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# IN VIVO BIOCOMPATIBILITY STUDY OF NITI STENTS

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## ABSTRACT

Coronary stents are tubular shape prostheses used to scaffold occluded coronary arteries. Most of these stents are made of stainless steel or tantalum. However, as an alternative material, NiTi offers many advantages, namely its shape memory, superelasticity and radiopacity properties. Previous studies on NiTi highlighted the dependency of the alloy biocompatibility and corrosion behavior to surface treatments. The improvement of NiTi stents corrosion resistance by different surface treatments (electropolishing, heat treatment and nitric acid passivation) has already been reported by our group. In the present study, *in vivo* biocompatibility of such stents is assessed by description and quantification of the fibrous response of rabbit paravertebral squeletal muscle in contact with the devices after periods of 2 and 6 weeks. After 2 weeks, no significant differences could be seen in the fibrous response among the stents with different surface treatments. However, after 6 weeks, a significant higher fibro-cellular response ($P<0.05$) was observed around the heat treated samples when compared to the others. This result may be related to the higher passivation current density and less uniform oxide layer generated by this surface treatment.

## INTRODUCTION

The use of Nickel-Titanium (NiTi) alloy as a biomaterial has increased in the last decade because of its unique properties of shape memory and superelasticity. These properties are very attractive for the improvement of different prosthesis [1,2] and particularly for cardiovascular devices[3]. In this field, NiTi offers many advantages for endovascular stents that are intraluminal scaffolds to maintain vessel patency after angioplasty. When stent expansion is taking place during the device implantation, superelasticity combined with shape memory properties of NiTi allow large deformation of the alloy in its elastic range. Also, optimal radiopacity of the metal, compared to a

more conventional stent material such as stainless steel, allows a more precise and easier positioning of the device at the site of occlusion.

However, the high content of Ni (50 at%) of the alloy and the potential role of Ni ions in toxicity and carcinogenecity limit its use for long-term implants[4]. Some studies have clearly shown that nickel can cause a significant inflammatory reaction while titanium causes minimal disruption to skeletal muscles when inserted in the back of rabbits[5]. These findings justify the importance to analyze the tissue reaction to NiTi to achieve knowledge of its biocompatibility. Still, the study of NiTi biocompatibility remains a controversial issue. Cutright et al., in a previous *in* vivo study of NiTi, demonstrated a minimal tissue reaction comparable to stainless steel around wire sutures in rats[6]. During a study on the biological reaction to NiTi implanted in rabbits tibias, Oonishi et al. have observed Ni elements in the tissues surrounding the implants even though no adverse biological reaction could be noticed[7]. A more recent study performed by Yahia et al., demonstrated a slower osteogenesis process associated to NiTi screws implanted in rabbits tibias compared to vitallium, titanium and stainless steel screws, suggesting that NiTi might be a less biocompatible material[8]. An *in vitro* study of NiTi in contact with rat spleen cells has shown the close dependency of the alloy biocompatibility to the surface conditions of the material[9], which might explain the variability in the *in vivo* studies.

Since biocompatibility is closely related to corrosion resistance and surface properties, surface treatment of NiTi should improve its performance. Passive metals, like NiTi, have a stable oxide layer on their surface which renders them corrosion resistant and relatively inert in physiological conditions. This passivity can be enhanced by modifying the thickness, topography and chemical composition of this oxide layer by different surface treatments[9-11]

We have already shown the improvement of the corrosion resistance of NiTi stents by different surface treatments: electropolishing, heat treatment (in air or in a salt bath) and nitric acid passivation[12]. In order to understand the effect of such surface treatments on the biocompatibility behavior, in relation with corrosion resistance of the devices, we have undertaken *in vivo* implantation of 4 surface treated stents in the skeletal muscles of rabbits. We report here the results obtained from this investigation.

## EXPERIMENTAL

### Samples

Stents (diameter of 4 mm, length of 14 mm in the expanded state) were manufactured by machining equiatomic (50.8 at% Ni) NiTi tubing and by laser cutting diamond shaped apertures. Four different groups of samples were prepared by Nitinol Devices and Components Inc. (Fremont, California, USA). Electropolished stents (EP) have been first, micro-abraded to remove mechanically the primary oxide layer, then, chemically polished at room temperature and finally, electropolished to obtain an oxide layer with a mirror-like surface finish. Air aged samples (AA) are electropolished and air aged at 450°C to produce a light yellow oxide layer. The heat treated stents (HT) are electropolished and heat treated in salt at 500°C to produce a dark blue oxide layer. The passivated stents (PA) are electropolished and passivated in a nitric acid solution at room temperature. They optically exhibit the same surface finish as the electropolished stents.

All samples were cleaned ultrasonically in soapy water (5 min.) and acetone (5 min.), rinsed in deionized water (2 X 5 min.) and air dried. The stents were longitudinally cut in two hemicylinders, to maximize the contact between the implant and the muscle, and autoclaved prior to surgery.

Animals

Ten healthy female New Zealand white rabbits, weighing 2-3 kg, were used in this study. The animals were divided in two groups for different periods of implantation: 2 and 6 weeks respectively. The rabbits were taken care of according to the Canadian Council on Animal Care Regulations and fed an unrestricted diet during the course of this study.

Implantation

The rabbits were anesthetized by intramuscular injection of a combination of Ketamine (35 mg/kg) and Xylazine 5 mg/kg (Rogar STD Inc., Montreal, QC, Canada). The low dorsal region of the rabbit was shaved and the skin was cleaned and disinfected with isopropylique alcohol and Stanhexidine. Longitudinal incisions of approximately 2 cm were made in the skin, on each side of the spine, near the paravertebral back muscle. Xylocaine (Astra Pharma Inc., Mississauga, Ont., Canada) was sprayed in the incision to locally anesthetized the implantation area. Four NiTi hemicylinders with different surface treatments were inserted (2 on each side with a distance of at least 2 cm between them) and the aponeurosis was closed with Dexon II 4-0 monofilament suture (Davis and Geck Cyanamid Inc., Montreal, QC, Canada).

Histology

The animals were sacrificed using an overdose of Penthotal (Abbott laboratories Ltd., Montreal, QC, Canada) administrated intravenously. Each implant and surrounding tissues were removed en bloc and immersed in 10% buffered formalin solution. Excess tissue was removed and then, the implants and remaining tissues were sectioned in the middle in order to study the cellular reaction in this region of the hemicylinder. Blocs of tissue surrounding the NiTi samples were then dehydrated in ascending grades of ethanol followed by a gradual infiltration with Hystocril (Marivac Ltd., Halifax, NS, Canada) and benzoyl peroxide paste. The specimens were embedded by *in situ* polymerizing of Hystocril in cold water to prevent the effect of temperature increase during the polymerization.

Transverse sections (approximately 5 μm) were cut perpendicular to the longitudinal axis of the implant and stained with Masson's trichrome to allow the evaluation of the tissular reaction to NiTi with different surface conditions. The inflammation was quantified by measuring, by optical microscopy (Leitz, Diaplan), the fibrous capsule thickness surrounding NiTi implants. An average of multiple measurements was made from each stent strut site to the limit of the the normal muscle fibres to characterize the cellular response around it.

## RESULTS AND DISCUSSION

2 weeks

All implant sites were square holes surrounded by a thin layer of fibro-cellular tissue near to normal or degenerative muscle fibres (figure 1). Degenerative muscle fibres could be observed when a stent strut was in direct contact with the endomysium membrane. The presence of a few red cells could also be noticed, indicating internal bleeding during the implantation of the devices. Thickness of the fibro-cellular capsule ranged from 0.07 mm to 0.17 mm as shown in figure 2. No significant difference could be observed in the fibrous response for the different treated surface stents (ANOVA test). For this early assessment after implantation, cellular response to local trauma seemed to predominate. The fibrous capsule could originate from this trauma or the inflammatory reaction caused by the implants themselves. In addition, muscle solicitation inducing minor implants movements may have contributed to exacerbate the local trauma.

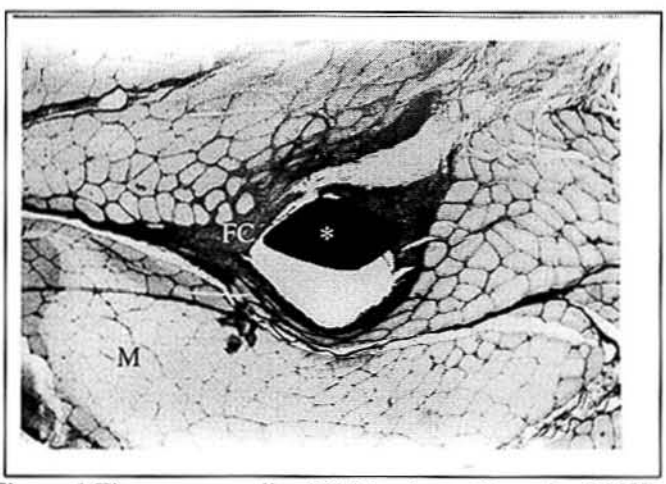


**Figure 1** Tissue surrounding NiTi implant at 2 weeks (300X)
*: portion of NiTi stent strut, FC: fibro-cellular capsule, M: muscle fibres

6 weeks

Histological observations show similarities for the NiTi implants sites between 2 and 6 weeks implantation periods. Fibro-cellular tissue and degenerative muscle cells could also be observed surrounding the implants. In the area in close contact with the NiTi struts, macrophages, fibroblasts and connective tissue were easily identified. Thickness of the fibro-cellular capsule ranged from 0.04 mm to 0.15 mm as shown in figure 2. An ANOVA statistical test demonstrated a significant difference ($P<0.05$) among the mean thickness values of fibro-cellular capsule around the stents with different surface treatments. A multiple comparison Tukey test indicated a significant higher fibrous capsule ($P<0.05$) around the HT stents compared to the other samples. At this level of confidence, no significant difference in inflammatory response can be assumed between the other surface treatments.

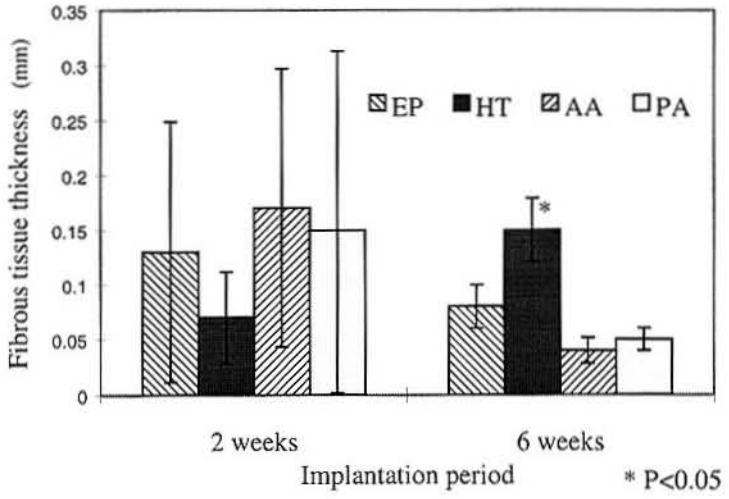


**Figure 2** Measurements of the fibro-cellular response

From our previous corrosion studies[12], HT stents exhibit an intermediate pitting potential and a higher passivation current density than the other treated surface stents indicating an intermediate resistance to localized corrosion at high potential and a greater corrosion rate in the passive range. *In vivo* biocompatibility results may suggest that the passivation current density plays a more important part in the fibro-cellular response around NiTi treated implants than the pitting potential. When these results are related to surface characterization, even though HT stents display a thicker oxide layer than the other treated stents, their corrosion resistance and *in vivo* biocompatibility seems inferior. This paradox could be caused by the observed non-uniformity of the oxide layer of these stents.

At this stage of the study, it is too early to conclude on the level of biocompatibility of the different treated surface stents. Incoming results for the 12 weeks post-implantation assessment should allow us to clarify the respective biocompatibility of these NiTi stents as a function of surface treatments and, to confirm the trend already observed.

## CONCLUSION

The main objective of this work was to study the *in vivo* biocompatibility of NiTi stents with different surface treatments at 2 and 6 weeks post-implantation in the paravertebral skeletal muscle of rabbits. Measurements of the fibro-cellular capsule thickness around the stents struts was done to assess the tissue response to the different treated surface implants. Results showed that after 2 weeks of implantation no significant differences could be observed. At 6 weeks after implantation, a significantly higher fibro-cellular capsule thickness was found for the HT samples compared to the other treated surface implants. It seems from our previous study on these NiTi stents[12] that passivation current density and uniformity of oxide layer play major roles in biocompatibility of treated NiTi implants. A 12 weeks post-implantation assessment is already scheduled to confirm the results obtained. A comparison between our results on NiTi and stainless steel stents biocompatibility is also planned.

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## REFERENCES

1. J.O. Sanders, A.E. Sanders, R. More, R.B. Ashman, Spine 18, 12, 1993, p.1640.
2. K.R. Dai, X.K. Hou, Y.H. Sun, R.G. Tang, S.J. Qiu, C. Ni, Injury 24, 10, 1993, p.651.
3. U. Sigwart, Endoluminal Stenting, Frontiers in Cardiology, W.B. Saunders Company Ltd., 1996, p. 154.
4. K.Takamura, K. Hayashi, N. Ishinishi, T. Yamada, Y. Sugioka, J. Biomed. Mat. Res. 28, 1994, p.583.
5. P.J. Laing, A.B., Ferguson, E.S. Hodge, J. Biomed. Mat. Res. 1, 1967, p.135.
6. D.E.Cutright, S.N., Bhasker, B. Perez, R.M. Jonhson, G.S.M. Cowan Jr., Oral Surg Oral Med Oral Pathol. 35, 1973, p.695.
7. H. Oonishi, E. Tsuji, M. Miyaga, T. Hamada, Y. Suzuki, T. Nabeshima, T. Hamagushi, N. Okabe, Biomaterials 1984, (Sec. World Congress on Biomat. Tech. Proc., Washington, D.C., 1984), p. 183.
8. M. Berger-Gorbet, B. Broxup, C. Rivard, L'H. Yahia, J. Biomed. Mat. Res. 32, 1996, p.243.
9. S.Shabalovskaya, J. Cunnick, J. Anderegg, B. Harmon, R. Sachdeva in Shape Memory and Superelastic Technologies, edited by A. Pelton, D. Hodgson and T. Duerig (Shap. Mem. Super. Tech. Proc., Pacific Grove, CA, 1994), p. 209.
10. F. Villermaux, M. Tabrizian, L'H. Yahia, M. Meunier, D.L. Piron, Appl. Surf. Sci., (in press).
11. S. Kimura, T. Sohmura, Osaka Univ. Dent. Sch. 27, 1987, p. 211.
12. C. Trépanier, M. Tabrizian, L'H. Yahia, L. Bilodeau, D.L. Piron in Materials for Smart Systems II, (Mat. Res. Soc. Tech. Proc., Boston, MA, 1996), (in press).