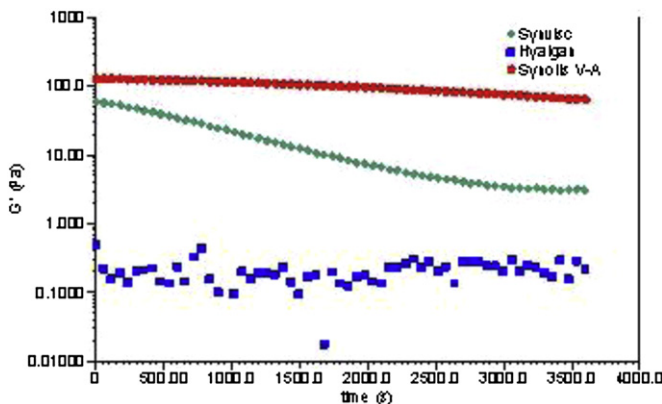
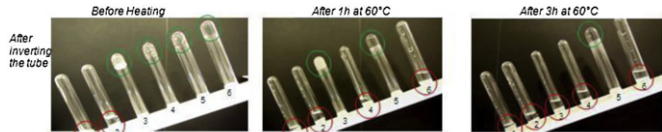


literature, free radical degradation is an important factor of HA degradation in the joint and is a key factor of the OA (free radicals is associated with chondrocyte death and cartilage destruction).



tyramine derivative containing an alkyl-based linker for the preparation of non-cytotoxic and biocompatible hydrogel-based materials with enhanced mechanical properties. In this work this HA derivative was further optimized to become a material with suitable properties for synovial fluid viscosupplementation. For this purpose, partially crosslinked solutions of this HA derivative were tested.

**Methods:** In this study partially crosslinked solutions on the basis of the HA derivative mentioned above were designed. We studied the influence of crosslinked density, HA derivative molecular weight and concentration of these solutions in order to optimize the viscoelasticity and to reach the appropriate properties corresponding with synovial fluid. The viscoelastic properties were measured by dynamic mechanical analysis in a frequency sweep oscillation mode (frequency 0,1-10 Hz at 0,5% strain) and by determination of storage modulus  $G'$ , loss modulus  $G''$  and loss angle  $\delta$ .

**Results:**

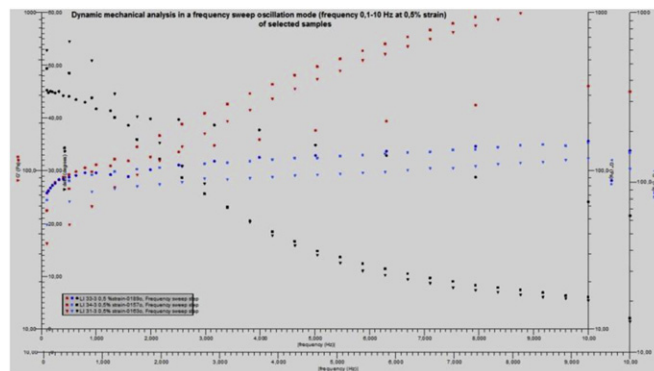
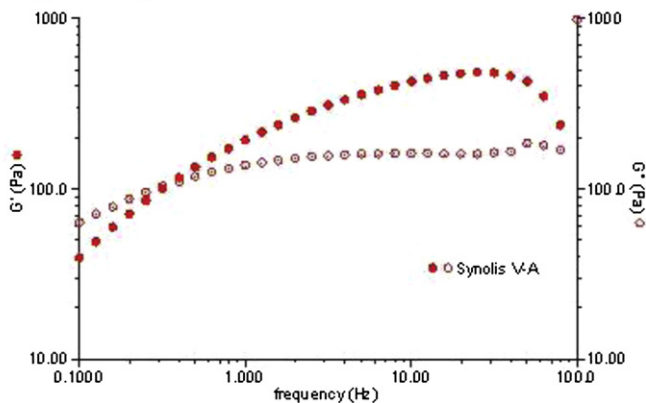


Fig.1: Representative results obtained from Dynamic mechanical analysis in a frequency sweep oscillation mode (frequency 0,1-10 Hz at 0,5% strain) of selected samples

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Images / Graphs



**MATERIAL ON THE BASIS OF HYALURONIC ACID FOR SYNOVIAL FLUID VISCOSUPPLEMENTATION WITH IMPROVED VISCOELASTIC PROPERTIES AND IN-VIVO BIOSTABILITY**

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**Purpose:** Synovial fluid is a joint lubricant and plays an important role in cartilage deformation caused by mechanical load. The main component of synovial fluid is hyaluronic acid with an approximate concentration of 2,5 mg/ml and the average molecular weight of 3-5 MDa in healthy joints. It is known, that in osteoarthritis the concentration and the average molecular weight of HA in synovial fluid are significantly reduced. This has a negative effect on the viscoelasticity of the synovial fluid, which leads to significant decrease of the mechanical protection of the articular system. Also, the articular cartilage function deteriorates. It is also known that preparations for medical treatment with smaller average molecular weight have not sufficient viscoelastic properties and undergo biodegradation too quickly. In our previous work we designed a new HA

**Conclusions:** The results obtained so far showed that we can design and prepare HA-based materials with suitable properties for synovial fluid viscosupplementation. Thanks to the partial crosslinking of the HA solutions we are also able to design materials with enhanced viscoelastic properties and prolonged biostability compared to HA-based treatment preparations with insufficient molecular weight.

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**NANOPARTICLE SYSTEM FOR THE LOCAL DELIVERY OF DISEASE MODIFYING OSTEOARTHRITIC DRUGS**

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**Purpose:** The purpose of this study is to develop the nanoparticles that i) can be injected intra-articularly ii) target to cartilage due to an opposite charge difference with the extracellular cartilaginous matrix and iii) due to their small size can penetrate into the cartilage. In this way retention time in the joint can be prolonged. By releasing disease modifying OA drugs (DMOAD) in the vicinity of chondrocytes such materials may be beneficial for restoring cartilage tissue homeostasis. Here we demonstrate the generation of drug-containing nanoparticles for intra-articular joint therapy.

**Methods:** We have prepared nanoparticles of biodegradable poly ethylene glycol- poly lactic acid PEG-PLA co-block polymers. The hydrophilic PEG and hydrophobic PLA ends of this polymer make it possible to generate micelles that contain drugs. The polymers are functionalized with UV-sensitive acrylate groups that can be stabilized by UV-crosslinking. These drug containing nanoparticles will be used for intra-articular joint injection and release of DMOADs. We have also established co-culture systems in vitro using MSCs and chondrocytes where the effect of these molecules and nanocarriers can be tested.

**Results:** Micelle type nanoparticles using PEG-PLA co-block polymers were prepared. The obtained dexamethasone loaded nanoparticles had diameters of 20-80 nm. These nanoparticles are photo-crosslinked at their hydrophobic cores which provides stability to the structure and resulted in a slight decrease in average particle size. Dexamethasone was successfully encapsulated in these nanoparticles. The current release profiles show initial burst release in the first 8 hours followed by a sustained release over at least 3 days.

**Conclusions:** We have generated nanoparticles that can serve as a carrier system to deliver clinically relevant disease modifying osteoarthritic drugs in a more effective way after intra-articular injection. We are currently investigating the retention of nanoparticles in the joint and are developing strategies to target these particles to cartilage.

### 593 MEDINGEL™: A CONTROLLED-RELEASE PLATFORM FOR INTRAARTICULAR DELIVERY OF OSTEOARTHRITIS DRUGS

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**Purpose:** Current osteoarthritis treatments have clear delivery limitations and are principally designed to treat symptoms. Drawbacks include inefficient penetration of drugs into the joint space, systemic side effects and short duration of action. Drugs administered for systemic release have poor synovial penetration that is required to provide relief from OA-related pain and inflammation. High plasma concentrations of many NSAID compounds lead to severe side effects such as stomach pain and ulcers. And in the case of orally administered celecoxib, warnings of increased risk for heart attack and stroke have been issued. MedinCell will present our company's experience in designing polymer-based delivery technology to circumvent these problems with the goal of improving osteoarthritis treatments. We will discuss the use of our MedinGel™ platform for multi-month local delivery of small molecules and biologics to help control pain, reduce inflammation and promote cartilage repair. Without controlled release capability, drugs injected directly into the joint spaces are often cleared within hours. Yet daily intraarticular injections are untenable, as they would have to be administered by trained clinicians and would severely impact the patients' quality of life. Additionally, a high frequency of injections into the joint could have increased risk of bacterial infection. By tailoring formulations to specific drugs for a range of delivery durations, MedinCell is confident that intraarticular formulations can deliver a sustained therapeutic drug concentration over multiple months to obviate current concerns and improve both treatment efficacy and patient compliance.

**Methods:** After evaluation of drug solubility and set up of in vitro-release assays, a series of sets of formulations evaluating combinations of polymers at different concentrations were prepared. The MedinGel matrix is composed of mixtures of drug molecules with solubilized, bioresorbable polymers. Within minutes after injection through a fine needle, the aqueous environment of the joint leads to a phase-separation of the formulated polymers and formation of a three-dimensional matrix. This resulting semi-solid hydrogel depot entraps and protects drug molecules within the joint space, and minimizes clearance to the plasma.

**Results:** Candidate formulations that provided a range of drug release rates in vivo were evaluated. The best candidates were then tested by intraarticular injection. MedinCell has designed intraarticular formulations of OA drug therapies that maintained therapeutic synovial concentrations for a 3-month duration in animals.

**Conclusions:** The MedinGel platform enables control over the initial drug burst characteristics, allowing the remainder of the drug to be released over a predetermined duration via polymer hydrolysis and drug diffusion until the depot is completely resorbed. No covalent modification of the active substance is required, which can simplify regulatory and development pathways. We will present data showing differential compartmentalization of drug between joint and plasma when comparing normal injection to MedinGel delivery. Additionally, MedinCell expects that this technology will provide effective delivery of disease-modifying caspase inhibitors and proteins (such as BMP7) for trauma-induced arthritis.

### 594 THE CHONDROPROTECTIVE ROLE OF CITED2 IN POST-TRAUMATIC OSTEOARTHRITIS

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**Purpose:** Joint injury is a major risk factor for osteoarthritis (OA); up to 50% of patients develop post-traumatic OA after joint injury. Cartilage degradation in OA is mediated primarily by proteolytic enzymes that

include members of the MMP (matrix metalloproteinase) and ADAMTS (A Disintegrin And Metalloproteinase with Thrombospondin motifs) families. There are still no effective treatments to prevent or slow progressive joint tissue degradation; however, studies in our laboratory suggest that the transcriptional regulator CITED2, which suppresses expression of multiple MMPs (-1, -3, -13), plays a chondroprotective role. In humans and mice, CITED2 expression is lower in OA cartilage than in healthy controls, suggesting that reduction in CITED2 may contribute to OA pathogenesis. Suppression of CITED2 by introducing CITED2 shRNA into joints also upregulated MMP-13 and ADAMTS-5 and led to OA-like histologic changes. In contrast, gene transfer of CITED2 into joints of rats with inflammatory arthritis limited cartilage matrix destruction. Therefore, in this study we tested whether CITED2 gene transfer would similarly prevent OA onset in a mouse model of post-traumatic OA due to meniscal injury.

**Methods:** CITED2 gene transfer in vivo and OA induction: Male C57BL/6 mice (8–10-wk old) received intra-articular injections of plasmids encoding CITED2 cDNA (10 mg/10  $\mu$ L PBS) in the right knee, followed by electroporation (225V, 100ms pulse length, 4 pulses each polarity) every 7 days; left (control) knees received 10  $\mu$ L PBS followed by electroporation (n=6). To induce osteoarthritis, destabilization of the medial meniscus (DMM) was then carried out by transecting the medial meniscotibial ligament (MMTL) of both limbs. Tissue analyses: Samples from each knee joint were prepared for histology and for RNA isolation. Formalin-fixed, decalcified sections were stained overnight at 4°C with anti-CITED2, anti-MMP-13, anti-ADAMTS-5 or irrelevant isotype-matched antibody controls, followed by anti-rabbit or anti-mouse secondary antibody and visualized with DAB chromagen. Safranin O-Fast green staining was used to detect proteoglycans. Expression of CITED2, MMP-13, and ADAMTS5 in articular cartilage was assessed by SYBR green real-time qPCR.

**Results:** Four weeks after DMM, joints receiving CITED2 by gene transfer (DMM+CITED2) showed elevated mRNA levels of CITED2 and reduced mRNA levels of MMP-13 and ADAMTS5 compared to DMM joints receiving PBS (DMM+PBS) (Fig 1). Histologically, DMM+CITED2 mice exhibited reduced cartilage degradation (OARSI score  $0.33 \pm 0.28$ ) compared to DMM+PBS controls (OARSI score:  $1.33 \pm 0.28$ ) (Fig 2). Immunohistochemistry showed that DMM+CITED2 mice had an increased number of chondrocytes expressing CITED2 and fewer chondrocytes expressing MMP-13 and ADAMTS-5 than DMM+PBS controls (Fig 3).

**Conclusions:** Our previous studies suggested that reduced CITED2 expression in cartilage contributes to OA pathogenesis. Our present results support the hypothesis that restoration of CITED2 in OA joints by gene transfer would limit disease progression and specifically implicate MMP-13 and ADAMTS5 as targets for CITED2 downregulation. This suggests that increasing CITED2 expression in articular cartilage may be a novel chondroprotective strategy for the treatment of OA.

