Purpose: Progressive articular cartilage degradation is central to osteoarthritis (OA), and is driven by mechanisms of cartilage matrix catabolic effects and anti-anabolic effects of chondrocytes. However, OA is a disease mediated by and affecting the entire synovial joint organ. Chronic, low-grade synovial inflammation contributes to symptoms and disease progression. We recently developed IL4-10 synkerine, a fusion protein composed of IL-4 and IL-10. It is demonstrated that these cytokines inhibit production of pro-inflammatory cytokines such as IL-1β, IL-6 and TNFα (Fiorentino, 1991; Hart, 1995; Lee, 2002), all important in the degeneration of cartilage and inflammation of synovium in osteoarthritis (Jiu-Bryan and Terkeltaub, 2014). In human and animal in vivo and in vitro experimental models the individual cytokines and IL-4 and IL-10 combined are shown to be effective. However, clinically effects are modest, most likely due to limited bio-availability and insufficient immunosuppression. IL4-10 synkerine might overcome these limitations and may successfully achieve structural repair in diseases like OA because of synergistic effects of the cytokines. The purpose of this study is to evate the cartilage protective and anti-inflammatory effects of IL4-10 synkerine ex vivo.

Methods: Cartilage from the condyles of seven end-stage knee OA patients selected for total knee replacement, was cultured for 4 days in presence or absence of IL-4 (10ng/ml), IL-10 (10ng/ml), a combination of IL-4 (10ng/ml) and IL-10 (10ng/ml), or IL4-10 synkerine (20ng/ml). Changes in proteoglycan (PG) synthesis and PG release were studied. Synovial tissue from six OA donors selected for total knee replacement, was cultured for 4 days in presence or absence of IL-4 (10ng/ml), IL-10 (10ng/ml), a combination of IL-4 (10ng/ml) and IL-10 (10ng/ml), or IL4-10 synkerine (20ng/ml). IL-4 alone, increased proteoglycan (PG) synthesis with 47.6% (p<0.018), 26.6% for IL-4 alone, 31.2% for IL-10 alone, or 6.5% for IL-4 and IL-10 combined. Only a small reduction of -8.7% (p=0.018) in PG release was found for the IL-4-10 synkerine (-9.5% for IL-4 alone, -8.6% for IL-10 alone, or -6.7% for IL-4 and IL-10 combined). The overall improved proteoglycan turnover was in cocurrence with a significant inhibition of IL-6, IL-8 and TNF-α cytokine production by the cartilage in presence of the IL4-10 synkerine (IL-6 -81.9%, IL-8 -65.9% and -2.0% TNF-α, resp.). Moreover, IL4-10 synkerine also reduced the production of IL-6, IL-8 and TNFα in osteoarthritic synovium (-83.5%, -85.6% and -25.9% resp., all p<0.01).

Conclusions: These data show that IL4-10 synkerine might induce both structural repair and reduce inflammation in OA. Ex vivo, IL4-10 synkerine has a direct effect on OA cartilage by affecting its proteoglycan turnover and cytokine production. Inhibiting the synovial cytokine production, adds indirectly to this structural repair of the cartilage. Together with the pain inhibiting effects found in animal models of OA, these data suggest disease modifying characteristics of the IL4-10 synkerine.

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KARTOGENIN LOADED PHOTO-CROSS-LINKABLE SCAFFOLD FOR CARTILAGE REGENERATION
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Purpose: Hyaline cartilage injury, a common disease is seriously affected the life quality of the patient and difficult to be cured. Recently, various kinds of methods have been conducted to solve this problem. Scaffold-based cartilage tissue engineering is a promising one. Although positive results were reported in many in vivo cartilage repair studies with cartilage tissue engineering, adverse effects were also detected because of using growth factors or exogenous cells. In this study, we used a small molecule Kartogenin (KGN) loaded photo-cross-linkable scaffold to restore cartilage defects through a cell homing way.

Methods: Small molecule KGN was synthesized and its chondrogenic ability was tested on human synovium derived mesenchymal stem cells (SMSCs). PLGA nanoparticles loaded with KGN were prepared by a single oil-in-water (O/W) emulsion/solvent evaporation method. Hyaluronic acid (HA) was modified with double bond by reacting with the methacrylic anhydride (MA). The HA hydrogel encapsulating KGN loaded PLGA nanoparticles (HA/KGN-NPs) can be formed by UV irradiation for 30 s, and in vitro drug released study was studied. Full-thickness cartilage defects were created in New Zealand White animals and the HA/KGN-NPs were used to repair them in vivo. The animals were sacrificed at 4 and 12 weeks after operation. Histology and immunohistochemistry were used to evaluate the repair quality.

Results: KGN had the ability to induce human SMSCs into chondrocytes in vitro. The sustained release of KGN from PLGA nanoparticles and hyaluronic acid hydrogel encapsulating PLGA nanoparticles at 37 °C was found. After HA/KGN-NPs hydrogel was formed in cartilage defects for 4 and 12 weeks, toluidine blue staining, safranin O staining and immunohistochemistry staining for type I and type II collagen all showed significantly better restoration.

Conclusions: This ultraviolet-reactive, rapidly cross-linkable scaffold with KGN releasing is a great therapeutic candidate for cartilage defects.

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POLYEOLECTROLYTE MICROENCAPSULES LOADED WITH C-TYPE NATRIURETIC PEPTIDE PROTECT CARTILAGE FROM IL-1B INDUCED DAMAGE
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Purpose: CNP exhibits potent anti-inflammatory and pro-anabolic effects in chondrocytes. For example, the peptide blocked production of nitric oxide (NO) and restored matrix synthesis in human chondrocytes cultured with IL-1b. These anti-inflammatory effects indicate that CNP has the potential to repair cartilage damage observed in osteoarthritis (OA). However, there is a lack of effective treatments for OA due to poor targeting of therapeutics into the articular joint, and agents that have a short half-life. Recently, layer-by-layer preparation of microcapsules has been developed as a depot system for the stable encapsulation and sustained delivery of bioactive agents. The present study fabricated multilayer polyelectrolyte microcapsules loaded with CNP, and examined whether this approach could have protective effects in cartilage explants treated with IL-1b.

Methods: Microcapsules were prepared using layer-by-layer assembly of a CNP labelled polyelectrolyte shell around a sacrificial calcium carbonate template loaded with 100μM FITC-conjugated CNP. Incorporation of labelled CNP was confirmed by fluorescence microscopy. Encapsulation efficiency and CNP release were determined by fluorescence assay in supernatants taken during preparation and after encapsulation, respectively. Microcapsule characteristics (morphology,
size) were determined by SEM. For cell culture studies, 5mm explants punched from bovine articular cartilage were wounded with a scalpel and equilibrated in culture for 24h. Explants were subsequently incubated with or 10ng/mL of IL-1β and/or microcapsules with or without unencapsulated CNP for 96h. At the end of the culture period, samples were analysed for NO release and GAG loss (%). Chondrocyte viability was assessed by incubation with calcein-AM and EtBr-1(5μM), and quantified by ImageJ analysis. Confocal microscopy was used to detect interaction of CNP microcapsules by incubating chondrocytes with antibodies to natriuretic peptide receptor (Npr) 2 and 3.

**Results:** SEM showed uniform, 2-3 μm spherical microcapsules with morphological characteristics similar in templates loaded with or without CNP. The protein was localized around the inner surface of the microcapsule shell with encapsulation efficiencies >82.9%. CNP release profiles were broadly similar following 1-9 days of culture. The presence of CNP microcapsules did not significantly affect cell viability (>80%), with DNA values that remained stable throughout culture conditions. Microcapsules were shown to localise to wounded areas of the explants, and confocal imaging showed clustering of microcapsules in chondrocytes to natriuretic peptide receptor (Npr) 2 and 3. Furthermore, treatment of cartilage explants with CNP microcapsules led to concentration-dependent inhibition of NO release in response to IL-1β and restoration of matrix synthesis.

**Conclusions:** The present study reproductively generated uniform microcapsules with homogeneous morphological characteristics suitable for delivery and retention in the articular joint. The microcapsule delivery system is highly effective for CNP since the peptide could be stabilised through electrostatic interaction with the polyelectrolyte layers sufficient to allow diffusion of active CNP after localisation to areas of damage. Furthermore, treatment of cartilage explants with bioactive concentrations of CNP led to protective effects with sustained release of CNP in a manner responsive to the local environment. In summary, we demonstrate for the first time controlled delivery of CNP to dampen inflammatory effects induced by IL-1β in cartilage explants that has the potential to promote cartilage repair in vivo.

### 204 OBESITY DOES NOT NEGATIVELY INFLUENCE CARTILAGE REPAIR OR CAUSE OSTEOARTHRITIS IN DBA/1 MICE


**Purpose:** Traumatic cartilage damage, if left untreated, could eventually increase the risk of developing osteoarthritis (OA). Narrow stimulation treatments, such as microfracture, are performed to stimulate intrinsic cartilage repair. The symptomatic improvements of microfracture are reported to be less in obese patients and these patients are now often excluded from treatment. Obesity is a major risk factor for OA and leads to low grade systemic inflammation and metabolic changes. There is however no convincing evidence that intrinsic cartilage repair is negatively influenced by obesity. To optimize cartilage repair treatments and to prevent OA development in obese patients, it is therefore essential to investigate whether and how obesity influences cartilage repair. In this study we investigated the effects of obesity on cartilage repair and OA development in the DBA/1 mouse strain. Our hypothesis was that obesity negatively influences intrinsic cartilage repair and accelerates the speed of OA development after cartilage damage.

**Methods:** Ten-weeks-old male DBA/1 mice were fed with control diet or obesity inducing high fat diet (HFD: 60% energy from fat). After two weeks, a full thickness cartilage defect was made in the trochlear groove of the left knee. Mice were sacrificed after 1 (n=6 mice per diet), 8 (n=9 mice per diet) and 24 (n=5 mice per diet) weeks. Cartilage repair was evaluated on histology using the Pineda scoring method and OA development was evaluated using the OARSI scoring method.

**Results:** Mice on HFD had higher bodyweight when making the defect (31.0±1.7gr versus 27.2±1.1gr; p<0.001) and the difference became even larger after 24 weeks (42.0±5.8gr versus 31.7±6.4gr; p<0.001). One week after defect creation, mice on HFD had a higher percentage of defect filling with fibroblast-like cells in the defect. After 8 weeks, mice on a HFD had more cartilage repair as indicated by a lower Pineda score (p=0.01). After 24 weeks, no mice had complete cartilage repair and we were not able to detect a statistically significant difference in Pineda score between mice on HFD and control diet with this group size. All mice at every time point had an OARSI score of 0, indicating no signs of osteoarthritis.

**Conclusions:** Obesity did not negatively affect intrinsic cartilage repair in DBA/1 mice. Obesity did not cause OA in DBA/1 mice, even in combination with induced traumatic cartilage damage. Future research into the inflammatory and metabolic changes after a high fat diet in this strain of mice could provide more insights into the link between obesity related changes, cartilage damage and OA development.

### 205 CARTILAGE TISSUE ENGINEERING USING ADAMTS-4/5 DEFICIENT MESENCHYMAL STEM CELLS

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**Purpose:** Aggrecan is one of the two major constituents of articular cartilage, and during diseases such as osteoarthritis (OA), it is subject to degradation by proteolytic enzymes. The primary proteases responsible for aggrecan cleavage are the aggrecanases, identified as members of the disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) family of proteases. Aggrecanase 1 (ADAMTS-4) and aggrecanase 5 (ADAMTS-5) have been reported as important therapeutic targets in osteoarthritis. However, there is still debate which of them is the major aggrecanase responsible for the degradation of aggrecan in human cartilage. The aim of this study was to generate ADAMTS-4 deficient and ADAMTS-5 deficient chondroprogenitors derived from human induced pluripotent stem cells (iPSCs) to enable the evaluation of the respective roles of ADAMTS-4 and ADAMTS-5 in human cartilage breakdown.

**Methods:** ADAMTS-4 deficient and ADAMTS-5 deficient human iPSCs were produced by knockout of these genes in iPSC cells using Transcription Activation-Like Effector nucleases (TALENs). These iPSC cells were differentiated into chondrogenic lineage via generation of mesenchymal stem cells (MSCs) using a multistep culture method, consisting of embryoid body (EB) formation, cell outgrowth from EBs, monolayer culture of sprouted cells from EBs, and 3-dimenisonal pellet culture. Interleukin-1 (IL-1) and tumor necrosis factor (TNF) treatment were applied after chondrogenic differentiation. The expression of type II collagen, aggrecan, ADAMTS4, and ADAMTS-5 was measured by quantitative real-time PCR, Western blotting, and immunofluorescence staining. The production of collagen and glycosaminoglycan (GAG) was quantified by dye binding assays.

**Results:** ADAMTS-4/5 deficient iPSC-derived MSCs exhibited fibroblast-like morphology similar to bone marrow MSCs and expressed surface markers for MSCs. After 4-6 weeks of pellet culture, cells in pellet exhibited a spherical morphology typical of chondrocytes. The expression of type II collagen and aggrecan in pellets progressively increased. Histological analysis revealed that ADAMTS-4/5 deficient MSC-derived pellets successfully underwent chondrogenic differentiation.

**Conclusions:** These results demonstrate a model system of chondroprogenitors from genetically modified human iPSCs. This work also provides potential iPSC progeny for developing cell-base approaches to repair joint cartilage damage.

### 206 EXTRACELLULAR MATRIX CHANGES IN RESPONSE TO SPRIFERMIN STUDIED IN EX VIVO CULTURES OF ARTICULAR CARTILAGE

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**Purpose:** Osteoarthritis (OA) is a degenerative disease with high prevalence, creating an unmet medical need for drugs to protect and regenerate cartilage. Sprifermin, a truncated form of fibroblast growth factor 18 (rhFGF18), is being investigated as a potential disease-modifying OA drug (DMOAD). Sprifermin has been shown to increase cartilage volume in the knees of OA patients. The few studies published about the mode of action behind its anabolic effects, indicate that full-