THE EFFECTS OF ANTI-OSTEOPOROTIC DRUGS ON A 3D DYNAMIC *IN VITRO* HUMAN BONE REMODELING MODEL

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Introduction

Osteoporosis is the most common bone remodeling disease causing a significant societal burden and an increased risk of mortality upon related fractures [1]. Novel drugs to prevent and treat osteoporosis are currently routinely tested in animal models with poor clinical translation [2]. *In vitro* bone remodeling models, employing osteoclast-osteoblast cocultures, could facilitate the investigation of human bone remodeling and thereby have the potential to improve preclinical testing while reducing the need for animal experiments. However, current osteoclast-osteoblast cocultures often lack physiological resemblance, including a 3D biomimetic environment and mechanical loading [3]. Here, we employed a coculture of human monocytes and mesenchymal stromal cells (MSCs) seeded on composite scaffolds and mechanically loaded with fluid shear stress to evaluate the effects of anti-osteoporotic drugs on human bone remodeling *in vitro*. As such, the capabilities and limitations of this *in vitro* model were studied.

Methods

Human MSCs and monocytes were seeded in a 1:5 ratio on mineralized silk fibroin scaffolds (N = 4-8 per group) [4] and cultured for 28 days in spinner flasks bioreactors, set at a rotation speed of 300 RPM, with coculture medium (α-MEM, 5% human platelet lysate, 1% antibiotic-antimycotic, 50 ng/ml RANKL and M-CSF, 10 nM dexamethasone and 50 µg/ml ascorbic acid) to stimulate osteoclastogenesis and osteogenesis. After 14 days, when cell differentiation was expected to be completed, anti-osteoporotic drugs alendronate and testosterone were added to the following groups: ALN: 2 µM alendronate, TEST: 75 nM testosterone, ALN+TEST: 2 µM alendronate and 75 nM testosterone. The control group remained untreated. To track remodeling and cytotoxicity, μCT scans were acquired and LDH activity was measured on medium samples taken weekly.

Results

On day 21, the treated groups all had a higher LDH activity than the control group (Figure 1A), indicating more cell death. Scaffold remodeling, tracked with μCT, revealed most mineral formation in constructs treated with only alendronate (Figure 1B+C). No clear treatment induced differences were found in mineral resorption (Figure 1B+C).

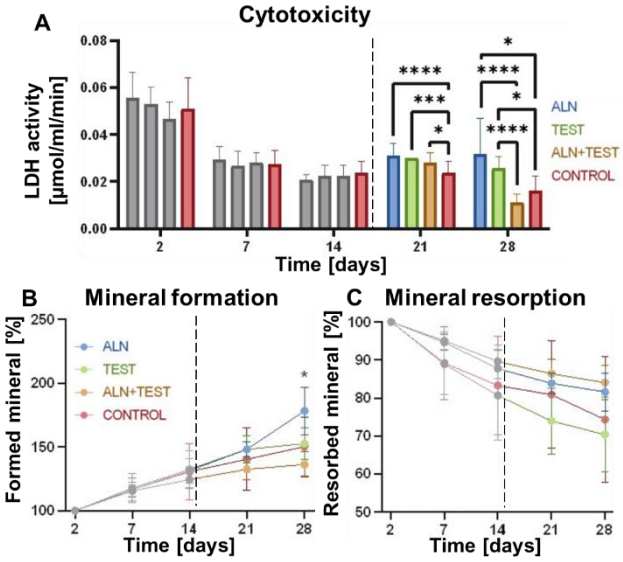

Figure 1: *Medium LDH activity indicative for cytotoxicity (A). Formed mineralized volume (B) and resorbed mineralized volume (C) obtained with µCT. The dashed lines represent the addition of the anti-osteoporotic drugs.*

Discussion and Conclusion

Cell death was mainly expected in groups treated with alendronate, since alendronate is known for inducing osteoclast apoptosis [5]. The increased mineral formation in the alendronate group might be explained by binding of alendronate to the mineralized scaffold, the little resorption in this group, or a combination thereof. Surprisingly, this was not observed in the groups treated with both testosterone and alendronate. As only minimal differences in remodeling were observed between the treated groups and control group, future experiments require optimization of treatment timing and dosing. Nevertheless, this *in vitro* bone model shows the potential to track remodeling and cell viability in response to drugs.

References

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