served with IL-1β+Ro treatment. Furthermore, western blot studies indicated that IL-1β+Ro did not induce the time-dependent activation of caspase-7 and -3 observed with TNF-α+Ro. The antiapoptotic protein bcl-2 expression has not been altered in a different manner by TNF-α+Ro or IL-1β+Ro. In order to elucidate the role of the caspases on the effect of TNF-α on synoviocytes cell death induced by Ro, caspase inhibitors (3, -3/7, -8 and general) effects were analyzed by cell death and activation of the executioner caspase-3 studies. Results show that preincubation for two hours with all inhibitors significantly decreased the percentage of apoptotic cells by treatment with TNF-α+Ro at 24 hours (1.88±0.29%, 3.76±2.04%, 2.28±0.77%, and 2.8±1.59% with caspase-3, caspase-3/7, caspase-8 and caspase general inhibitor, respectively, n=2, p<0.0001). When caspase-3 activation was evaluated by western blot, it was found that preincubation of caspase-8 inhibitor was effective to prevent caspase-3 induced by TNF-α+Ro. By contrast, caspase-8 inhibitor not prevented bcl-2 decrease induced by TNF-α+Ro or IL-1β+Ro.

Conclusions: These results confirm that the cytokines TNF-α and IL-1β differently regulate machinery apoptotic activation in synoviocytes. In addition, this difference is dependent on caspase-8 expression levels. These data could be important for a better understanding of the participation of TNF-α and IL-1β in the OA pathogenesis.

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**SCA-1 POSITIVE CELLS FROM THE SYNOVIIUM OF AN ADULT JAPANESE WHITE RABBIT CAN DIFFERENTIATE INTO NEURON-LIKE CELLS**


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**Purpose:** Bari et al. showed that fibroblast-like cells from synovial tissue could differentiate into osteoblasts, chondrocytes, adipocytes, and muscle cells. However, there are no reports showing the differentiation ability of stem cells from the synovium. In this study, we detected Sca-1 positive cells from synovium of adult Japanese white rabbit, and tried to differentiate into neuron-like cells.

**Methods:** 1. Presence of Sca-1 positive cells;
   To demonstrate the presence of Sca-1 positive cells, we performed the immunohistological analysis of the synovium using Sca-1 antibody.
   2. Differentiation into neuron-like cells with Sca-1 positive cells and culture of Sca-1 positive cells;
   We isolated and cultured the synovium cells and performed FACS analysis with Sca-1 antibody. To evaluate the differentiation ability of Sca-1 positive cells into neuron-like cells, we cultured the Sca-1 positive cells using the nerve progenitor cell maintenance medium, and performed morphological and biochemical analysis.

**Results:** 1. Presence of Sca-1 positive cells;
   In the immunohistological staining, Sca-1 positive cells were observed in the synovium of adult Japanese white rabbit.
   2. Differentiation into neuron-like cells with Sca-1 positive cells and culture of Sca-1 positive cells;
   The result of the FACS analysis showed that the average 3.99±1.20% of total cultured cells formed synovium was Sca-1 positive cells.

Morphological analysis showed neurosphere formation in the culture. Biochemical analysis also showed that nestin-positive cells were observed in the neurosphere, suggesting that Sca-1 positive cells could differentiate into nestin-positive cells. Moreover, we cultured nestin-positive cells using the medium with b-FGF and serum, and performed the morphological and biochemical analysis. In this culture, a successful differentiation into neurofilament-M-positive cells were apparently achievable, suggesting that Sca-1 positive cells from synovium differentiated into neuron-like cells via nestin-positive cells.

**Conclusions:** Sca-1 positive cells from synovium of adult rabbit could differentiate into neuron-like cells.

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**BIOFUNCTIONAL POLY(PROPYLENE SULFIDE) NANOPARTICLES FOR TARGETED DRUG DELIVERY: IDENTIFICATION OF A NOVEL TARGETING PEPTIDE FOR ARTICULAR CARTILAGE BY PHAGE DISPLAY**

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**Purpose:** Increasing knowledge of molecular mechanisms in the pathogenesis of osteoarthritis leads to the identification of possible drug targets and drug development. As systemic drug administration is associated with a low bioavailability in the cartilage matrix and direct injection results in a fast clearance out of the joint, nanoparticle-based localized intra-articular drug delivery with an active targeting mechanism may enhance the compound’s bioavailability in articular cartilage and thus the treatment efficacy while reducing adverse systemic effects. This may particularly be favorable in clinical entities with early cartilage degradation, such as femoro-acetabular impingement in the hip.

**Methods:** In order to surface-functionalize poly(propylene sulfide) (PPS) nanoparticles, a phage display library has been screened against bovine cartilage grafts to find a novel targeting peptide for the cartilage matrix. Five cycles of biopanning have been carried out with increasing stringency of binding conditions in each cycle. The sequence of the displayed peptide was obtained by DNA sequencing after cycle 5 of retained and amplified phage clones. Binding assays have been carried out in vitro against bovine cartilage in physiological conditions (37°C, with and without synovial fluid). The selected peptide and its mismatch were synthesized using standard Fmoc-chemistry with a cysteine at the C-terminus. The targeting peptide was then conjugated via the thiol group of the cysteine by Michael-type addition to Pluronic F-127 which was previously functionalized with vinylsulfone. PPS nanoparticles were prepared in an inverse emulsion polymerization with conjugated (10%) and non-conjugated (90%) Pluronic F-127 as the emulsifier, such that an average size of 38nm was obtained. After polymerization of the PPS core, the Pluronic with the conjugated targeting peptide remains displayed on the particle surface, thereby forming a surface-functionalized nanoparticle.

**Results:** DNA sequencing revealed three putative peptide sequences after 5 cycles. All of these sequences have been shown to be specific to cartilage versus synovial membrane by two orders of magnitude. A competitive binding assay between the three phage clones and the original library retained only two phage clones. The free peptide of the clone with the highest titer per ml was subjected to a competitive binding assay against the corresponding phage clone, which resulted in an IC50 of the free peptide of 200nM, suggesting a fairly high affinity to its target. Similarly, the surface-functionalized nanoparticle was subjected to a competitive binding assay against the corresponding phage clone and compared to the free peptide and its mismatch. The conjugated nanoparticles at a concentration of 2.5% w/v exhibited similar binding as the free peptide at a concentration of 10M (10.4±6% vs. 13.9±2.8% of control), whereas the mismatch peptide did not bind competitively and thus did not reduce the phage titer (92±12%).