

# The Bone Inducing Principle: Paving the Way Between Bench Top and Bed Side

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**INTRODUCTION:** Bone morphogenetic proteins (BMPs) are the key cytokines in bone formation and repair. Since the cloning of the first members of the BMP superfamily in 1988 recombinant BMPs failed to substitute autologous bone as gold standard in clinical treatments for bone repair, mainly because the first clinical trials showed, that milligram doses of rhBMP-2 are required for effect [1]. Therefore, BMP-presentation and release kinetic mainly determined by the delivery system have to be optimized for an efficient clinical application of rhBMP. Another strategy to decrease the BMP dose in clinical applications is to combine the cytokine with enhancers of BMP activity. Here we show that NMP (N-methylpyrrolidone) is an enhancer of BMP activity and can be used to generate biomaterials of the 3<sup>rd</sup> generation, where biocompatibility, biodegradability, and bioactivity are combined.

**METHODS:** MC3T3-E1 pre-osteoblastic cells were tested for different cell maturation responses: ALP (Alkaline phosphatase activity) and Alizarin Red mineralization assay. At the molecular level, cell extracts were analyzed by Western Blotting for Smad 1,5,8 phosphorylation, as well as by quantitative real time PCR (qRT-PCR) for specific osteoblastic markers (Osteocalcin-OCN, Bone Sialoprotein-BSP). Histological and histomorphometric analysis of bone repair *in vivo*: non critical size 6 mm defects were created in the rabbit calvaria and subsequently treated with three different membranes, namely PLGA, Osseo quest and NMP-PLGA, or left untreated (control). Membranes were placed both on top and underneath the defects, and the regenerated bone within the defect was determined 4 weeks after the operation.

**RESULTS:** NMP increased ALP activity of MC3T3-E1 cells concentration dependent. Compared to controls 2.5 mM NMP increased ALP  $2.5 \pm 0.3$  times (N=6,  $P < 0.001$ ) and mineralization of MC3T3-E1 cells determined after 4 weeks by Alizarin-red staining was enhanced  $1.5 \pm 0.05$  times (N=6,  $P < 0.001$ ). NMP action depended on extracellular bone morphogenetic protein (BMP), because in the presence of 2.5 mM NMP it was reduced below control levels ( $67 \pm 10\%$ ) by the addition of the BMP antagonist Noggin ( $1 \mu\text{g/ml}$ ). In combination

with rhBMP-2 NMP showed a synergistic effect on ALP activity, mineralization and Smad 1,5,7 phosphorylation. The *in vivo* results in the guided bone regeneration model showed that in the presence of NMP healing of the defect was  $79.17 \pm 5.61\%$  compared to  $49.31 \pm 8.75\%$  without NMP.

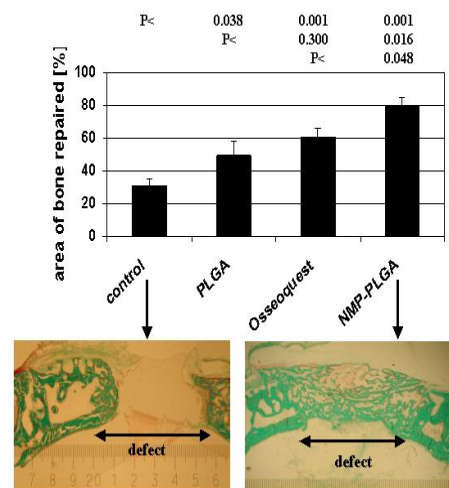


Figure 1: Enhancing of bone repair by guided bone regeneration and NMP: Defects of 6 mm in diameter created in the calvarial bone were treated with three different membranes (PLGA, Osseo quest and NMP-PLGA) or left untreated (control). The upper panel shows the percentages of the area where bone regeneration in the defect has occurred in relation to the original defect area.

**DISCUSSION & CONCLUSIONS:** The results show that NMP improves the biological activity of BMP *in vitro* and *in vivo* by enhancing the kinase activity of the BMP-BMP-receptor complex. In the end, our finding could translate into novel treatment strategies for bone regeneration under the influence of autologous BMP for non-critical size defects and recombinant human BMP for critical size defects. Since NMP can be delivered by PLGA-based materials these materials become bioactive and are turned into materials of the 3<sup>rd</sup> generation.

**REFERENCES:** <sup>1</sup> Schmitt, J. M., K. Hwang, et al. (1999). J Orthop Res : 17(2): 269-78

**ACKNOWLEDGEMENTS:** This study was in supported by Inion OY, Tampere Finland.