

HISTOLOGICAL AND BIOLOGICAL ANALYSIS OF TISSUE DIFFERENTIATION DURING BONE HEALING AROUND TITANIUM IMPLANTS

E. Slaets^{1*}, J. Duyck¹, G. Carmeliet² & I. Naert¹

¹*Department of Prosthetic Dentistry, BIOMAT Research Group, KULeuven, Belgium*

²*Department of Developmental Biology, Laboratory of Experimental Medicine and Endocrinology, KULeuven, Belgium*

INTRODUCTION: Due to the insertion of an implant, a cascade of bone healing events occurs such as blood clot formation, inflammatory reaction eventually followed by the formation of a fibrous callus [1]. The latter will be replaced by woven bone, that in turn will be remodeled into lamellar bone. At the end, this leads to direct bone-to-implant contact coined "osseointegration". However, detailed information on the cellular processes and tissue differentiation that occur early during bone healing around endosseous implants is very limited. In the perspective of immediate implant loading, this insight becomes important. The aim of the present study was to investigate these healing processes surrounding a titanium implant and mainly during the first days and weeks after implant placement.

METHODS:

Animals: Five mature New Zealand White rabbits were selected. Both in trabecular bone of the epiphysis and in cortical bone of the diaphysis of the tibia, implants were placed at different time points, respectively 42, 28, 14, 7, 3 and 1 day before sacrifice.

Implants: Cp wrought titanium implants, with a diameter of 1 mm and lengths between 6 and 10 mm, according to the thickness of the bone, were placed press-fit.

Specimen preparation and analyses: The samples (implants with surrounding bone) from 2 rabbits were fixed in 2 % paraformaldehyde, whereafter the implants were removed and the bone samples decalcified and embedded in paraffin. The samples from the 3 other rabbits were fixed in an 4% buffered formaldehyde solution, after which the implants were removed from the samples in 2 out of 3 rabbits only. The samples were dehydrated in a graded series of ethanol and embedded in polymethylmetacrylate (VWR International, Leuven). The removed implants were screened with a scanning electron microscope (FEI-Philips XL30 ESEM FEG) to ensure that no tissues were ripped off during implant removal.

The following stainings were performed:

Paraffin sections: hematoxylin–eosine staining for a general overview, TRAP staining (tartrate

resistant acid phosphatase) for the visualisation of osteoclasts and safranin O-Fast Green staining for the visualisation of cartilage.

Polymethylmetacrylate sections: toluidin blue staining for a general overview, Von Kossa staining for the visualisation of mineralised bone and Goldner staining for the visualisation of osteoid.

RESULTS & DISCUSSION: Presently we are analysing the histology. 12 implants per animal were introduced according to an inverse time scheme, limiting the subject-dependent variation. Time points of 1, 3 and 7 days were chosen to focus on the early events of bone healing around the implants, with special attention to the different cell types that allow correct healing. 42 days was taken as maximum time point because the remodeling cycle takes 6 weeks in rabbits. The implants were removed in 4 out of 5 rabbits to allow embedding in paraffin and/or production of micro-thin sections resulting in a higher number of sections per sample. Histological sections up to 4 µm (polymethyl-metacrylate) or 7 µm (paraffin) were cut when the implants were removed. Cutting the samples with the titanium implants still present resulted in a limited number of sections per sample and in thicker sections up to 20 µm.

In future experiments, histomorphometric and molecular analyses will be performed as well. As mentioned earlier, there is no detailed information on the types of cells that sequentially are present during the first steps of healing of bone around an implant. This study is part of a project on, bone healing and bone response around loaded implants.

REFERENCES:

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ACKNOWLEDGEMENTS:

This study is supported by the Research Council KULeuven and by the Fund for Scientific Research, Flanders.