

Small chemicals to enhance bone repair

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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 3rd 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 and p38 phosphorylation, as well as by quantitative real time PCR (qRT-PCR) for specific osteoblastic markers (Osteocalcin-OCN, Bone Sialoprotein-BSP). Histological and histo-morphometric analysis of bone repair *in vivo*: non critical size 6 mm defects were created in rabbit calvarias and subsequently treated with three different membranes, namely PLGA, and NMP-PLGA, or left untreated (control).

RESULTS: NMP increased ALP activity of MC3T3-E1 cells concentration dependent. and mineralization of MC3T3-E1 cells. NMP action depended on extracellular bone morphogenetic protein (BMP), because in the presence of the BMP antagonist Noggin ALP activity in the presence of NMP was reduced below control levels (67±10%). In combination with rhBMP-2 NMP showed a synergistic effect on ALP activity, mineralization p38 and Smad 1,5,7 phosphorylation. This synergistic effect depends on active PKD. The synergistic effect on p38 phosphorylation is also PKC dependent. The *in vivo* results in the guided bone regeneration model showed that in the presence of NMP healing of the defect was 79.17±5.61% compared to 49.31±8.75 % without NMP.

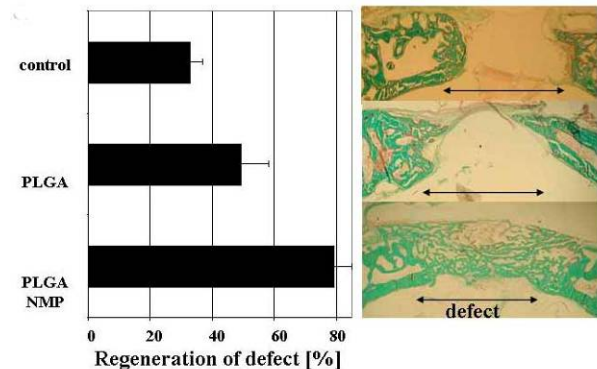


Figure 1: Enhancing of bone repair by guided bone regeneration and NMP: Defects of 6 mm in diameter created in the calvarial bone were treated with PLGA membrane, NMP-PLGA membrane or left untreated (control). The left panel shows the percentages of the area where bone regeneration in the defect has 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. Since p38 and Smad 1,5,7 phosphorylation is increased, both the preformed receptor complexes and the BMP induced receptor complexes exhibit an NMP dependent enhanced in kinase activity. In contrast to normal BMP activity which is only PKD dependent, the synergistic effect for p38 is also PKC dependent. Thus, NMP not only enhances the BMP activity but also induces other, PKC dependent pathways, and creates in the presence of BMP an orchestrated signalling pattern favouring bone repair and regeneration. 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.

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

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