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In vivo Histomorphometric and Corrosion Analysis of Ti-Ni-Cr Shape Memory Alloys in Rabbits

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***Abstract*—**A series of Ti based shape memory alloys with composition of $Ti_{50}Ni_{49}Cr_1$, $Ti_{50}Ni_{47}Cr_3$ and $Ti_{50}Ni_{45}Cr_5$ were developed by vacuum arc-melting under a purified argon atmosphere. The histometric and corrosion evaluation of Ti-Ni-Cr shape memory alloys have been considered in this research work. The alloys were developed by vacuum arc melting and implanted subcutaneously in rabbits for 4, 8 and 12 weeks. Metallic implants were embedded in order to determine the outcome of implantation on histometric and corrosion evaluation of Ti-Ni-Cr metallic strips. Encapsulating membrane formation around the alloys was minimal in the case of all materials. After histomorphometric analyses it was possible to demonstrate that there were no statistically significant differences between the materials. Corrosion rate was also determined in this study which is within acceptable range. The results showed the Ti-Ni-Cr alloy was neither cytotoxic, nor have any systemic reaction on living system in any of the test performed. Implantation shows good compatibility and a potential of being used directly *in vivo* system.

***Keywords*—**Shape memory alloy, Ti-Ni-Fe, histomorphometric, corrosion.

I. Introduction

AMONG shape memory alloys (SMAs), Ti-Ni based alloys are the most admired because they have better properties in shape memory effect and superelasticity [1]. They are usually ductile and can be deformed practically in good physical shape. Furthermore available high strength, excellent corrosion behavior, long fatigue life and better mechanical properties are considerable advantages over Cu and Fe based shape memory alloys [2]. Nickel–Titanium alloys have been widely used in the sectors of biomedical engineering, such as endovascular stent, orthodontic archwire and orthopedic implants due to its unique biocompatible and bioactive characteristics [3]. In recent times, surface modification techniques have been used in order to improve the biological properties of Ti-Ni alloys. By addition of third element to Ti-Ni, its mechanical and other shape memory properties can be altered. Cr, Ta, Zr, Pd and many other elements are used as ternary element is this system. Ti-Ni-Cr is one of the important superelastic and shape memory alloys being used as biomedical, aircraft, engine and high temperature structural materials [4]-[6]. Due to its high corrosion resistance it may also be used for biomedical applications. Ti-Ni-Cr is a recently developed alloy which has not been studied for biomedical applications. Moreover the object of this work was histomorphometric and corrosion investigation of solution treated (homogenized) Ternary Ti-Ni-Cr alloys implanted in rabbits.

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II. Materials and Methods

A. Metallic Implants and Surgical Procedure

$Ti_{50}Ni_{49}Cr_1$, $Ti_{50}Ni_{47}Cr_3$ and $Ti_{50}Ni_{45}Cr_5$ (Samples ID: S1, S2 and S3 respectively) polycrystalline ingots were prepared using vacuum arc-melting furnace in argon atmosphere. The ingots were homogenised at 973K for 60mins followed by water quenching to a temperature of 292K. A 3mm×3mm×2 mm sample was taken from the centre of the ingot for the purpose of implantation. Twelve male rabbits of two to three years ages were used in this research work. They were kept under standard laboratory diet and atmosphere. All rabbits were slaughtered by using volatile anesthetic, an overdose of diethyl ether. Alloy samples were immersed in 20% (v/v) HNO_3 for 20 minutes following by washing and drying (ASTM F86 – 12) [7], then lastly autoclaved for sterilizing. Following the standard anesthesia, incisions were completed under aseptic circumstances. Subsequently the S1, S2 and S3 alloys were implanted subcutaneously in right limb.

B. Histomorphometric and Corrosion Analysis

Tissue samples from surrounding subcutaneously were gained from rabbits after slaughtering and instantly kept in neutral buffered formalin (10%). After elimination of formalin during overnight tap water washing, tissue sections were carried through a series of alcoholic water solutions as follows; 70% ethanol, 80% ethanol, 90% ethanol, designated as ethanol 1, 2 and 3 containers respectively. Tissues were processed for hisotmorphological assessment by paraffin implanting method. Section thickness was precisely fixed by cutting at 4 μm which were marked with haematoxyllin & eosine [8]. The tissue blocks were captured using a digital camera and optical microscope. The encapsulating membrane thickness around the implants was precisely calculated using computer-assisted histomorphometry. All digital images were processed using image-analyses (Axiovision computer software) to calculate the encapsulating membrane thickness around the metallic implants. The histomorphometric study was carried out in all images obtained. Any apparent

unfavorable result present inside the subcutaneous tissue was noted. Statistical analysis was performed to verify the mean of all encapsulating membrane thickness and corrosion rates through one way ANOVA by using Mini Tab software, followed by z-test, which determined the mean values ± Standard deviations. The decrease in volume of metallic implants can be expressed in term of corrosion rate by using the weight loss technique [9], where the weight loss (W) is replaced by the reduction in volume (DV) multiplied by the standard density (q) resulting in CR = ΔV / A. t : where CR, A, t and ΔV represent the corrosion rate, surface area of metallic implant, the exposure time, the reduction in volume respectively [10]. In this vivo corrosion study microstructural study was also taken by SEM of post implanted materials.

III. Results and Discussion

There was no considerable variation in the mean the encapsuling membrane thickness around the metallic implants between the materials (Fig. 1).

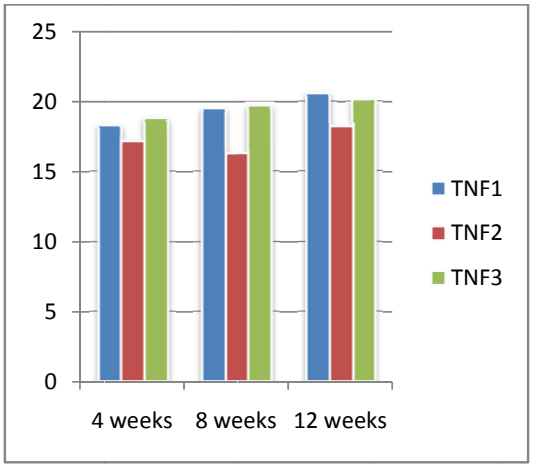
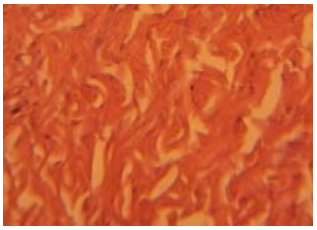
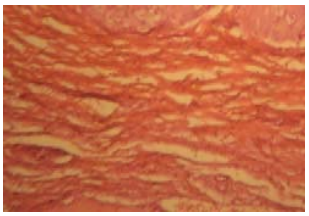


Fig. 1 The encapsulating membrane thickness post implantation. Along Y-axis, thickness is shown in micrometers whereas the time elapsed is indicated along X-axis

The encapsulating membrane thickness was observed as time-dependent and with the passage of time it was enhanced. The encapsulating membrane thickness of S1, S2 and S3 were compared with each other at 4, 8 and 12 weeks. There was, however, an increase in thickness with S1 at 4, 8 and 12 weeks (mean ± 1 SD); 18.3 ± 5.6, 19.5 ± 5.9, 20.6 ± 6.1.The variations in encapsulating membrane thickness were not found to be statistically different. S2 capsule was thickest at 4 weeks (S1 18.3 ± 5.6mm vs. S2 17.2 ± 7.5mm vs. S3, 18.8 ± 8.1mm). At 8 weeks, there were no clear differences (S1 19.5 ± 5.9mm vs. S2 16.3 ± 7.9mm vs. S319.7 ± 7.7mm). At 12 weeks, all capsules around the test implants were found to be almost equally thin (S1 20.6 ± 6.1mm vs. S2 18.2 ± 8.2mm vs. S3 20.1 ± 7.3mm), and the morphological studies were also found similar (Fig. 2).



(a)



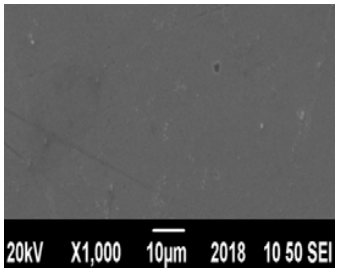
(b)

(c)

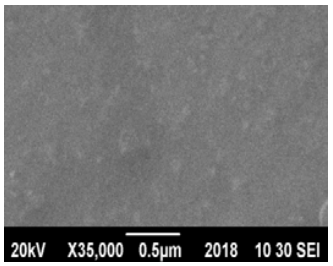
Fig. 2 Example of a photo used for histomorphometry showing minimum surrounding Tissue response. (a) S1 (b) S2 (c) S3, after 12 weeks of implantation There was no considerable variations between the three implanted materials in the thickness of the encapsulating membrane

There was neither necrosis nor granulom as soft tissue calcification around any of implanted strips. Evaluation of toxicity may be caused by physicochemical interaction of material with environment and host [8]. Weight loss calculations are frequently used to explore the uniform deterioration as well as pitting corrosion of materials in a specific environment. It also gives a complete picture about corrosion rate of material with the passage of time. Weight loss measurement technique is used for this purpose. In this study, vivo corrosion has also been investigated. The S1, S2 and S3 degradation was not significant. It was observed that the alloys corroded *in vivo* had an appropriate slow corrosion rate (Table I).

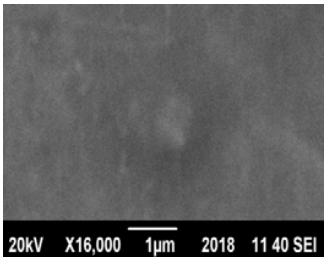
It has restricted the localized corrosion rapidity and if alloys are being corroded, it may alter blood parameters and influence blood compatibility. However, corrosive changes, as shown by SEM analysis, no pits were seen in the Ti-Ni-Cr metallic implants. Some surface contaminants may be present in S2 and S3 materials, which influence the formation of cell debris (Fig. 3 (b) and (c)).



(a)



(b)



(c)

Fig. 3 SEM images of metallic implants (a) S1 8 weeks after implantation. No marks of corrosion (b) S2 8 weeks after implantation. No evidence of corrosion but some cell debris is seen (c) S3 8 weeks after implantation. Smooth surface with some cell debris is seen

This work concluded that the alloy has excellent corrosion behavior in term of resistance in biological systems. In Table I, it can be observed that all the values of test statistics have no significant effect on their mean.

TABLE I
IN VIVO CORROSION RATES (MM PER YEAR) OF THE CORRODING METALLIC IMPLANTS*

Test Materials	S1		S2		S3		Significance
	F-Test Statistics	CR(mmy^{-1})	F-Test Statistics	CR(mmy^{-1})	F-Test Statistics	CR (mmy^{-1})	
	N= 6						
4 weeks	0.013	0.49±0.09	0.061	0.53± 0.07	0.058	0.51 ± 0.11	NS
8 weeks	0.021	0.40±0.08	0.032	0.45±0.06	0.047	0.42 ± 0.14	NS
12 weeks	0.023	0.32±0.06	0.029	0.44± 0.13	0.055	0.31 ± 0.09	NS

* Summary of corrosion rates, presenting mean values and standard deviations (SD).Values given in CR (mmy^{-1}) column are given as mean of all samples of one time interval ± standard deviation (SD). The values of SD are greater than value of $p < 0.05$, all values are insignificantly different, So No significant differences were found for Corrosion rate (CR).

IV. CONCLUSION

In the present study, an *in vivo* histomorphometric and corrosion evaluation was carried out by implantation of materials in subcutaneous tissues of rabbits for 4, 8 and 12 weeks. Encapsulating membrane formation around the alloys was minimal in the case of all materials. There were no statistically significant differences of encapsulating membrane thickness between the implanted materials and the corrosion rate of alloys *in vivo* implantation is within an appropriate slow corrosion rate and these presented an acceptable host response without the appearance of subcutaneous gas cavities and fibrous tissue encapsulations. Alloys are presenting insignificant corrosion rate and there was no evidence of deterioration in SEM analysis for any of the alloy implants. It demonstrates the bioactivity and biocompatibility of materials in biological systems.

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