

Porous β -TCP activation with a chitosan/rh-BMP-2 coating.

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INTRODUCTION: Porous cement materials are widely used in biotechnology and medicine as bone filling materials. Calcium phosphates are being increasingly used as bone substitutes because of biocompatible and osteoconductive properties. Combination of porous β -TCP scaffold with other BMP-2 carrier biomaterial, could allow to obtain a compound with enhanced characteristics for bone tissue engineering. Chitosan, a copolymer of N-acetylglucosamine and glucosamine, has interesting biological properties (biocompatible, bioresorbable and bioactive) was chosen as a BMP carrier. In a previous work we demonstrated that chitosan film activated with rhBMP-2 are able to support cellular growth and induce cell differentiation towards osteoblastic phenotype and *in vivo* induce bone regeneration [1]. In this work, we developed biodegradable β -TCP ceramic coated with chitosan film carrying rh-BMP-2. The osteoinductive effect of this material was both *in vitro* and *in vivo* evaluated.

METHODS: Porous calcium phosphate cement consists of a mixture of 61.3wt% tricalcium phosphate (β -TCP), 34.4wt% calcium phosphate monobasic, 4.3wt% calcium carbonate. An aqueous solution of acetic acid (50 mM) was used as the liquid component. Discs were cut and sterilized by heating at 300°C for 3 h. 1% (w/v) chitosan solution in 50 mM acetic acid was employed for coated them. The rhBMP-2 activation of film was done as previously described [2].

The ceramics were seeded with C2C12 cell line (muscle myoblast, mouse, CRL 1772) and an environmental scanning electron microscope was used to characterize the surface topography and cell adhesion. The activity of the adsorbed protein on C2C12 cells was quantified through the colorimetric alkaline phosphatase activity assay.

Non critical cranial defect in New Zealand rabbit as *in vivo* experimental model was selected. After 3 weeks, samples were retrieved and fixed for radiological, micro TC (Skyscan 1172, Trabeculae, Empresa de Base Tecnológica, Spain) and histological evaluation.

RESULTS: The developed discs have 5,8 mm in diameter and 2,9 mm thick, and 53% porosity. μ CT-based image analysis shows initially a wide range of pores size distribution. In the other hand, ceramics coated with chitosan film show a redistribution of porous diameter.

ESEM inspection showed cells covering whole surface, forming a thin layer and many of them penetrated inside the porous.

The increase in the alkaline phosphatase activity of C2C12 cell culture demonstrated that chitosan/BMP2 cement allows *in vitro* cell differentiation towards osteoblastic lineage.

In vivo results seem indicate an enhanced bone tissue ingrowth in the defect filled with chitosan/BMP2 cement (*Figure1*).

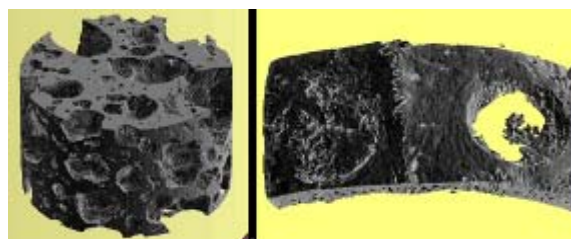


Fig. 1: First image shows ceramic porous structure. Second image show in vivo effect of activated ceramic in the left side and empty defect as control in the right side.

DISCUSSION & CONCLUSIONS: The porous structure of developed chitosan coated ceramics allows cells growth. rhBMP-2 activation induce cell differentiation onto this material. Finally, rhBMP-2 activated cement shows osteoconductive properties, allowing the growth of healthy bone tissue *in vivo*.

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ACKNOWLEDGEMENTS: This work was supported by the NORICUM S.L., Ministerio de Sanidad (PIO-41048) (Spain) and MMA Biomedical Research Foundation (Spain).