

## Enhanced bone regeneration by a bioresorbable and bioactive PLGA membrane

B. San Miguel<sup>1,2</sup>, M. Ehrbar<sup>1</sup>, C. Ghayor<sup>1</sup>, M. Textor<sup>2</sup>, F. Weber<sup>1</sup>

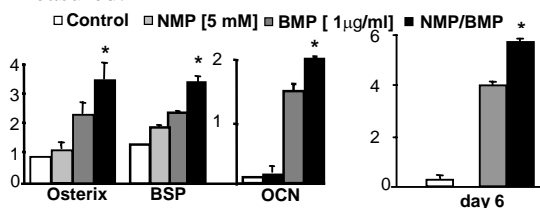
<sup>1</sup>Institute of Oral Biology-Bioengineering and Cranio-maxillofacial Clinic-University Hospital Zürich, Switzerland <sup>2</sup>ETH Zürich, BioInterfaceGroup/LSST Zürich, Switzerland

**INTRODUCTION:** In Guided Bone Regeneration, a membrane is implanted to create a compartment that can easily be occupied by bone. In order to avoid a second surgery required for its removal, a bioresorbable poly (lactide-co-glycolide) membrane was developed. The intrinsic rigidity of the polymeric material was overcome by using the plasticizer NMP (N-methyl-2-pyrrolidone), which has recently been shown to be bioactive, as it enhances osteoblastic maturation in vitro as well as bone regeneration in vivo.

**METHODS:** In vitro, MC3T3-E1 pre-osteoblastic cells seeded onto TCPS plates were tested for early and late osteoblastic responses, namely: ALP (Alkaline phosphatase activity), osteocalcin protein secretion and Alizarin Red calcium nodule formation respectively. At the molecular level, cell extracts were analyzed by Western Blots for Smad 1,5,8 phosphorylation (related to the initiation of the BMP-2 signalling pathway), as well as mRNA levels via quantitative RTPCR (Q-PCR) for specific osteoblastic markers. (BSP:bone sialoprotein, OCN:Osteocalcin)

In vivo, PLGA membranes containing NMP were implanted into a non-critical size rabbit calvarial defect, in parallel with similar membranes without NMP, commercially available Ossequest® e-PTFE, and empty defects as controls (n=6). 4 weeks after implantation, bone regeneration was analyzed both qualitatively and quantitatively by histology, histomorphometry as well as micro computer tomography (micro-CT) analysis.

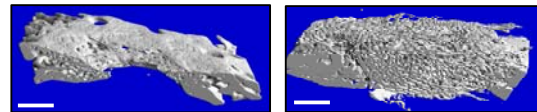
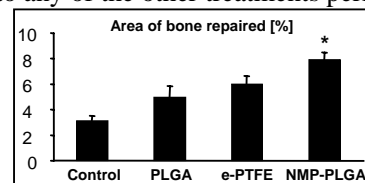
**RESULTS:** NMP acting synergistically with rhBMP-2 increases ALP activity, mRNA levels of various bone related transcription factors and ECM proteins, correlating with efficient translation into osteocalcin protein secretion into the medium. Already after 5 min. of stimulation, NMP/rhBMP-2 combination also promoted the acceleration of Smad 1,5,8 phosphorylation. At later time points, an increase in calcium deposition was also measured.



*Fig. 1: Q-PCR of osterix after 24h, BSP and OCN after 6 days of stimulation respectively (left). OCN*

*protein concentration (ng/ml) in cell medium after 6 days of stimulation (right).*

In vivo, empty control samples failed to bridge the bone defect, while membrane-implanted defects successfully filled it with newly formed bone to different extent. The in vivo results illustrate that the guided bone regeneration membrane releasing NMP enhances bone formation significantly with respect to any of the other treatments performed.



*Fig.2: Histomorphometrical evaluation of the different treatments performed (top). Micro-CT cross-sections of the empty defect and NMP-PLGA membranes respectively. (Scale bar = 1mm)*

**DISCUSSION & CONCLUSIONS:** The acceleration of Smad 1,5,8 phosphorylation, Q-PCR, expression of ALP and OCN results might indicate an interaction between NMP and the BMP pathway. The mechanism of NMP action is still unclear, however further investigations are being carried out in this respect.

This findings could be translated into novel treatment strategies for bone regeneration, where NMP improves the biological activity of autologous/exogenous BMP.

**REFERENCES:** <sup>1</sup>F.E. Weber, H.G. Schmökel, E. Pirhonen (2003) The first bioactive, degradable membrane for guided bone regeneration, Abstractbook 1854, IADR, Gothenburg. <sup>2</sup>C.H. Hammerle and R.E. Jung (2000) Bone augmentation by means of barrier membranes. *Periodontology* 33:36-53. <sup>3</sup>H.D. Zegzula, J.M. Wozney, J.O. Hollinger (1997) Bone formation with use of rhBMP-2, *Journal of Bone and Joint Surgery. American Vol.* 79: 1778-90.

**ACKNOWLEDGEMENTS:** This study was supported by a grant from Inion OY, Tampere-Finland. Micro-CT analysis was performed with technical assistance at the Institute of biomedical engineering ETH Zurich.