IN VIVO STUDY OF THE EFFECT OF A STRONTIUM-RICH INJECTABLE SYSTEM ON BONE REGENERATION, USING A SHEEP MODEL

C. Machado[1,2], A.H. Lourenço[1,2,3]
N. Neves[1,2,4] , N. Alexandre[5,6]
M. Lamghari[1,2] , A.T Cabral[4]
M.A. Barbosa[1,2,7], C.C. Ribeiro[1,2,8]

[1] INEB – Instituto de Engenharia Biomédica, Universidade do Porto, Portugal
[2] I3S – Instituto de Investigação e Inovação em Saúde
[3] Faculdade de Engenharia , Universidade do Porto, Portugal
[4] Faculdade de Medicina, Universidade do Porto, Portugal
[5] Departamento de Zootecnia, Universidade de Évora, Portugal
[6] ICAAM – Instituto de Ciências Agro-ambientais Mediterrânicas, Universidade de Évora, Portugal
[7] ICBAS – Intituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal
[8] ISEP – Insituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Portugal.

INTRODUCTION Bone has the capacity to regenerate as part of the repair process, being newly formed bone indistinguishable from the adjacent uninjured bone. However, there are cases in which bone regeneration is required in large quantity, beyond the normal potential for self-healing, such as for lesions caused by trauma, infection, tumour resection or cases in which the regenerative process is compromised such as avascular necrosis and osteoporosis. Biomaterials such as alginate are very promising due to its ability to form hydrogels *in situ* under mild conditions in the presence of divalent cations. The combination with ceramic microspheres results in a mechanically improved injectable system, adequate for minimally invasive procedures. Moreover, the combination with chemical elements such as strontium, described as promoter of bone formation, inhibiting bone resorption [1], provides ion exchange between the implanted biomaterial and surrounding tissue, enhancing bone regeneration. Our goal is to study in an *in vivo* sheep model, the effect of an injectable system composed of strontium doped hydroxyapatite microspheres, delivered in an alginate vehicle, crosslinked with strontium.

RESULTS & DISCUSSION

Micro-CT analysis

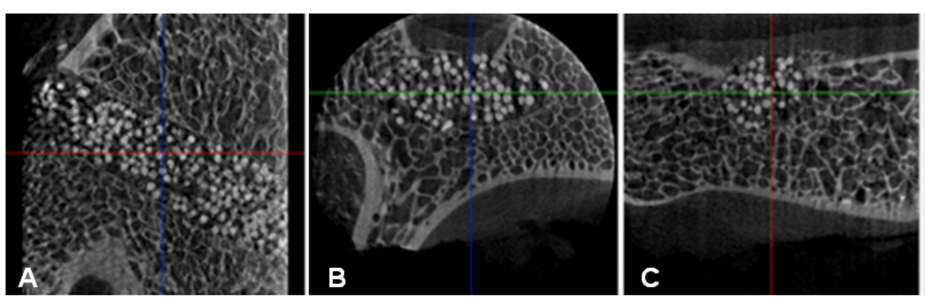

Fig 1 Orthogonal reconstructed slices of micro-computed tomography (micro-CT) (A, B, C), of the defects filled with the hybrid system, after one week of implantation.

The hybrid system **perfectly filled the defect.**

During the injection, no separation of the alginate and the microspheres was observed.

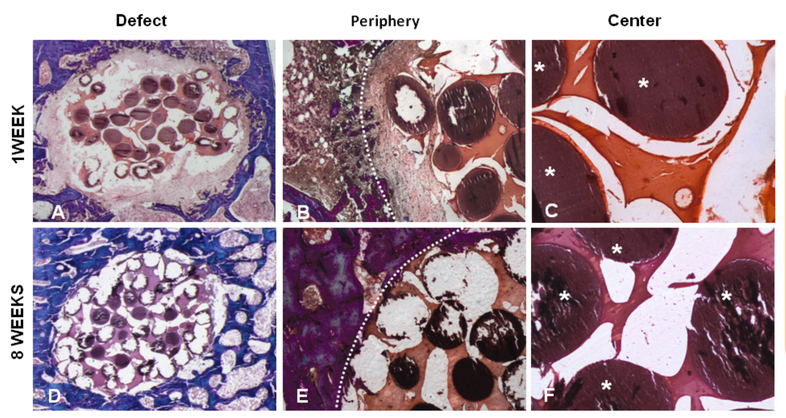

Fig 2 Safranin/Light Green stained histological sections of sheep vertebra defect filled with the hybrid system, one week (A, B, C) and eight weeks (D, E, F) post-implantation. Global view of the defect (A, D, 20x) ; details of the periphery (B, E, 40x, dashed line delimit the defect) and details of the center of the defect (C, F, 100x, microspheres are indicated with the asterisk).

The degradation of the alginate allowed the **migration of inflammatory cells,** a reaction commonly seen in the first phase of bone repair.

No signs of degradation of the microspheres was observed (lower degradation rate than alginate).

New bone formation and resorption

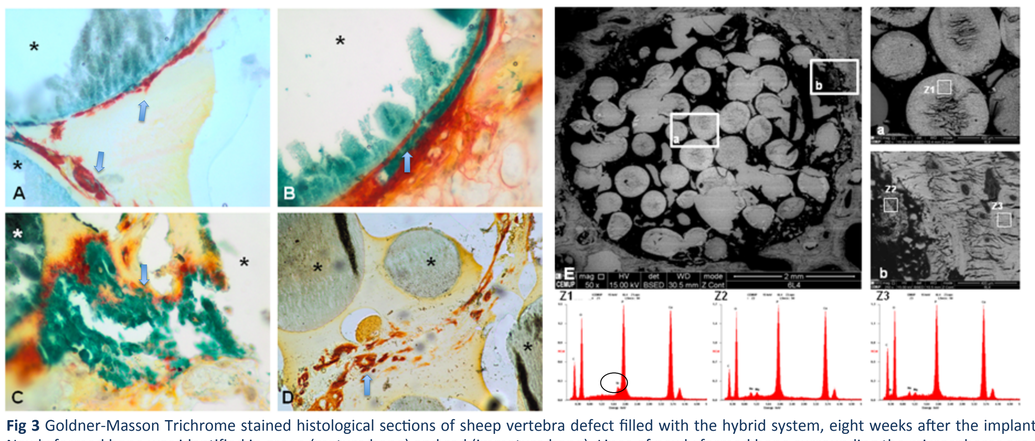

Fig 3 Goldner-Masson Trichrome stained histological sections of sheep vertebra defect filled with the hybrid system, eight weeks after the implantation. Newly formed bone was identified in green (mature bone) and red (immature bone). Lines of newly formed bone surrounding the microspheres are visible in **A** and **B** (400x) and between two microspheres in **C** (400x). Immature bone is shown in the center of the defect in **D** (100x). Microspheres are identified with *. Images of scanning electron microscopy and energy-dispersive X-Ray spectra (E) of 8 weeks post implantation histological section. Z1, Z2 and Z3 indicate the different analysed areas and correspondent EDS spectra.

Bone surrounded the microspheres, both in the periphery and in the center of the defect.

EDS analysis confirmed the presence of newly formed bone in the defect (no Sr was detected excluding the possibility of being microspheres fragments).

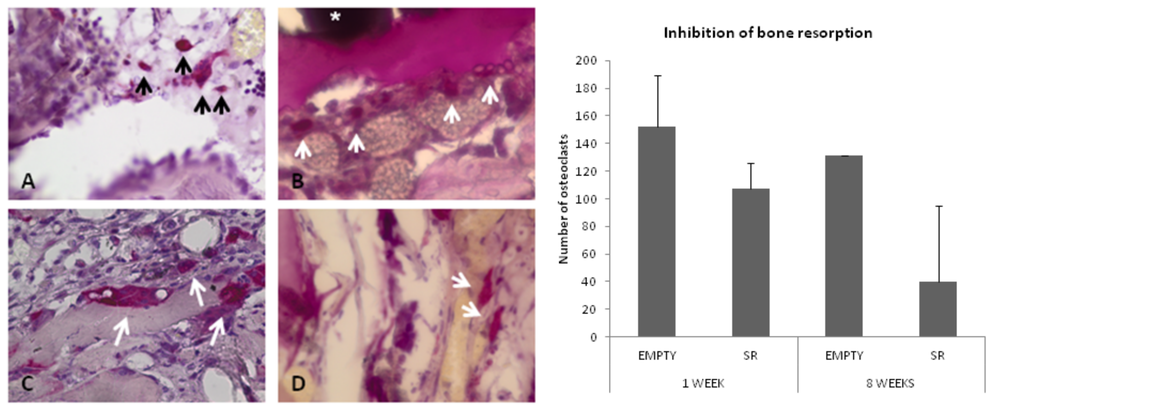

Fig 4 Tartrate-resistant Acid Phosphatase stained histological sections of sheep vertebra defects. Arrows indicate osteoclasts in the empty defect after eight weeks of implantation (A, 400x), in the filled defect after one week (B and C, 400x), and after eight weeks (D, 400x). The graphic represents the number of osteoclasts in the empty and in the filled defects at 1 and 8 weeks time-points.

After eight weeks the **strontium effect at decreasing the number of osteoclasts** was evident, possibly by inhibiting its recruitment and promoting apoptosis.

Morphologic evaluation of soft tissues

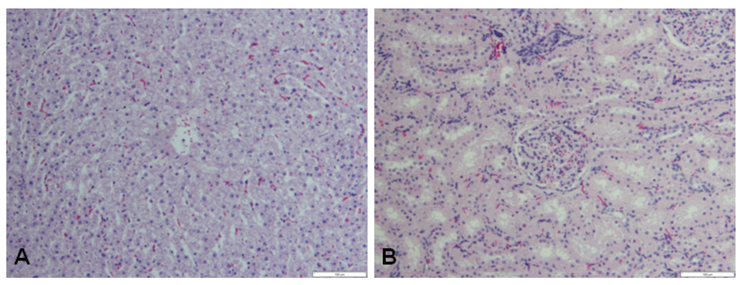

Fig 5 H&E stained histological sections of liver (A) and kidney (B), at 100x magnification.

The morphology of kidneys and liver were macro and microscopically normal, suggesting the **absence of major toxic systemic effects caused by strontium.**

CONCLUSIONS

The studied system has the ability to promote local bone formation as well as inhibiting bone resorption, without major systemic effects. Our results suggest that this material may be a promising alternative for bone regeneration, particularly in osteoporotic patients.

MATERIALS & METHODS In this study, a critical size-defect (4.5x3.6 mm), adapted from Lamghari *et al [2],* was made in the body of 3 lumbar vertebrae of 5 years old Merino Branco sheeps and filled with the injectable hybrid system. An empty defect was used as control. Bone regeneration was evaluated at one and eight weeks post-implantation by micro computed tomography and histological analysis after methylmethacrylate embedding of bone specimens. Soft tissues analysis was also performed to evaluate strontium systemic effects.

REFERENCES
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