

A bioactive biodegradable guided bone regeneration membrane: from the bench to the dental practice

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INTRODUCTION: Bone morphogenetic proteins (BMPs) are the key cytokines in bone formation and repair. A possible strategy to utilize BMP activity in clinical applications is to enhance the activity of autologous BMP. Here we show that NMP (N-methyl-pyrrolidone) is an enhancer of BMP activity and can be used to generate a guided bone regeneration (GBR) membranes of the 3rd generation, where biocompatibility, biodegradability and bioactivity are combined. To identify possible other pro osteogenic pathways induced by NMP we, then, applied micro array techniques and found that in addition to the BMP signalling, the natriuretic hormone system is also tuned into a pro osteogenic state.

METHODS: MC3T3-E1 pre-osteoblastic cells were tested for different cell maturation responses: ALP (Alkaline phosphatase activity) and Alizarin Red mineralization assay. Micro array experiments were performed with C2C12 cells. The effect of NMP was determined *in vivo* in a guided bone regeneration model. Histological and histomorphometric 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: In preosteoblastic cells, NMP increases ALP and mineralization concentration dependent. NMP action depends on extracellular BMP, because it is sensitive to BMP antagonist Noggin. In combination with rhBMP-2, NMP shows a synergistic effect on ALP activity, mineralization and Smad 1,5,7 phosphorylation. Although BMP activity depends on protein kinase D, the synergistic effect is protein kinase C dependent. The *in vivo* results in a GBR model show that at 4 weeks in the presence of NMP healing of the defect is 79.17% complete compared to 49.31 % without NMP.

Micro array experiments performed with the multi potent mesenchymal stem cell revealed that 4 h exposure of C2C12 cells to 5mM of NMP halved the expression of natriuretic peptide receptor type

(npr3) and increase in the expression of the natriuretic peptide precursor type B (BNP) 1.5 fold. These results were confirmed by low density arrays and RT-pcr.

DISCUSSION & CONCLUSIONS: The results suggest 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 a protein kinase C dependent way. The *in vivo* results show that the content of autologous BMP in bone is sufficient for NMP to enhance bone healing. Since NMP can be delivered by PLGA-based materials the combination of PLGA and NMP generates a 3rd generation GBR membrane, since this GBR membrane combines biocompatibility, biodegradability, and bioactivity. Interestingly, NMP shows also effect in the absence of BMP. The plasticizer NMP decreases npr-3 expression and increases BNP expression, mimicking the knock-out and over-expression of those genes in mice which is manifested in formation of longer bones and increased bone formation¹. Therefore, the direct effect of NMP on the transcription of 2 elements of the natriuretic peptide hormone system in a pro osteogenic way could at least partially account for the accelerated bone healing seen under the influence of NMP *in vivo*.

REFERENCES: ¹Bartels CF, Bükülmez H, Padayatti P, et al. Mutations in the transmembrane natriuretic peptide receptor NPR-B impair skeletal growth and cause acromesomelic dysplasia, type Maroteaux. *Am J Hum Genet.* 2004 75(1):27-34

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