inflammatory state of the knee. However, little is known whether obesity influences adipocytes in the IPFP. The aim of this exploratory study was to determine whether obesity influences the size of the adipocytes, as a measure of their inflammatory activity, in the IPFP.
**Methods**: IPFP was obtained from 18 patients undergoing knee arthroplasties with a mean body mass index (BMI) of 29.7 kg/m2 (range 21.5–48.47 kg/m2). Subcutaneous fat was obtained from 12 donors undergoing total hip or knee replacement with a mean BMI of 33.0 kg/m2 (range 24.2–48.5 kg/m2). Fat tissues were cryosectioned and stained with haematoxylin and eosin and imaged at x100 magnification. To determine the size of the adipocytes, the cross-sectional area of the imaged adipocytes was calculated using Fiji Is Just ImageJ software with the additional Adiposoft plugin. Three separate sections, with a minimum of 25 adipocytes in each section were measured per donor. The Shapiro-Wilk test was conducted to assess the distribution of the adipocyte sizes for each donor and a Kruskal-Wallis with multiple comparisons was used to compare adipocyte sizes between groups. Spearman's rho was determined to relate BMI to adipocyte size in IPFP and subcutaneous fat.
**Results**: The adipocyte sizes for each individual donor were not normally distributed. Median subcutaneous adipocyte size positively correlates with donor BMI (r=0.63, p=0.028), however this relationship was not observed in IPFP adipocytes (r= −0.06, p=0.82). For non-obese donors (BMI &lt 0.99). In obese donors (BMI &gt 30) on the other hand, subcutaneous adipocytes were significantly larger than the adipocytes in the IPFP (p=0002).
**Conclusions**: In contrast to subcutaneous adipose tissue, obesity does not affect the size of adipocytes in the IPFP. Previous reports have shown that adipocyte hypertrophy is directly linked to a higher secretion of pro-inflammatory cytokines and the recruitment of pro-inflammatory macrophages. Our results may highlight the functional differences between these two adipose tissues. In the context of obesity and OA, our data suggests that adipocytes in the IPFP are not responsible for obesity related joint environment changes. Further investigations are necessary to determine whether IPFP adipocyte secretion pattern is influenced by BMI.

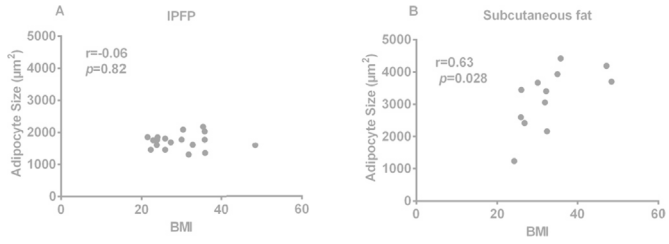


Figure 1. Spearman's test for correlation between donor BMI and adipocyte size in the infrapatellar fat pad (IPFP) (A) and subcutaneous fat (B).

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## TISSUEGENE-C (INVOSSA™) INDUCES AN ANTI-INFLAMMATORY ENVIRONMENT IN THE ARTHRITIC KNEE JOINT VIA MACROPHAGE POLARIZATION

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**Purpose**: TissueGene-C (Invossa™) is a novel gene and cell therapy for osteoarthritis (OA). The phase II human clinical studies showed that Invossa™ improved pain, sport activities and structural changes of cartilage in patients with OA. However, the exact mechanism underlying how Invossa™ works has not been revealed. Here we hypothesized that treatment of Invossa™ may induce an anti-inflammatory environment, especially M2 macrophage, which leads to the reduction of the pain and cartilage regeneration. In this study, we tested the effect of Invossa™ treatment on pain and structural modification with a rodent OA model and investigated whether Invossa™ could induce an anti-inflammatory environment in the arthritic knee joints.
**Methods**: The *in vivo* efficacy of Invossa™ or each component of the Invossa™(hChonJ: human chondrocytes and hChonJb#7: chondrocytes expressing TGF-β) and the vehicle control (CS-10) was analyzed with rat MIA model. To evaluate the anti-inflammatory effect of Invossa™, various cytokines were analyzed by a multiplex assay using the synovial fluid. The synovial macrophage differentiation profiles were investigated by immunohistochemistry with CD86 as a M1 marker and Arginase I as a M2 marker, respectively. Gene expression profiles were analyzed by quantitative RT-PCR to assess the mRNA levels related to macrophage polarization in the knee joints following either Invossa™ or vehicle treatment. Finally, Pain behavior was analyzed by the *von frey* filament test.
**Results**: The pain relief by Invossa™ treatment started at early time point (day 15) and maintained until 56 days post treatment, while hChonJb#7 cells showed improved behavior at earlier observation time point (d15), however, its pain-relieving effect was reduced at later time point. Cytokine expression profiles in synovial fluid showed that Invossa™ induced IL-10 at day 4, which was from CD68-positive monocytes in the synovial membrane. Furthermore the Invossa™ attracted more arginase I-positive cells which were reported as M2 macrophages in the synovial membrane. On the other hand, the number of CD86-positive cells which is known as M1 macrophage in the Invossa™ group was comparable to the control group. Quantitative RT-PCR results also showed that M2 macrophage related markers were highly up-regulated in the Invossa™ treated synovial membranes.
**Conclusions**: Invossa™ treatment improved pain and cartilage regeneration and induced anti-inflammatory environment. The anti-inflammatory cytokine IL-10 and M2 macrophages were highly escalated in Invossa™ treated knee joints. Therefore, Invossa™ treatment induced the anti-inflammatory environment, which may have contributed to the analgesic effect and cartilage improvement.

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## EFFECTS OF APPA ON HUMAN NEUTROPHIL FUNCTION

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**Purpose: APPA** (a fixed dose combination of apocynin, **AP** and paeonol, **PA**) has shown efficacy in several animal models of osteoarthritis, decreasing pain and meniscal cartilage damage. Its efficacy is due, at least in part, to the effects of both AP and PA on the regulation of the transcription factor, NF-κB. This plays a key role in both constitutive and inducible gene expression, particularly in immune cells and, as such, is an attractive therapeutic target. Neutrophils are the most abundant white blood cell and are an essential part of the innate immune system, migrating to inflammatory sites where they release cytotoxic enzymes and reactive oxygen species (ROS) to fight infection. Many neutrophil agonists (e.g. cytokines) can activate NF-κB, while ROS are themselves activators of NF-κB. The purpose of this study was to investigate if APPA affected key neutrophil functions.
**Methods**: Neutrophils were isolated from the blood of healthy subjects. Cells were incubated in the absence (control) and presence of various concentrations of APPA (up to 3mM in initial experiments) prior to the measurement of cell functions, such as ROS production, chemotaxis, apoptosis and surface receptor expression. In addition, protein and RNA extract were collected and expression levels of key proteins and transcripts were determined by western blotting and quantitative PCR, respectively.
**Results**: APPA did not significantly affect the rates of constitutive or cytokine delayed apoptosis. Chemotactic migration towards IL-8 and fMLP were only affected at the highest concentrations of APPA (>3 mM). Neutrophil phagocytosis, bacterial killing and expression of surface receptors that facilitate these processes (integrins and Fc receptors) were not significantly altered. However, APPA down-regulated neutrophil degranulation and ROS levels, although the latter was due to the scavenging of ROS rather than inhibition of production. APPA also decreased the formation of neutrophil extracellular traps (NETs), probably via its effects on ROS levels. Cytokine stimulated (GM-CSF, TNFα, IFNγ, & IL-1β, alone and in combination) gene expression of TNFα, IL-1, IL-8 & Nrf2 was also measured and, while TNF levels decreased with APPA, this did not reach statistical significance. TNFα-induced NF-κB signalling was inhibited with APPA (600 μM and above) as was GM-CSF signalling via ERK1/2.
**Conclusions**: APPA did not affect the overall functions of neutrophils at concentrations of up to 6 mM, but is a potent ROS scavenger, in addition to affecting neutrophil degranulation. Other phenolics with high reactive oxygen species (ROS) scavenging activities have been shown to exhibit multiple biological effects, including antiallergic, antibacterial, antidiabetic, anticancer, and anti-inflammatory activities that may be mediated via oxidative stress and resultant inflammation. While APPA does not interfere with the normal 'first line of defence' activities of neutrophils, it effects cytokine driven signalling pathways (e.g. NF-κB),

which may be an additional way that it exerts an anti-inflammatory effect other than via ROS scavenging.

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### INFLAMMATORY SULFATION ON SYNOVIAL LUBRICIN

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**Purpose**: We have previously identified that synovial lubricin, involved in the lubrication in cartilage lubrication of the joints, is sulfated. This sulfation is covalently attached to the highly glycosylated mucin domain of lubricin and has been indicated to interfere with inflammatory responses in the joint tissue. In order to exploit this furher, we are interested in idenifying the enzymes involved in the altered glycosylation. This would help us to genarate glyco versions of lubricin to explore the functional consequences of altered lubricin during inflammation.
**Methods**: Recombinant expression of O-linked glycoprotein oligosaccharides was performed in chinese hamster ovary (CHO) cells. Sulfation and elongation of the CHO cells commonly expressed core 1, Galb1-3GalNAc, oligosaccharides were performed using human core 2 and elongation core 1 GlcNAc transferases together with sulfotranferases specific for 3 linked and 6 linked sulfation. O-linked oligosaccharides were released from reccombinant mucin glycoprotein carrier an analysed using graphitized carbon chromatography coupled to mass spectrometry.
**Results**: By transfection of CHO cells with sulfotransferases and GlcNAc-transferarses we could show that we could mimic the sulfation found on lubricin in normal an inflammed states. This included the unusual sulfation of core 1 O-linked oligosaccharides and the more ubiquitously expressed 6 linked sulfation on core 2 O-linked structures.
**Conclusions**: Sulfation of O-linked oligosaccharides on lubricin is an unexplored feature of this important molecule. Using recombinant technology we can can start to understand the phenomenon of the inflammatory processes involved in the degradation of the cartilage.

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### THE ROLE OF CHONDROCYTE PRIMARY CILIA IN OSTEOARTHRITIS

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**Purpose**: Bardet-Biedl Syndrome (BBS) is a pleiotropic disorder resulting in retinal degeneration, obesity, intellectual disability, and polydactyly. Data indicate that BBS is also associated with osteoarthritis (OA). This makes BBS mouse models useful for studying the role of primary cilia as they pertain to OA. Certain proinflammatory cytokines are known to be up-regulated through the Receptor for Advanced Glycation End Product (RAGE) pathway during the onset and progression of OA. We hypothesized that dysfunctional chondrocyte primary cilia result in OA through the up-regulation of this pathway.
**Methods**: We performed immunohistochemistry to evaluate the level of Htra1 and TGF-b1, cellularity and histology in BBS mutant and wild type mice of various ages.
**Results**: We observed a significant increase in HTRA1 and decrease in TGF-B1 expression in BBS mice when compared to age matched controls. Interestingly, there was no difference in OARSI scoring between BBS mutant and wild type mice.
**Conclusions**: These data indicate that the RAGE pathway is integral to all OA cases, and that the expression of specific RAGE-related biomarkers precede cartilage degradation in the cilia disorder, BBS. These findings also provide support of the novel idea that primary cilia are an integral part of the RAGE pathway.

## Joint Tissue Anabolism & Catabolism

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### SYNOVIAL MACROPHAGES PROMOTE TGF-B SIGNALING BUT PROTECT AGAINST INFLUX OF S100A8/S100A9-PRODUCING CELLS AFTER INTRA-ARTICULAR INJECTIONS OF OXIDIZED LOW-DENSITY LIPOPROTEINS

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**Purpose**: In previous studies we found that synovial macrophages regulate joint pathology during experimental osteoarthritis (OA) and, more recently, we found that high systemic levels of LDL aggravate joint pathology during experimental OA with synovitis. LDL in inflamed synovium is oxidized and taken-up by macrophages via scavenger receptors, leading to an activated macrophage phenotype. In this study, we investigate whether direct injection of oxLDL into a normal murine knee joint induces joint pathology and elucidate the role of synovial macrophages in that process.
**Methods**: Knee joints of C57BL/6 mice were injected at five consecutive days with 6 μL (1.2 mg/mL) oxLDL, LDL, or an equal volume of vehicle (PBS). This same procedure was done in mice which were depleted of synovial macrophages by intra-articular injection of clodronate liposomes seven days prior to the (ox)LDL or vehicle injections. Joint pathology was investigated by immunohistochemistry and FACS analysis, and RNA expression and protein production by synovium were determined using RT-PCR and luminex, respectively. Active TGF-β was measured using a functional CAGA-luciferase assay. Data are depicted as mean ± standard deviation.
**Results**: LDL and oxLDL injection in naïve knee joints did not increase synovial thickening, or production of pro-inflammatory factors (IL-1β, IL-6 and S100A8/9) compared to vehicle injection. Levels of active TGF-β in synovial wash-outs was, however, significantly increased by 33% (from 84.7 ng/mL/g synovium ± 14.4 to 113.0 ng/mL/g synovium ± 33.3; $p<0.05$). Immunohistochemistry of total knee joints showed that oxLDL injection decreased formation of aggrecanase-induced neo-epitopes (NITEGE) compared with vehicle injections (3.6 times; $p<0.05$). In contrast, repeated injections of oxLDL in macrophage-depleted knee joints led to a 3.1 fold increase of synovial thickening, compared with injection of vehicle ($p<0.01$), while LDL injections did not alter synovial thickening. Protein and RNA levels of chemokines CCL2 (Mcp-1) and CCL3 (Mip-1α) were significantly upregulated in macrophage-depleted joints after oxLDL injections (6.7 fold and 4.6 fold, respectively; $p<0.01$). FACS-analyses revealed increased presence of monocytes and polymorphonuclear granulocytes (PMN) in the synovium, which was confirmed with immunohistochemical staining for NIMP.R14. Also protein levels of S100A8/A9, markers for inflammation, were significantly increased in synovial wash-outs of oxLDL injected joints, compared with LDL (fold increase 5.6; $p<0.05$) or vehicle (fold increase 8.3; $p<0.01$) injection, as was NITEGE expression (fold increase 1.92; $p<0.05$). Interestingly, no raise in active TGF-β was measured in these macrophage-depleted joints.
**Conclusions**: Synovial macrophages promote anabolic effects after oxLDL injections in knee joints, supporting earlier studies which show increased ectopic bone formation during LDL-rich conditions in experimental osteoarthritis. In absence of synovial macrophages, however, oxLDL induces monocyte and PMN influx, production of pro-inflammatory mediators and aggrecanase activity, thereby increasing catabolic activity.

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### IL37 SUPPRESSES IL-1β-INDUCED PRO-INFLAMMATORY CYTOKINE AND CATABOLIC ENZYME PRODUCTION IN PRIMARY HUMAN OA CHONDROCYTES: PROTECTION AGAINST CARTILAGE DEGRADATION

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**Purpose**: Cartilage degradation is a critical feature of osteoarthritis (OA). In chondrocytes, pro-inflammatory cytokines induce the production of extracellular matrix degrading enzymes such as MMPs and ADAMTSs, leading to cartilage damage. Therefore, inhibition of the production and signaling of pro-inflammatory cytokines is a promising approach to slow down OA pathology. Recently, IL37 has come into view as a potent anti-inflammatory cytokine, which is able to reduce the expression of pro-inflammatory cytokines in several human inflammatory disease models, such as psoriasis, inflammatory bowel disease and systemic lupus erythematosus. Until now, the anti-inflammatory potential of IL37 in OA has not been investigated.
Therefore, in this study we investigated the potential of IL37 to decrease the expression of pro-inflammatory cytokines and catabolic enzymes in primary human OA chondrocytes.
**Methods**: Human OA cartilage was obtained from eight patients undergoing total knee or hip joint arthroplasty. Cartilage pieces were analyzed for IL37 expression by immunohistochemistry and qPCR. After isolation, chondrocytes in monolayer were stimulated with IL-1β, OA