

# Animal inoculation tests on the sintered titanium biocompatibility

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The use of titanium and its alloys in medicine is justified by the beneficial properties it has. In order to verify the biocompatibility of the sintered titanium achieved through modern powder metallurgy methods tests of animal inocuity on sheep. Titanium powder treated by powder metallurgy specific procedures was used for this purpose. Three incisions of three cm were made in the following regions: the retroscapular area for a subcutaneous implant, the tibial diaphysis for the subperiosteal implant and the latissimus dorsi area for the intramuscular implant. Three samples of tissue releaved in the three distinct areas mentioned were subjected to histopathological tests. The metal samples and the three tissue samples of adjacent periosteal, muscular and subcutaneous tissue were immersed in liquid nitrogen for 5 minutes at  $-196\text{ }^{\circ}\text{C}$ ; tissular fragments were prelevated longitudinally with the microtome to expose the interface of biological tissue – sintered Ti part.

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## 1. Introduction

The use of metals and alloys in the reconstructive surgery has undergone remarkable progress during the last decades, in so far their diversification and the promotion of new, unconventional technologies and procedures are concerned [1, 2].

Titanium and its alloys represent a challenging option as these materials can be found in the medical and therapeutic applications for decades now; initially they were used mainly for implants, but, in the course of the time they began being used more and more frequently in other fields and for other purposes too, with a special emphasis in surgery. The utilisation of titanium in the orthognate surgery and traumatology is justified by some exceptional properties these materials have: good resistance to corrosion, excellent biocompatibility, mechanical strength, low density, low heat conductivity, low thermal expansion coefficient, X-ray translucence, inodorous and tasteless character, low costs.

In dentistry, titanium was first introduced in 1968, by Leonard Linkow, for implant related purposes. In 1969, Branemark and, in 1985, Hofmann, contributed with the research with the titanium alloy  $\text{TiAl}_6\text{V}_4$ . At that time, the titanium based products were mainly produced by cold working [3].

The exceptional behaviour exhibited by this material that is still considered as “exotic” [4] is due to its elementary properties, with respect to physical, chemical and biological reactions.

## 2. Experimental and laboratory results

In order to check for the biocompatibility of the titanium sintered by modern methods and procedures belonging to powder biological inocuity tests were carried out on ovines. Six sintered titanium samples of purity 99,89%, with surface microporosities and nanoporosities, were used. The samples were sterilised at  $130^{\circ}\text{C}$  and 2,5 atmospheres in wet heat.

The animal used for inoculation was a three year old half-breed sheep, with no pathological problems.

The animal was given anesthetics, the protocol was fulfilled and, dependent upon implant location, three 3-cm long incisions were performed in the following regions:

- the retroscapular region, for the subcutaneous implant;
- the tibia diaphysis for the subperiosteal implant;
- the latissimus dorsi area, for the intramuscular implant.

The subcutaneous tissue was with drawn and two sintered titanium plates of size  $5/4/2$  mm were introduced in the retroscapular region. The aponevrosis of the latissimus dorsi muscle was selected and dissociated, the muscle was dissected, the aponevrosis of the left illeospinal muscle is dissociated and two sintered titanium plates of size  $5/4/2$  were implanted. The left pretibia periosteum is dissociated in the diaphysis area and two sintered Ti plates of size  $5/4/2$  are implanted subperiosteally. The incisions are sutured anatomically and in points Vycril 3.0 and D-tek 2.0 silk. Postoperatively, a three day long antibiotherapy were performed. The postoperative evolution was positive,

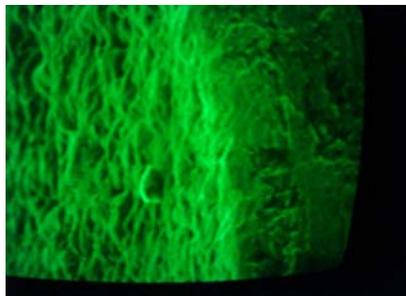
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except for a haematoma occurring in the pretibia lesion, that was cleaned and drained for two days. Three weeks after the surgery, the implants were revealed along the former incision lines. The six plates and the accompanying adjacent tissue were extirpated, that is the pretibial periosteum, the ileospinal muscle and the retroscapular subcutaneous fat tissue.

Three samples of tissue taken from the three mentioned areas were sent for histopathological tests. The metal specimens and the rest of three accompanying contiguous tissue, i.e. periosteal, muscular and subcutaneous were immersed in liquid nitrogen for 5 minutes at the temperature of  $-196^{\circ}\text{C}$ , then tissular fragments with a microtome in longitudinal section were taken out, to expose the biological tissue-sintered titanium part interface. The parts were metallised in vacuum and analysed with the scanning electronic microscope (SEM) type TESLA BS 300. The images were captured with a digital camera, type Fuji fine pix S 7000.

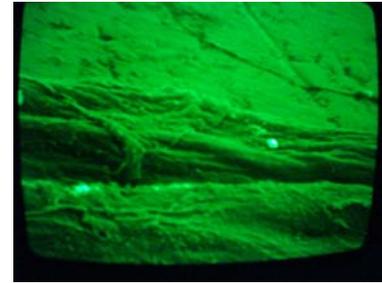
Macroscopically, the plate was well integrated in the contiguous muscular tissue and no inflammatory reaction to the foreign body was found. In this surface section, the reactive inflammatory tissue is absent between the Ti plate and the contiguous muscular tissue (Fig. 1). The macroscopic absence of the inflammatory type granulation tissue is also visible. A slight suprapariosteal oedema was, however, perceived, and it was interpreted as the consequence of the postoperative haematoma. The adherence of the periosteal tissue to the titanium plate and the periosteal organisation with respect to the microporosity of the sintered surface (Fig. 2). The integration of the titanium implant in the adipose tissue is seen at macroscopic analysis and no inflammation reaction tissue can be observed. The microporosity in the section of implanted titanium can be noticed and the tight joint between the titanium plate and adipose tissue, adipocytes being interposed in the surface microporosities.

The contact surface between the titanium plate and adipose tissue presents no microscopic pathological modifications, and no reactive conjunctive tissue is interposed in the section (Fig. 3).



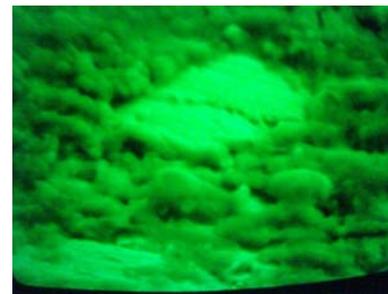
x1000

Fig. 1. Titanium plate-muscular tissue interface–SEM image.



x1500

Fig. 2. Titanium plate-periosteum interface–SEM image.



x2000

Fig. 3. Surface of Ti plate covered by adipocyte –SEM image.

The histopathological test in case 1 consisted in both microscopical analysis: (part of size 1/0.7/0.5 cm, grey, firm) and macroscopical analysis; the preparation was stained with haematoxiline- eosine (HE), Tricon Masson (TCM) and Sirius red (RS). In HE: The material is represented by conjunctive and vascular tissue, adipose tissue and nerve ends.

At this level some areas of granulation tissue exhibiting abundant infiltrates of inflammatory nuclear-round cells as well as polzmorphic cells.

Granulation tissue with a conjunctive tendency are also found. In Tricrom Masson: The TCM stain points out conjunctive tissue in green-blue. In Sirius red: the conjunctive strips are highlighted in the bright red colour (Figs. 4 -6).

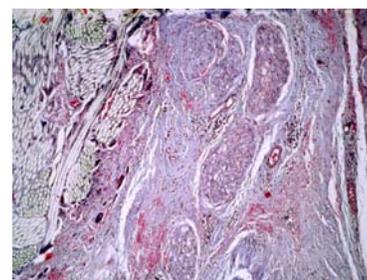
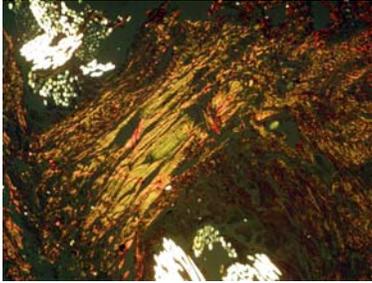
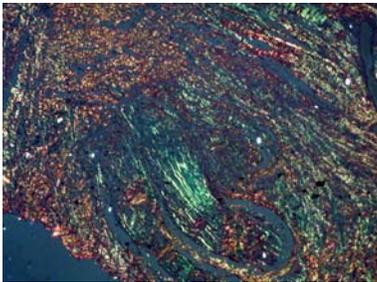


Fig. 4. TCM (x100) stain Nerve end incorporated in granulation tissue at the junction between titanium and suprascapular tissue.



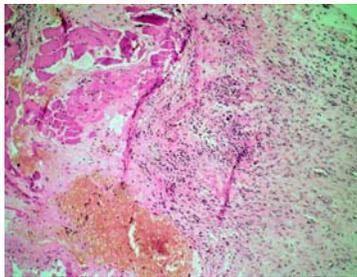
*Fig. 5. Sirius Red (x100) stain in polarised light. The suture threads are very bright. Small separate fragments can be seen as incorporated in the basic tissue. The intermediate bunch (conjunctive tissue) has a green brightness=Young conjunctive tissue where the titanium plate meets the subscapular tissue. Orange - yellowish stains = mature conjunctive tissue.*



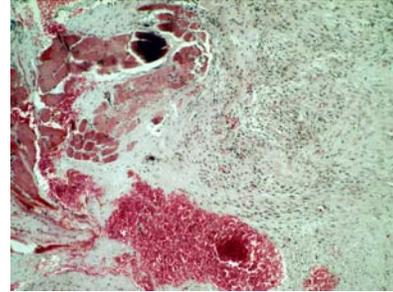
*Fig. 6. Polarised light Sirius Red (x100). Green (young) conjunctive tissue in the middle, outlined by a capsule of mature (yellow) conjunctive tissue where the titanium plate joins the subscapular tissue.*

The histopathological test in case two consisted in both microscopic analysis: a 1.5/1/1 cm sample, grey brown in colour, firm, and macroscopic analysis: the preparation the preparation was stained with haematoxiline-eosine (HE), Tricon Masson (TCM) and Sirius red (RS).

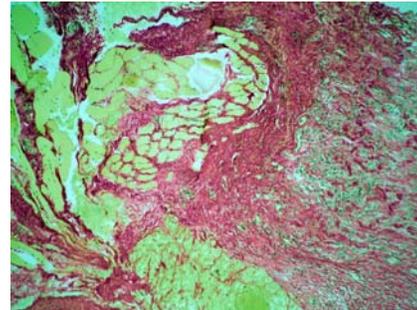
In HE: The material under test contains areas of striated muscular tissue with ceonic moderate inflammatory infiltrates and areas with granulation tissue preponderant nuclear round cells as well as polymorphnuclear cells of inflammatory nature. In some areas, the granulation tissue exhibits aspects of conjunctive presentation. In Tricrom Masson: the areas of conjunctive tissue are coloured in bluish green. In Sirius red: The conjunctive fibres are coloured in bright red (Figs. 7-9).



*Fig. 7. HE (x100) stain Striated muscular tissue + granulation tissue in the joining area of the muscle/titanium.*

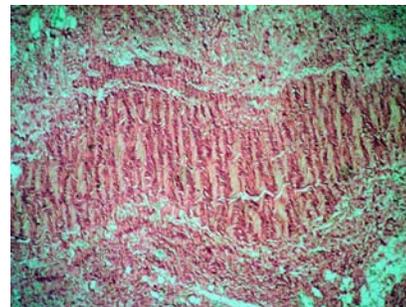


*Fig. 8. TCM x100 stain. Striated muscular tissue + granulation tissue in the joining area of the muscle/titanium.*



*Fig. 9. Sirius Red x100 stain. Striated muscular tissue + granulation tissue in the joining area of the muscle/titanium.*

The histopathological test in case 3 consisted in both microscopical (part of about 1 cm  $\Phi$ , grez, ferm) and macroscopical analysis (the preparation was stained with haematoxiline- eosine (HE), Tricon Masson (TCM) and Sirius red (RS). In HE: The material is represented by a dense conjunctive tissue and adipose tissue. At this level some areas of granulation tissue exhibiting moderate infiltrates of inflammatory nuclearround cells. In Tricrom Masson: The conjunctive tissue is stained with green-bluish. In Sirius red: the conjunctive strips are highlighted in the bright red colour (Figs. 10-12).



*Fig. 10. Sirius Red x100 stain. Collagen conjunctive tissue with various compaction levels – compact, related.*

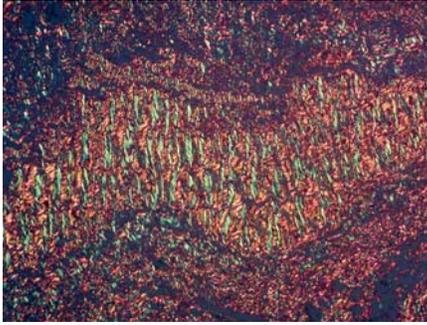


Fig. 11. Sirius Red x100 stain. The same polarisation light field. Young and mature collagen in alternating strips.

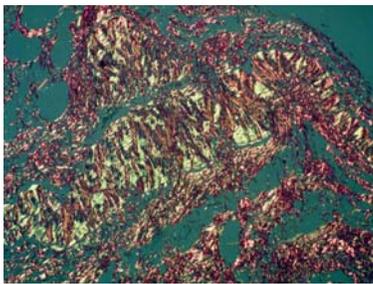


Fig. 12. TM x100 stain. Collagen conjunctive tissue with various compaction levels – compact, related.

### 3. Discussion and conclusions

The results of the biological inocuity tests carried on ovines both subcutaneously and intramuscularly and mainly in the subperiosteal area reveal that, besides the properties of a bioinert and biocompatible material, sintered titanium achieves a firm interface with the contiguous tissue that tends to incorporate it due to its surface and structural microporosity. This property can be seen both in the macroscopical analysis of the subcutaneous, intramuscular and subperiosteal samples and in the electronic microscope analysis, made at surface and cross section level. No foreign body typical inflammatory reactions were observed in any of the locations mentioned, which was confirmed by the histopathological test of the tissue in the vicinity of the implants made.

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