Bioactivity and Osteoinductivity of Magnesium-Based Alloys *in vitro*

Roxane Bonithon[1], Arianna De Mori [2], Tosca Roncada[2], Marta Roldo[2], Gordon Blunn[2]
[1]USchool of Mechanical and Design Engineering, University of Portsmouth, Portsmouth, UK, [2]School of, University of Portsmouth, Portsmouth, UK
roxane.bonithon@port.ac.uk

Disclosures: Roxane Bonithon (N), Arianna De Mori (N), Tosca Roncada (N), Marta Roldo (N), Gordon Blunn (N).

INTRODUCTION: Magnesium (Mg) alloys are gaining increasing interest in the orthopaedic field due to their mechanical properties, which are the closest to bone tissue than any other biomaterial, and their ability to degrade in vivo [1,2]. It has also recently been shown that the release of Mg ions during its biodegradation process is actively linked with the activation of cellular signaling pathways leading to the differentiation of human bone marrow stroma cells (hBMSC) into osteoblasts [3]. However, its direct effect on bone cells (e.g. osteoblasts or osteoclasts), as well as on the process of bone mineralization (i.e. bioactivity) remains unknown. This study aims at quantifying for the first time the bioactivity of aluminum-free porous Mg-based alloys as well as their osteoinductive properties in contact with osteoblasts. The main purpose is to investigate the influence of Mg alloys on bone growth and mineralization.

METHODS: Primary ovine osteoblasts (N=100,000) from 3 different animals (with three replicates per animal) have been grown on Mg-based alloys and in 6-well plate (control) for 1, 3 and 7 days in DMEM/F12 supplemented with 1% p/s, 10% FBS and 20 mM of HEPES at 37°C and 5% of CO_2. Cell viability, proliferation and differentiation were assessed by Live/Dead staining, Presto Blue assay, LDH assay and RT-qPCR. Magnesium bioactivity was quantified with ImageJ based on SEM images after immersion into simulated body fluid (SBF) for 1 day, 7 and 14 days. Statistical differences were assessed by either two-ANOVA, followed by a Tukey's multi-comparison, or Kruskal-Wallis tests, using GraphPad Prism 8.0.2. Data were considered significant when $p<0.05$.

RESULTS: A significant increase in cell viability and proliferation was observed based on Live/Dead ($90.2 \pm 7.1\%$ to $99.1 \pm 0.6\%$, $p<0.05$) and Presto Blue (2.7 ± 1.2 to 8.1 ± 2.5, $p<0.005$) assays from 1 to 7 days of incubation (Fig 1). Additionally, a significant increase ($p<0.001$) in RUNX2 gene expression was computed for cells grown on the Mg alloys from 1 to 7 days. The immersion in SBF showed a significant ($p<0.005$) increase in CaP deposition from $1.6 \pm 0.3\%$ at 1 day to $3.9 \pm 0.4\%$ at 14 days. CaP deposits in the form of spherulitic structures formed on contact with osteoblasts and allow them to anchor to the surface of the Mg alloy (Fig 2).

DISCUSSION: Mg alloys demonstrated a positive effect on the viability, proliferation and differentiation of osteoblasts *in vitro* suggesting the potential for substantial bone growth and osteointegration *in vivo*. This study also quantified for the first time the bioactivity of magnesium-based alloys immersed in SBF solution for up to 2 weeks. It appears that the biodegradation of the Mg alloys participates in the deposition of CaP-based particles in the vicinity of osteoblasts *in vitro* which favors the mineralization process at a later stage *in vivo*. Therefore, these results seem to imply that Mg-based alloys promote bone growth, , as well as its mineralization to form a hard callus that will later be remodelled to bone tissue during the bone healing process after injury[4].

SIGNIFICANCE/CLINICAL RELEVANCE:
This study aims to further investigate the biological properties of Mg-based alloys in order to validate their clinical use as bone regeneration treatments.

REFERENCES:
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3. Hung, C. C., Chaya, A., Liu, K., Verdelis, K. & Sfeir, C. The role of magnesium ions in bone regeneration involves the canonical Wnt signaling pathway. *Acta Biomater.* **98**, 246–255 (2019).
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IMAGES AND TABLES:

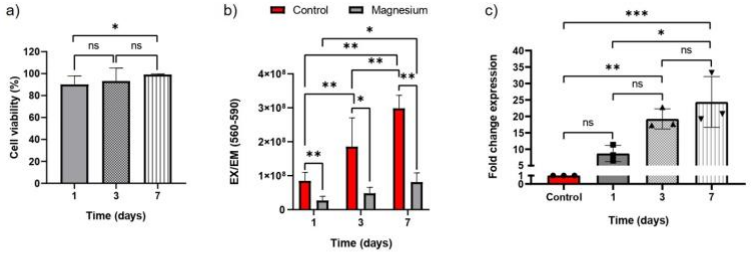

Figure 1: a) Cell viability with time based on Live/Dead assay. *p<0.05 significant difference. b) Cell proliferation with time between 2D controls and Mg-based alloys based on Presto Blue assay. *p<0.005 and **p<0.0001 significant difference. c) RUNX2 gene expression with time between 2D controls cultivated for 1 day and Mg-based alloys based on RTqPCR. *p<0.01, **p<0.005 and ***p<0.001 significant difference.

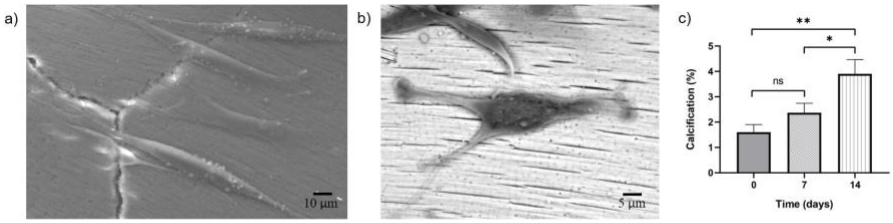

Figure 2: a) Secondary electron image (SE) of CaP-based particles deposition on top of osteoblasts at 2 kX magnification and b) backscattered electron image (BSE) of CaP-based particles as cells filopodia anchor at 3.5 kX magnification and c) Bioactivity (Ca and P co-deposition) with time. *p<0.05 and **p<0.005 significant difference.