

A novel antimicrobial Iodine-treated orthopaedic implant surface demonstrates biocompatibility and improved osseointegration in a sheep ingrowth model

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This novel Iodine surface treatment is biologically safe and does not affect osseointegration

Background

Periprosthetic joint infection (PJI) remains one of the most challenging unmet needs in orthopaedics. A novel antimicrobial Iodine surface treatment, applied to Ti6Al4V implants, may support the management of PJI by reducing microbial attachment and biofilm formation on the implant surface through the gradual release of iodine into the surrounding joint space.

With the addition of an antimicrobial to the surface of a device, its biological safety must not be compromised. This study evaluated the local tissue response, acute systemic toxicity, genotoxicity and osseointegration effects of this new technology.

Objectives

The goal of this study was to assess the biocompatibility of Iodine-treated implants following the principles of ISO 10993 standards. The sheep implantation study included also blood and urine iodine levels and thyroid function markers.

Materials and Methods

Iodine treatment: All test samples received the Iodine surface treatment via an electrochemical process developed from the work of Takaya et al.¹

Cytotoxicity, irritation and sensitization: Extracts were obtained using a pour-to-cover method* from Iodine-treated hip stems. For cytotoxicity, cells were incubated at 37°C for 48h, and then graded based on cellular characteristics and % lysis. For irritation, extracts were delivered via intracutaneous injections and observations for erythema and edema were noted at 24, 48 and 72 hours after injection. For sensitization, extracts were delivered intradermally and as soaked patches, and animals were scored based on dermal reactions.

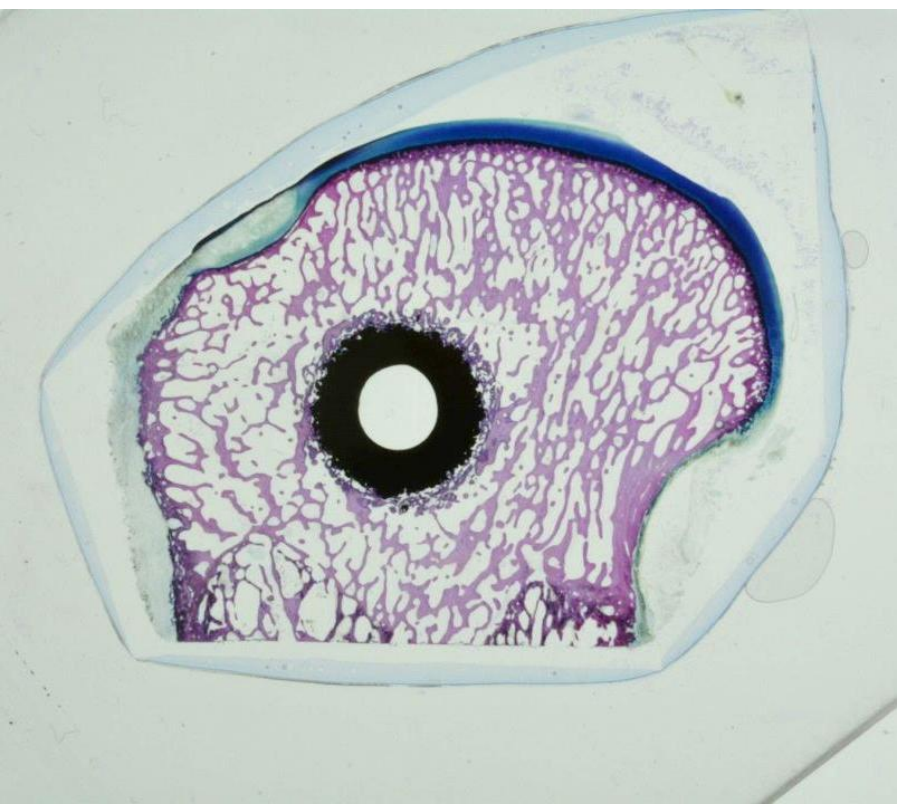
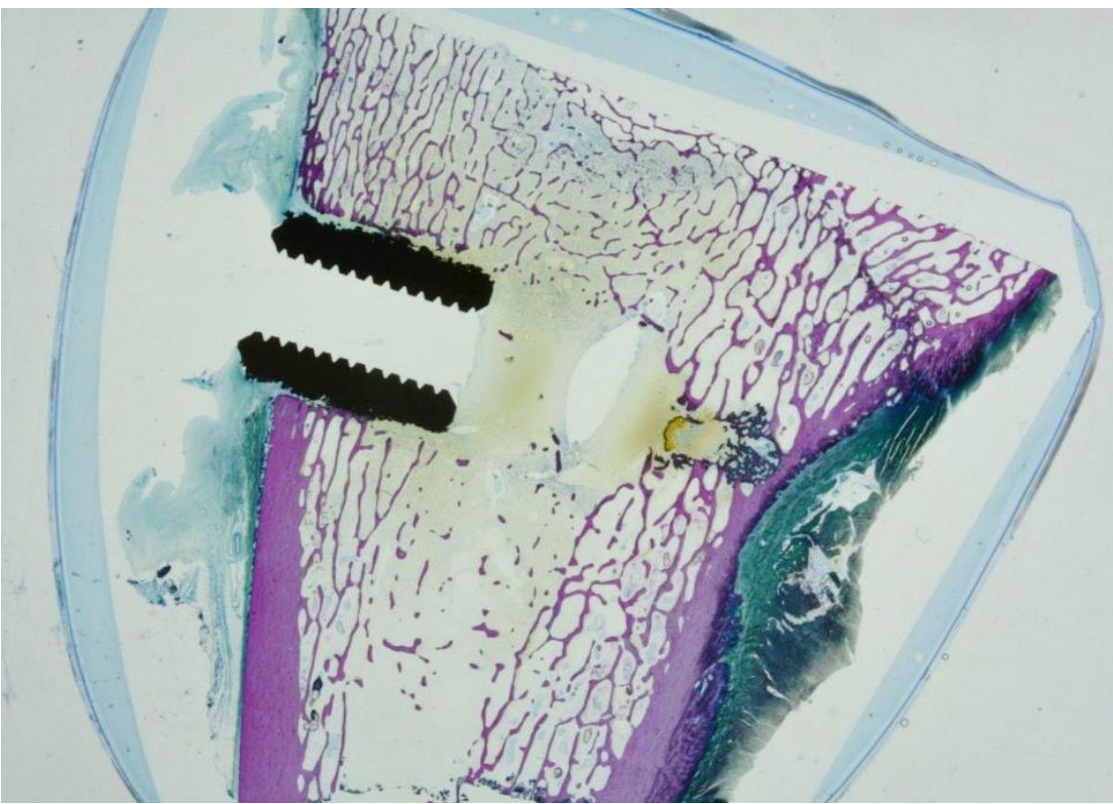
Acute systemic toxicity: Iodine-treated acetabular cups were extracted using a 3 cm²/mL ratio. The extracts were injected intravenously (saline) or intraperitoneally (sesame oil), and the animals were observed and weighed for 72h.

Genotoxicity: Iodine-treated acetabular cups were extracted using a 3 cm²/mL ratio. Both the Mouse Lymphoma Assay (MLA) and the Reverse Mutation Assay (Ames) were performed.

Biological Endpoint	ISO standard	Extraction media	Extraction conditions	Biological Test Model
Cytotoxicity	10993-5 ²	Minimum Essential Medium (1x MEM)	37°C for 72h	L929 fibroblast cells
Irritation	10993-23 ³	Saline and sesame oil	50°C for 72h	Rabbits
Sensitization	10993-10 ⁴	Saline and sesame oil	50°C for 72h	Guinea pigs
Acute systemic toxicity	10993-11 ⁵	Saline and sesame oil	50°C for 72h	Mice
Genotoxicity	10993-3 ⁶	MLA: Cell culture medium and DMSO	37°C for 72h	L5178Y mouse lymphoma cells
		Ames: Saline and DMSO		Four strains of <i>S. typhimurium</i> and one strain of <i>E. coli</i>

Sheep implantation study: Cylindrical Ti6Al4V samples were used in this study that had Porous Plasma Spray (PPS) coating, as representative of cementless devices. The test followed the principles of ISO 10993-6⁷. Three treatment groups were included based on iodine concentration – Low (25-50 µg/cm²), Medium (80-120 µg/cm²) and High (160-200 µg/cm²) – with reference to the technology engineering specification. Untreated samples were used as controls. All animal received four samples in each hind leg: cortical femur, cancellous femur, cortical tibia and cancellous tibia. The effects on the local tissue response, osseointegration and systemic toxicity were evaluated: histological assessments with % bone-in-contact (%BIC) evaluations, histomorphometry, distant-organs histology, and mechanical push-out testing were conducted at 4, 12 and 26 weeks. Iodine serum levels, as well as thyroid markers TT4, TT3 and FT4 were also monitored during the study.

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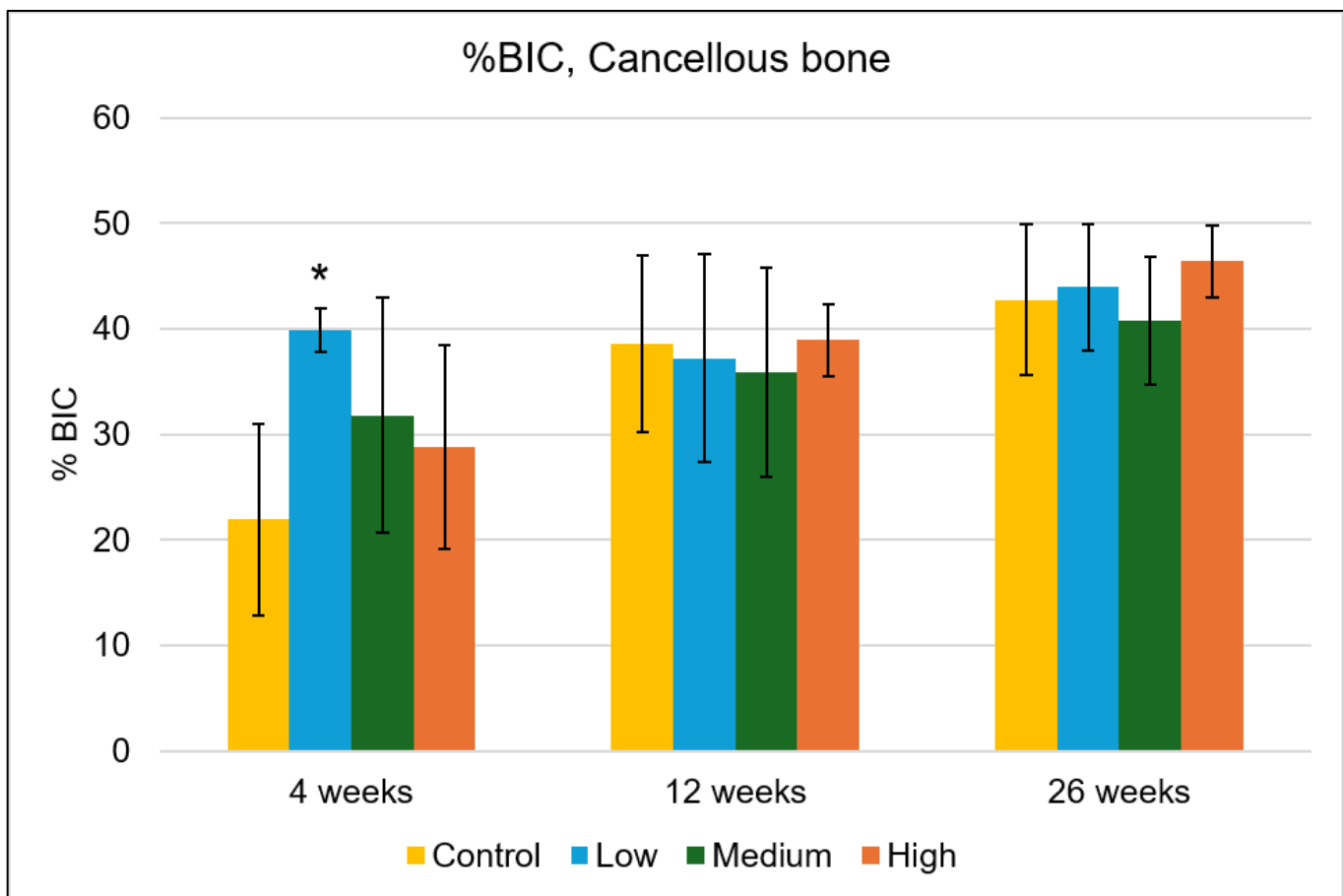
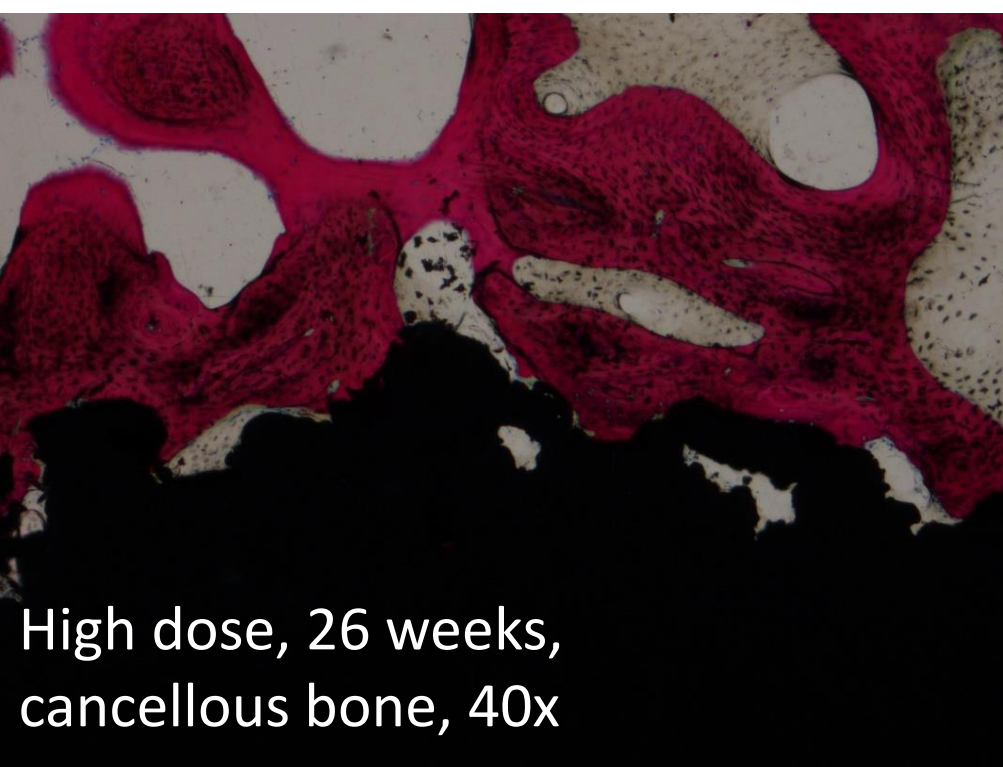
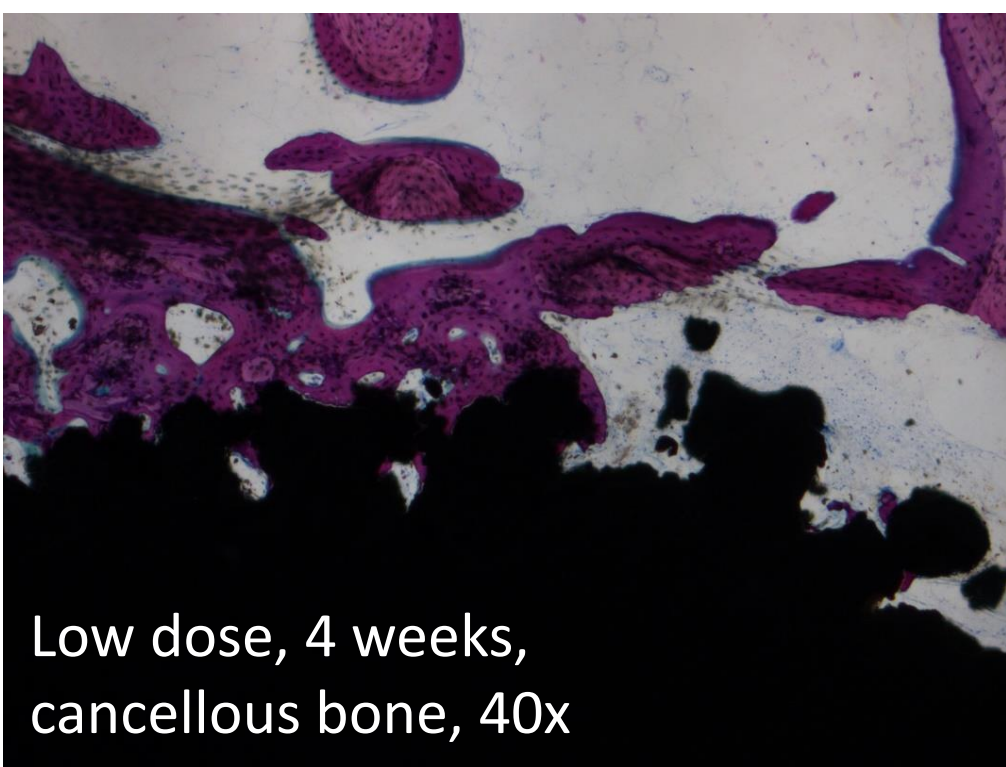
Results

Devices with the novel antimicrobial Iodine surface treatment passed cytotoxicity, irritation, sensitization, acute systemic toxicity and genotoxicity testing.

Biological Endpoint	Test results	Pass/Fail
Cytotoxicity*	Grade 0 for cell rounding, cells without intracytoplasmic granule and % lysis.	Pass
Irritation*	0.0 score difference between test and control group	Pass
Sensitization*	Score 0 for dermal reactions at 24 and 48h.	Pass
Acute systemic toxicity	No mortality and no evidence of systemic toxicity	Pass
Genotoxicity	MLA: No biologically relevant increase of mutants. Ames: No gene mutations by base pair changes and no frameshifts in the genome of the tester strains.	Pass

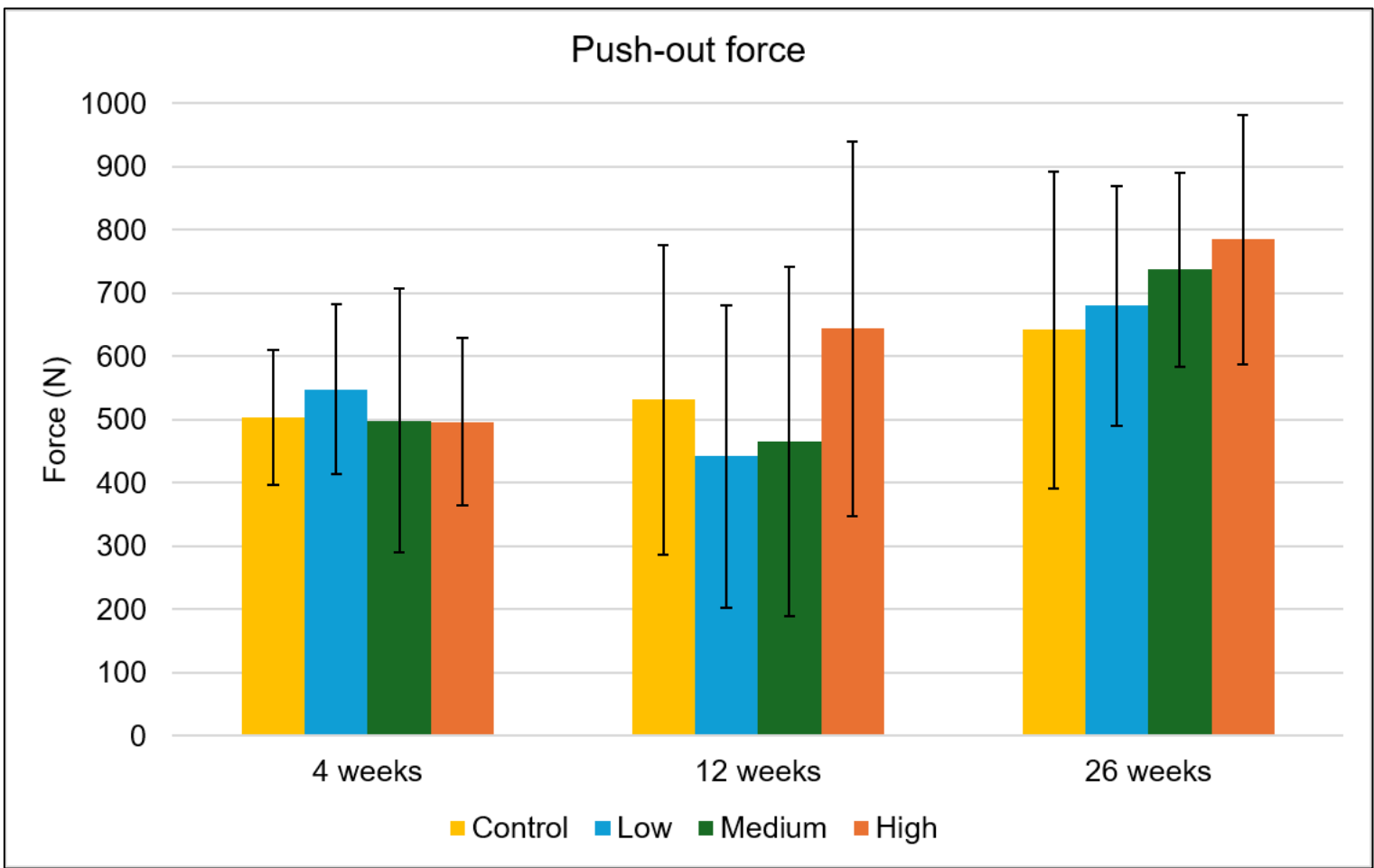
Results of the sheep implantation study:

- Distant organs histology:** no relevant findings in the heart, lungs, liver, kidneys, popliteal lymph nodes or spleen.
- Clinical pathology:** Thyroid markers TT4, TT3 and FT4, as well as serum iodine did not develop any pattern. No change in urine iodine was noted.
- Histology:** Newly-formed bone can be seen within the Low dose implant pores and in contact with the surfaces of the implant at 4 weeks; bone in contact with the High dose implant is evident at 26 weeks. Stevenel's blue/van Gieson stain.



- Histomorphometry:** no evidence of local toxicity, irrespective of iodine dose. At 4 weeks, %BIC values were higher for the treated groups, and then comparable to controls at the later time-points. No inflammation, necrosis, fibrosis or hemorrhage were seen.

- Push-out force:** Within each group, the average push-out force tended to increase with time, although no statistically significant differences were found between time-points within groups, or between groups within each time-point.



* Due to the irregular nature and porosity of the PPS Iodine-treated surface, a pour-to-cover extraction approach was applied instead of the standard 3 cm²/mL extraction ratio.

Conclusions

Implants with a novel antimicrobial Iodine surface treatment passed ISO 10993 testing for cytotoxicity, irritation, sensitization, acute systemic toxicity or genotoxicity effects. The sheep implantation study confirmed normal local tissue response, with no adverse effects on osseointegration relative to control implants, regardless of initial iodine concentration. No evidence of systemic effects or distant organ toxicity was found, and thyroid markers were not affected. These findings, together with chemical characterization data, toxicological risk assessments, and a clinical literature review, support the overall biological safety of this Iodine surface treatment.

References and Disclosures

¹ K. Hashimoto, M. Takaya et al. (1999), *Antimicrobial characteristics of anodic oxidation coating of aluminium impregnated with iodine compound*, Inorg. Mater. 6(283): 457-462
² ISO 10993-5 *Biological evaluation of medical devices – Part 5: Tests for in vitro cytotoxicity*
³ ISO 10993-23 *Biological evaluation of medical devices – Part 23: Tests for irritation*
⁴ ISO 10993-10 *Biological evaluation of medical devices – Part 10: Tests for Skin Sensitization*
⁵ ISO 10993-11 *Biological evaluation of medical devices – Part 11: Tests for systemic toxicity*
⁶ ISO 10993-3 *Biological evaluation of medical devices – Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity*
⁷ ISO 10993-6 *Biological evaluation of medical devices – Part 6: Tests for local effects after implantation*