Colloids and Surfaces B: Biointerfaces 143 (2016) 111–117

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Biocompatibility and degradation of gold-covered magneto-elastic biosensors exposed to cell culture

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ARTICLE INFO

Article history:
Received 4 December 2015
Received in revised form 17 February 2016
Accepted 10 March 2016
Available online 14 March 2016

Keywords:
Biocompatibility
CHO cells
Magneto-elastic alloy
Au-coating
MTT assay
AO/EB staining
ICP-OES

ABSTRACT

Magneto-elastic materials (ME) have important advantages when applied as biosensors due to the possibility of wireless monitoring. Commercial Metglas 2826MB3™ (FeNiMoB) is widely used, however sensor stabilization is an important factor for biosensor performance. This study compared the effects of biocompatibility and degradation of the Metglas 2826MB3™ alloy, covered or not with a gold layer, when in contact with cell culture medium. Strips of amorphous Metglas 2826MB3™ were cut and coated with thin layers of Cr and Au, as verified by Rutherford Backscattering Spectroscopy (RBS). Using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), the presence of metals in the culture medium was quantitatively determined for up to seven days after alloy exposure. Biocompatibility of fibroblast Chinese Hamster Ovary (CHO) cultures was tested and cytotoxicity parameters were investigated by indirect means of reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) at 1, 2 and 7 days. Cell death was further evaluated through *in situ* analysis using Acridine Orange/Ethidium Bromide (AO/EB) staining and images were processed with ImageJ software. Ions from Metglas® 2826MB3™ induced a degradation process in living organisms. The cytotoxicity assay showed a decrease in the percentage of live cells compared to control for the ME strip not coated with gold. AO/EB *in situ* staining revealed that most of the cells grown on top of the gold-covered sensor presented a normal morphology (85.46%). Covering ME sensors with a gold coating improved their effectiveness by generating protection of the transducer by reducing the release of ions and promoting a significant cell survival.

1. Introduction

Magneto-elastic (ME) sensors [1,2] have attracted considerable interest within the sensor community as an excellent platform for measuring a wide range of chemical and biological parameters. From the detection of hydrocarbons [3] to the determination of glucose in urine sample [4] to the evaluation of contaminants in milk [5,6], ME sensors have proven their usefulness. ME materials have advantages when applied as biosensors in the biomedical area due to the possibility of wireless monitoring [7,8]. Amorphous ME ribbons have been widely used in various studies, including determined efforts to detect food contaminants [9,10]. They have also been used in several applications, like monitoring growth in cell cultures [11], evaluating cell behavior such as adhesion [12] and controlling restenosis in peripheral artery stents [13].

The application of magnetic materials in biological problems naturally leads to the question of the compatibility of the magnetic material with the biological system under consideration. Magnetic materials in the form of nanoparticles [14,15] or thin films [16] have recently been examined from the point of view of possible toxic or injurious effects which might be caused. This is a more restricted form of biocompatibility and does not contemplate any positive interactions between biological tissues and the magnetic materials.

The ME amorphous alloy Metglas® 2826MB™, for example, is readily available for use in the development of biomedical devices, but requires special care, since ion release can promote cell cytotoxicity [12,17]. That alloy has approximate composition $Fe_{45}Ni_{45}Mo_3B_7$ and molybdenum and nickel are reported to have harmful effects on organisms. Molybdenum has been associated with various toxic effects on the body, causing damage to various

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http://dx.doi.org/10.1016/j.colsurfb.2016.03.034
0927-7765/

organs such as kidney, liver and spleen [18,19]. Nickel is known to be carcinogenic and toxic [20]. The dose-dependent cytotoxic effect of nickel has been reported in cultured fibroblasts [21]. A recent study showed that molybdenum nanoparticles (Mo-NPs) induced cytotoxicity in mouse skin fibroblast cells (L929), with decrease in cell viability and alterations in cell morphology [22]. The search for coatings that promote biocompatibility and stability in ME alloys for biological systems has been recently reported [17]. However, the performance of a biosensor for the wireless monitoring of biological samples can be affected by the application of these coatings. Different types of analyte solutions, such as the ones based on buffered salt solutions, which preserve living organisms, can induce accelerated corrosion of the magneto-elastic material and the release of toxic ions. The coating of these materials with nanometric chrome and gold films provides a surface capable of immobilizing the bioactive component. In addition, the preservation of the physical and mechanical properties of these sensors [23] has been attributed to the presence of a gold coating.

In order to investigate cytotoxic effects of ions in cell cultures, this study considered the effects of biocompatibility and degradation of the Metglas® 2826MB3™ alloy, as well as the effects of a gold covering layer, when in contact with cell culture medium. Cell cytotoxicity was evaluated through MTT, which measures the reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) according to the methodology described by Denizolt and Lang [24]. Cell death induction was further evaluated through Acridine Orange/Ethidium Bromide (AO/EB) staining. Finally a quantitative determination of the presence of metal ions in the cell culture medium was made. This allowed an evaluation of the beneficial effects of the presence of a gold covering layer on the ME material. It also allows us to determine the time scale for these effects.

2. Material and methods

2.1. Substrate preparation

The amorphous alloy Metglas® 2826MB3™ was supplied by the Metglas Corp. of Conway SC, with approximate composition in wt.% of $Fe_{45}Ni_{45}Mo_3B_7$. The alloy was supplied in the form of 2 in. wide ribbons. The ribbons were first mechanically polished on both sides using a Struers Tegramin 20 polishing system with 0.05 μm alumina and water. After 1.5 h of polishing, their thickness was reduced to about ~15 μm. The debris or grease retained from the polishing process was removed by cleaning the strips ultrasonically in 100% methanol for 30 min. After the cleaning process, tapes were sputtered-coated (AJA, model ATC 2000) on both sides with a protective layer of chromium and then with gold. The Au-coated strips were cut to dimensions 5 mm × 1 mm × 15 μm. Bare alloy strips were cut to dimensions 5 mm × 1 mm × 30 μm with a microdicing saw. ME ribbons were sterilized at 200 °C for 120 min before exposure to cell culture medium.

2.2. Substrate characterization

The thicknesses of the chromium and gold layers were evaluated by Rutherford Backscattering Spectroscopy (RBS). Simulations of the spectra associated with deposited layers were made with the usual RUMP software routines [25,26]. The covered sensor surfaces were examined using Scanning Electron Microscopy (SEM).

2.3. Cell culture

Chinese Hamster Ovary cell lines were purchased from American Type Culture Collection (CCL-61, ATCC). Cells were cultured in Dulbeccoís Modified Eagleís Medium (DMEM), supplemented with 10% of Fetal Bovine Serum (FBS) and 1% of penicillin-streptomycin BRL; Life Technologies (Van Allen Way, Carlsbad, CA, USA). Cell lines were kept in a humidified atmosphere at 37 °C and 5% of CO_2.

2.4. Cytotoxicity assay

Cell cytotoxicity was assessed through MTT, an indirect cytotoxic test based on the formation and colorimetric quantification of an enzyme reaction product, which evaluates the reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) according to the methodology described by Denizolt and Lang [24]. Briefly, 8×10^4 cells were inoculated for analysis after one day, 10^3 cells for analysis after two days, and 5×10^2 cells after seven days exposure to the strip surface. Afterward cells were seeded with 1 mL of supplemented culture medium in a 24-well plate and incubated for 24 h at 37 °C. The ME alloy was placed in the well for different incubation times (1, 2 and 7 days), while the solution of MTT (1 mg/mL in serum-free medium) was added for two hours after the incubation period. The formazan crystals were dissolved with dimethyl sulfoxide (DMSO) for 30 min, and the absorbance was measured using a microplate reader (Spectra Max M2e, Molecular Devices) at 570 nm. The absorbance of the negative control (cells without ME alloy) represents 100%. The percentage of growth inhibition was calculated as: cell viability (%) = (absorbance of experimental wells/absorbance control wells) × 100. Each experiment was performed in triplicate.

2.5. Acridine Orange/Ethidium Bromide (AO/EB) staining and image processing

The AO/EB technique is suitable to visualize viable cells and cell death induction. Initially, 8×10^4 cells/well were seeded in 24 well plates and incubated for 24 h with bare or Au-covered magneto-elastic strips. After incubation, the strips were washed twice with phosphate buffer saline (PBS). Cell death was evaluated through a direct *in situ* analysis on the surface of the sensor using Ethidium Bromide and Acridine Orange (Sigma-Aldrich). Each surface received 2 μL of AO and EB (100 μg/mL). Images were taken with a fluorescent light microscope (BX43—Olympus) with 10× and 40× objective magnification. The correlation between the intensity of green and red pixels were analyzed using ImageJ software v. 1.50 (http://rsb.info.nih.gov) and processed as described by Mironova et al. [27]. Briefly, AO/EB images were divided into three channels (R-red, G-green, B-black). Images of the green and red channels were quantified and the correlation plot of co-localized and non colocalized fluorescence was processed. Non-correlated pixels looked green and red and were attributed to living and dead cells, respectively. Correlated pixels looked yellow-orange and were also attributed to dead cells. The percentage of live cells was determined by dividing the number of green pixels by the total number of red and green pixels. The threshold was fixed at an intensity of 75, determined by the 8 bits extraction of channel images.

2.6. Ions concentration in media culture

To evaluate ion detachment into the medium from the surface of ME strips not covered with Au, the strips were placed in 160 mL of DMEM. After 7 days, the strips were removed and weighed dry. The incubation medium was sent for analysis by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) method. Analysis was performed by the laboratory *Greenlab® Análises químicas e toxicológicas Ltda.*