

Effect of therapeutic IL1Ra and IL10 mRNA vehiculated by polymeric nanoparticles in osteochondral ex-vivo model: a new approach for osteoarthritis treatment.

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INTRODUCTION: Currently the most widely used drugs for the treatment of Osteoarthritis (OA) are glucocorticoids and non-steroidal anti-inflammatory drugs administrated in local and systemic ways that aim to attenuate the inflammation¹. However, this approach can give rise to several side effects that can end, in the extreme case, in liver and kidney toxicity. Local administration is limited due to the low penetrability of the drugs through the various tissues in the joint with extracellular matrices. Cartilage tissue, not being vascularized, is less accessible to treatments in a situation of inflammation. Therefore, new types of treatments, which can target all tissues within the joint, are of extreme necessity and urgency. A recent innovation is based on the use of biological therapies, in which nucleic acids are administered using nanocarriers².

AIM: In this work we aimed to develop an OA explant model to test the efficacy of nanoparticle-delivered mRNA as an anti-inflammatory therapeutic.

METHODS: Stifle joints of 6-12 months old calves were acquired from a local abattoir. Cylindrical osteochondral plugs were obtained using a custom-made coated trephine drill. After 24h the explants were treated with 1 ng/ml IL1b (Kingfisher) and respectively incubated with nanogel (NG) loaded with IL1Ra or IL10 mRNA, NG, only "inflammatory medium" only (Inflamed CTR) or culture medium (control no Inflammation). NG based on multifunctional poly(amidoamines) (20Med) at concentration of 1.5 mg/ml were loaded with mRNA before the transfection; therapeutic mRNAs were diluted to 120 ug/ml in 20mM Hepes to obtain a final mRNA:NG ratio (w/w) of 1:12.5. mRNAs containing chemically modified nucleotides were produced by using in vitro transcription with T7 RNA polymerase (Ethis). For capping, anti-reverse cap analogue (ARCA) was also included in the in vitro transcription reaction. mRNAs were carrying a 120 polyA-tail. Content of nitric oxide (NO) and glycosaminoglycan (GAG) in conditioned medium was measured using Griess reagent and Dimethylmethylene Blue Assay (DMMB). Real-Time qPCR was performed using gene expression assays for IL8, IL6, IL10, ADAMTS5 and RPLP0 as endogenous control. ELISAs of IL6, IL10 and IL1Ra in conditioned medium were performed at 24h, 7 and 14 days. Histological evaluation was carried out by Saf-O / Fast Green staining at day 0 and day 14. Viability test was performed after 24h using AlamarBlue assay.

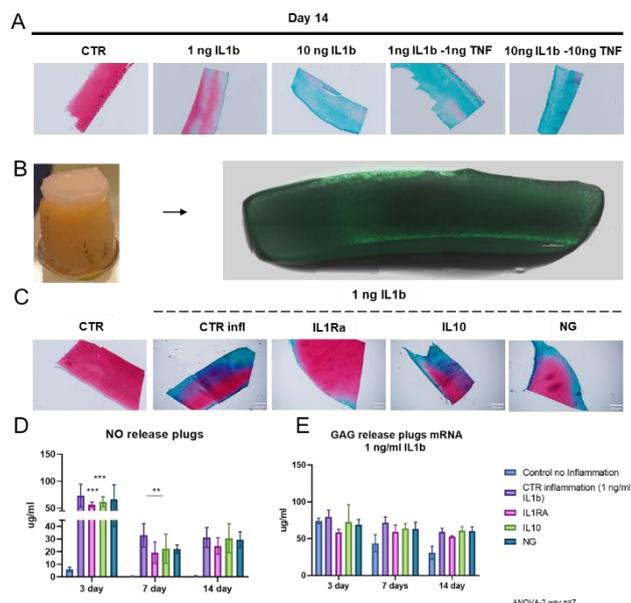


Figure 1 Osteochondral plug OA model (A), SEM picture of NG and transfection of cartilage with GFP-mRNA (B) Effect of IL1Ra and IL10 on inflamed plugs over 14 days (C) Nitric oxide release (D) and GAG release into medium (E)

nanoparticles and therapeutic mRNA able to penetrate the cartilage. The IL1Ra and IL10 mRNA could be a possible alternative in local treatment for the modulation of inflammation that can possibly counteract the progression of inflammation and cartilage degeneration.

SIGNIFICANCE/CLINICAL RELEVANCE: Using an optimized ex vivo organ culture system, we provide a suitable OA model for drug testing, Reducing the necessity for in vivo experiments. Additionally, we showed how a new approach based on NG and therapeutic mRNA can modulate the inflammation under chronic conditions. Further studies are ongoing to evaluate the beneficial composition of NG and mRNA able to specifically target the cartilage and the capability of counteracting the progression of inflammation related to OA.

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RESULTS: The use of 10 ng/ml IL1b and combination of 1-10 IL1b and TNF for 14 days induced a high loss of matrix and vast inflammation compared to 1 ng/ml IL1b alone (Fig. 1 A). An upregulation of IL6, IL8, and ADAMTS5 gene expression was observed after 7 days which persisted over 14 days, confirming that the treatment with inflammatory cytokines was constant over time, also in terms of NO and GAG released into the conditioned medium compared to control. The use of GFP- labeled mRNA showed the capacity of penetration of mRNA encapsulated within NG (Fig. 1 B). Toxicity assay on 2D cultured chondrocytes for IL1Ra and IL10 mRNAs with NG did not show any effect compared to control after 24h, as previously observed³. We further tested the effect of therapeutic IL1Ra and IL10 mRNAs on inflamed explants. Interestingly, mRNA treatment reduced the GAG and NO ($p < 0.001$ IL1Ra, IL10) release (Fig.1 D-E) into the medium compared to the inflamed control. The concentration of IL6 was as well attenuated by the administration of IL1Ra mRNA, though no significant effect was observed for IL10 mRNA. Histology showed larger Safranin-O positive stained area after treatment with IL1Ra compared to all other groups (Fig. 1 C) over the 14 days in constant presence of inflammation.

DISCUSSION: In this work we provided a suitable and stable model able to mimic the human chronic inflammation of OA, furthermore we showed the feasibility of using alternative drugs based on