ABSTRACT INTRODUCTION:
In osteoarthritis (OA), cartilage damage is one of the main pathological features. There is, however, a significant involvement of the synovium in a large proportion of OA-patients. The mechanism through which the synovium contributes to OA pathology is not yet known. In cartilage, it is proposed that developmental processes important in embryonic development are reactivated. Associations have been identified of the occurrence of OA with polymorphisms of genes from the wnt/β-catenin pathway, a pathway that is involved in normal cartilage development. In the wnt/β-catenin pathway soluble wnt-proteins are secreted by cells and bind to Frizzled (Fzd) receptors, which sets into motion a signaling cascade leading to intra-cellular β-catenin accumulation and the transcription of a plethora of genes, like several proteases. The levels of β-catenin in the chondrocytes are crucial for the stability of chondrocyte phenotype.

The aim of the present study is to investigate the contribution of the synovium to OA pathology via wnt signaling.

METHODS:
To demonstrate the presence of canonical wnt signaling in murine models for OA, murine knee joints isolated at several time point after induction of collagenase induced OA (CIOA), surgically induced OA and STR/ort mice were stained for the presence of β-catenin. CIOA was generated by intra-articular injection of collagenase, which induces joint instability. Surgical OA was induced by destabilization of the medial meniscus, by transecting the medial meniscotibial ligament. A longitudinal expression analysis was performed in 2 of the models, one with clear synovial involvement, CIOA, and the spontaneous OA model in STR/ort mice, which shows less synovial involvement. Synovial expression of components of the wnt signaling pathway was determined at several time points. From these results, targets were selected for the generation of adenoviral vectors to overexpress specific genes. To study in vivo effects, adenoviral vectors were injected intra-articularly in murine knee joints and joints were isolated and processed for histological examination at day 1, 3, and 7 after injection. To study the effect of these genes on chondrocytes, human chondrocytes were isolated from cartilage that was obtained from joint replacement surgery. One day after isolation, these cells were transfected with the adenoviral vectors, and incubated for 7 and 14 days. Hereafter Q-PCR was performed at the mRNA that was obtained from these cultures, to detect expression of aggrecan, collagens type I, II and X and several MMPs. Cartilage and synovial specimen were obtained at the time of joint replacement surgery after a signed informed consent was obtained. All animal experiments were approved by the institutional review board conforming to the local laws and regulations.

RESULTS SECTION:
In all experimental murine models for OA, β-catenin stained stronger in naive mice, especially in the superficial cartilage layer (Figure 1). In the deeper, calcified layer cartilage stained positive in naïve mice as well. Also in synovium, β-catenin staining increased when OA developed (not shown). This indicates that during experimental OA, canonical wnt signaling occurs in the cartilage and synovium.

Strong upregulation of the canonical wnts wnt16 (up to 256-fold) and wnt2b (up to 90-fold) was found in both models, although regulation was stronger in CIOA. Expression in the synovium was clearly higher compared to the cartilage. In the cartilage, no clear upregulation of canonical wnt-proteins was found. Clear intracellular accumulation of β-catenin was found in both synovium and cartilage, which indicates the activation of wnt/β-catenin in both tissues. This suggests that wnt-proteins that are expressed in the synovium, diffuse to the cartilage and induce wnt-signaling in chondrocytes. Wnt-1 induced signaling protein (WISP-1), a protein downstream canonical wnt signaling, was highly expressed in the synovium as well, again indicating activation of this pathway in the synovium. To determine whether canonical wnt expression in the synovium has the potency to cause cartilage damage, canonical wnt8a was overexpressed specifically in the synovium by intra-articular injection of an adenoviral vector. At day 1 and 3, no significant differences were observed in the cartilage from wnt8a overexpressing knee joints compared to joints transfected with control virus. Remarkably, at day 7, a strong induction of cartilage pathology was observed at the medial margin of the medial tibial plateau (Figure 2), a preferential site for the start of cartilage damage in our models. This shows that expression of canonical wnt in the synovium causes cartilage degeneration. In addition, overexpression of WISP-1, a mediator that is induced by canonical wnts induces MMP- and aggrecanase mediated cartilage damage, already 4 days after transfection.

Due to their size, wnt proteins and WISP-1 can reach the chondrocytes in the cartilage matrix and may alter the chondrocyte phenotype. This was underlined by increased β-catenin staining in cartilage of wnt8a overexpressing mice. Adenoviral overexpression of wnt8, wnt16 and WISP1 in human primary chondrocytes led to a significant increase with 14 days of Collagen type I, and a significant decrease of Collagen type II, suggesting loss of the articular chondrocyte phenotype.

DISCUSSION:
Canonical wnt expression and subsequent WISP-1 production is increased in the synovium during experimental OA. This canonical expression may lead to the degradation of cartilage as soon as 7 days after transfection, possibly by inducing changes in the articular chondrocyte phenotype. This identifies synovial wnt expression as a potential target for OA therapy. Future studies should focus on the inhibition of the canonical wnt8a and WISP-1, to underline its efficacy as a target for OA therapy.