EFFECT OF UNI-AXIAL STRETCH OF OSTEOBLASTS ON COLLAGEN MATRIX

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Introduction. Osteoblasts cultured in biomaterial scaffolds in tissue engineered constructs are subjected to micromovement. The function of osteoblasts and osteoclasts are coupled to each other. One of the preconditions for the initiation of the ARF cycle is that the bone surface to which the osteoclasts are attached has to be cleaned from collagen. During reversal, the bottom of the resorption pit has to be cleaned of collagen debris before osteoblasts start de novo formation of bone. It was hypothesized that physical strain in form of stretching might provide the stimulus for the activation of the collagenolytic MMP cascades.

Methods. This hypothesis was tested using uniaxial stretching in a computer-controlled stretching apparatus of osteoblasts cultured on type I collagen matrix on silicon rubber support at a density of $1 \times 10^4$ cells/cm$^2$. Effect of stretching was assessed by a quantitative RT-PCR, which was used to measure some key matrix metalloproteinases (MMPs), including the classical interstitial collagenase MMP-1, its endogenous activator MMP-3 and its tissue inhibitors TIMPs.

Results. Impact of the stretch on osteoblasts was confirmed by demonstrating alignment of the osteoclast perpendicular to the axis of the stretch as a function of time. This effect was seen with 105% stretch, but not with 101% stretch (Figure 1).

Figure 1. Left panel: cells on collagen under stretch. Right panel: Cell angle with respect to the stretch axis in 101% and 105% stretched cells. Although 101% stretched cells tended to align, there was no significant difference from control, which was in contrast to the 105% stretched cells. *p <0.05 versus control, ** p <0.01 versus control, #p <0.05 versus 101% stretched cells.

Impact of the stretch was also confirmed by assessing COX-2 mRNA levels, known to be regulated by uniaxial stress (Figure 2, upper left). One Hz uni-axial 105% stretch in 3D-matrix induced coordinated up-regulation of MMP-1 (lower left) and its activator MMP-3 (upper right) ($p <0.01$), whereas MMP-2, MMP-9 and collagenase inhibitors (only TIMP-1 is shown, lower right) were not affected and MMP-13 was not expressed at all. This response was down-regulated as the osteoblasts aligned in a more favourable orientation in the stretch field.

Conclusion. It is concluded that stretch-induced collagenase can serve as a factor coupling physical stress to bone metabolism. Therefore, the biomaterial and physical environment has to be taken into consideration upon implant and scaffold design and application.