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Connexin43 is downregulated in sclerotic human subchondral osteoblasts:

A possible explanation in decrease of mechanosensitivity in these cells

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PURPOSE. In osteoarthritis, subchondral bone remodeling is increased leading to bone sclerosis. In a previous work, we have reported that osteoblasts coming from the sclerotic subchondral bone express a particular phenotype characterized by an increased production of IGF-1, IL-6, IL-8, PGE2, RANKL, MMP-3 and a decreased of OPG. We have also reported that all these genes are mechanosensitive and that osteoblast coming from the sclerotic zone were less sensitive to compression than osteoblasts of the non-sclerotic zone. In this study, we have compared the effects of compression on the expression of membrane receptors by osteoblasts coming from sclerotic and not sclerotic area in response to compression. ■

METHODS. Osteoblasts were isolated from sclerotic (SC) or non-sclerotic (NSC) zones of OA subchondral bone of five men. After 28 days, osteoblasts in culture were surrounded by a newly synthesized matrix and formed a strong membrane. This osteoblasts-containing membrane was then placed onto a Biopress Flexercell plate and submitted to compression (10%) for 4 hours at the frequency of 1 Hz. The expression of integrin α5, αV, β1, β3, CD44 and connexin43 (Cx43) was evaluated by real time RT-PCR. Cx43 was also visualized by IHC in the osteoblasts. ■

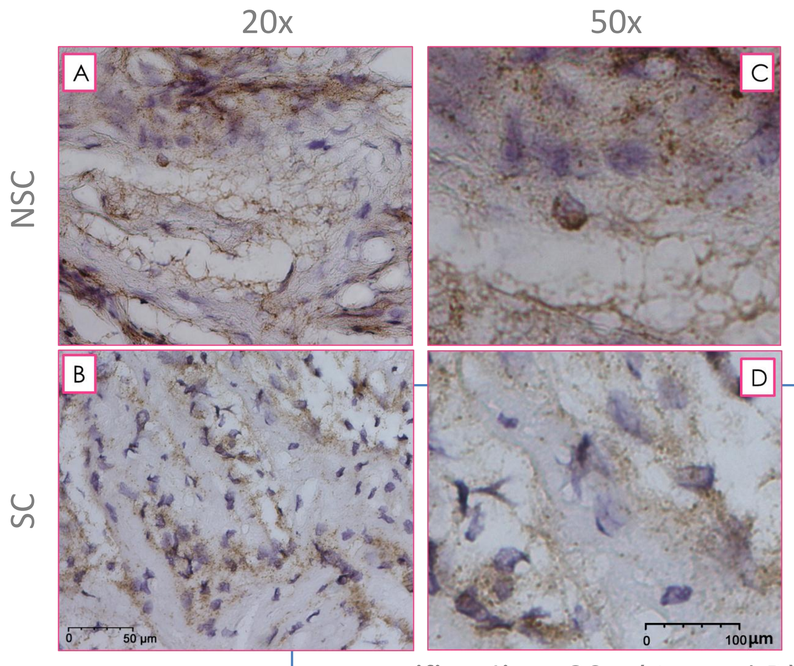


Fig. 1
Connexin43 IHC localization using Cx43 specific antibodies on Ob membrane, magnification 20x (A and B) or 50x (C and D).
A and C: Unloaded NSC Ob,
B and D: Unloaded SC Ob.

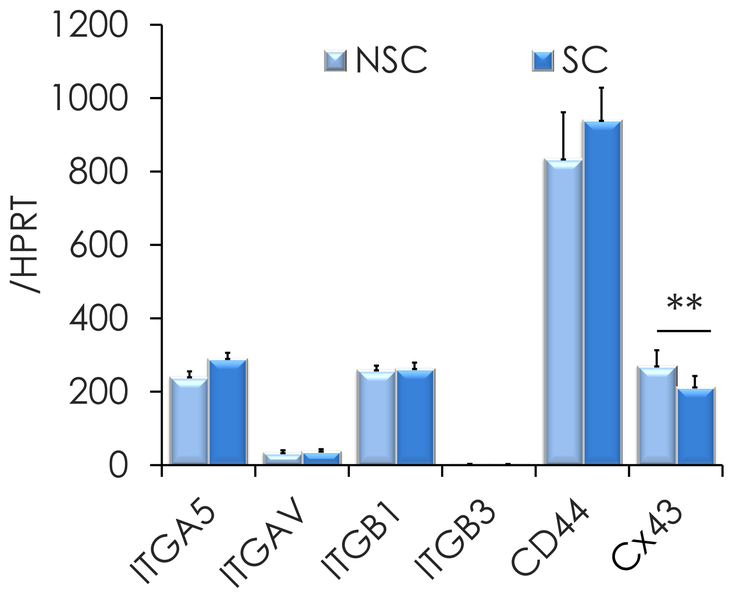
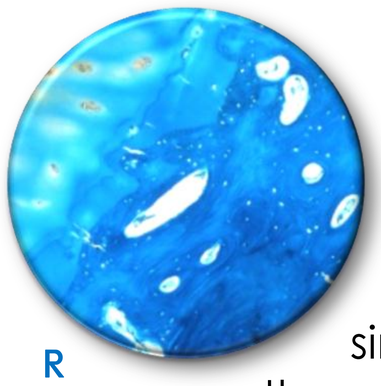


Fig. 2
Basal expression of mechanoreceptors in human subchondral osteoblasts.
Normalized by HPRT gene expression.



RESULTS. IHC analysis showed less Cx43 proteins in SC than NSC membranes (Fig. 1).
In the absence of mechanical stimuli, integrin α5, αV, β1, β3, and CD44 mRNA levels were similar in NSC and SC osteoblasts. Integrin α5 and β1 expression was 10 fold more elevated than αV and β3 and Cx43 mRNA levels were significantly lower (-30%) in SC osteoblasts than in NSC osteoblasts ($p<0.01$, Fig. 2). Further, Compression (4h, 10%, 1Hz) stimulated integrin α5 (1.42-fold), αV (1.37-fold) and β3 (2.27-fold) expression in NSC osteoblasts ($p<0.01$) but didn't significantly alter integrin β1 or Cx43 expression. In SC osteoblasts, only integrin β3 gene expression was increased by compression ($p<0.001$), while integrin α5, αV or β1 genes were not modified and Cx43 expression decreased (0.70-fold, $p<0.01$). CD44 expression was increased by compression similarly both in NSC and SC osteoblasts (1.4-fold, $p<0.01$). ■

CONCLUSION

Cx43 plays a major role in osteoblast mechanotransduction. It is less expressed by SC than NSC osteoblasts. Further, Cx43 is decreased by compression in SC osteoblasts. This downregulation of Cx43 could be responsible for the decrease in mechanosensitivity of SC osteoblasts. This finding offers a new perspective of research to explain the role play by mechanical stimuli in OA pathogenesis.