Laser microtome for site-specific sectioning of the interface and sub-sequent qPCR analysis

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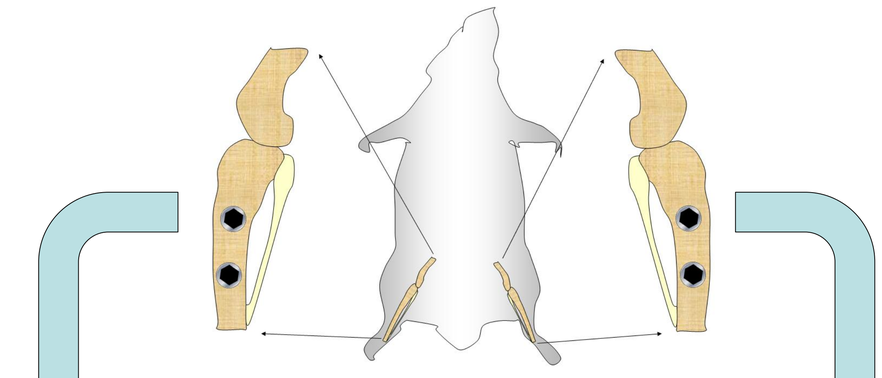
Background

The mechanisms governing tissue healing at the *in vivo* implant-tissue interface are not fully understood. This is largely attributed to the lack of reliable procedures to collect samples from within the interface in the way that their spatial distribution can be determined. Recent studies revealed that gene expression of implant-adherent cells is a more sensitive indicator of the biological response elicited by implant materials than that obtained by analyzing the peri-implant bone collar [1, 2]. Therefore, it was suggested that a site-specific analysis of gene expression at the interface would provide a powerful tool for further studies on the integration of materials in tissues.

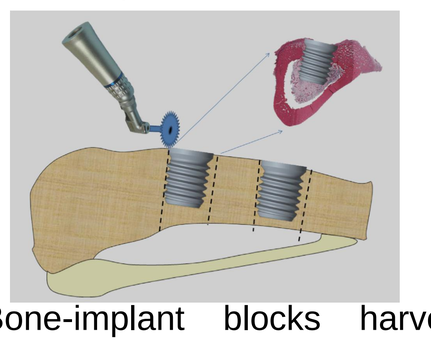
Purpose

The aim of the present *in vivo* work was to apply a laser-cutting microtome for retrieving predetermined samples, from bone-titanium implant interface, that are suitable for gene expression analysis.

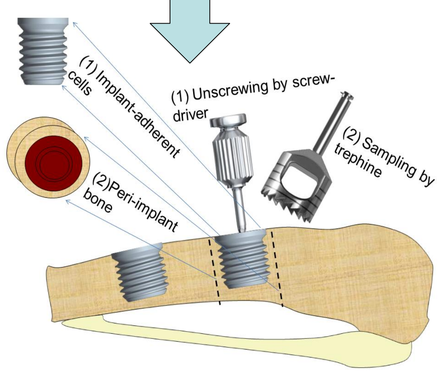
Materials & Methods



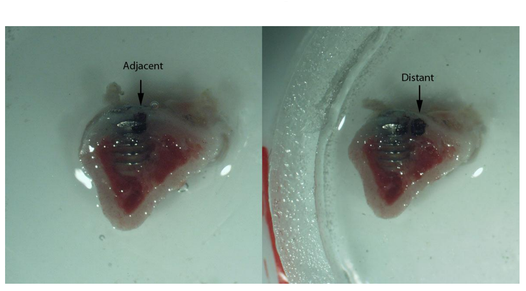
Machined implants were inserted in rat tibia for 21 days



Bone-implant blocks harvested using dental disc cutting, transversely, immediately along the side of the implant



Implants (implant-adherent cells) & peri-implant bone collars retrieved by unscrewing and trephine, respectively



Micro-section retrieval using a femtosecond laser (TissueSurgeon; Rowiak)

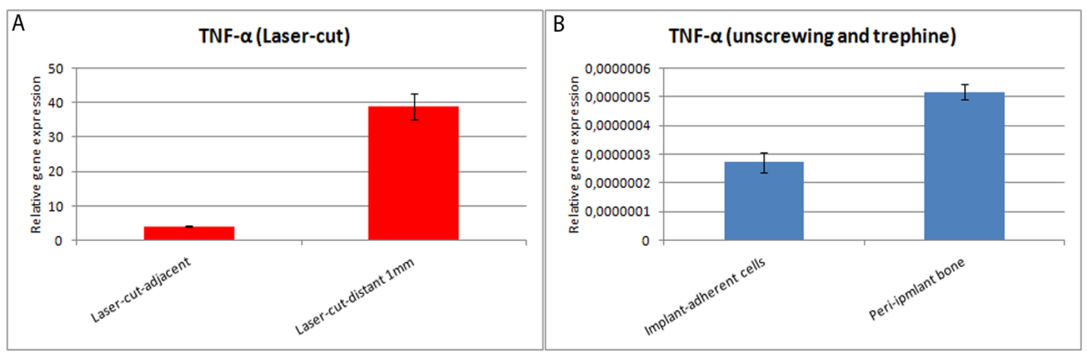
Homogenization & RNA extraction

Preamplification

Gene expression analysis (qPCR)

Results

It was possible to extract and analyze the RNA in the samples retrieved by laser microtome.



The analysis indicated higher levels of expression of the analyzed markers in 1-mm distant samples compared to implant-adjacent ones (A), which was comparable to the expression pattern in the peri-implant bone and implant-adherent cells, respectively (B).

Discussion & Conclusion

For the first time, a laser-cutting microtome was applied for retrieving specific samples within the interface between undecalcified bone and metal implants, with the possibility to analyse the gene expression in these samples.

This provides a new approach to investigate site-specific regulation of genes in relation to biomaterials of different designs and/or surface properties, *in vivo*.

Acknowledgements

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References

1. Omar O, Lenneras M, et al, *Biomaterials, 2011,* 32(2): 374-86
2. Omar O, Svensson S, et al, J Biomed Mater Res A. 2010, 15;92(4):1552-66

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