CHOLESTEROL-MODIFIED HYALURONIC ACID NANOHYDROGELS FOR TARGETED DELIVERY OF ANTI-INFLAMMATORY AGENTS

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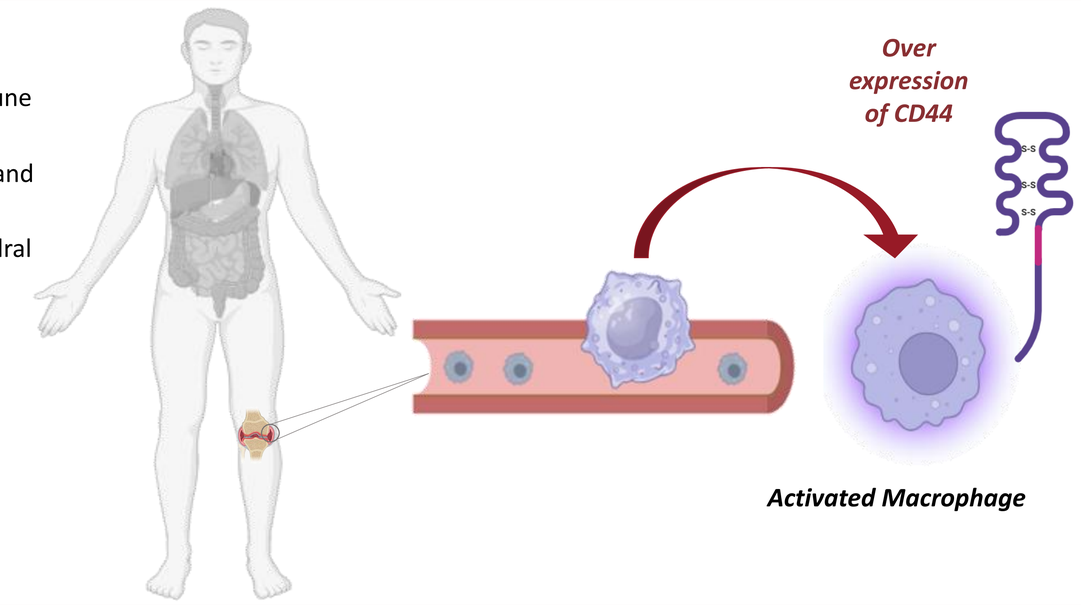
INTRODUCTION

Rheumatoid arthritis (RA) is an inflammatory autoimmune disease characterized by:

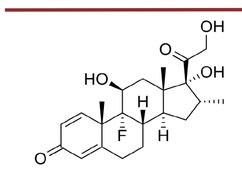
- immune cell infiltration into the synovial membrane and formation of hyperplastic synovium
- progressive cartilage destruction and subchondral bone erosion
- joint ankylosis

In the disease condition of RA, the inflammed joint and the synovial fluid express **CD44 receptors** on the surface of activated macrophages. Hyaluronic acid (HA) acts a as natural ligand for this receptor so a delivery approach using CD44 as the target can help minimizing off-target drug distribution [1].



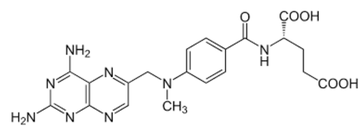
Available therapeutics for the **treatment of RA**:

- **CORTICOSTEROIDS**

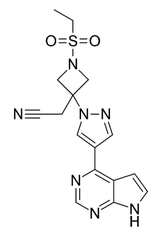


Dexamethasone

- Disease-modifying anti-rheumatic drugs **DMARDs**



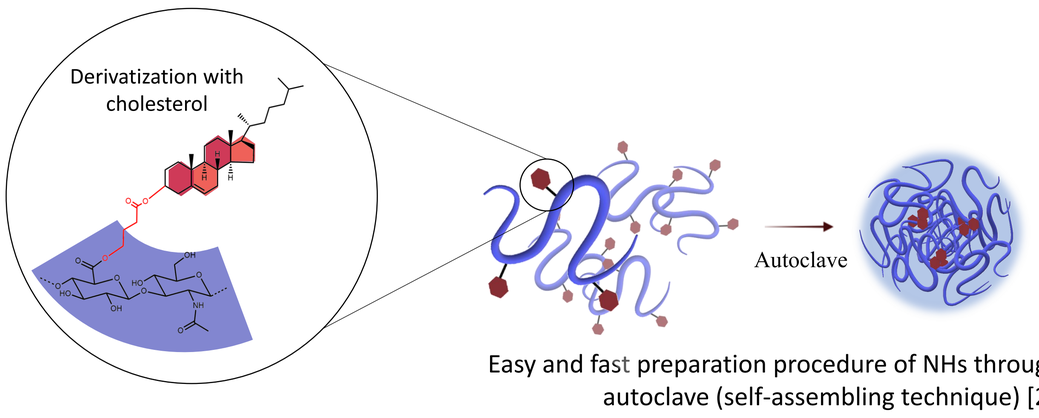
Methotrexate



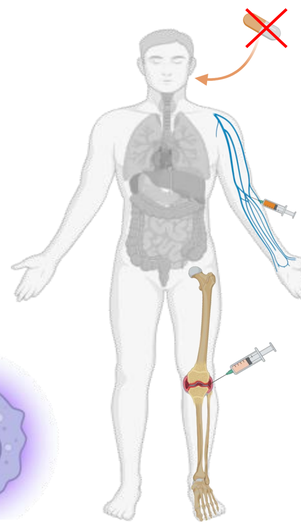
Baricitinib

AIM OF THE WORK

Delivery of anti-inflammatory drugs through **Nanohydrogels (NHs)** based on a **HYALURONIC ACID** derivative grafted with **CHOLESTEROL** (HACH)



Easy and fast preparation procedure of NHs through the use of autoclave (self-assembling technique) [2].



ORAL

Lack of target specificity and various side effects.

INTRAVENOUS

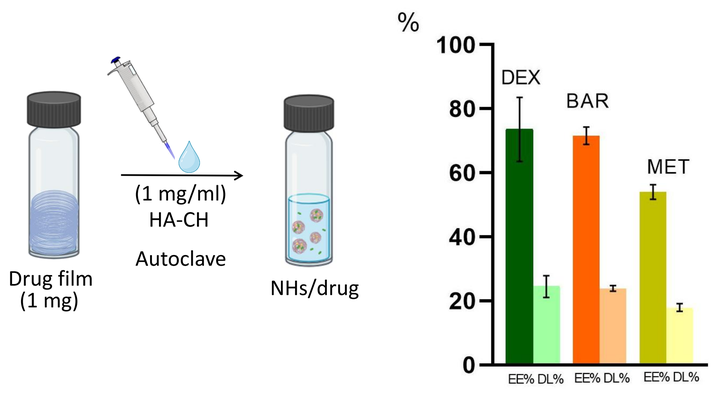
NHs loaded with DEX were studied for intravenous administration. This route is **safe** and **prevents the inactivation** of the drug by the gastrointestinal enzymes.

INTRA-ARTICULAR

NHs loaded with MET and BAR were studied for intra-synovial administration. This route offers direct access to the joint space, thus **increasing the bioavailability** while reducing systemic exposure.

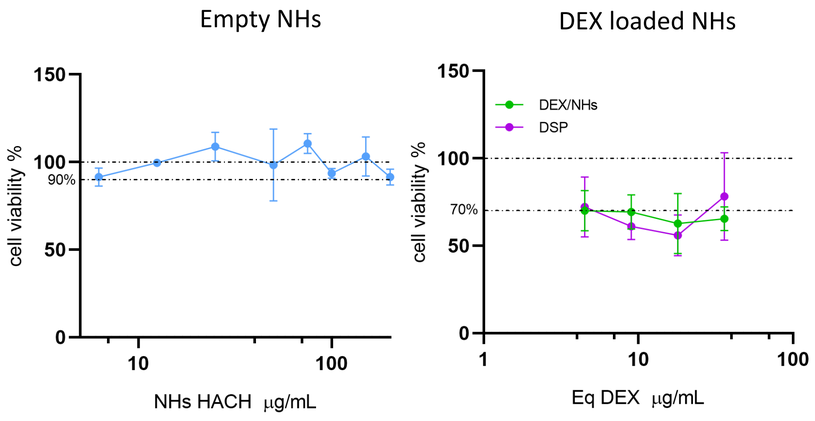
Safety and efficacy of NHs loaded with dexamethasone

Loading of anti-inflammatory drugs into NHs encapsulation efficiency



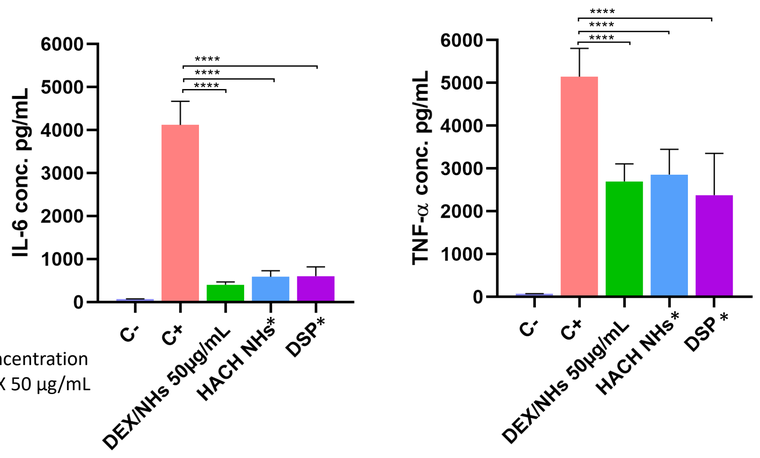
The anti-inflammatory drugs were loaded through a film forming technique into NHs with a high values of drug loading (DL%) and encapsulation efficiency (EE%).

Viability assay to assess the safety of the formulation on macrophages RAW 264.7



The MTT test shows that empty **HACH NHs** are **safe** up to a concentration of 200 µg/mL. For the NHs loaded with DEX there is a decrease in cell viability due to the drug cytotoxicity as shown by **DSP** control (water soluble form of DEX).

Cytokine inhibition in LPS-activated macrophages treated with NHs/DEX

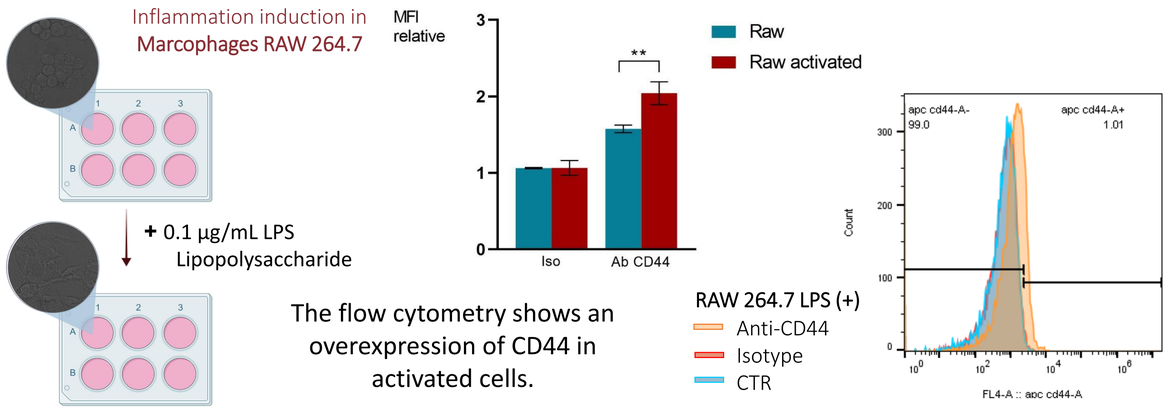


*Same concentration of NHs/DEX 50 µg/mL

The cytokine inhibition assay shows a **strong reduction** of inflammatory citokines (**IL-6** and **TNFα**) in all conditions.

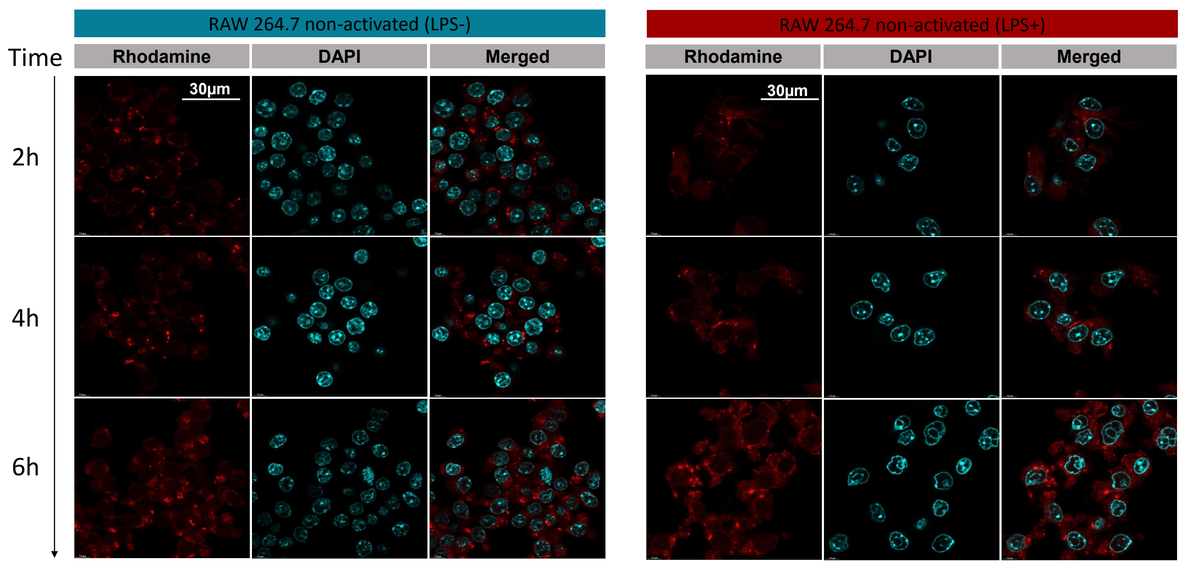
HACH NHs internalization in activated and non activated RAW 264.7

Effect of inflammation induction on the expression of CD44 receptor

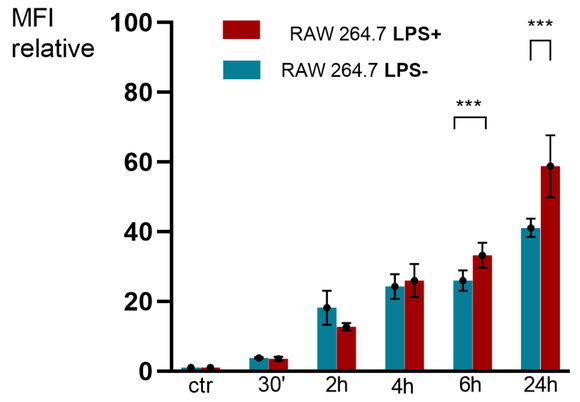


The flow cytometry shows an overexpression of CD44 in activated cells.

Internalization studies of NHs into macrophages RAW 264.7



Confocal microscopy images and flow cytometry studies reveal a difference in terms of fluorescence between the two populations of cells **after 6h**, indicating an **increase of internalization** for activated RAW at higher time point. This can be attributed to a **receptor mediated internalization** which is slower than other mechanism of uptake.



Conclusions

HACH NHs formulations are safe and can efficiently load anti-inflammatory compounds, resulting in a reduction of the release of inflammatory cytokines in activated macrophages. Internalization studies showed the ability of such designed NHs to enter in the cells of the immune system, therefore representing a promising strategy for the treatment of rheumatoid arthritis.

References

[1] S. Priya et al., Int. J. of Biol. Macromol., 2024, 271, 132586
[2] E. Montanari et al – Advanced healthcare materials., 2018, 7 (12)