Hybrid nanocomposites based on magnetic nanoparticles embedded in polymer brushes as stimuli-responsive substrate for cell culture

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Introduction

Numerous efforts have been made to understand and control chemical, physical and biological properties of scaffolds for cell cultures, especially for the last two decades. A rapid development in the design of interfaces enabling controlled adsorption of biological structures is stimulated by applications requiring attraction of cells, proteins or DNA, followed by their triggered detachment from surfaces. It significantly affects the need for more sophisticated surfaces, especially to ensure the non-invasive cell detachment, preserving cellular integrity and create opportunities for further study of cell behaviour.

In this report we present a facile and efficient method of controlled embedding of inorganic nanoparticles into a thin (~100 nm) and flat polymer brush coating covered with poly-L-lysine that prevents their unwanted aggregation. This hybrid nanocomposite was characterized using atomic and magnetic force microscopy (AFM/MFM), secondary ion mass spectrometry (SIMS), X-ray photoelectron spectroscopy (XPS), optical microscope and in vitro tests on *neuroblastoma* cells. In particularly, the response of cells cultured on those nanocomposites to magnetic field was studied indicating their promising performance as magnetically-controllable scaffold for cell culture.

Synthesis of the hybrid nanocomposite

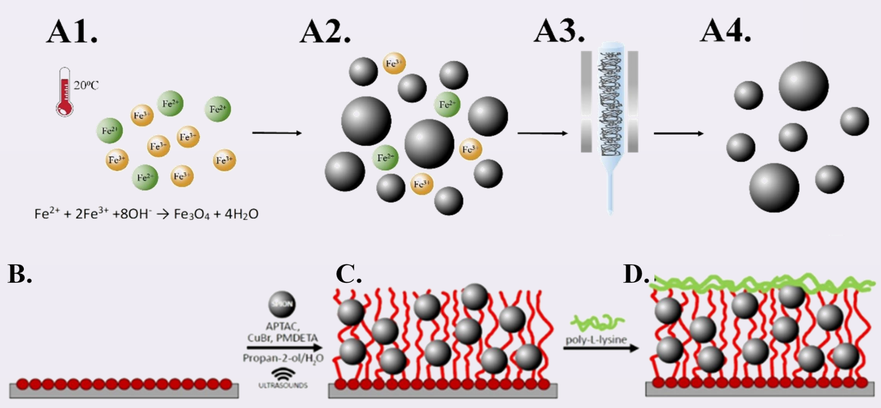

Figure 1. Schematic draw of the fabrication of poly(APTAC)+SPIONs scaffolds: A. Synthesis of magnetic nanoparticles: 1. Fe^{2+}, Fe^{3+} salts mixture at RT in H_2O, 2. Mixture of nanoparticles and post-reaction remains, 3. Magnetic chromatography, 4. Purified nanoparticles. B. Thin layer of surface-grafted initiator (APTES+BIB), C. Poly(APTAC) brushes obtained in simultaneous ATRP in the presence of SPIONs, D. Poly(APTAC) brushes with incorporated SPIONs coated with PLL layer.

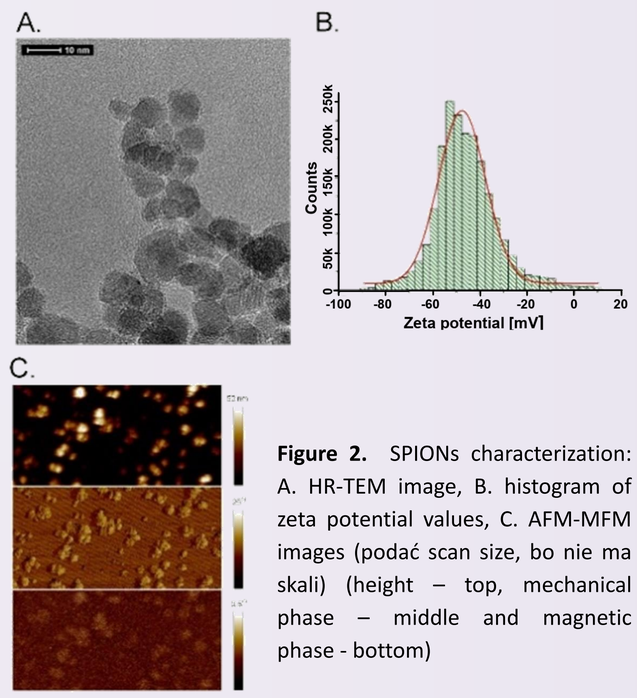

Figure 2. SPIONs characterization: A. HR-TEM image, B. histogram of zeta potential values, C. AFM-MFM images (podać scan size, bo nie ma skali) (height – top, mechanical phase – middle and magnetic phase - bottom)

Characterization of nanoparticles embedded in polymer brushes

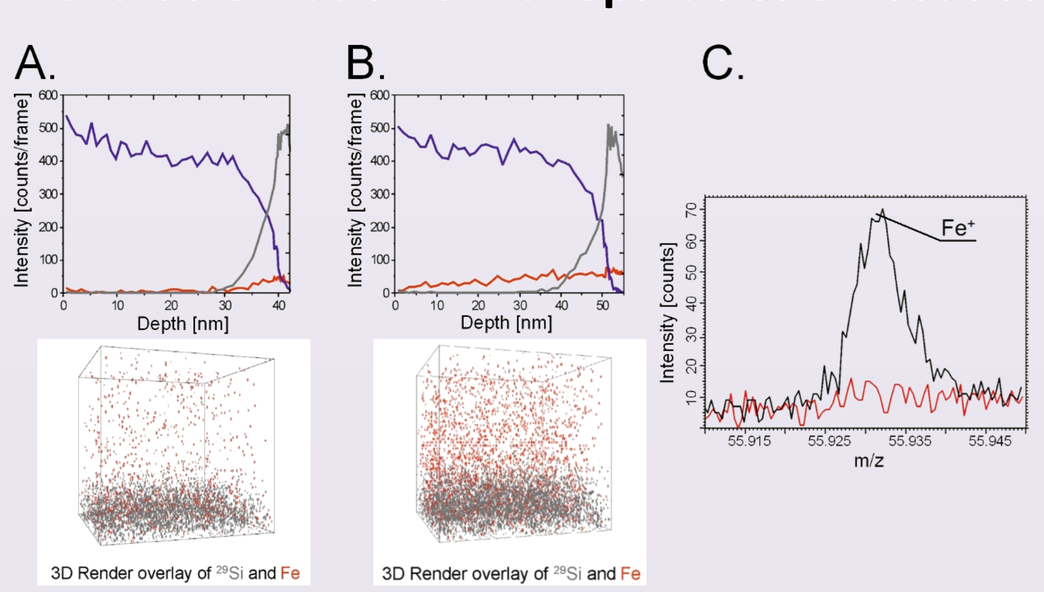

Figure 4. Secondary ion mass spectrometry (SIMS) depth profiles for poly(APTAC) (A.) and poly(APTAC)+SPIONs (B.) covered with PLL (gray color represent ^{29}Si signal, violet $^{13}CC_2H_{10}N$ and red Fe) with corresponding 3D images of spatial distribution of iron (red) and silicon 29 isotope (gray), (C.) – fragment of collected mass spectrum that includes an iron signal, integrated from space above silicon, for poly(APTAC)+SPIONs brushes (black line) and bare poly(APTAC) brushes (red line).

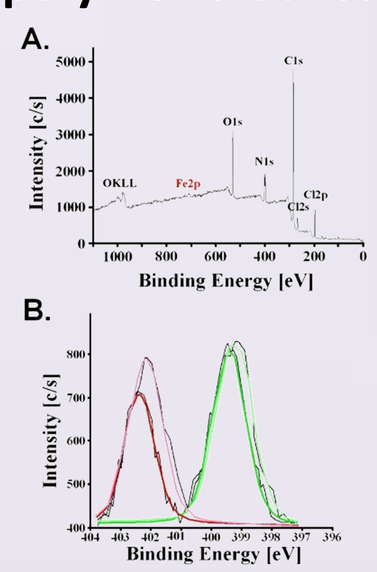

Figure 5. X-ray photoelectron spectroscopy (XPS) spectrum of poly(APTAC)+SPIONs (A.) and fragment of spectrum corresponding to binding energies for N1s for poly(APTAC)+SPIONs with (bright lines) and without (dark lines) PLL cover (red line: –C(NH)C-, green line: $–N^+(CH_3)_3$).

AFM/MFM analysis

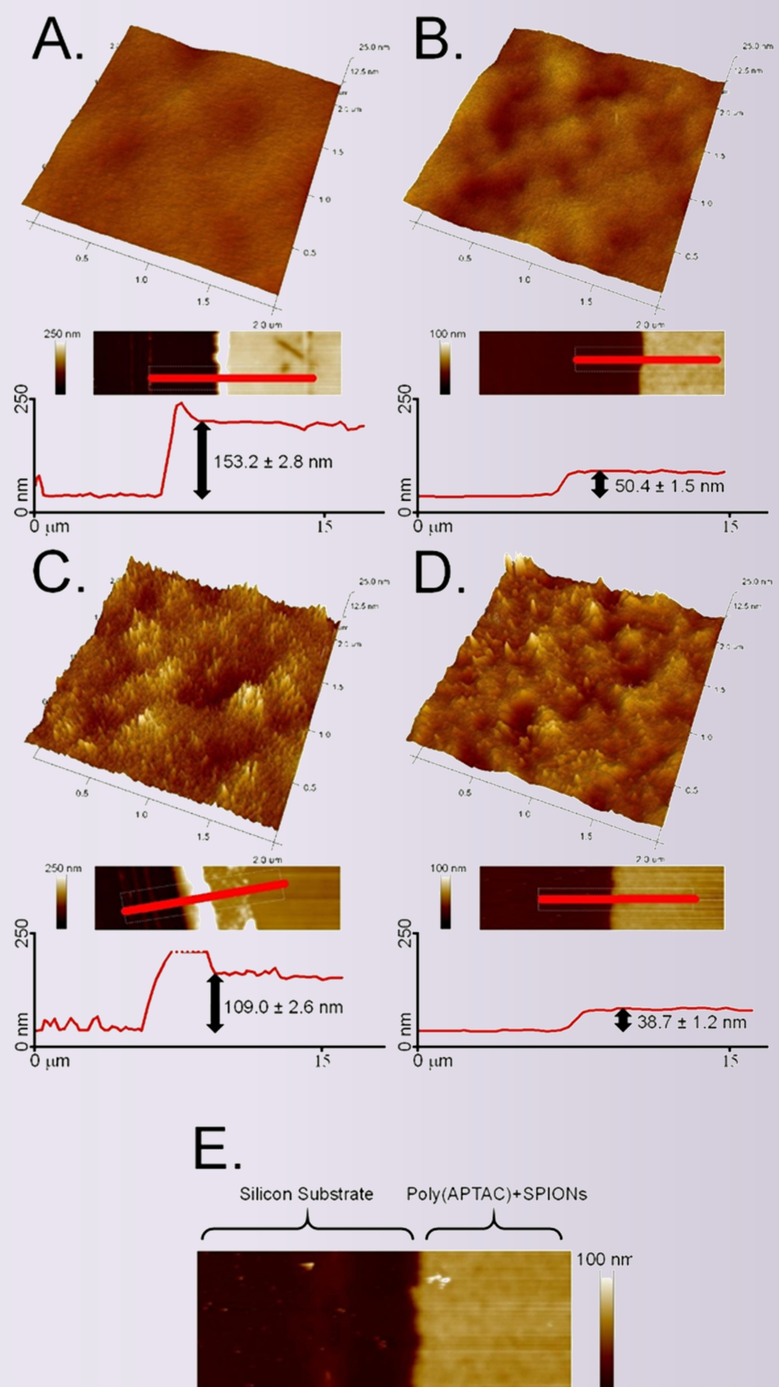

Figure 3. AFM topography images with corresponding cross-sections of polymer brushes: A. bare poly(APTAC), B. poly(APTAC) coated with PLL, C. poly(APTAC)+SPIONs brushes and D. poly(APTAC)+SPIONs brushes coated with PLL, E. MFM phase image of poly(APTAC)+SPIONs brushes coated with PLL thin film (height image – top, magnetic phase – bottom).

In vitro experiments on *neuroblastoma* cells

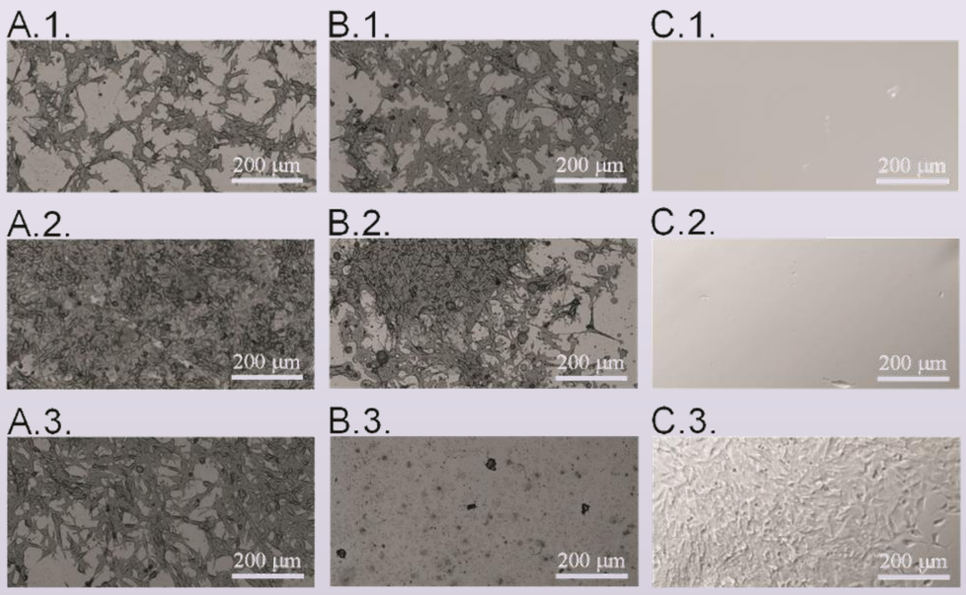

Figure 6. Optical microscopy images of neuroblastoma cells cultured before (A.) and after (B.) applying external magnetic field and cells detached from the surface (C.) of: 1. Silicon modified with PLL, 2. Poly(APTAC) brushes with PLL, 3. Poly(APTAC)+SPIONs brushes with PLL. (D.) Cell viabilities results (MTT assay) for murine neuroblastoma cell lines, illustrating the amount of cells remaining on the surface of the poly(APTAC) brushes and poly(APTAC)+SPIONs brushes after exposure to neodymium magnet (H).

Conclusions

- A strong magnetic signal from the entire surface of hybrid scaffold poly(APTAC)+SPIONs brushes was determined by magnetic force microscopy;
- Homogeneous incorporation of SPIONs in brushes was also confirmed using 3D images of spatial distribution of iron and silicon 29 isotope;
- Neuroblastoma cells cultured on bare poly(APTAC) and poly(APTAC)+SPIONs adhered well to the surface and exhibited ordinary morphology. However, after the 48 h in the applied magnetic field only cells cultured on poly(APTAC)+SPIONs have shrunk and have significantly decreased their amount adhering to the surface;
- This magnetic-responsive poly(APTAC) - SPION hybrid material may be a promising controllable stimuli-responsive scaffold for cell culture.

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