Cytotoxicity of Titanium Oxynitride Obtained by Reactive Magnetron Sputtered on Endothelial Cell Line EA.hy 926

Maria Surovtseva , Olga Poveshchenko[1], Alexander Lykov , Irina Kim, Vladimir Pichugin[2], Irina Zhuravleva[1]

Research Institute of Clinical and Experimental Lymphology- Branch of the Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia

[1]*E. Meshalkin National Medical Research Center of the RF Ministry of Health,* Novosibirsk, Russia

[2] *Tomsk Polytechnic University,* Tomsk, Russia

INTRODUCTION

Nickel-Titanium (NiTi) alloys are an important material for biomedical applications and are widely used for cardiovascular implants. The development of coated stents with bioinert, biologically active properties is an urgent problem. Reactive magnetron sputtering (RMS) is a method for preparing thin films based on nitrogen-containing compounds of titanium oxides (Ti-O-N) on the surface of the stent. The coatings obtained by this method improve the biomedical properties of the surface of stents, are able to play the role of a depot of nitric oxide, locally release its molecules in the area of contact with vascular cells. Endothelial cells are inner line of the vessels. In this report, we screened cytotoxicity of samples of nitinol obtained treated with different RMS modes on endothelial cell line EA.hy 926.

MATERIALS AND METHODS

Medical grade stents made 316L stainless steel were used to obtained coatings titanium oxynitride (TiOxNy) via RMS using UVN-200 MI. During RMS oxygen and nitrogen have been supplied as a reactive gas. Was prepared 6 samples (≈11 x 3 mm): TiO_2 only oxygen as reactive gas (N=1); Ti-O-N with oxygen and nitrogen (N_2/O_2) in proportion 1/1 and negative bias voltage (U_{bias})= -100V (N=2); Ti-O-N with oxygen and nitrogen (N_2/O_2) in proportion 2/1 and U_{bias}= 100V (N=3); Ti-O-N with oxygen and nitrogen (N_2/O_2) in proportion 3/1 and U_{bias}= 100V (N=4); Ti-O-N with oxygen and nitrogen (N_2/O_2) in proportion 2/1 and U_{bias}= 0V (N=5) and Ti-O-N with oxygen and nitrogen (N_2/O_2) in proportion 1/1 and U_{bias}= 0V (N=6). Three samples of each test group were used for each experiment (n=3). Untreated nitinol samples, glass and the cells with no contact with any samples were as a control. The endothelial cell line EA.hy 926 was cultured in DMEM/F12 medium supplemented with 10% fetal bovine serum, 80 μg/mL of gentamycin, 2 mM L-glutamine under 37^0C in a 5% CO_2 and high humidity environment. The levels of the persistent nitric oxide metabolites (NO)-nitrites (NO_2) production by EA.hy 926 cell line (24, 72, and 120 hours) was determined by Griess reagent. Cytotoxicity of the samples of nitinol treated with RMS on EA.hy 926cell line was done by MTT-test after 24, 72 and 120 hours. For this $4x10^4$ cells/well were seeded

in a 24-well plate on 24 hours, then media was removed and samples of nitinol (untreated and treated with RMS), glass were placed on the cells, and fresh media was done. For apoptosis of EA.hy 926 cell line was stained in a 1:1 proportion of 100 µg/mL ethidium bromide and 100 µg/mL acridine orange and observed under an Axio Observer Z1 microscope (Zeiss, Germany).

RESULTS

Fig. 1 shown different rate of the viability of EA.hy 926 cell line on 24, 72, and 120 h exposure with the nitinol samples. So, on 24 h EA.hy 926 cell lines a live from 86% to 100% (N4 and N6 respectively) compared with basal rate of survival (EA.hy 926 cell line without any samples).

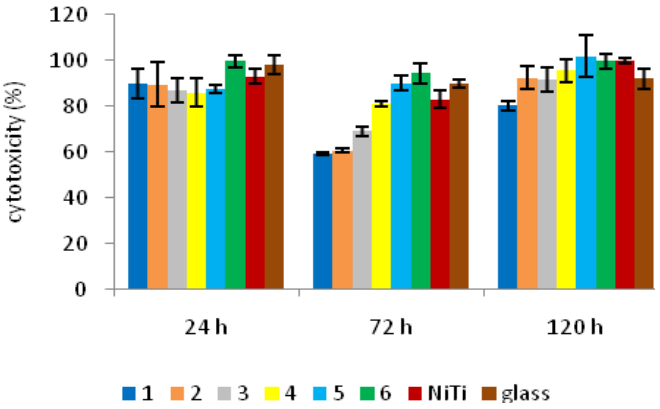

Fig. 1. Cytotoxicity of EA.hy 926 cell line under the influence of Titanium oxynitride samples after 24, 72 and 120 hours (Mean ± SD, n=3)

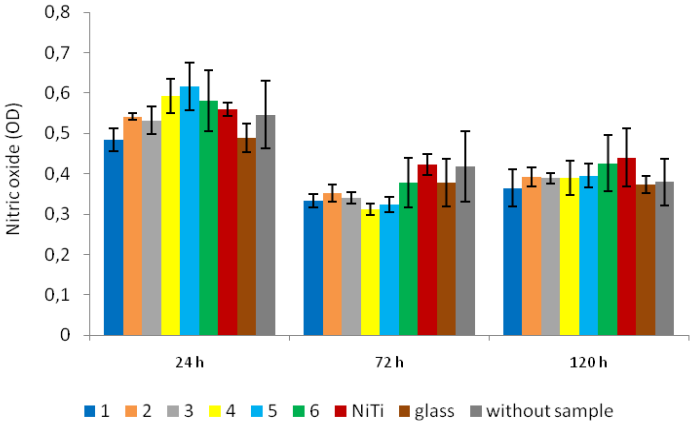

Fig. 2. Effect of Titanium oxynitride samples on nitric oxide production by EA.hy 926 cell line (Mean ± SD, n=3)

On 72 h the viability of the EA.hy 926 cell line markedly decreased, the lowest viability was detected for sample N1 (59%), whereas sample N6 showed high viability (94%) compared with control (cells without samples).After 120 h EA.hy 926 cell line survival rate was a significant increased for all tested nitinol samples: 80% for sample N1 and 100 % for samples N5, sample N6, and untreated sample of nitinol.

Nitric oxide production by EA.hy 926 cell line was not a significant changed on 24 h exposure with nitinol samples compared with basal levels of the NO production by cells samples. On 72 h exposure EA.hy 926 cell line with nitinol samples NO production a significant decreased compared with basal levels. So, exposure EA.hy 926 cell line with sample N4 reduced NO production upon 39%, whereas samples N1, N2, N3 and N5 reduced NO production in range from 31% to 36% compared with basal level of NO production (Fig.2). The level of NO production by endothelial cells on 120 h was comparable between groups of the treated samples of nitinol, untreated sample of nitinol, glass and control wells (without samples).

The studied samples of titanium oxinitride did not stimulate apoptosis of EA.hy926 cell line compared to the level of apoptosis control without any sample (Fig. 3)

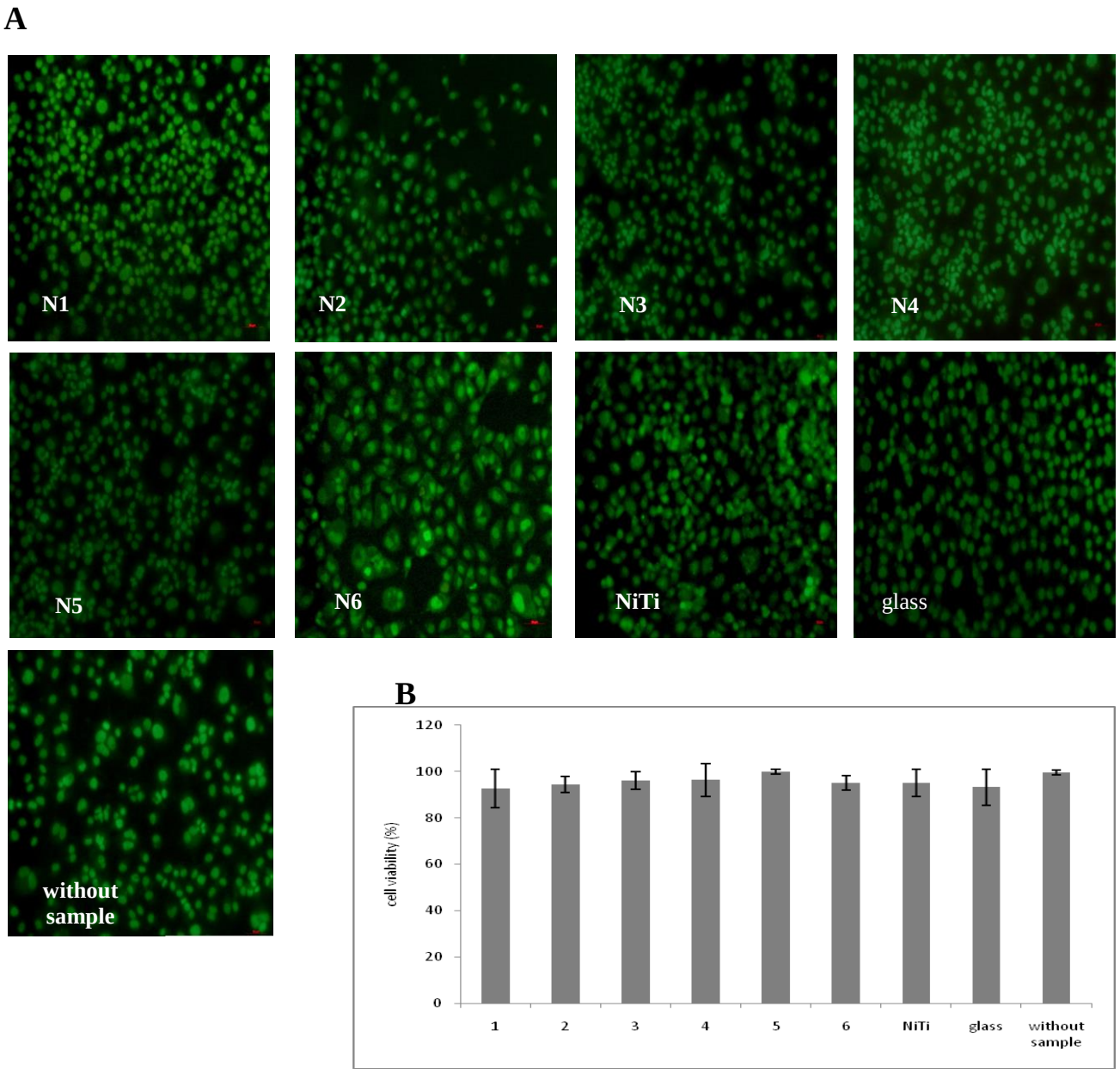

Fig. 3. Effect of Titanium oxynitride on EA.hy 926 cell line apoptosis after 120 h exposure. A- live cells at the bottom of the wells stained green (acridine orange, green fluorescence) (magnification 200, sale bar = 50 µm); B- the number of live cells counted in the photos (Mean ± SD, n =3)

CONCLUSION

This study demonstrated that nitrogen-coating of titanium oxide obtained under various proportion of the reactive gas of oxygen and nitrogen by reactive magnetron sputtering showed not a significantly alteration functional properties on endothelial cell line EA.hy 926 *in vitro*.