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Influence of sodium hypochlorite treatment of electropolished and magnetoelectropolished nitinol surfaces on adhesion and proliferation of MC3T3 pre-osteoblast cells

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Abstract The influence of 6 % sodium hypochlorite (NaClO) treatment on adhesion and proliferation of MC3T3 pre-osteoblast cells seeded on electropolished (EP) and magnetoelectropolished (MEP) nitinol surfaces were investigated. The chemistry, topography, roughness, surface energy, wettability of EP and MEP nitinol surfaces before and after NaClO treatment were studied with X-ray photoelectron spectroscopy (XPS), profilometry, and contact angle meter. In vitro interaction of osteoblast cell and NaClO treated EP and MEP nitinol surfaces were assessed after 3 days of incubation by scanning electron microscopy. The XPS analysis shows that NaClO treatment increases oxygen content especially in subsurface oxide layer of EP and MEP nitinol. The changes of both basic components of nitinol, namely nickel and titanium in oxide layer, were negligible. The NaClO treatment did not influence physico-morphological surface properties of EP and MEP nitinol to a big extent. The osteoblast cells show remarkable adherence and proliferation improvement on NaClO treated EP and MEP nitinol surfaces. After 3 days of incubation they show almost total confluence on both NaClO treated surfaces. The present study shows that NaClO treatment of EP and MEP nitinol surfaces alters oxide layer by enriching it in oxygen and by this improves bone cell–nitinol interaction.

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1 Introduction

Nickel titanium intermetallic compound differs from other implantable alloys by its unique and unusual properties of thermal shape memory, superelasticity (strain recovery) and good damping properties. Its low elastic modules, between 20 and 90 GPa, close to that of cortical bone, between 15 and 25 GPa [1], makes it very attractive as orthopedic implantable material as bone plates, staples, spine fracture fixation devices, maxillofacial and dental implants and intramedullary nails for repair of fractured elongated bones [2], etc. The first and most important interaction between implantable material and host organism is cell adhesion. The cell adhesion initiates further cellular functions such as growth, spreading, proliferation, and interaction with adjacent cells. As we know, cells do not interact with bare biomaterial. The prerequisite for cell adhesion are proteins presence on the implant surface which adsorption occurs immediately upon the contact with surrounding tissue [3]. The adsorbed proteins form focal contacts with cell membrane and immobilize them on the implant surface starting cells adhesion on biomaterial surface. This process is conducted by cell membrane integrins connected to cytoskeleton which binds certain protein amino acid sequences [4]. It is well documented that performance of nitinol as implantable device (vascular or orthopedic) can be compromised by nickel leaching to surrounding tissue of host organism [5]. The ability of Ni^{2+} cations to interact with proteins and form complexes creates possibility of altering proteins conformation on the implant surface [6]. Those proteins conformation and further interaction with other proteins can lead to

changes in their functionality and cellular homeostasis producing a variety of pathogenic effects [7] including a rejection of implant. To minimize this risk countless surface modification methods were proposed and introduced to greater or lesser extents [8]. Some of those methods depend only on refining sole composition of nitinol as etching, pickling, mechanical polishing, sand or grit blasting, anodizing [9], electropolishing [10], or recently proposed magnetoelectropolishing process [11–14]. Another ones use additional inorganic or organic materials to coat native nitinol surface. The most popular inorganic coatings are diamond-like carbon (DLC) [15], titanium nitride (TiN) [16], silicon carbide (SiC) [17]. Organic coatings include wide variety of polymers with recently proposed nanocomposite (POSS-PCU) polymer [18]. From all above mentioned nitinol surface modification techniques directed to removing nickel from outer oxide layer the best are electropolishing and magnetoelectropolishing processes. But even electropolishing, which is recognized as a gold standard for finishing implantable devices, is unable to remove totally all nickel compounds from nitinol surface [19]. The only finishing method able to remove totally nickel from the surface of nitinol is magnetoelectropolishing process [11, 13]. This is done by interaction of external magnetic field with ferromagnetic nickel which speeds up its dissolution leaving only titanium in oxidized form of TiO_2 in most outer passive layer. This corresponds only to nickel inclusion-free nitinol surfaces. However, it is not possible to produce 100 % inclusions (consisting of nickel as well as of titanium elements) free nitinol. Those inclusions are randomly distributed through volume of nitinol and finding them on the surface is of pure luck. Even if raw material surfaces by chance are free from intermetallic inclusions it does not mean that finished product surfaces will also be free from them. They can be revealed from the interior of material by mechanical, chemical or electrochemical production operations or introduced by them from outside. To look for them on the surface of nitinol, simple and 100 % reliable method was introduced [20]. The method consists in submersing nitinol in 6 % sodium hypochlorite (NaClO) for 15 min and visually observing it for any traces of black flocculent oozing from the surface [21]. As this method is able to prevent implantation of medical devices with intermetallic inclusions on its surface (except of biologically harmless titanium dioxide TiO_2 inclusions) which could have catastrophic outcome when implanted (fracture and enhanced corrosion with substantial nickel release) nothing is known how these chemically modified surfaces will interact with living cell. In this study we undertook evaluation of EP and MEP nitinol surfaces which underwent NaClO treatment [21] with negative results (no surface intermetallic inclusions except of TiO_2 found) on MC3T3 pre-osteoblast cells adhesion and proliferation.

2 Materials and methods

2.1 Preparation of the sample

2.1.1 Abrasive polishing

The samples 10 mm in diameter 3 mm thick were stamped from austenitic flat annealed, in oxidized state SE 508, nitinol plate which was obtained from Nitinol Devices and Components (NDC Fremont, CA); the chemical composition and transformation temperature of this nitinol alloy are given elsewhere [22]. To remove oxide before electropolishing and magnetoelectropolishing, samples were successively polished mechanically using grades 340, 600, 1500 silicon carbide paper, and then cleaned ultrasonically in distilled water.

2.1.2 Electropolishing (EP)

The mechanically polished samples underwent electropolishing process (EP) under oxygen evolution regime. The electrolytic cell for EP consisted of transparent 500 ml beaker. The nitinol sample (anode) was vertically suspended in the middle of the beaker 10 mm under the surface of electrolyte solution. The cathode consisted of 316L stainless steel screen positioned around internal beaker wall. The EP was performed for 5 min in room temperature of 25 °C under constant potential of 10 V. After EP the samples were ultrasonically cleaned in distilled water.

2.1.3 Magnetoelectropolishing (MEP)

The magnetoelectropolishing process was performed exactly as electropolishing with addition of externally applied constant magnetic field of around 100 mT. The external magnetic field was imposed on the system by placing electrolytic cell inside four ring magnets stack together.

2.1.4 Sodium hypochlorite (NaClO) treatment

Half of electropolished and magnetoelectropolished nitinol samples underwent submersion in 6 % NaClO at room temperature of (25 °C) for 15 min. The 6 % NaClO solution was prepared from solution purchased from Sigma-Aldrich Co.; Merck Index 12,8773 RTECS# NH3486300 OXIDIZER CORROSIVE. The samples were submersed in vertical position in 500 ml glass beaker for the purpose of visually monitoring them. After NaClO submersion the samples were ultrasonically washed in distillated water and dried.

2.2 Roughness measurement

Surface roughness measurements of EP, MEP, EP + NaClO and MEP + NaClO nitinol samples were performed using the automated optical 3D profiler Talysurf CCI 6000 by Taylor Hobson [23]. The measurements of roughness were conducted for each sample without consecutive treatment. Obtained parameters are based on scanning size of 360 × 360 μm. The meaning of roughness parameters are explained here: root mean square height (S_q) represents the standard deviation of the height distribution of the measured surface, (S_a) is the arithmetical mean height which represents the mean roughness, S_p, S_v and S_z are the maximum peak height, the maximum pit depth from the mean plane and the maximum height between the highest peak and the deepest valley, respectively.

2.3 Contact angle measurements

To evaluate influence of NaClO treatment of EP and MEP nitinol samples the wettability contact angles were measured using a sessile drop method. For comparison, contact angles were also measured on EP and MEP nitinol samples. The Kyowa contact angle meter was used for contact angle measurements.

2.4 XPS study

The chemical composition of the surface layer of differently finished nitinol samples (EP, MEP, EP + NaClO, MEP + NaClO) was analyzed by X-ray photoelectron spectroscopy (XPS). The surface was analyzed using PHI Quantera scanning XPS microprobe, employing monochromatic Al Kα X-ray radiation. After survey was done, high resolution Ti 2p, Ni 2p and O 1s was acquired at 55 eV pass energy in order to determine their chemical states. Wide energy survey spectra were collected in a large area analysis mode to determine which elements were present at the surface. The larger area analysis mode probes an area of 200 μm in diameter with a 50 W monochromatic Al Kα X-ray beam. Sputter depth profiles were obtained using the large area analysis mode and 2 kV argon ions rastered over 3 × 3 mm area. The sputter etch rate of 6.5 nm/min was determined using SiO_2 film of known thickness.

2.5 In vitro pre-osteoblast cell–material interaction

The cell culture media was prepared by adding 10 % fetal bovine serum (FBS), 1 % penicillin, 1 % B-glycerophosphate, 1 % dexamethasone and 0.5 % ascorbic acid-2-phosphate into MEM media, Alpha 1X (Invitrogen 12571071).

The MC3T3 cells, subclone 4, were first cultured in a 75 cm^2 cell culture flask using F-12K as the medium. When confluence was achieved by the cells, they were trypsinized, centrifuged and re-suspended in culture media for cell counting and cell seeding.

In order to assess the cell proliferation, nitinol alloys were placed into a 12-well plate and seeded with 50 × 10^3 cells per well. Cell culture plates with nitinol alloys were incubated for 72 h at 37 °C, 5 % CO_2 in cell culture media.

Later, cell culture media was removed and the nitinol alloys were gently washed with de-ionized water and then with PBS. Finally, the cells were fixed using 2.5 % glutaraldehyde in PBS and placed in refrigerator for 90 min. Nitinol alloys were then taken out from refrigerator and rinsed twice with PBS. Afterwards, nitinol alloy samples were dehydrated using 30, 50, 70, 85 and 100 % ethanol for 15 min. After dehydration, samples were placed in hexadimethylsilazane (HDMS) and allowed to dry overnight in the hood.

2.6 Cell counting

Cells were counted by randomly choosing an area of 500 μm^2. The consistency of the result was ascertained by performing experiments at least three times.

3 Results

3.1 Sodium hypochlorite (NaClO) treatment

Sodium hypochlorite is a very powerful oxidizer and reacts with lots of metals and their compounds including nickel. In nitinol alloy, nickel and titanium atoms are kept together by very strong inter-atomic bonds. How long those bonds are intact nitinol is immune to NaClO. But when those bonds are broken nickel is immediately dissolved by NaClO with a characteristic black color flocculent oozing from reaction site. In our case no black flocculent and gas bubbles were detected during 15 min of observation. The lack of those events proves that samples surfaces are free from nickel containing inclusions or matrix sites enriched in nickel.

3.2 Surface roughness and morphology

Figure 1 shows micrographs of differently finished nitinol surface. All of those differently treated surfaces look very similar with prominent feature of retained titanium intermetallic inclusions protruding from them. The intermetallic inclusions which unfortunately are inseparable with nitinol also are visible on 2D photo simulation (Fig. 2). The results of roughness measurement are shown in Table 1.

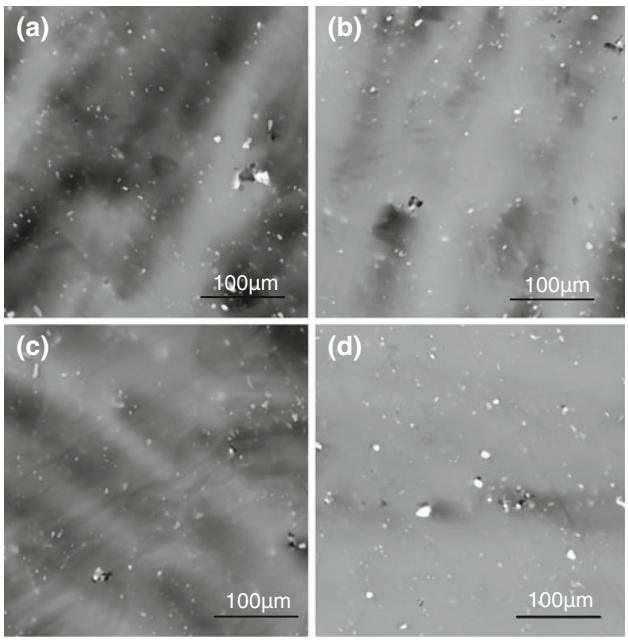

Fig. 1 Micrographs of nitinol surface after: **a** EP, **b** MEP, **c** EP + 6 % NaClO, **d** MEP + 6 % NaClO

Generally, magnetoelectropolished nitinol has the smallest surface roughness (arithmetical mean roughness (S_a)) when compared to electropolished one. As was mentioned previously, roughness parameters were collected for every sample with particular treatment separately. Taking under consideration the influence of randomly distributed surface intermetallic inclusions and initial manual surface polishing it is impossible to conclude the influence of NaClO treatment on samples roughness. The roughness parameters of every sample are in the same range.

3.3 Contact angle

To evaluate difference between EP and MEP nitinol wettability and further influence of 6 % NaClO treatment of EP and MEP on the wettability of nitinol the contact angle between nitinol surface and three different liquids were measured. The results are shown in Table 2. As could be expected MEP nitinol surface showed lower water contact angle of 82.9° compared to 88.4° of EP nitinol sample. The 6 % NaClO treatment of EP and MEP showed inconsistent results. In the case of EP nitinol sample the water contact angle drops from 88.4° of EP sample to 82.2° of EP + 6 % NaClO. For MEP sample 6 % NaClO treatment shows totally different results. The water contact angle increased from 82.9° for MEP sample to 95.4° for MEP + 6 % NaClO sample.

3.4 XPS study

Depth profiles of differently finished nitinol are shown in Fig. 3. They present changes in elemental composition from the top layer in-depth after: (a) EP, (b) MEP, (c) EP + 6 % NaClO, and (d) MEP + 6 % NaClO (Table 3).

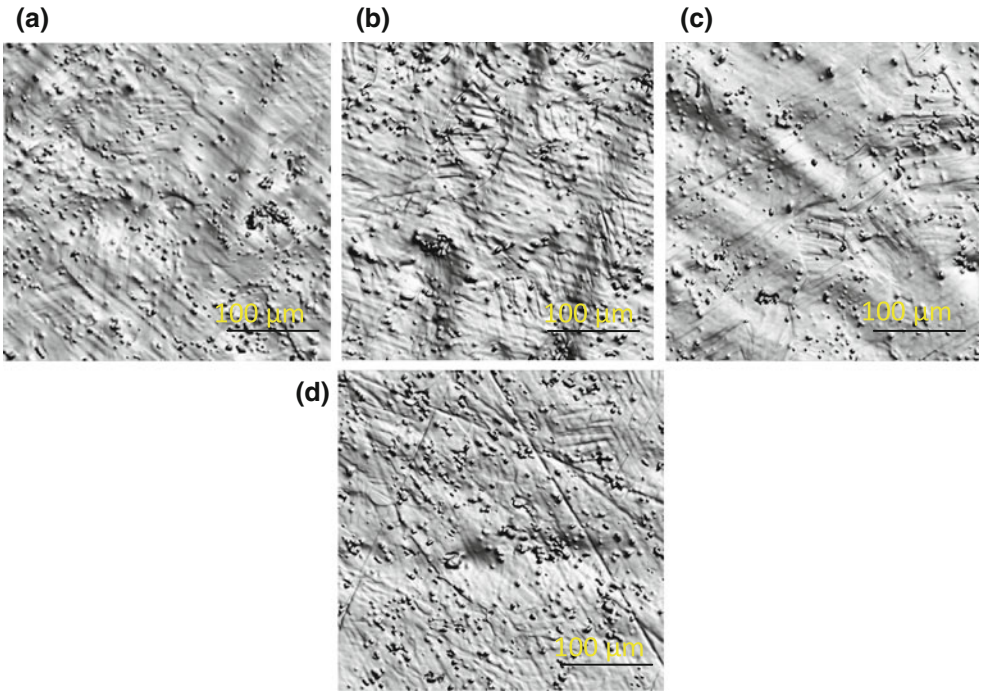

Fig. 2 2D photo simulation of morphology of nitinol surface after: **a** EP, **b** MEP, **c** EP + 6 % NaClO, **d** MEP + 6 % NaClO

Table 1 Surface height parameters from Talysurf CCI 6000 tests based on the scanning size of 360 × 360 μm

Sample index (nm)	Treatment			
	EP	MEP	EP + 6 % NaClO	MEP + 6 % NaClO
S_q	524	325	325	368
S_p	5,827	3,277	6,045	3,870
S_v	1,203	1,263	1,366	1,740
S_z	7,026	4,540	7,410	5,600
S_a	386	249	237	216

As can be expected, the dominant elements detected on the surface of all samples are oxygen and carbon. The presence of carbon is inevitable contamination which is adsorbed from air born carbon molecules. The oxygen exhibits its peak on the surface and subsurface layer and then sharply decreases. The Ni and Ti profiles indicate the lowest concentration near surface and then return to steady state of bulk of alloy. In the case of MEP and MEP + 6 % NaClO treated samples, no Ni in any form was detected in the most outer layer of samples (Fig. 3b, d).

3.4.1 XPS survey

Figure 4 presents XPS survey of nitinol surface after consecutive treatments. In Fig. 4a the XPS results after EP are presented, in Fig. 4b—after MEP, in Fig. 4c—after EP + 6 % NaClO, and in Fig. 4d—after MEP + 6 % NaClO. All they served to gain high resolution spectra.

3.4.2 Titanium

All titanium spectra (Fig. 5) were dominated by peak at 458.9 eV, which are assigned to Ti^{4+} (TiO_2). The highest intensity of this peak was visible on MEP + 6 % NaClO sample. Ti^0 peak between 453.5 and 454.1 eV was detected on the surface of any samples, which means that Ti on the surfaces of all samples was in the oxidation state.

3.4.3 Nickel

A peak at 852.8 eV was assigned to nickel in the metallic state Ni^0. In plots of all four studied samples (Fig. 6) this peak was very weak or completely not detected. However, subsurface layers of all four samples revealed comparatively weak nickel peaks when compared with their bulk peaks. This subsurface peak of nickel was less intensive in EP + 6 % NaClO treated sample. The depth profiles of both MEP samples (Fig. 3b, d) indicate lack of nickel in any state in most outer layer.

3.4.4 Oxygen

The oxygen spectra of all four samples show the characteristic double peak configuration (Fig. 7). The lowest peaks below 530 eV are attributed to oxygen contained in organic contaminants of the surface which are only detected on most outer layer of samples oxides. The second arm of the double peaks is shifted to higher energy above 530 eV and represents oxygen in metallic oxides. After the first layer is etched away the second layer shows only a single oxygen peak which is totally attributed to metal oxide. It is worth noting that oxygen peak in sublayer of MEP sample is stronger than that of EP one. The both samples (EP and MEP) which underwent 6 % NaClO treatment show also the strongest oxygen peaks (more noticeable in the case of EP sample) in their sublayers those in most outer layers. The XPS of combined oxygen signals results show oxidation enhancement of EP and MEP samples which underwent 6 % NaClO treatment compared to their not NaClO treated counterparts (Table 4) and amount approximately to: EP ≈7,800 c/s, EP + 6 % NaClO ≈10,000 c/s, MEP ≈8,800 c/s, MEP + 6 % NaClO ≈11,400 c/s, correspondingly.

3.5 In vitro pre-osteoblast cell–material interaction

The MC3T3 pre-osteoblast cell adhesion and proliferation on nitinol samples finished in four different ways (EP, MEP, EP + 6 % NaClO, MEP + 6 % NaClO) after 72 h of culture are shown in Fig. 8. At qualitative level the pre-osteoblast

Table 2 Wettability components

Sample	Contact angle (°)			Interfacial free energy (mJ/m^2)			Work of adhesion (mJ/m^2)		
Nitinol treated	Water	Ethylene glycol	Diiodo-methane	Water	Ethylene glycol	Diiodo-methane	Water	Ethylene glycol	Diiodo-methane
EP	88.4	55.5	45.9	35.6	10.6	2.4	74.9	75.0	86.1
MEP	82.9	55.4	46.9	28.4	10.2	2.7	81.8	75.1	85.5
EP + 6 % NaClO	82.2	59.9	46.7	26.3	12.0	1.3	82.6	72.0	85.6
MEP + 6 % NaClO	95.4	57.9	49.2	42.2	9.9	2.1	65.9	73.3	84.0

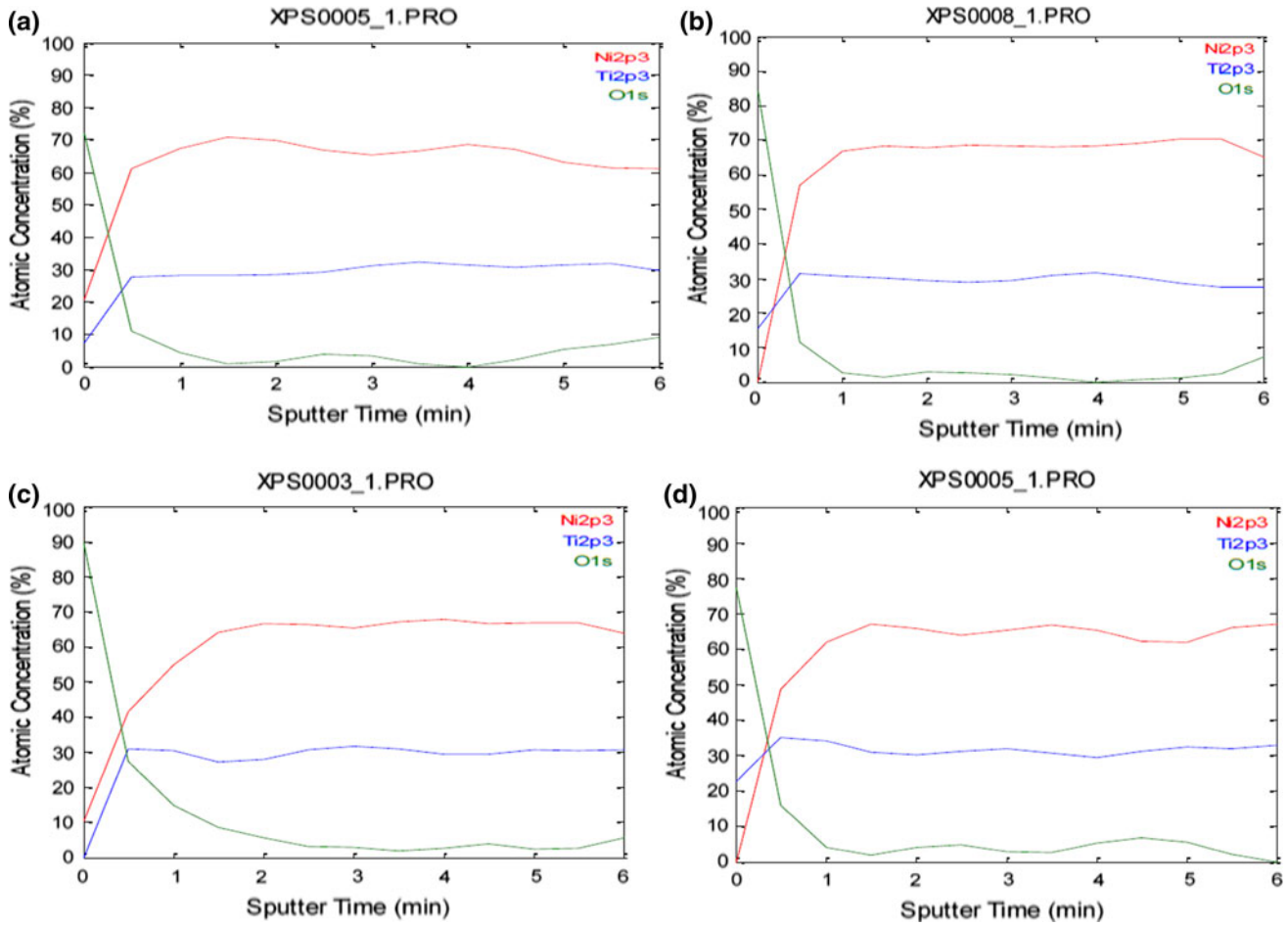

Fig. 3 XPS depth profiles of nitinol obtained after: **a** EP, **b** MEP, **c** EP + 6 % NaClO, **d** MEP + 6 % NaClO

Table 3 Elemental concentration of nitinol oxide layer (at.%) in depth of ≈13 nm detected using XPS

Sample	Component				
Nitinol	C	O	Ti	Ni	Ti/Ni
EP	67.9	21.4	1.7	<0.1	17
MEP	71.2	20.1	1.7	<0.1	17
EP + 6 % NaClO	74.6	17.5	1.7	<0.1	17
MEP + 6 % NaClO	62.1	27.0	2.9	0.4	7.25

cells had a healthy response showing the same morphology on all four samples. However, at quantitative level a profound difference between group of NaClO treated samples and their untreated counterparts was found. The NaClO treated samples reached almost 100 % confluency compared to about 40 % confluence of NaClO untreated samples. It is worth to note that all four samples are dotted with titanium oxide—TiO_2 native inclusions (rounded white spots). They seem to be totally harmless toward the osteoblast cells. As can be seen from micrographs (Fig. 8), osteoblast cells adhere to those inclusions and grow on top of them.

The results of cells counting (average of three samples) are as follows: (a) 65 on EP sample, (b) 118 on EP + 6 % NaClO sample, (c) 58 on MEP sample, (d) 120 on MEP + 6 % NaClO sample.

4 Discussion

4.1 Influence of nitinol surface properties on pre-osteoblast attachment and proliferation

It is obvious that physicochemical properties of implant surface influence the attachment and conformation of proteins, interaction of other extracellular molecules as soon as implant surface comes to the contact with biological environment. In the skeletal system, especially bones, a prerogative for implant to succeed is proper osseointegration. The first phase of the process is attachment of osteoblast cells followed by their proliferation. To fulfill those requirements the surface of implantable material has to possess physicochemical properties which osteoblast

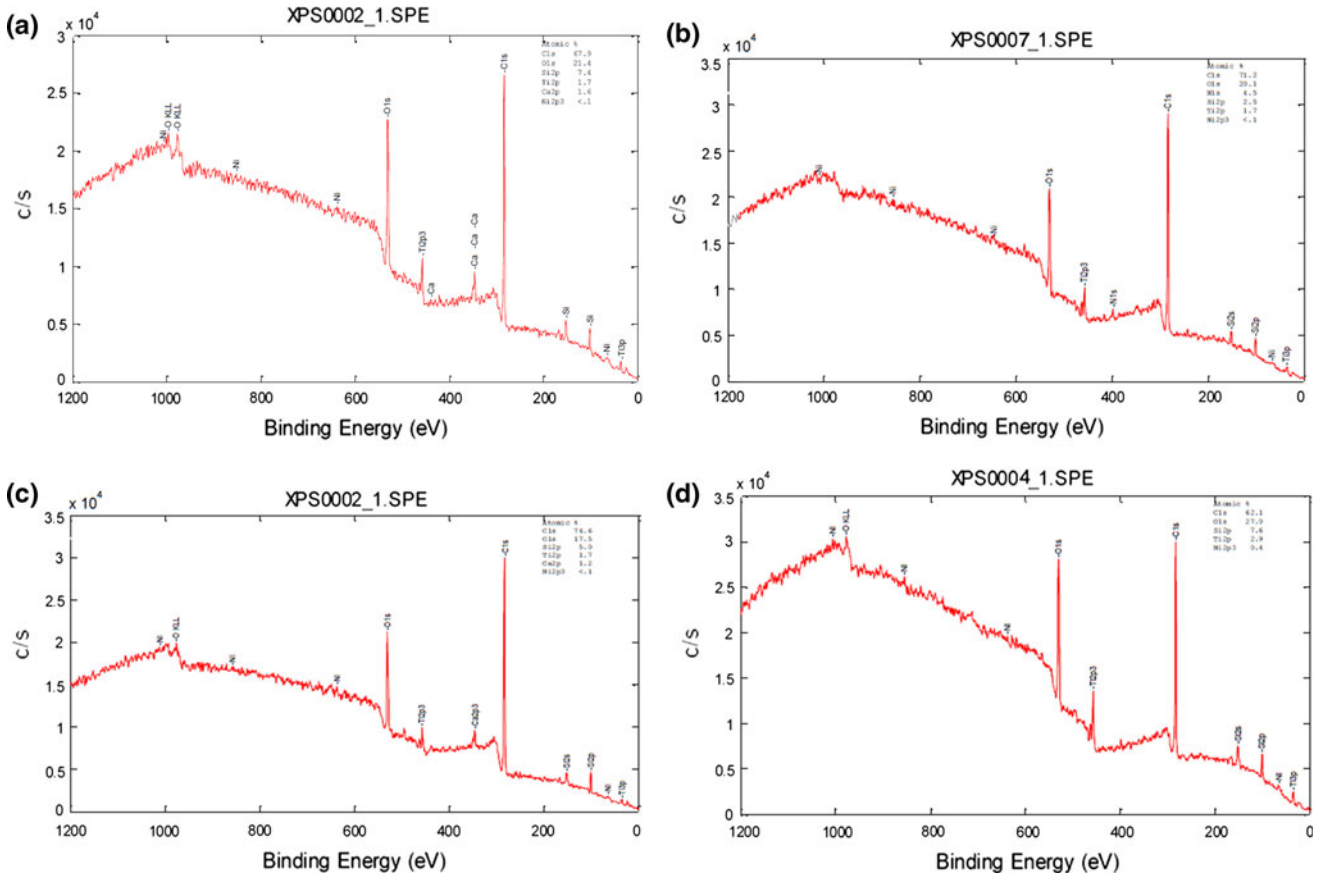

Fig. 4 XPS survey after: **a** EP, **b** MEP, **c** EP + 6 % NaClO, **d** MEP + 6 % NaClO

cells could tolerate. The most important properties are: roughness, topography, wettability, surface energy, and chemistry of outer layer of implantable material.

The main factor which prevents a broader application of nitinol as orthopaedic implants material is high content of nickel which can be released to surrounding tissues. The amount of 30 ppm can trigger any cytotoxic response during in vitro experiments [24]. The primary goal of this study was evaluation of NaClO treatment of EP and MEP nitinol surfaces on attachment and proliferation of osteoblast cells. The results of our study did not show too many differences in physical properties for differently finished samples. The surface roughness was of the same order of magnitude for all four samples (Table 1). Topography and morphology of the samples were not changed either (Figs. 1, 2). The wettability of all samples remained in the hydrophobic range. As was reported in our earlier work the MEP sample was less hydrophobic than the EP one [11]. The NaClO treatment showed no consistency on changes of wettability. The wettabillity of EP sample treated with NaClO was improved contrary to MEP sample which became even more hydrophobic after the treatment (Table 2). In spite of no noticeable changes in physical properties of treated surfaces (roughness, wettability, surface energy, etc.) the osteoblast cells attached and proliferated much better on EP and MEP surfaces which underwent NaClO treatment (Fig. 8). The main factor responsible for different osteoblast cells behavior on studied nitinol surfaces is altered chemistry of the passive layer of differently finished samples.

It is very important to notice that in our study the elemental concentrations of nickel in oxide layer of all four differently finished samples are very low (Table 3). The surfaces of samples which underwent MEP are totally free from nickel (Fig. 3b, d). This low concentration of nickel is attributed to wet electrochemical finishing techniques applied in this study. Contrary to strictly physical method of thermal oxidation used to passivate nitinol the wet electrochemical methods (EP, MEP, NaClO passivation) do not result in the formation of Ni-rich Ni_3Ti intermetallics and pure Ni particles at the interface between the surface oxide and the bulk of the alloy [25] which after implantation could become the source of nickel leaching to surrounding tissue. The study of Trepanier et al. [26] has clearly shown that thermal oxidation promotes the growth of mixed titanium oxide and nickel-rich phases which can significantly lower

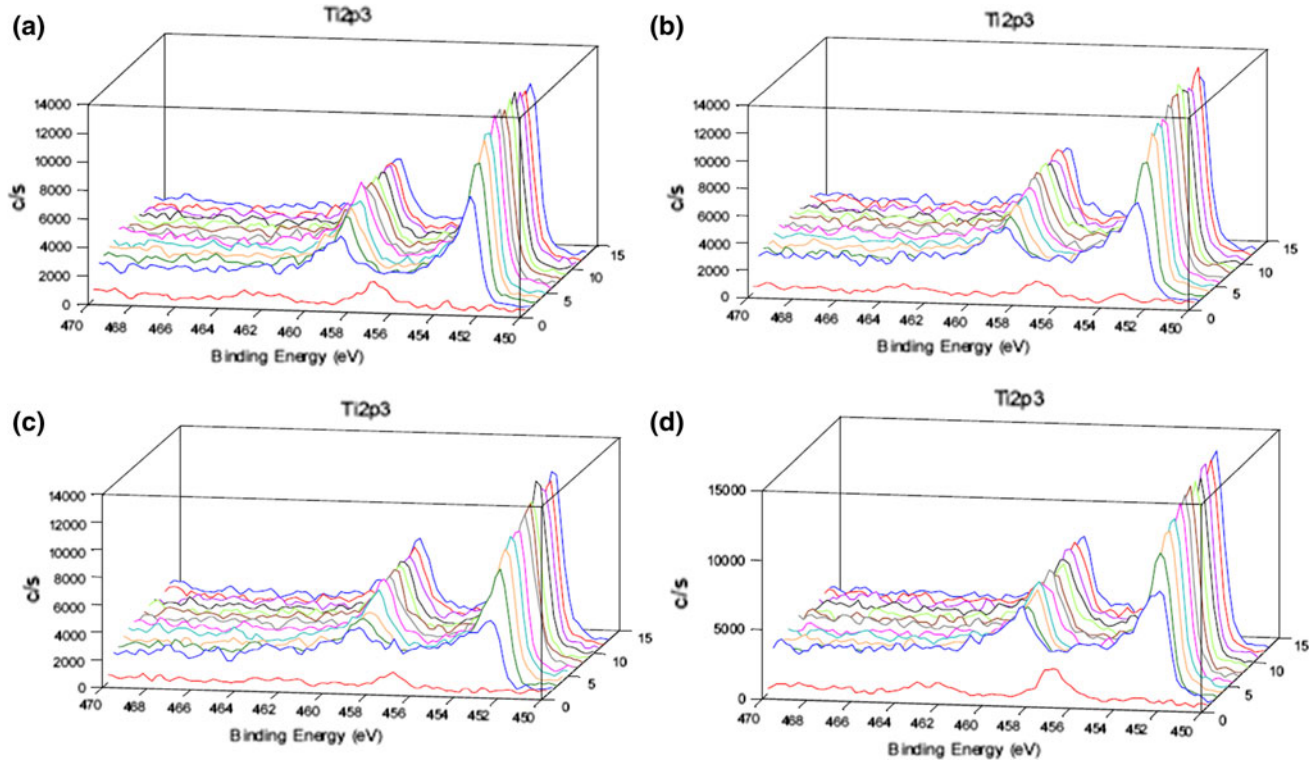

Fig. 5 XPS titanium montage plot: **a** EP, **b** MEP, **c** EP + 6 % NaClO, **d** MEP + 6 % NaClO

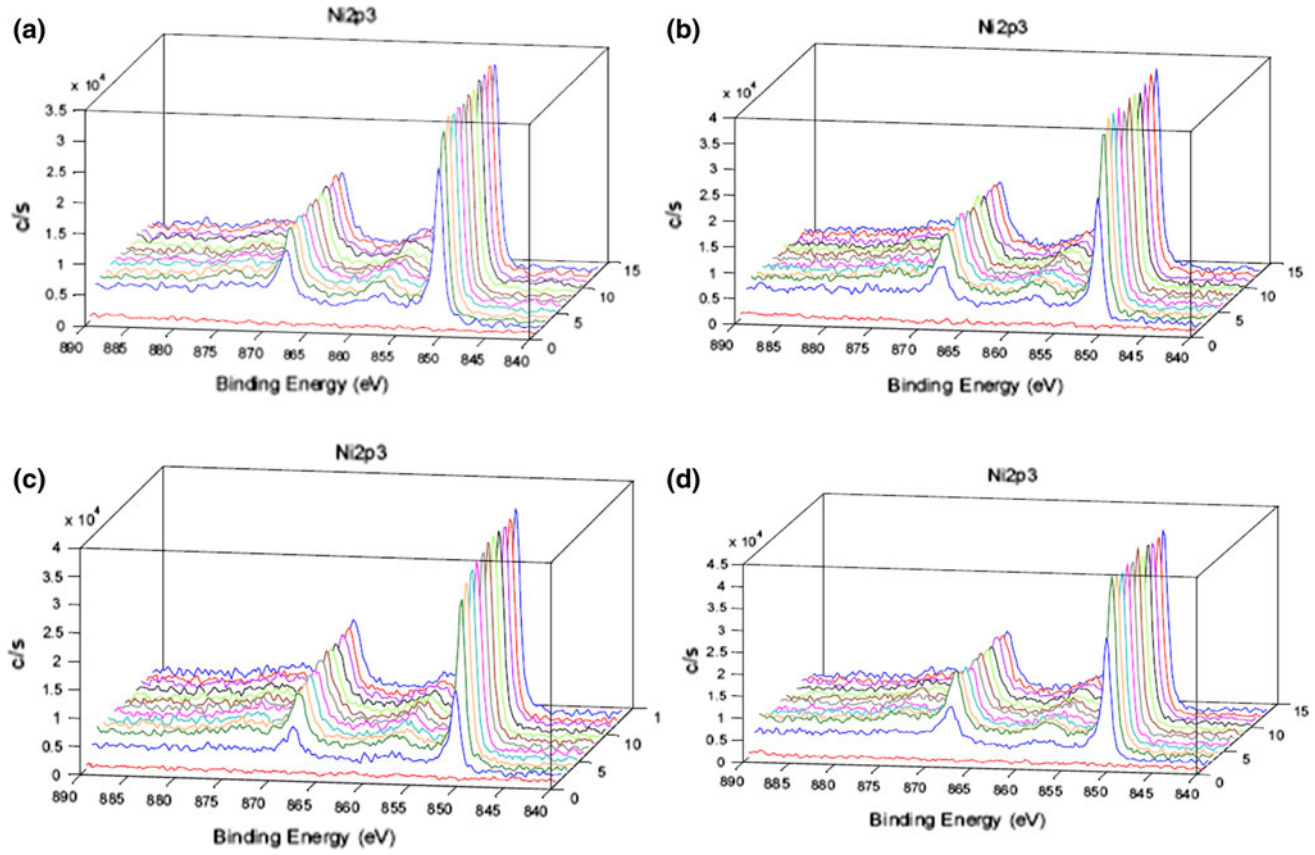

Fig. 6 XPS nickel montage plot: **a** EP, **b** MEP, **d** EP + 6 % NaClO, **d** MEP + 6 % NaClO

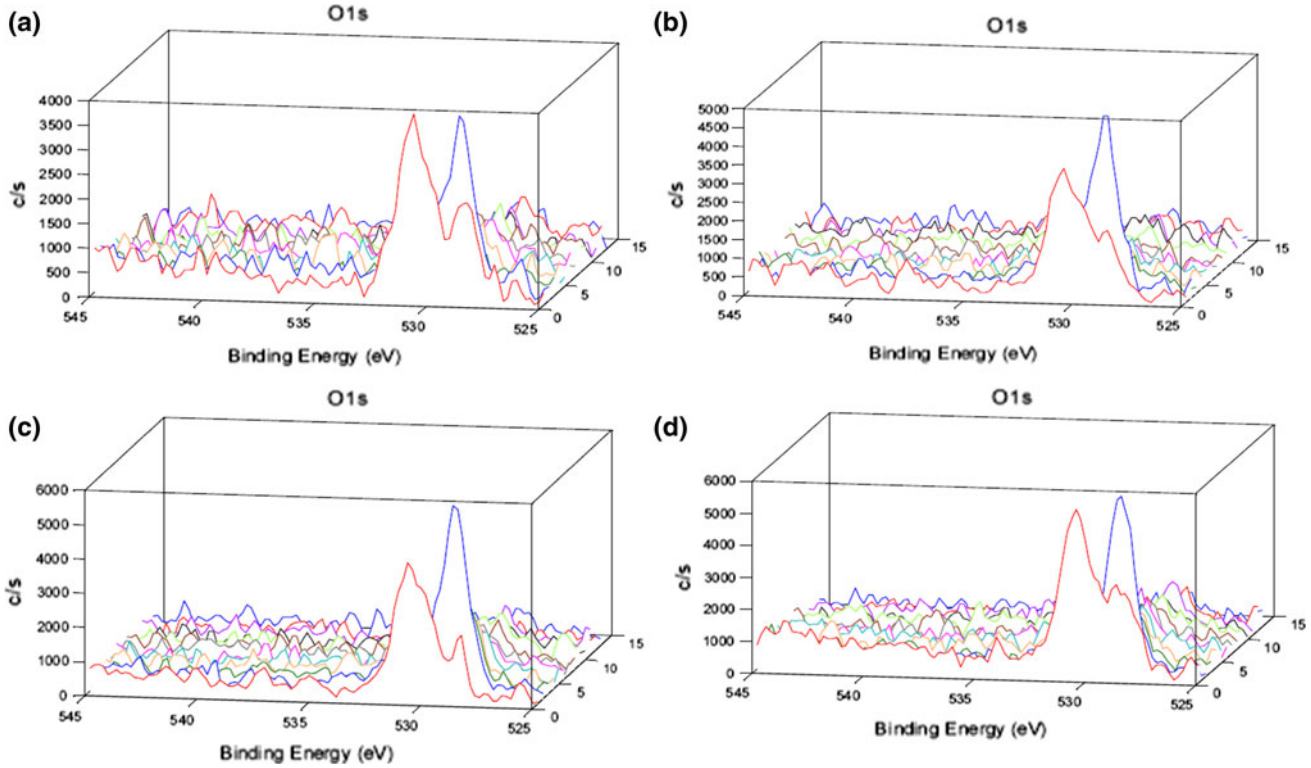

Fig. 7 XPS oxygen montage plot for nitinol samples after: **a** EP, **b** MEP, **c** EP + 6 % NaClO, **d** MEP + 6 % NaClO

Table 4 XPS oxygen intensity signals in c/s

Sample treated	Oxygen signals in top oxide layer (c/s)	Oxygen signals in sublayer (c/s)	Combined oxygen signals to the depth of 13 nm (c/s)
EP	3,900	3,900	7,800
MEP	3,700	5,100	8,800
EP + 6 % NaClO	4,200	5,800	10,000
MEP + 6 % NaClO	5,500	5,900	11,400

the corrosion resistance of nitinol and by this compromise its biocompatibility. It is also worth of noting another difference between thermal oxide and oxide produced by wet chemistry/electrochemistry methods (EP, MEP, NaClO treatment), namely the oxide thickness. The thickness of oxide produced by thermal oxidation is measured in micro meters (μm) in contrast with nanometers (nm) of oxide produced by wet chemical methods. The probability of cracks in micrometer thick oxide is much more higher than of nanometer thick one which is able to withstand bending and deformation of underlying nitinol alloy without cracking [27]. Similarly, low temperature techniques (temperature between 60 and 160 °C) which are commonly used as pre-treatment before coatings depositions on nitinol lead to similar result of Ni enrichment of interface layer through diffusion pathway. The pure titanium and its alloys, especially those with high Ti content when exposed to the ambient atmosphere at room temperature, are spontaneously covered by thin Ti-based oxide which is responsible for their high corrosion resistance in various environments [25]. In the case of nitinol this process breaks inter-atomic bonds between Ti–Ni. The titanium undergoes immediate oxidation and Ni atoms become lattice defects and interstitial atoms in the structure of the Ti-based surface oxides [25, 28, 29]. The titanium oxide created by ambient atmosphere at room temperature is non-stoichiometric with lot of vacancies. In the case of pure titanium and most probably of all high content titanium alloys those vacancies are filled in time by additional oxygen atoms which lead to its thickening [30]. But in the case of nitinol the free Ni atoms which are already positioned in the volume of Ti-based oxide are easily finding migration path through oxygen vacancies toward the bulk of the alloy. As can be seen high temperature oxidation and even low temperature dry physical processes unavoidably lead to Ni accumulation in the interface layer in the form of Ni_3Ti intermetallics, Ni-oxide and pure Ni particles. Those forms of nickel accumulated in interface layer of nitinol medical device become reservoirs for Ni ions released upon implantation which leads to prolong inflammation, allergic reaction, delayed cells adhesion and tissue integration or total implant rejection.

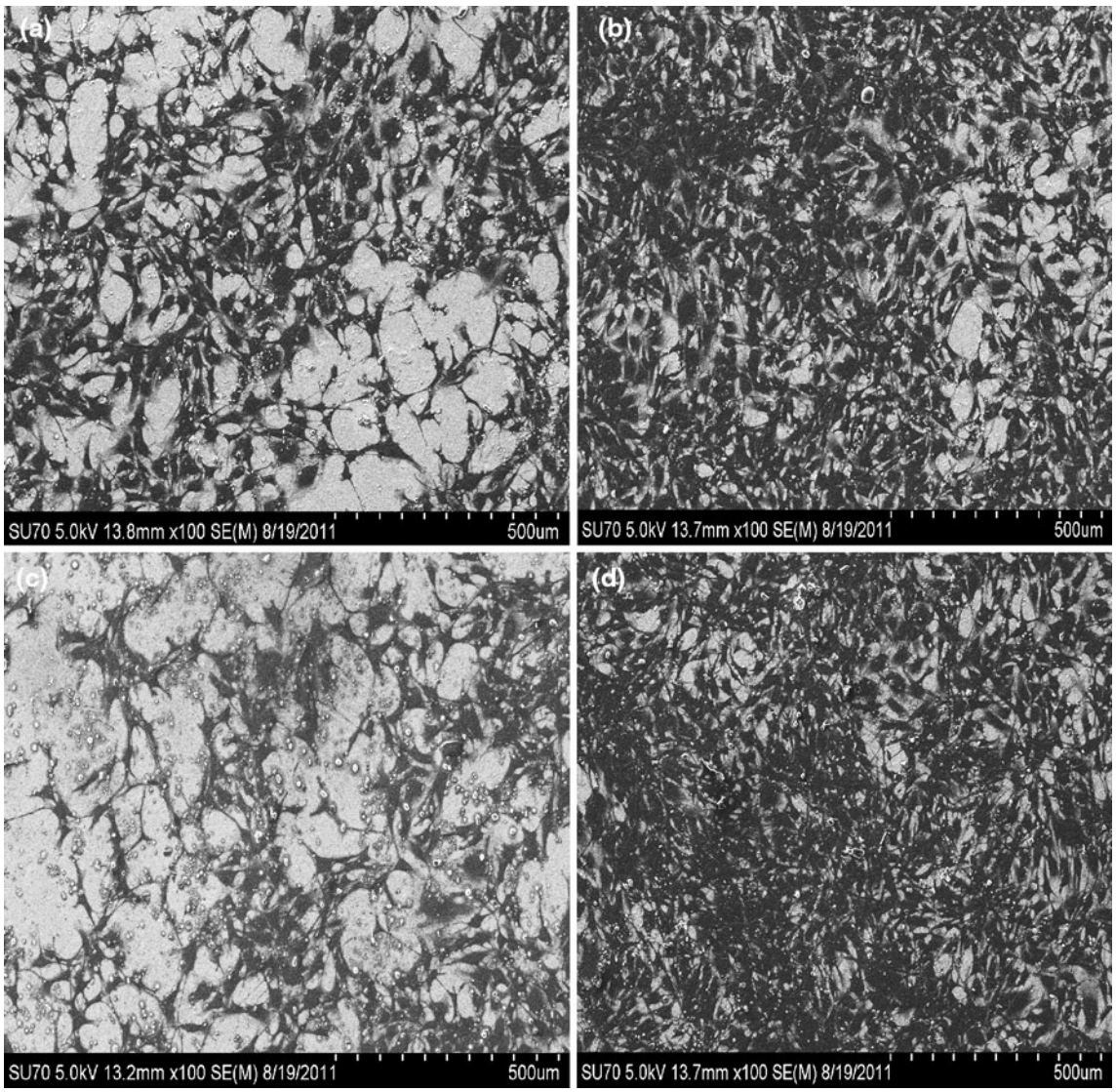

Fig. 8 Pre osteoblast cell on nitinol surface after 72 h of incubation after: **a** EP, **b** EP + 6 % NaClO, **c** MEP, **d** MEP + 6 % NaClO

To resolve the problem of nickel retention and accumulation on the surface of nitinol as well as in interface wet electrochemical techniques have to be employed. The wet electrochemical techniques not only can remove existing oxide layer but also can create oxide which can be depleted or even totally deprived of Ni content as in case of MEP process (Fig. 3b, d). The ionization of Ni upon breaking inter-atomic bounds with Ti during electrolysis and its dissolution into electrolyte makes its diffusion toward bulk of alloy impossible. But as can be seen from micrographs (Fig. 8) of pre-osteoblast cells cultures incubated on very low (<0.1 at.% of Ni) or even totally Ni-free surfaces do not show the same cell adhesion, proliferation and confluence. The XPS analysis concludes that oxygen content of passive layer is the factor of main importance in cell behavior on nitinol surfaces. Combined oxygen signals (Table 4) to the depth of 13 nm correlate with samples cells confluence. The EP and MEP (Fig. 8a, b) nitinol surfaces are showing the lowest pre-osteoblast cells coverage of about 40 %. The cells are spread unevenly with visible patches of bare nitinol surface. It is worth to note that total absence of Ni on the surface of MEP (Fig. 8b) sample do not make much difference in pre-osteoblast coverage. The combined oxygen signals for EP sample are the lowest. The higher oxygen signals of MEP sample are attributed to the influence of magnetic field on oxygen during magnetoelectropolishing process [11]. The micrographs of EP + 6 % NaClO (Fig. 8c) and MEP + 6 % NaClO (Fig. 8d) treated samples are showing almost 100 % cells confluence. Their combined oxygen signals are approximately 30 % stronger compared to their NaClO untreated counterparts. The cells are showing flattened

morphology, adhere closely to each other and are anchored to the substrate by visible filopodia. It is worth noting that contrary to another studies [9, 31] wettability which is in the range of hydrophobicity (Table 2) does not show any influence on attachment and proliferation of pre-osteoblast cells in present work.

The XPS oxygen spectra analysis (Fig. 7) gives clear picture of the influence of NaClO treatment of both EP and MEP nitinol surfaces. In both cases of NaClO treated samples the sublayer oxygen peak is more intensive than that of its untreated counterpart. That more intensive oxygen peak in oxide sublayer of EP as well as MEP of NaClO treated samples indicates that oxygen from powerful oxidizer (NaClO) was able to penetrate most outer oxide layers which have to be still non-stoichiometric. The difference of oxygen peaks intensity between sublayers of EP and MEP untreated samples and their NaClO treated counterpart amount to ≈ 50 and ≈ 15 %, correspondingly. The smallest difference of MEP samples indicates more stoichiometric oxide of MEP untreated sample which should also be more corrosion resistant.

4.2 Sterilization propose rationale

Sterilization of implantable medical device refers to any appropriate process that kills or removes all forms of microbial life (such as fungi, bacteria, viruses, spores, etc.) and transmissible agents as prions. Terminal sterilization is defined as the "process whereby product is sterilized within its sterile barrier system" [32]. The terminal sterilization process is considered a manufacturing process step itself and usually takes place at, or near the end of, the manufacturing process [33]. Many physical and chemicals methods can be used for sterilization. Presently most often used methods for terminal sterilization of medical implantable devices are radiation and ethylene oxide processes. It is well recognized that sterilization can dramatically change physicochemical properties of sterilized material and consequently influence device biocompatibility. In fact sterilization of nitinol surface is a final surface treatment before implantation [34]. Neglecting the sterilization effect on studied implantable medical device biocompatibility makes such work irrelevant, but unfortunately this is the common practice which also applies to nitinol. Besides the study of Shabalovskaya [35], Thierry et al. [36] there is not much data concerning this problem. In present work the described process of modification of EP and MEP nitinol by 6 % NaClO for improvement of adhesion and proliferation can simultaneously serve as terminal sterilization technique. The NaClO is the most widely used chlorine disinfectant with a broad spectrum of antimicrobial activity. The 6 % NaClO contains 60,000 ppm available chlorine and is able to kill: *M. tuberculosis*—1,000 ppm, *Salmonela choleraesuis*, *P. aeruginosa*—100 ppm, Clostridium difficile spores—5,000 ppm, etc. [36].

Justification for proposed sterilization process for nitinol medical device follows. The nitinol medical implantable device is subjected to EP or MEP process which electrochemically dissolves surface layer where suspected microbial organisms thrive attached to the surface. Unavoidably microorganisms attached to material surface which is in the state of dissolution are losing substrata support and fall to the very acidic electrolyte ($pH < 1$) where they perish or become inactivate. After EP or MEP process is terminated a device is ultrasonically washed in sterile water and dried in clean room environment. After drying nitinol device is submersed in 6 % NaClO for 15 min in room temperature. Removed from NaClO solution nitinol is ultrasonically washed in sterile water dried and packed in sterile environment.

The main factor which has prevented usage of NaClO for sterilization of metallic implantable medical devices is improper assumption of its corrosiveness to metals in high concentrations (>500 ppm) [37]. This assumption is partly true, for example stainless steels which are widely used for production of orthopaedic medical devices are corroded by NaClO, in contrast pure titanium is totally prone to corrosion and is the best material which is used for storage of NaClO. The work of Praisarnti et al. [38] on fatigue resistance of nitinol endodontic rotary files tested in NaClO clearly shows no detrimental effect on their corrosion behavior.

Misconception that NaClO is corrosive toward nitinol came from misunderstanding the significance of surface intermetallic inclusions which are not separable with nitinol [21].

5 Conclusion

The study shows that surface chemistry dominates interaction between nitinol and pre-osteoblast cells. Physical properties of surface, such as roughness, wettability, surface energy, have in this case second order effect. The main factor responsible for enhancement of osteoblast cell adhesion and proliferation in the case of almost Ni free passive layer of nitinol is increased oxygen content in the whole oxide profile. Trace amounts of Ni in the most outer oxide layer do not have any effect on pre-osteoblast cell adhesion and proliferation. The elevated oxygen content in the sublayers of EP and MEP NaClO treated samples shows that NaClO is able to penetrate existing oxides of EP-treated and to a lesser extent MEP-treated which indicate higher homogeneity of former one. The enhanced adhesion and proliferation of pre-osteoblast cell on NaClO treated surface directly indicates enhanced biocompatibility.

The presented study clearly indicates that simplicity, effectiveness and multi-purposiveness of described method for enhancement of adhesion and proliferation of osteoblast cells, intermetallic inclusions detection and propose sterilization of EP and MEP nitinol medical implantable devices by 6 % NaClO treatment have a chance to become the procedure of choice for every implantable nitinol orthopaedic device. We hope that this method permanently opens the door for a broader use of nitinol as material for medical implantable orthopaedic devices.

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References

1. Shabalovskaya SA. On the nature of the biocompatibility and on medical applications of NiTi shape memory and superelastic alloys. Biomed Mater Eng. 1996;6:267–89.
2. Hallab N, Merritt K, Jacobs JJ. Metal sensitivity in patients with orthopedic implants. J Bone Jt Surg. 2011;83(3):428–36.
3. Horbett TA. Principles underlying the role of adsorbed plasma-proteins in blood interaction with foreign materials. Cardiovasc Pathol. 1993;2(3):137–48.
4. Ruoslahti E, Pierschbacher MD. New perspectives in cell adhesion: RGD and integrins. Science. 1987;238:491–7.
5. Petrini L, Migiavacca F. Review article: biomedical applications of shape memory alloys. Hindawi Publishing Corporation, J Metall. 2011. pp. 15. Article ID 501483.
6. Haider W. Enhanced biocompatibility of NiTi (Nitinol) via surface treatment and alloying. Electronic Theses and Dissertations. 2010. Florida International University, Miami, Paper 177.
7. Sigel A, Sigel H, Sigel RKO. Metal ions in life. Science. 2007;2:619–60.
8. Haider W, Munroe N, Tek V, Pulletikurthi C, Puneet K, Gill S, Pandya S. Review on surface modifications of nitinol. J Long Term Eff Med Implants. 2010;19(2):113–22.
9. Bernard SA, Balla VK, Devies NM, Bose S, Bandyopadhyay A. Bone cell-materials interaction and Ni ion release of anodized equiatomic NiTi alloy. Acta Biomater. 2011;7:1902–12.
10. Fushimi K, Startmann M, Hassel A. Electropolishing of NiTi shape memory alloys in methanolic H_2SO_4. Electrochim Acta. 2006;52:1290–5.
11. Rokicki R, Hryniewicz T. Nitinol surface finishing by magneto-electropolishing. Trans Inst Metal Finish. 2008;86(5):280–5.
12. Hryniewicz T, Rokicki R, Rokosz K. Corrosion and surface characterization of titanium biomaterial after magnetoelectropolishing. Surf Coat Technol. 2009;203(10–11):1508–15.
13. Hryniewicz T, Rokosz K, Rokicki R. Surface investigation of NiTi rotary endodontic instruments after magnetoelectropolishing. MRS proceedings of the 18th international materials research congress, 9 on biomaterials, Cancun, Mexico, 16–20 August 2009. 2009;244E:21–32. ISBN 978-1-60511-221-3. http://www.mrs.org/s_mrs/sec_subscribe.asp?CID=24949&DID=280254.
14. Hryniewicz T, Rokosz K, Rokicki R. Magnetoelectropolished titanium biomaterial, in biomaterials science and engineering. Chapter 11. In: Rosario Pignatello, editor. ISBN 978-953-307-609-6, InTech, 2011; 227–248. http://www.intechweb.org/, http://www.intechopen.com/articles/show/title/magnetoelectropolished-titanium-biomaterial.
15. Quinones R, Gawalt ES. Study of the formation of self-assembled monolayers on nitinol. Langmuir. 2007;23(20):10123–30.
16. Lifeng Z, Yan H, Dayun Y, Xiaoying L, Tingfei X, Deyuan Z, Ying H, Jinfeng Y. The underlying biological mechanisms of biocompatibility differences between bare and TiN-coated NiTi alloys. Biomed Mater. 2011;6:0250012 (12pp).
17. Schmehl JM, Harder C, Wendel HP, Claussen CD, Tepe G. Silicon carbide coating of nitinol stents to increase antitrombogenic properties and reduce nickel release. Cardiovasc Revasc Med. 2008;9(4):255–62.
18. Bakhshi R, Darbyshire A, Evans JE, You Z, Lu J, Seifalian AM. Polymeric coating of surface modified nitinol stent with POSS-nanocomposite polymer. Colloids Surf B. 2011;86(1):93–105.
19. Wu W, Liu X, Han H, Yang D, Lu S. Electropolishing of NiTi for improving biocompatibility. J Mater Sci Technol. 2008;24(6):926–30.
20. Rokicki R. Method for surface inclusions detection in nitinol which are primary corrosion and fatigue initiation sites and indicators of overall quality of nitinol material. US Patent Application number: 2010, 0274077. http://www.faqs.org/patents/app/20100274077.
21. Rokicki R. Detecting nitinol surface inclusions. Mater Device Diagn Ind. 2010;32(2):44–8.
22. Nitinol Devices & Components Inc. http://www.qmed.com/supplier/nitinol-devices-components.
23. Talysurf CCI 6000—The world's largest resolution automated optical 3D profiler. Brochure, Taylor Hobson, 2005.
24. Bour H, Nicolas JF, Garrigue JL, Demiden A, Schmitt D. Establishment of nickel specific T cell lines from patients with allergic contact dermatitis: comparison of different protocols. Clin Immunol Immunopatol. 1994;73:142–5.
25. Firstov G, Votchev R, Kumar H, Blanpain B, van Humbeeck J. Surface oxidation of NiTi shape memory alloys. Biomaterials. 2002;23:4863–71.
26. Trepanier C, Zhu L, Fino J, Pelton AR. Corrosion resistance of oxidized nitinol. In: Pelton AR, Duerig T, editors. SMST-2003 proceeding of the international conference shape memory and superelastic technologies, 5–8 May 2003. SMST Society Inc. Asilomar Conference Center, Pacific Grove.
27. Hryniewicz T, Rokosz K. On the wear inspection and endurance recovery of nitinol endodontic files. PAK (Measurement Automation and Monitoring). 2009;55(4):247–50.
28. Haider W, Munroe N. Assessment of corrosion resistance and metal ion leaching of nitinol alloys. J Mater Eng Perform. 2011;20(4):812–5.
29. Shabalovskaya SA, Tian H, Anderegg JW, Dominique U, Schryvers U, Carroll WU, van Humbeeck J. The influence of surface oxides on the distribution and release of nickel from nitinol wires. Biomaterials. 2009;30:468–77.
30. Rokicki R. The passive oxide film on electropolished titanium. Metal Finish. 1990;88(2):69–70.
31. Webb K, Hlady V, Tresco PA. Relative importance of surface wettabillity and charged functional groups on NIH 3T3 fibroblast attachment, spreading and cytoskeletal organization. J Biomed Mater Res. 1998;41:422–30.
32. ISO/TS 11139:2006. Sterilization of health care products—vocabulary, 2006.
33. Lambert BJ, Mendelson TA, Craven MD. Review: radiation and ethylene oxide terminal sterilization experiences with drug eluting stent products. AAPS Pharm Sci Tech. 2011;12(4):1116–26.
34. Shabalovskaya SA, Anderegg J, van Humbeeck J. Review: critical overview of nitinol surfaces and their modifications for medical applications. Acta Biomater. 2008;4:447–67.
35. Shabalovskaya SA. Surface, corrosion and biocompatibility aspects of nitinol as an implant material. Biomed Mater Eng. 2002;12(1):69–109.

36. Thierry B, Tabrizian M, Savadogo O, Yahia L. Effect of sterilization processes on NiTi alloy: surface characterization. J Biomed Mater Res. 2000;49:88–98.
37. Guidline for disinfection and sterilization in healthcare facilities, 2008, Department of Health & Human Services, USA.
38. Praisarnti C, Chang JWW, Cheung GSP. Electropolishing enhances the resistance of nickel-titanium rotary files to corrosion fatigue failure in hypochlorite. J Endod. 2010;36:264–7.