A novel approach for the production of human recombinant BMP-2 for bone tissue engineering applications

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INTRODUCTION

Bone tissue engineering has been an increasing field of research during the last years. The ideal approach for a regenerative application would consist in the use of cells from the patient, scaffolding materials and differentiation growth factors. Bone morphogenetic protein-2 (BMP-2) is one such growth factor, with a strong ability to induce new bone and cartilage formation and has been used as a powerful osteoinductive component of several late-stage tissue engineering products for bone grafting. In this work, we aimed at obtaining high yields of human recombinant BMP-2 in a stable, pure and biologically active form by use of a new bacteria expression system that circumvents the disadvantages of conventional recombinant protein preparation methods and to perform a study of the stability conditions and the functionality of these peptides in vitro in human mesenchymal stem cells and C2C12 murine cell line.

MATERIALS & METHODS

Purification of rhBMP-2 by high affinity chromatography

rhBMP-2 was then purified by high affinity chromatography and size exclusion chromatography and tested in C2C12 cell line. This is a well-studied and stable model for testing the in vitro biological activity of recombinant BMPs.

RESULTS & DISCUSSION

Purification by size exclusion chromatography

Size exclusion chromatography permitted partial separation of monomer, dimer and polymer fractions, as analysed by Western blot.

Biological activity assays

MTS cytotoxicity assay

Fig. 6. MTS assay revealed no cytotoxicity of purified rhBMP-2

Morphology of human MSCs

Fig. 7. Addition of 5 500ng/ml mBMP-2 to human adipose mesenchymal stem cells resulted in changes of morphology

RT-PCR for specific markers

Fig. 8. RT-PCR shows increase of specific markers of osteogenic differentiation (ALP, Smad5, runx2, osteocalcin) when C2C12 cells were stimulated with 500ng/ml of our mBMP-2 stabilized at pH 10.

CONCLUSIONS

The novel approach described herein shows to be a promising way for obtaining large amounts of partially purified rhBMP-2 which shows evidence of bioactivity, capable of inducing some markers of specific osteogenic (bone) differentiation and showing no relevant cytotoxicity.

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