Influence of Nitinol Wire Surface Preparation Procedures, on Cell Surface Interactions and Polymer Coating Adherence

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Abstract

As the use of nitinol continues to grow in medical applications, so also does the need to acquire additional fundamental information regarding the stability of the material, in specific areas of deployment in the body. For devices fabricated from wires, data relating to the influence of wire surface state is an important consideration. In the present study a range of wires, all in the shape set condition, but with subtle variations in surface preparation procedures are characterised in terms of surface roughness, cell surface interactions and polymer coating adhesion . Pickling of the wire leads to the roughest surface state while mechanical polishing after etching and pickling considerably reduces the surface roughness. Cell growth on all the different wire surfaces is good with the smoothest surface leading to the lowest degree of cell spreading. Quantitative procedures for measuring the adherence of polymer coatings on stents has become a very important experimental necessity as drug eluting stents become more widely used. This work reports on the first data measured for polymer coatings on nitinol wire substrates. The measured values of adhesion energy clearly show that adhesion is best when the wire surface state is at its roughest.

Introduction

Nitinol because of its unique properties of shape memory and superelasticity is a very valuable material for the manufacture of medical devices. Whether they be stents (vascular and non-vascular) or other devices or instruments, their stability in the complex body fluid environment relies almost excluvisely on the stability of their surface oxides, which in the main is composed of TiO_2.

The natural oxides formed on the nitinol samples also contain some nickel (1), which can alter the stability of the material and to a large extent effect the corrosion resistance (2). Because of the known toxicological effects of nickel, it is very important to understand the factors which might lead to excessive release of nickel in the body. Much of the work which has been carried out in this area has involved the use of highly polished samples which result in data of little practical significance. In order to obtain reliable and usable data it is important that work be carried out using wires whose surface properties mirror those used in actual stent production. Since all of the devices receive a final shape setting heat treatment which results in a very considerable thickening (perhaps by as much as 8-9 times) of the surface oxide (3), it is also important that this be considered when assessing the overall stability of the material. Indeed it has been shown that this thickening of the oxide results in a dramatic decrease in the corrosion resistance of nitinol wires, measured (1) in simulated body fluids such as Ringers solution, 0.9% NaCl, bile and gastric juice.

In order to ascertain if changes in nitinol wire surface preparation procedures could have an influence on the subsequent performance of the wire when used in a given application, a range of wires have been prepared, varying slightly in surface texture but all subsequently shape set at 500^0C. For the present study, cell surface interactions and polymer coating adhesion are used as parameters to highlight any surface induced effects. Subsequent work will detail the influence of surface state on wire corrosion resistance, passivation procedures to improve corrosion resistance and gene expression pathways.

For any implanted medical device, cell surface interactions are an important marker as to the possible biocompatibility of the material. While cell proliferation may not always be desirable, cell death is a positive cause for concern. As polymer coatings to improve biocompatibility and coatings for localised drug delivery become widely used, quantitative methods to measure the adhesion of these coatings becomes a very important consideration. For the present study, a nanoindentation technique is used which may have important applications particularly in stent studies.

Experimental

Nitinol wire samples with a diameter of 0.030 inches (0.762 mm) were fabricated from binary nickel-titanium alloy with a nominal composition of 50.8 atomic percent nickel and an austenite start temperature in the fully annealed condition of -31^0C as measured by DSC per ASTM F 2004-00. Standard reduction and thermal processing was used to draw the wire to 0.0403 inches (1.02 mm). Additional processing to achieve 45 % cold work at 0.030 inches followed by a heat straightening step to produce superelastic properties at room to body temperature were performed. The active A_f temperature of the final wire was measured using the Bend and Free recovery method per ASTM-F 2082-01 to confirm that a superelastic condition had been achieved.

All specimens were cold drawn using either synthetic polycrystalline (Syn.Poly.) diamond dies or single crystal natural diamond (ND) dies. Heat straightening was performed at 500^0C under various levels of an oxygen rich atmosphere such that the resulting oxide could be adjusted from a light (LO) condition (gold to light brown in appearance) to a heavy (HO) condition (black in appearance). Before heat straightening, some of the wires were subjected to additional chemical and mechanical treatments in order to achieve the desired surface states.

Table 1(a) Wire surface condition, light oxide

Sample No.	Surface Condition
L1	Syn. Poly./LO
L2	Syn. Poly./LO/E/P
L3	Syn. Poly./LO/E/M
L4	Syn. Poly./LO/E/P/M
L5	ND/LO
L6	ND/LO/E/M
L7	ND/LO/E
L8	ND/LO/E/P
L9	ND/LO/E/P/M

Removal of the oxide by etching (E) using a solution proprietary to Fort Wayne Metals Research Products Corporation (FWMRPC) was performed for a series of specimens with the intent of attacking only the oxide itself. Additional specimens were exposed to a pickling process (P), after the initial etching, again using a solution of a proprietary nature to FWMRPC. This second chemical exposure allowed attack of the base material. To achieve additional test conditions for the study, specimens that had seen only etching or both etching and pickling were mechanically polished (M) using a mechanical wire polishing machine fixed with abrasive pads. Details of the wire surface preparation procedures are outlined in Tables 1 (a) and (b) for the light oxide and heavy oxide samples respectively.

Table 1 (b) *Wire surface condition, heavy oxide*

Sample No.	Surface Condition
H1	Syn. Poly./HO
H2	Syn. Poly./HO/E/P/M
H3	ND/HO/E/M
H4	ND/HO/E/P

3T3 fibroblast cells were used for all the cell experiments reported in this work. Cells were grown in DMEM (4) supplemented with 1% penicillin/streptomycin antibiotic and 10% foetal bovine serum. The cells were seeded on to the wires at a concentration of 3.0×10^4 cells cm^2. All samples were then incubated for a time period of 24 hours. F-actin content was determined by fluorescence measurements of rhodamine-phalloidin labeled samples, according to the method described in (5) with some minor modifications.

Working with wires as opposed to flat metal specimens presents a number of experimental difficulties when working with cells. However it is again important to work with specimens which are as close as possible to actual devices. Special Teflon holders were used to hold the wire samples in a fixed position so as to facilitate observations in the SEM and confocal microscopes.

N-isopropylacrylamide (NiPAAm) and N-*tert*-butylacrylamide (NtBAAm) co-polymer with monomer ratio 65: 35 was developed by T. Golubeva and A. Gorelov (Department of Chemistry, University College Dublin, Ireland), and were synthesized as outlined (6).

The wires were coated with this polymer using a 10% solution in ethanol and a Nima dip coater. Immersion and withdrawal rates were chosen in order to obtain the desired coating thickness. Once coated the samples were placed in an atmosphere of ethanol for 24 hours and dried in an oven at 55^0C overnight.

Nanoindentation tests were conducted with a nano-hardness tester (NHT, CSM Instruments, Switzerland) using a spherical diamond indenter, with a tip radius of 20 μm. For all tests the load used was 250 mN. Indentation induced de-lamination and fracture were characterised using visible-light microscopy.

The image of the de-lamination zone was analysed using NIH Scion Image Software.

SEM examination of the wire surfaces was carried out using a Hitachi S-4700 field emission microscope. For the cell surface examinations the cells were first fixed to the surface and the slowly dehydrated (4) before gold coating

A DI Dimension atomic force microscope (AFM) was used to measure the surface roughness of the various wires. The values quoted (R_z nm) are the difference between the maximum and minimum surface heights measured from the mean plane within the box cursor. The width of the box was approximately 2 μm and the length 15μm, aligned parallel to the wire direction. Six different areas per sample were examined. Confocal images of cells on the wire surface were obtained using a Zeiss Confocal Laser Scanning Microscope, model 510.

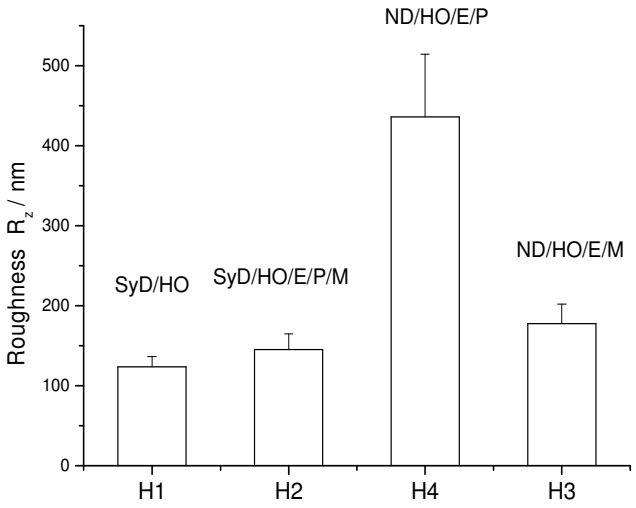

Figure 1 (a) Surface roughness values for the heavy oxide wires estimated from AFM measurements.

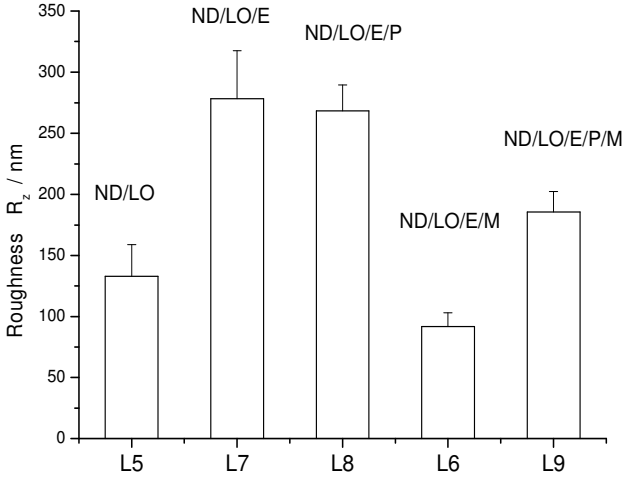

Figure 1. (b) Surface roughness values for the light oxide wires estimated from AFM measurements.

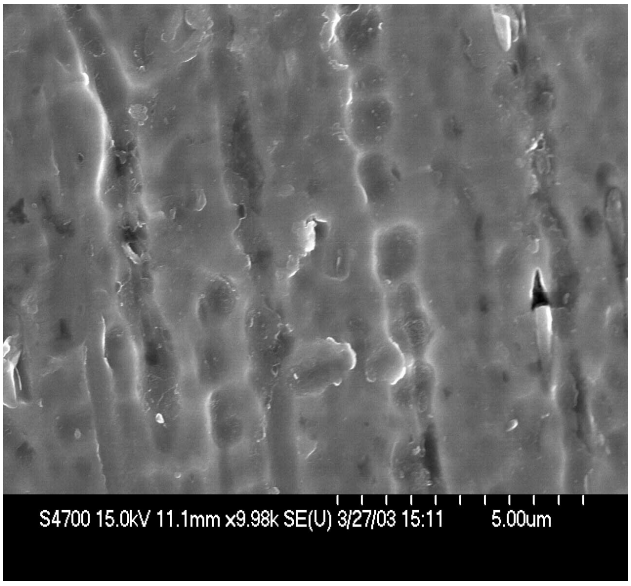

Figure 2 (a) SEM image of pickled surface for sample L 8

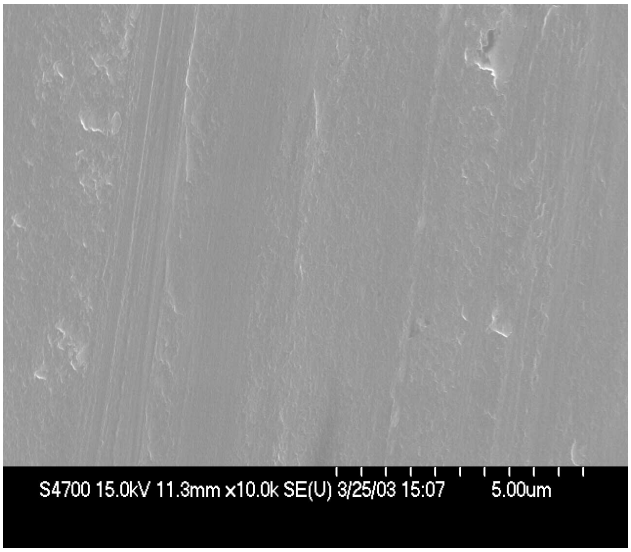

Figure 2. (b) SEM image of mechanically polished surface of sample H2.

Results

All of the data which is reported in this work has essentially been obtained at the micron measurement level. This is a very important consideration when comparing data particularly as related to stents and the dimensions of wires and struts in fabricated devices for deployment in real situations. As an initial criteria for surface characterization, all of the wires were subjected to a detailed examination using scanning electron microscopy (SEM). This was followed by surface roughness measurements obtained using the atomic force microscope. Surface roughness measurements, expressed as R_z values are presented for a range of samples (LO and HO) in Figures 1(a) and (b) respectively. It is immediately clear from this data that samples which are etched and pickled have the roughest surface profiles with the pickled samples having some of the highest values recorded. Mechanical polishing of both the etched and pickled samples results in a significant smoothing of the surface. Samples drawn using natural diamonds have somewhat similar surface topographies to those drawn using synthetic polycrystalline diamonds, particularly in relation to the more prominent surface morphologies of the etched and pickled surfaces. This quantitative data is borne out by the qualitative data obtained using the SEM. As indicated in Figure 2 (a) at a magnification of close to x10K, the etched and pickled wire surface is clearly rough when compared to the etched, pickled and mechanically polished surface as presented in Figure 2 (b) at a similar magnification. Mechanical polishing, although not what one might expect clearly leads to a surface which is definitely smoother in appearance.

As already alluded to, cell growth on wire samples presents a number of experimental difficulties. For the present work cell growth, adhesion and spreading was observed on virtually all of the wire samples studied. All samples (except ND/HO/E/P) demonstrate reasonable properties for cell adhesion and spreading . In most instances the wire surface topography did not have any influence on the direction of cell growth. Therefore, the "contact guidance" of the cells on the wire surfaces was not observed. This is clearly evident in the SEM image for wire L3, shown in Figure 3.

Estimates of cell surface spreading obtained from SEM and confocal images for a range of the wires Figure 4 (a) and (b) indicate that all of the surfaces act as good substrates for cell growth. Samples L3 and L6 both with a light oxide and etched and mechanically polished show the lowest degree of cell spreading.

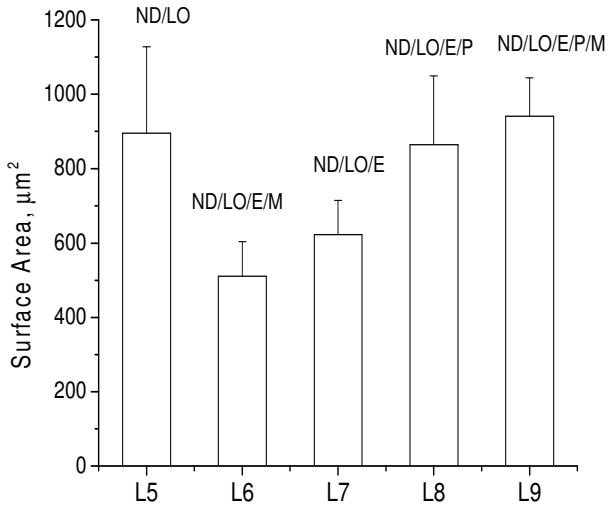

Figure 4 (a) Cell spreading on the natural diamond drawn wires.

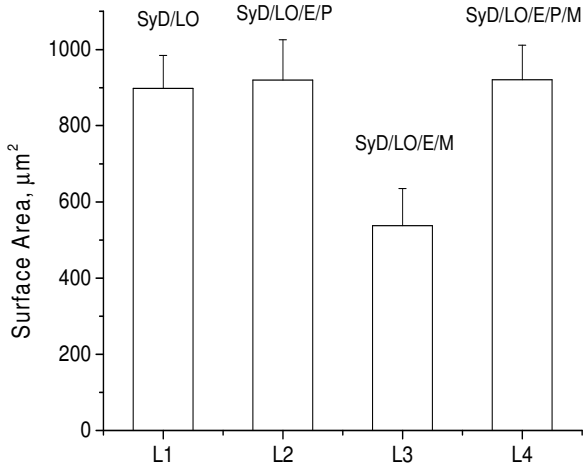

Figure 4 (b) Cell spreading on the synthetic diamond drawn wires

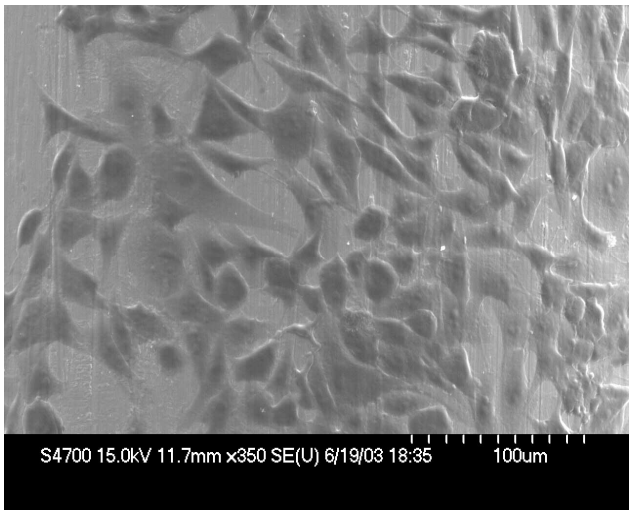

Figure 3. Cell growth on the surface of wire L3.

Adhesion of polymer coatings

As polymer coatings become important everyday parts of stent technology and more importantly as vehicles for drug delivery, methods of assessing coating adhesion to stent struts becomes a very important factor. Thus quantitative as opposed to qualitative methods of determining coating adhesion becomes a necessity. The data presented in this study is a first, in the sense that wire samples so important in stent studies are used in this procedure for the first time. The data calculated will be refined in further studies and polymer coating type will become an important variable in all estimations. For the present study the adhesion strength of the polymer/nitinol interface was evaluated using the annular-plate analysis (7,8) which was developed for soft compliant films interfaced to rigid substrates.

This analysis gave the interfacial fracture toughness or strain-energy release rate as

$$G = \frac{K\,(1-\nu^2)\,H^2\,h}{E\,(1+\nu+(c/a)^2(1-\nu))^2}$$

where c is the de-lamination radius, a is the tip radius; E, H, and ν are the elastic modulus, hardness and Poisson 's ratio of the film respectively; and h is the film thickness. Figure 5. shows a representative light microscope image of an indent with the tip and de-lamination radii clearly visible. The values of elastic modulus, hardness and Poisson's ratio of a PNIPAA/NtBA film are 4.2 GPa and 0.21 GPa and 0.33 respectively. K is a numerical coefficient. These values have been obtained for the present polymer from indentation data recorded for the polymer on stainless steel substrates. According to Oliver and Pharr (9), as long as indents into the film are of the order of 10% of the overall film thickness, any influence of the substrate on the measurements is at a minimum. According to (7) the value of K is 1.04. The coating thickness measurements are in the range 5 µm to 18 µm for the different wires. The interfacial fracture toughness, or practical work of adhesion for PNIPAM/NtBA coatings on the nitinol wires are presented in Table 2.

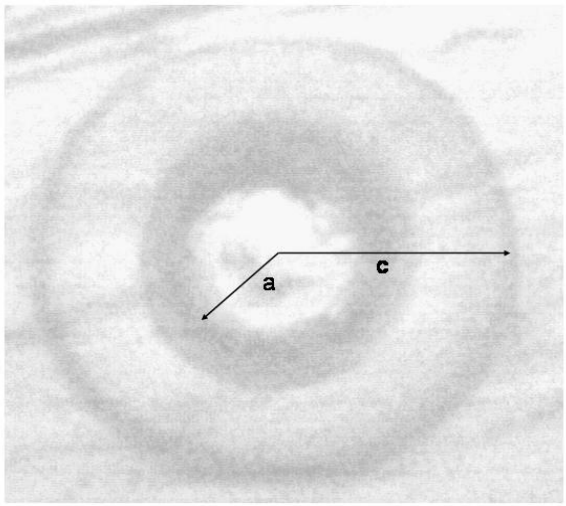

Figure 5. Light microscope image of indent showing the tip and de-lamination radii.

Table 2. Adhesion energy values for the various wires

Wire sample	Adhesion energy/G, Jm^{-2}
L1	0.58
L2	0.59
L3	0.75
L4	0.49
L5	0.58
L6	0.37
L7	0.75
L8	0.91
L9	0.11
H1	0.32
H2	0.19
H3	0.47
H4	0.64

Discussion

As the data presented in this study indicates, wire surface preparation procedures can influence the surface topography

of the finished material. In general, the etched and pickled samples have the roughest surfaces with mechanical polishing reducing the surface roughness to a considerable degree. Depending on the particular application for a device this is an important consideration. All of the wires perform reasonably well in terms of cell growth and spreading with the mechanically polished samples in general showing the lowest spreading rates. The results of *in vitro* studies of fibroblast response to NiTi are contradictory. For example, it was found that NiTi reduces significantly the cell growth in human foetal fibroblasts and modifies the morphology of the cells (10). On the contrary, no inhibition of mitosis of human fibroblasts by NiTi has been observed (11) and the latter was considered to be biocompatible and comparable to titanium.

Our results show that NiTi wires (except the ND/HO/E/P sample) demonstrate reasonable properties for cell adhesion and spreading. The dependence of cell behavior from the development process is insignificant. Again, in terms of choice for a particular application, wire samples with the lowest surface roughness may be the preferred choice. As already indicated quantitative methods for estimating polymer coating/metal or alloy adhesion properties is now a very important consideration in the medical device industry. This is not surprising as coatings and drug delivery vehicles become the norm in stent manufacture. The adhesion of coatings during stent deployment, the influence of drug loading on polymer adhesion properties and any possible shelf life problems associated with changes in polymer properties will ensure that this remains a topical problem. Because of nano-indentation techniques, possible answers to this problem may now become available. As already indicated, the results presented in this work on surface adhesion values are preliminary in nature. The values quoted in Tables 2. are typical for values measured for thin films on rigid substrates. As an example the value measured (7) for polystyrene films of the order of 3-5μm on glass substrates are in the range 0.6 Jm^{-2} . As is indicated in Figure 1 (a) polymer adhesion values seem closely related to the surface roughness of the wire. Thus on going from the ND/LO sample to the NO/LO/E sample to the NO/LO/E/P sample it seems that as the surface roughness increases so also does the degree of coating adherence. This is indeed not surprising, and as indicated by the data for sample L6 (which is similar to sample L7 but with the addition of mechanical polishing) the smoother surface results in a lower adhesion value. For the samples with the heavy oxide it is important to also note that it is the sample with the pickled and hence roughest surface which exhibits the best adhesion properties. Thus for the polymer used in the present study, it appears that coating adherence is at its best with the roughest surface. As most stent surfaces are now routinely subjected to electropolishing treatments after laser cutting, perhaps this smoothing of the surface while eliminating possible balloon penetration sites and improving fatigue life could also lead to premature de-lamination of coatings.

Conclusion

Changes in wire surface properties as a result of different preparation procedures have only a very minor influence on cell growth and spreading. The surface topography of the wires however can have a considerable influence on the adhesion of polymer coatings applied to them.

Acknowledgements

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