INTRODUCTION:
In western countries, low back pain is one of the major health problems, causing the individual patient major pain and discomfort. Although low back pain is often correlated with disc degeneration, degeneration does not necessarily induce pain sensation. The reasons why some degenerated discs are symptomatic and others are not are unknown to date. It is known that pain can be linked to nerve irritation, e.g. due to irritation by nucleus pulposus tissue during disc prolapse. Nerve roots and nerve endings may also be irritated by proinflammatory mediators, which can be produced by disc cells themselves and can diffuse out of the disc through clefs and tears in the degenerated tissue. It is still unclear why, under certain conditions, disc cells produce increased amounts of proinflammatory mediators, but it is assumed that specific degradation products can induce a proinflammatory cascade. Fibronectin is an extracellular matrix glycoprotein of many tissues that is frequently present in its fragmented form in degenerated discs. In chondrocytes, fibronectin fragments stimulated expression of proinflammatory cytokines and matrix degrading enzymes. Based on these findings in chondrocytes, we hypothesized that fibronectin fragments can induce the production of proinflammatory cytokines in intervertebral disc cells, thus maybe being the missing link between disc degeneration and discogenic back pain.

METHODS:
Human intervertebral disc cells were isolated from biopsies of patients undergoing spinal surgery due to disc degeneration, herniation or sequestration (n=7). The study was approved by the ethics committee and informed consent was obtained from all patients. The tissue was enzymatically digested over night using collagenase NB4 and dispase II and expanded in monolayer. In passage 2-4, cells were stimulated with fibronectin fragments (500 nmol/l) or interleukin-1β (IL-1β): 5 ng/ml or 10 ng/ml. IL-1β was used as a positive control as it is known to induce a proinflammatory cascade in disc cells.

RESULTS:
RNA (n=5) and protein (n=2) were isolated from stimulated cells as well as from untreated control cells after 2, 6 or 18 hours. Expression of TNF-α, IL-1β, IL-6, IL-8 and IL-10 was analyzed on the mRNA and protein level using real-time RT-PCR and immunoblotting technique. Changes in gene expression were quantified using the comparative CT-method (data shown as Mean ± SEM) and changes in protein expression were analyzed semi-quantitatively. Quantitative data was statistically evaluated using a one sample T-test with a significance level of p < 0.05.

DISCUSSION:
Increased levels of certain cytokines, especially IL-6 and IL-8, are known to be present in symptomatic degenerated intervertebral discs. These proinflammatory cytokines are assumed to diffuse through clefs and tears and can therefore be able to irritate nerve endings in the outer annulus fibrosus or nerve roots in the epidural space, causing pain sensation.

The fact that treatment with fibronectin fragments and IL-1β in this in vitro cell culture model of intervertebral disc cells resulted in increased expression of IL-1β, IL-6 and IL-8 indicates that a proinflammatory cascade typical for painful intervertebral discs could be initiated. Thus, the accumulation of fragmented fibronectin may constitute a link between disc degeneration and development of discogenic back pain. Whether other matrix degradation products (e.g. fragmented collagen) also do have the potential to induce a proinflammatory cascade, therefore enhancing the negative effects of fibronectin fragments, will have to be analyzed in further studies.

This study was supported by AOSPINE (SRN 02/103, AOSBRC-07-03).