Chondrogenic Differentiation Capacity of Mechanically Stimulated MSCs Is Increased via Downregulation of CD73

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Introduction
Bone regeneration is influenced by mesenchymal stem cells (MSCs) and mechanical conditions. How healing outcome and mechanical stability are linked on the cellular level remains elusive. Cyclic-compressive loading of MSCs in vitro, based on in vivo data, affects the expression of molecules that are involved in angiogenesis and matrix assembly and downregulates the expression of CD73. The aim of this study was to determine the consequences of cyclic-compressive loading on the MSC differentiation capacity and the putative involvement of CD73.

Materials and Methods
Rat-MSCs were embedded in a fibrin/trabecular bone construct and mechanically stimulated (20% compression, 1Hz, 72h). Gene expression was investigated by qRT-PCR and normalized to β-actin, Eef1a1 and GAPDH. Differentiation assays were performed as described elsewhere with adenosine and adenosine 5’-(α,β-methylene)diphosphate (APCP; CD73 inhibitor). Differentiated cells were stained for alkaline phosphatase and alizarin red (osteogenic), sudan IV (adipogenic) and alcian blue (chondrogenic). Statistics: n=5, Student's t test, p < 0.05

Results
Directly after loading, among the chondrogenic (Sox9, Col2a1, Acan, Fmod), osteogenic (Runx2, Alpl, Spp1, Ibsp, Col1a1, Bglap) and adipogenic (Pparγ, Lpl, Fabp4, Fas) markers, Fmod was downregulated in loaded compared to unloaded MSCs. After differentiation in appropriate media, however, chondrogenic differentiation ability of loaded MSCs was significantly increased. Differentiation media with APCP stimulated chondrogenic differentiation of MSCs of both loaded and unloaded MSCs (Fig. 1).

Discussion and Conclusions
Chondrogenic differentiation is increased in loaded MSCs, hence following current models. Here, through CD73 antagonist treatment, MSC chondrogenic differentiation was further increased. This data points towards a role of CD73 in chondrogenic differentiation of MSCs possibly via A2AR signaling, which is mutually regulated with CD73 in MSCs. Moreover, A2AR signaling activates the cAMP-PKA and PKC but also the MAPKs pathways and thereby possibly regulates MSC differentiation. In conclusion, we hypothesize that CD73 is a novel regulatory factor in chondrogenic differentiation of MSCs.

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Disclosures
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