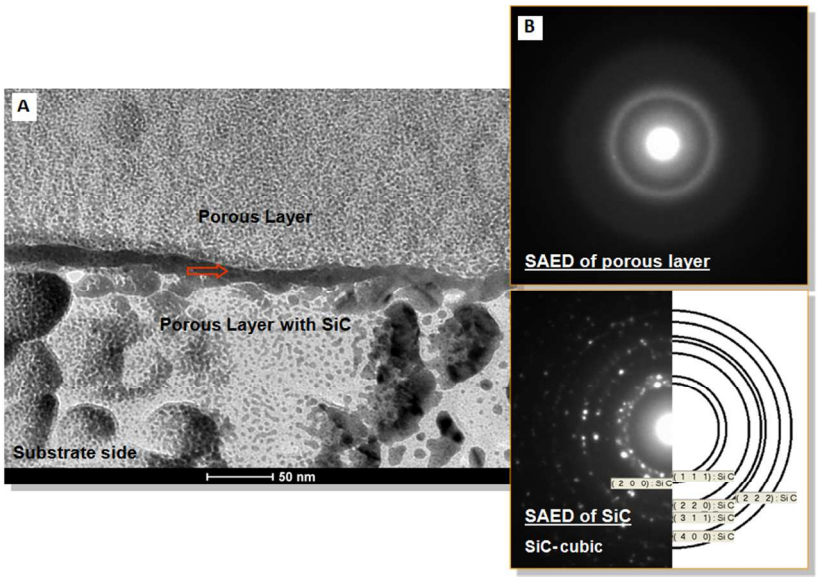



Fig. 2. Cross section TEM analysis of the silicon carbide nanoparticles introduction into porous polymer coatings: a) bright field image; b) selected area electron diffraction patterns. The red arrow indicates a border between polymer porous layers.
310x177mm (96 x 96 DPI)

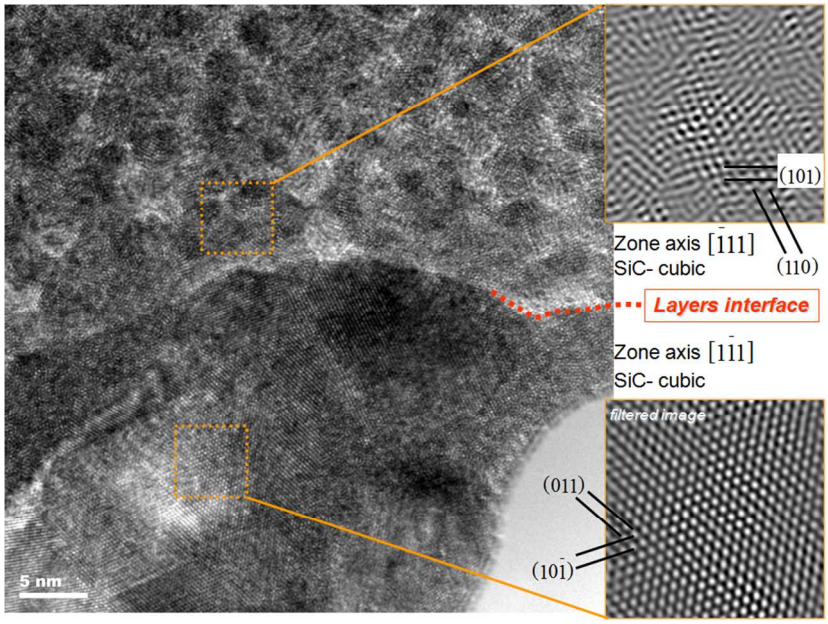
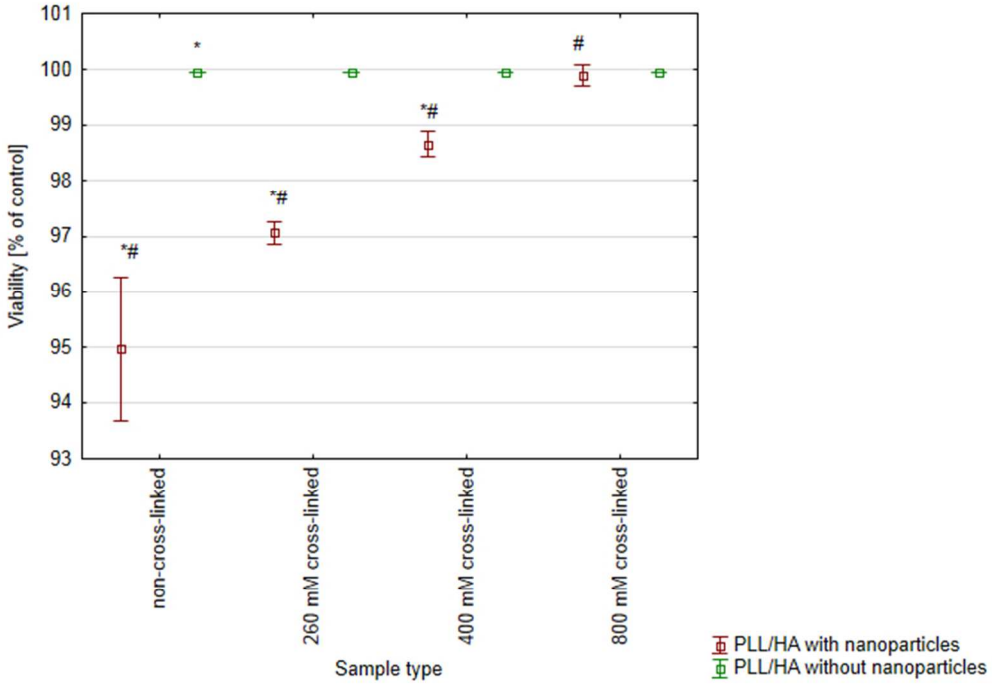
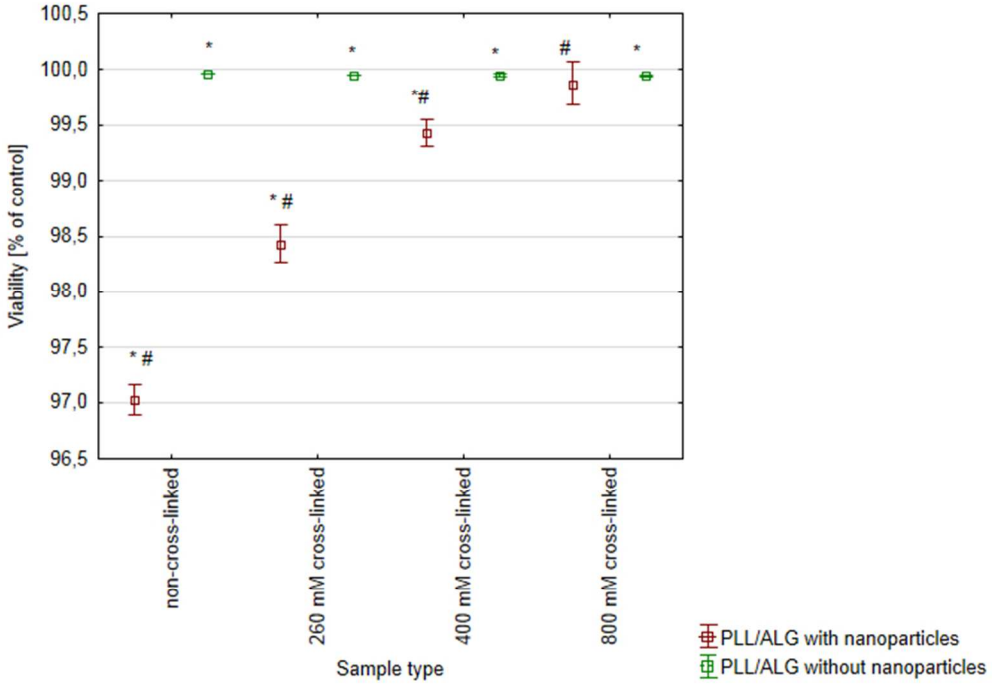


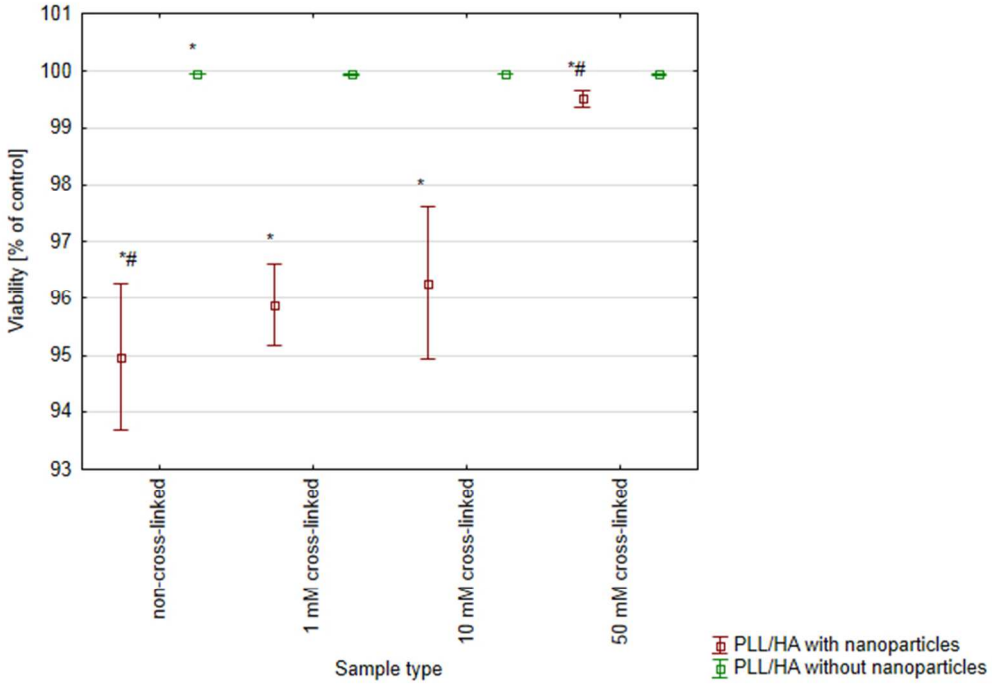

Fig. 3. HRTEM analysis of the silicon carbide nanoparticles introduction into porous polymer coatings.
310x191mm (96 x 96 DPI)



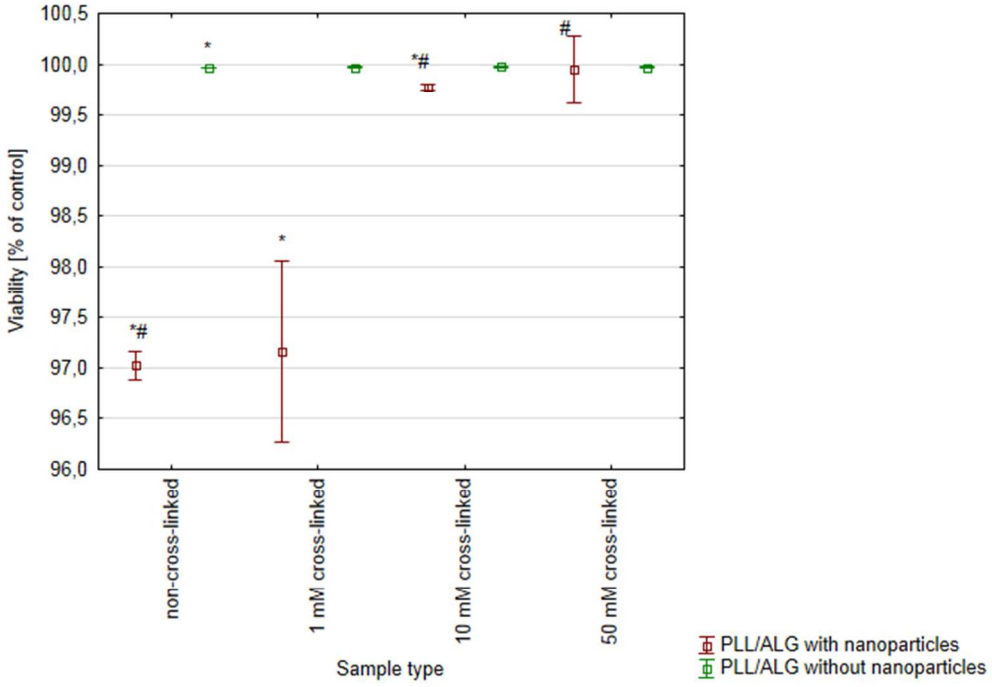

Cytotoxicity analysis of PLL/HA films with or without incorporated SiC nanoparticles and cross-linked by variousconcentration of NHS/EDC. Data represent mean ±SD; n=3; *P< 0.05 vs. sample non-cross-linked without NPs, #P< 0.05 vs. sample non-cross-linked with NPs.
177x123mm (96 x 96 DPI)




Cytotoxicity analysis of PLL/ALG films with or without incorporated SiC nanoparticles and cross-linked by various concentration of NHS/EDC. Data represent mean ±SD; n=3; *P< 0.05 vs. sample non-cross-linked without NPs, #P< 0.05 vs. sample non-cross-linked with NPs.
177x123mm (96 x 96 DPI)




Cytotoxicity analysis of PLL/HA films with or without incorporated SiC nanoparticles and cross-linked by various concentration of genipin. Data represent mean ±SD; n=3; *P< 0.05 vs. sample non-cross-linked without NPs, #P< 0.05 vs. sample non-cross-linked with NPs.
177x123mm (96 x 96 DPI)




Cytotoxicity analysis of PLL/ALG films with or without incorporated SiC nanoparticles and cross-linked by various concentration of genipin. Data represent mean ±SD; n=3; *P< 0.05 vs. sample non-cross-linked without NPs, #P< 0.05 vs. sample non-cross-linked with NPs.
177x123mm (96 x 96 DPI)