

## Bone marrow multipotential stromal cell colonisation of natural bone substitute Orthoss® – Osteoconductivity, osteoinductivity and graft expander potential

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**INTRODUCTION:** In the Orthopaedic and Trauma discipline, both autografts and allografts have been used for the treatment of impaired fracture healing and the management of critical size bone defects<sup>1</sup>. In spite of its superiority, harvesting of autologous bone grafting has been associated with a number of comorbidities and limited volume availability<sup>2</sup>. For this reason, the concept of ‘graft expanders’ (combining grafts) has been recently popularised to increase the volume and potential biological activity of the implanted material. In addition, new strategies based on autologous multipotential stromal cells (MSCs) seeded on osteoinductive bone substitute scaffolds have been also developed<sup>3</sup>. The aim of this study was to test the properties of Orthoss® granules to support exogenously seeded MSCs as well as to attract neighboring host MSCs, thus serving as a potential graft expander for repairing large bone defects in trauma patients.

**METHODS:** Following ethics committee approval, bone marrow (BM) MSCs (passage 3, p3) harvested from 6 patients admitted for elective orthopaedic procedures were studied for their proliferative and differentiation capacities in 2-D cultures. Bone autograft was also harvested for the purpose of migration experiments. In 3-D cultures, Orthoss® granules (2-4 mm) were seeded dynamically with  $2 \times 10^5$  cells/granule p3 MSCs and further maintained either in MSC expansion or differentiation media for up to 21 days. In homing/migration experiments, bone autografts (size 4 mm) were placed in Matrigel in close proximity to Orthoss® to study cell egress from autografts and their penetration into the Orthoss®. Scaffold colonisation and MSC differentiation were assessed by confocal microscopy, standard electron microscopy, and energy-dispersive X-ray spectroscopy.

**RESULTS:** Cultured cells exhibited morphology, growth kinetics, phenotypic profile and tripotentiality consistent with MSCs. MSC attachment to Orthoss® was donor-independent showing excellent colonisation after both 4 and 7 days (Figure 1). Long-term incubation (21 days) resulted in formation of multiple cell-matrix layers lining the scaffold pores as well as outer surfaces.

MSC differentiation to osteoblasts was evident as strong deposition of Calcium and Phosphorus on the top of cell layers was detected (Figure 1). Importantly, high Orthoss® osteoinductivity was revealed following culture in both MSC expansion and osteogenic conditions. Cell egress experiments demonstrated the migration of cells from neighbouring autografts and their attachment and re-settlement on Orthoss®.



Fig. 1: MSC colonisation of Orthoss® granules at days 7 (left) and 21 (middle). Calcium (upper right) and Phosphorus (bottom right) deposition by developed osteoblasts on day 21.

**DISCUSSION & CONCLUSIONS:** Orthoss® scaffolds support MSC attachment, growth and osteogenic differentiation. Furthermore, our *in vitro* 3-D modelling experiments showed that resident bone subpopulations can rapidly migrate towards, attach, and expand on Orthoss® scaffolds. These results indicate that Orthoss® is not only an excellent natural bone substitute material but can be also used as graft expander in the discipline of trauma and orthopaedic surgery in cases where bone regeneration is desirable.

**REFERENCES:** <sup>1</sup> Kinney, R. C., Ziran, B. H., Hirshorn, K., et al. (2010) *Journal of Orthopaedic Trauma* **24**:S52-S55. <sup>2</sup> Giannoudis, P. V., Dinopoulos, H., and Tsiroidis, E. (2005) *Injury* **36**:S20-S27. <sup>3</sup> Pittenger, M. F., Mackay, A. M., Beck, S. C., et al. (1999). *Science* **284**:143-147.