INTRODUCTION:
VEGF (vascular endothelial growth factor) is not only one of the most important angiogenesis factors, but is involved also in inflammatory and degenerative processes. Recent studies have shown that VEGF as well as its receptor VEGFR-2 are expressed on osteoarthritic chondrocytes, but not on normal adult chondrocytes (1). VEGF expression is upregulated by hypoxia-inducible factor-1 (HIF-1) and HIF-1 activity depends on the amount of Hif-1α subunit, which is tightly regulated by oxygen tension. Moreover, after mechanical overload chondrocytes were strongly immunopositive for Hif-1α, suggesting an activation of Hif-1α (2). In order to investigate whether there is an interdependency between Hif-1α activation and the increased expression of VEGF and if there is a causation with the emergence of osteoarthrosis we challenged chondrocytes with mechanical stress.

METHODS:
Luciferase assays. 1.5 μg of the Hypoxia Responsive Element (HRE) reporter plasmid containing the firefly luciferase reporter gene, and 0.5 μg of the pRL-TK plasmid, containing the Renilla luciferase gene as an internal control, were cotransfected into C-28/I2 chondrocytes in a 10 cm plate by the lipotransfection method (Lipofectamine2000, Invitrogen). 24 h after transfection the cells were seeded to a silicon 6-well plate. The activities of both firefly and Renilla luciferases were determined 48 h after transfection with the dual luciferase reporter assay system (Promega, Madison, Wis.). The luciferase activities were normalized to the Renilla luciferase activity of the internal control.

Mechanical Stress. For mechanical stress application we used a FlexCell and different protocols for strain (for example: cells were subjected to 10% cyclic biaxial strain at a frequency of 0.3 Hz.)

Small Interference RNA: The mammalian expression vector pGE1 (Stratagene) was used for the expression of siRNA. The gene-specific insert which is specified by a 29-nucleotide sequence 5’-GTCTTCAGCATGTTACGTGATGAGGATGG-3’ of the human Hif-1α, is separated by a 8-nucleotide non-complementary spacer (GAAGCTTG) from the reverse complement of the same 29-nucleotide sequence. This construct was inserted into the pGE1 using BamHI and XbaI restriction sides, and referred to as pGE1-siHif2. A control vector (pGE1-negative) serves as a non-silencing control (Stratagene).

RT-PCR RT–PCR Total RNA was extracted from equal numbers of Chondrocytes plated on 6-well dishes using an RNeasy Mini kit (QIAGEN). Semi-quantitative RT-PCR to analyze Hif-1α gene transcription. Taking together, these findings indicate that Hif-1α is induced in chondrocytes by mechanical overload and may contribute to the pathology of osteoarthrosis. Utilising the Hif-1α siRNA technique, we are able to investigate the role of Hif-1α in the in this context.

RESULTS:
Luciferase Assay demonstrated, that the expression of Luciferase driven by HRE was enhanced in chondrocytes after mechanical stress application compared with control cells. The activation of Luciferase expression is approximately equall to chondrocytes cultured under 5% O₂ (Figure 1).

DISCUSSION:
VEGF and VEGF receptor 2 are expressed in severe human osteoarthritits (Mankin score 7-14, Pufe et al. 2001). Mechanical overload seems to lead in cartilage explants via hypoxia inducible factor (HIF) to an expression of VEGF (Pufe et al. 2004). We show here, that Hif-1α activation is induced under non-hypoxic conditions by mechanical stress in immortalised chondrocytes. Since mechanical overload is one of the causative factors for osteoarthritis. Furthermore, we constructed siRNA against the mRNA of Hif-1α to downregulate the transcription factor and block the Hif-1α gene transcription. To test the constructed siRNA against the mRNA of Hif-1α in order to downregulate the transcription factor we perform RT-PCR with mRNA of chondrocytes expressing the control-or the Hif-1α-siRNA. The Figure 2 shows reduced Hif-1α-mRNA in cells bearing the Hif-1α-siRNA, indicating the functionality of the siRNA.

To further confirm the functionality of the siRNA we performed a Luciferase Assay with chondrocytes expressing either the control-siRNA or siRNA against Hif-1α. As shown in Figure 3, chondrocytes expressing the Hif-1α-siRNA are no longer in a position to induce expression of Luciferase driven by HRE under hypoxic condition.

REFERENCES:
(1) Pufe T et al. The splice variants VEGF121 and VEGF189 of the angiogenic peptide vascular endothelial growth factor are expressed in osteoarthritic cartilage. Arthritis Rheum. 2001, 44:1082-8

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