viability using a fluorescent in situ double-staining technique. In order to identify the number of MC, male-derived MC with PLA were transplanted into osteochondral defects of female femurs. The male-derived sex-determining region Y (SRY) gene was used as a marker of MC detecting by polymerase chain reaction (PCR), and matrix metalloproteinase-1 (MMP-1), which exists in male and female autosomal gene, was used as a control gene. The total cell and MC number were calculated using NIH imaging software as a quantitative method.

**Results:** MC survived in the PLA (Fig. 1), and produced a matrix of collagen fibers (Fig. 2) and glycosaminoglycans (Fig. 3) at 1 week after culturing in vitro. Following that, at 1 week after the transplantation of the construct, 79% of total cells in the defect were MC. However, the ratio of MC decreased with time, and finally, transplanted cells were not detected in the defects at 24 weeks (Fig. 4).

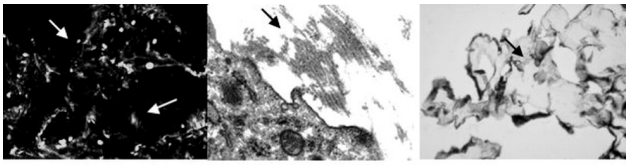

Fig. 1 Fig. 2 Fig. 3

Fig. 1. Arrows indicate cellular viability of MC in PLA assessed by confocal laser scanning microscopy.
Fig. 2 Presence of glycosaminoglycan stained with safranin O.
Fig. 3. Presence of collagen fiber detected by electron microscope.

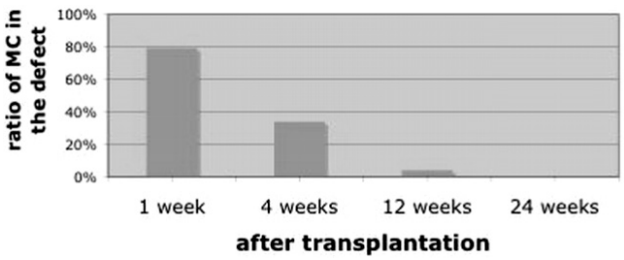


Fig. 4. Variable of MC ratio in the defect with time in vitro.

**Conclusions:** MC attached and produced cartilage matrix in the PLA in vitro. In vivo condition, MC could survive in osteochondral defect, and then were replaced by host cells within 24weeks. This study to evaluate the survivability of transplanted MC in vivo is essential for further tissue engineering strategy to enhance cartilage regeneration in osteochondral defect.

## A24

### MURINE SURGICALLY-INDUCED OSTEOARTHRITIS IS SEX AND STRAIN DEPENDENT

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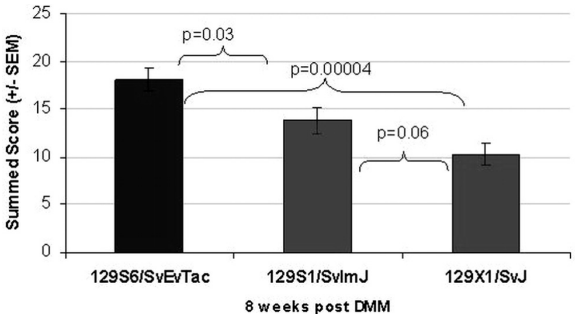
**Purpose:** Many inbred strains of mice exist and are used for specific disease models. Evaluation of wild-type mice of different genetic background has found huge variability in susceptibility to OA following surgical destabilization of the medial meniscus (DMM). 129SvEv, C57BL6 and C57BL10 mice have more OA than FVB/n, which has more OA than DBA/1J. Sex differences are also observed in this model, with females having less OA than males. To extend upon these observations, 3 different 129 strains (commonly in use for knock out (KO) creation), as well as castrated mice (with and without testosterone replacement), were evaluated in the DMM model.

**Methods:** All animal studies were performed in accord with Wyeth IACUC protocols. Three 129Sv strains (129S6/SvEvTac, 129S1/SvImJ and 129X1/SvJ) underwent DMM surgery at 10 weeks of age with 20 mice/group. Intact and castrated 129S6/SvEvTac mice were purchased from Taconic and underwent the DMM surgery as well as addition of testosterone or placebo slow-release pellets, implanted subcutaneously. Following sacrifice at 8 weeks post-operatively, the knees were decalcified, stained with Safranin-O/Fast green and scored blindly by 2 observers using a semi-quantitative system in which a higher score reflects greater OA severity.

**Results:** 129X1/SvJ had far less OA than 129S6/SvEvTac. The scores of 129S1/SvImJ were intermediate. Castration of male mice resulted in significantly less OA (compared to intact males), which was reversible with the addition of testosterone.




**Conclusions:** Susceptibility to OA varied dramatically across different murine strains, including different 129 strains. Distinguishing exactly which background a KO mouse is created and back-crossed onto is critical for accurate interpretation of any OA KO studies. In addition, intact male mice have more OA than female mice or castrated male mice, and addition of testosterone to castrated males increases OA severity. Further studies are needed to assess if testosterone enhances activity levels, or has adverse effects on musculoskeletal and/or cartilage properties.

## A25

### COLLAGEN TYPE II DEGRADATION AND FORMATION ASSESSED IN EX VIVO AND IN VIVO IN MODELS OF RA

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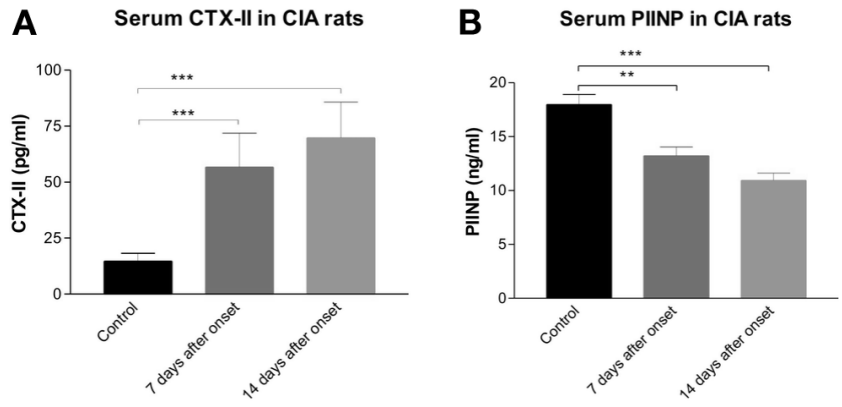
**Purpose:** Cartilage erosion in rheumatoid arthritis (RA) is a result of over-expression of pro-inflammatory cytokines that leads to protease expression and cartilage destruction. We investigated whether collagen type II degradation and formation in serum and locally in the joint would reflect disease status in an animal model of RA. Furthermore, we used an *ex vivo* model of articular cartilage degradation to assess the direct effect of pro-inflammatory cytokines on collagen type II turnover.

**Methods:** *In vivo:* Arthritis was induced in 10 female Lewis rats (150-175 g) by intradermal injection of porcine type II collagen at the base of the tail at day 0 and 7. Hind paw score (0-4, 4=most severe) and hind paw volume was measured from day 8 after immunization. Serum samples was collected at baseline and day 7, onset+1 day, onset+7 days and onset+14 days, where after the animals were terminated. One hind paw was snap-frozen in nitrogen, homogenized and extracted for proteins, while the other hind paw was fixed in formaldehyde and decalcified in EDTA for histology and immunohistochemistry.*Ex vivo*: Bovine articular cartilage explants were cultured in presence of oncostatin M (OSM) 10 ng/mL, tumor necrosis factor-$\alpha$ (TNF) 20 ng/mL for

**A25**




21 days with refreshment of medium every other day. Levels of collagen degradation was measured by ELISA recognizing the C-terminal telopeptide fragments of type II collagen (CTX-II), cartilage formation was measured by N-terminal pro-peptide of type II collagen (PIINP), and BMP-2 levels was measured by ELISA. Measurements were performed in serum, protein extracts from paws and in the conditioned medium of articular cartilage explants.

**Results:** *In vivo* collagen induced RA, was accompanied by an 500%, $P<0.001$ increase in type II collagen degradation (CTX-II) and a 40% decrease in collagen type II formation (PII), at disease onset +14 days, Figure 1 A&B. In the paw extracts, collagen degradation (CTX-II) was increased by 4000% compared to control, $P<0.001$. Collagen formation (PII) was increased by 100% compared to control, $P<0.001$.

In the *ex vivo* cartilage explants, OSM + TNF at all time-points before day 16 resulted in more than 75% reduction in collagen type II formation as measured by PIINP, $P<0.01$. At later time points (day 16-21), collagen type II formation was significantly increased by 200%, $P<0.01$. Collagen type II degradation (CTX-II), was induced by more than 5000%, $P<0.001$, after day 14 of culture. BMP-2 levels were increased by 50%, $P<0.01$, after day 16 compared to that of earlier days.

**Conclusions:** *In vivo,* in serum, induction of RA results in highly increased levels of collagen type II degradation and decreased levels of collagen type II formation. Locally in the infected joints, cartilage degradation was increased by 4000%, and formation by 200%, demonstrating pathological cartilage metabolism.*Ex vivo*, pro-inflammatory cytokines resulted in highly increased collagen type II degradation and decreased collagen type II formation, which at later time points resulted in significant more BMP-2 and collagen type II synthesis. These results strongly support the *in vivo* findings.

We clearly show that inflammatory cytokines initially result in decreased cartilage formation and increased degradation. We suggest that the measurements of both cartilage formation and degradation under pathologic situations may provide essential information on disease status and risk of progression.

## A26

### DEVELOPMENT OF A NEW CANINE MODEL OF OSTEOARTHRITIS: THE GROOVE MODEL

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**Purpose:** The frequently used anterior cruciate ligament transection (ACLT) model of osteoarthritis in the dog makes use of a permanent trigger (joint instability) for inducing degenerative changes. The present study evaluates a relatively new canine model of osteoarthritis, which is induced by a one-time trigger: the groove model.

**Methods:** The articular cartilage of the weight-bearing areas of the femoral condyles in one knee of 30 Beagle-dogs was damaged by making grooves without damaging the subchondral bone. Surgery was followed by 3, 10, or 20 weeks intensified loading of the affected joint. The severity of osteoarthritis was evaluated at 3 (n=10), 10 (n=10), 20 (n=5) and 40 weeks (n=5) after surgery. Cartilage integrity, chondrocyte activity, and synovial inflammation were determined by macroscopy, histochemistry and biochemistry.

**Results:** Ten weeks after surgery osteoarthritic features were found. Proteoglycan synthesis, percentage release of newly formed proteoglycans, the total amount of proteoglycans, and amount of denatured collagen were enhanced, whereas proteoglycan content was diminished (all $p<0.05$). Based on these parameters there was a slow progression over time from 20 and 40 weeks after induction, statistically significant for synthesis, content and Mankin grade. Importantly, three weeks after surgery these characteristics of osteoarthritis were not yet evident. In contrast, synovial inflammation was mild at 3 and 10 weeks and diminished slightly in time.

**Conclusions:** The present results show that characteristics observed at 10 weeks or later after induction of osteoarthritis in the groove model:
– comparable to those found the canine ACLT model.
– slowly progressive over time in the first year.
– not primarily mediated by synovial inflammation.
– induced by a one-time trigger.
– not just the expression of the surgically applied damage.

In this model the effect of treatment of cartilage damage is not counteracted by permanent joint instability or hampered by inflammation. Therefore, the groove model might be more sensitive to detect effects of therapy, aimed at cartilage protection and repair.

## A27

### CHONDROCYTE SENESCENCE IN OSTEOARTHRITIS

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**Purpose:** Articular chondrocyte senescence is responsible, at least in part, for the age-related increased incidence of osteoarthritis (OA). Recently, it was suggested that caveolin-1, a 21-24 kDa membrane protein, participates in the premature cellular senescence. Caveolin-1 is the principal structural component of caveolae, vesicular invaginations of the plasma membrane. We studied whether or not catabolic factors, oxidative stress and IL-1β, induce premature senescence of articular chondrocytes through the up-regulation of caveolin-1 expression.