Activity Report 2011

Preliminary Results of Titanium Diffusion on Shinbone of Mice with Osseointegrated Implants

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INTRODUCTION

The use of Ti-based dental implants to replace lost dental elements is increasingly frequent. Dental implants are composed of Ti IV-type which is an alloy of titanium, aluminum and vanadium. When the implant is contacted with the tissue, a modification in its surface undergoes, releasing particles of titanium in different concentrations. Titanium is a transition element extremely resistant to corrosion; it is present at trace levels in tissues and body fluids in concentrations not well established. The metabolism of free titanium is not well known. Titanium up-taking mode or movement in the body is not known, nor its toxic dose.

This research group has studied the behavior of titanium concentration of dental supra- and infra-gingival calculus at different stages of maturation. The studies involved analyses of saliva and gingival fluid in different local situations, corresponding to healthy and ill subjects, smokers and non-smokers, postmenopausal women with osteopenia and osteoporosis, patients affected by periodontal disease with and without treatment, people with different calcium intake in the diet, etc.. The results show large variations in the concentration of titanium. Another study measured saliva and gingival fluid of patients with dental implants by TXRF with synchrotron radiation. The mean concentration measured in saliva was 3.5 $\pm$ 0.5 ppm in patients without implants and 2.8 $\pm$ 0.5 ppm in patients with implants. In gingival fluid of patients without implants the concentration was 1.3 $\pm$ 0.4 ppm, while in gingival fluid it was found 22 $\pm$ 7 ppm. Levels of confidence (ANOVA) between the groups were $p > 0.33$ for saliva and $p < 0.005$ for gingival fluid. There is a greater tendency to increase the concentration of titanium in gingival fluid of patients with implants, while there are no significant differences in saliva [1].

The micro-XRF technique is a suitable method for measuring trace elements in small samples whether liquid or solid. After studied the behavior of titanium in oral fluids, we interested to know the titanium behavior in bone tissue. In this work we analyze the titanium diffusion through the shinbone bone of mice.

EXPERIMENT

Six adult male Wistar mice of 180 $\pm$ 60 g were studied. General anesthesia was performed with 2% xylazine / ketamine 5%. Surgical site was prepared in a shinbone of the animals, the area was disinfected with alcohol. A vertical incision of 1.5 cm was performed, and a hole in the cortex with sterile round bur with manual pressure was carried out. A single pure titanium foil of 1.5 mm by 0.3 mm and 4 mm thick was introduced in the perforation, inside the bone, in the longitudinal direction [2]. Finally, nylon suture 4-0 was used for suturing.

Euthanasia of animals was done separating them in two groups of three animals randomly. This procedure was performed one month (1st group) and two months (2nd group) after titanium implantation. Dried tibiae were preserved in 10% formalin. Soft tissue was removed, RXs were taken, and bones were included in a self-curing acrylic for subsequent cross-section cuts. Animals care and surgery were performed under International Animal Protection Rules [3].

The experiments were carried out at the microfluorescence station of the XRF beamline [4] of the LNLS. A fine conical capillary, which allows condensing the white beam into a small area, produces the x-ray microbeam. All measurements were performed selecting the sampled area with an optical microscope, that is placed perpendicular to the sample surface, and using the standard excitation geometry ($45^0 + 45^0$). An energy dispersive Si(Li) solid state detector was used to collect the fluorescent and scattered radiation coming from the samples. The actual beam size on the sample was 10 x 10 microns.

For each sample, XRF spectra were taken by linear scanning in area near the new bone formed around the Ti implant.

Figure 1 shows a microphotograph of the implant, the newly formed bone around and the line of irradiation under analysis. Depending in the sample, among 15 and 40 points were measured during the linear scanning. The measuring time per point varied from 700 s to 1000 s.

The AXIL program for spectrum fitting [5] was used to obtain the net intensity of the elements present in the samples and standards.

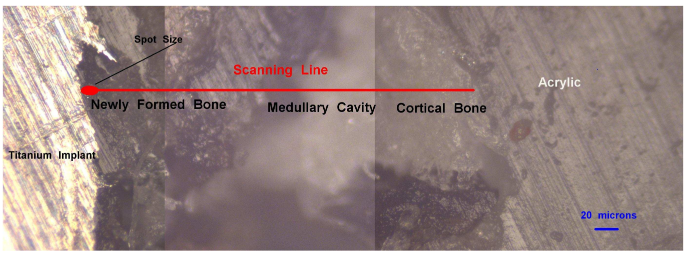

FIG. 1: Figure 1. Microphotograph of the scanning area near the titanium implant.

RESULTS AND DISCUSSION

Firstly, it was verified that during healing none wound became infected and all titanium sheets were osseointegrated. Figure 2 shows a RX of one of the samples shinbone demonstrating a complete osseointegration of the shinbone. The same was observed for all the samples.

Figure 1 shows a microphotograph of the cross section of one sample with the titanium implant and the newly formed bone around. The scanning line is marked in the figure. Figure 3 and 4 show the net intensity of Ti and Ca corresponding to the sample observed in Figure 1. As can be seen there is a clear effect of titanium diffusion while calcium intensity present a different behavior. Moreover, a clear correlation among the different structures of bones is observed in the Ti and Ca intensities.

All the samples presented more or less a similar pattern of diffusion.

FIG. 2: Figure 2. RX of a shinbone showing implant osseointegration.

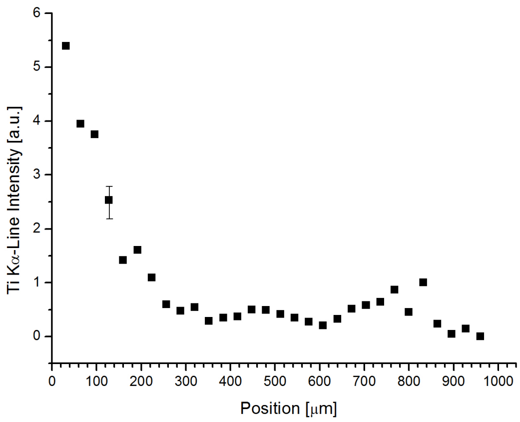

FIG. 3: Figure 3. Ti Kα-Line intensity as a function of the position.

CONCLUSION

At this stage of investigation we have found a clear evidence of titanium diffusion in the newly formed bone. A more complex data analysis is required in order to find quantitative

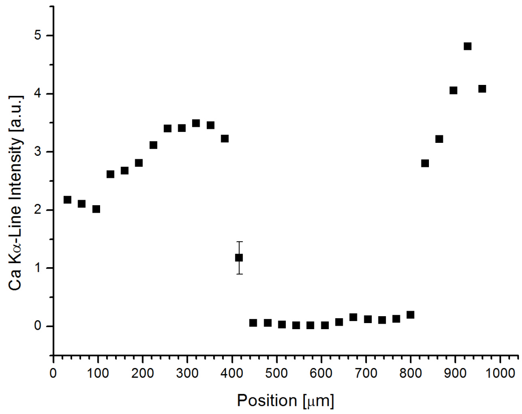

FIG. 4: Figure 4. Ca Kα-Line intensity as a function of the position.

parameters of diffusion rates. These analyses will require intensities normalization of titanium peaks. This process could be attained by considering the net intensity of calcium, which represents somehow the compact degree of the octocalcium phosphate structure. Another alternative to normalize the titanium intensity will be a densitometry by ACT around the implant. After normalization, the diffusion rates will be quantified by derivatives procedures.

ACKNOWLEDGEMENTS

This work has been supported by the Brazilian Synchrotron Light Laboratory (LNLS) / Brazilian Biosciences National Laboratory (LNBio) under proposal XRF 12520

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