ПЯТЫЙ ЮБИЛЕЙНЫЙ ЕВРАЗИЙСКИЙ ОРТОПЕДИЧЕСКИЙ ФОРУМ

EURASIAN ORTHOPEDIC FORUM

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Взаимодействие гиалуроновой кислоты и внеклеточных везикул: взаимное влияние патологического и терапевтического компонентов при остеоартрите

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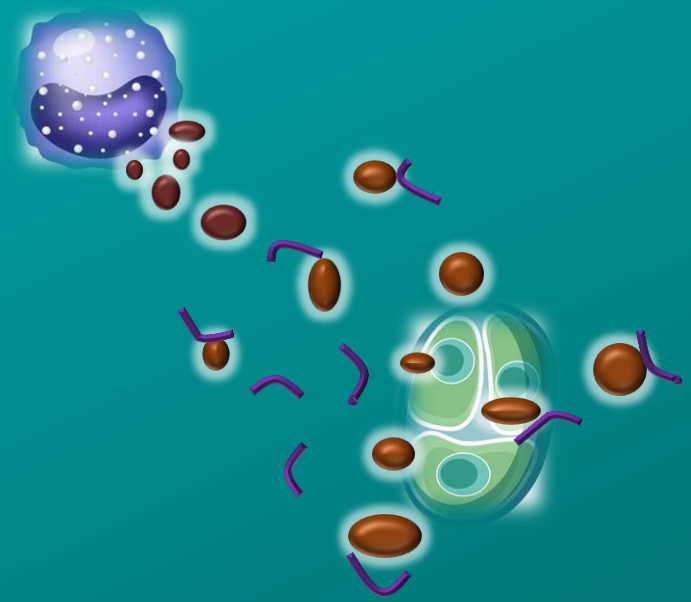

ПОСТЕРНЫЙ ДОКЛАД

Hyaluronic acid-extracellular vesicles interactions: pathological and therapeutic implications in osteoarthritis

Piero Sestili[1], Vittoria Carrabs[1,2,3], Isabel Guillén[2,3], Marisa Ferrándiz[2], Fabio Ferrini[1], Rachele Agostini[1], Elena Barbieri[1], Carmela Fimognari[4], and Michele Guescini[1]

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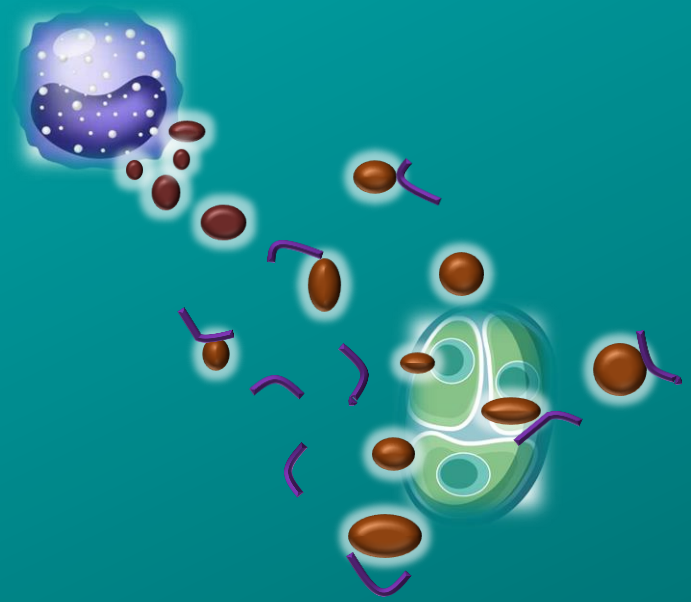


E-POSTER

PATHOPHISIOLOGY OF OSTEOARTHRITIS (OA)

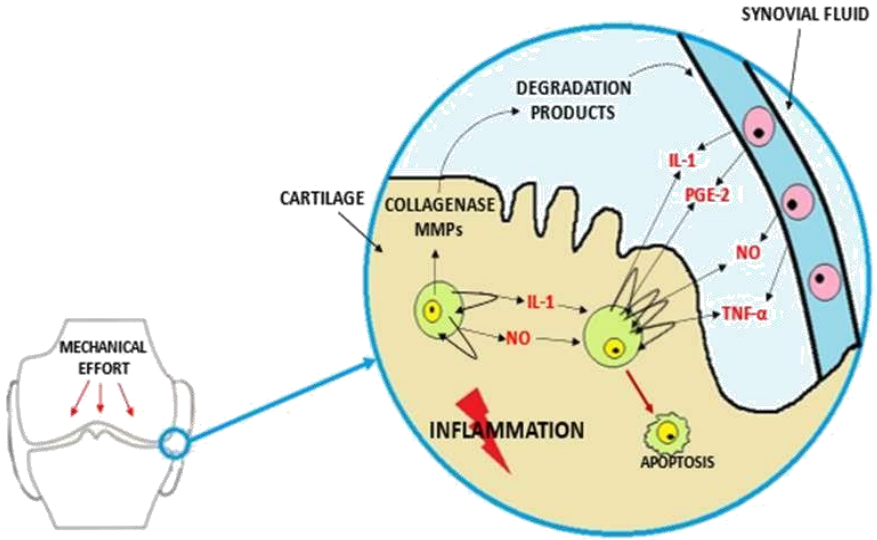


Fig.1. Response of the arthritic joint to the mechanical effort. This generates the activation of the degradative enzymes of cartilage and the release of proinflammatory mediators that participate in the aetiopathogenesis of OA.

Pathogenesis of OA

Mechanical effort/use of joint

↓

Reversible ECM degradation

↓

Attempts at repair (unsuccessful)

↓

Irreversible matrix degradation

↓

Accompanying synovitis

↑ ↓

Release of OA effectors (Il1b, IL-6, TNFα, MMP-13, collagenase, hyaluronidase, $ONOO^-$….)

↑ ↓

Loss of cartilage

↑ ↓

Sclerosis/osteophytes

↑ ↓

Misalignment/loss of congruence → **PAIN**

↓

Progressive osteoarthritis

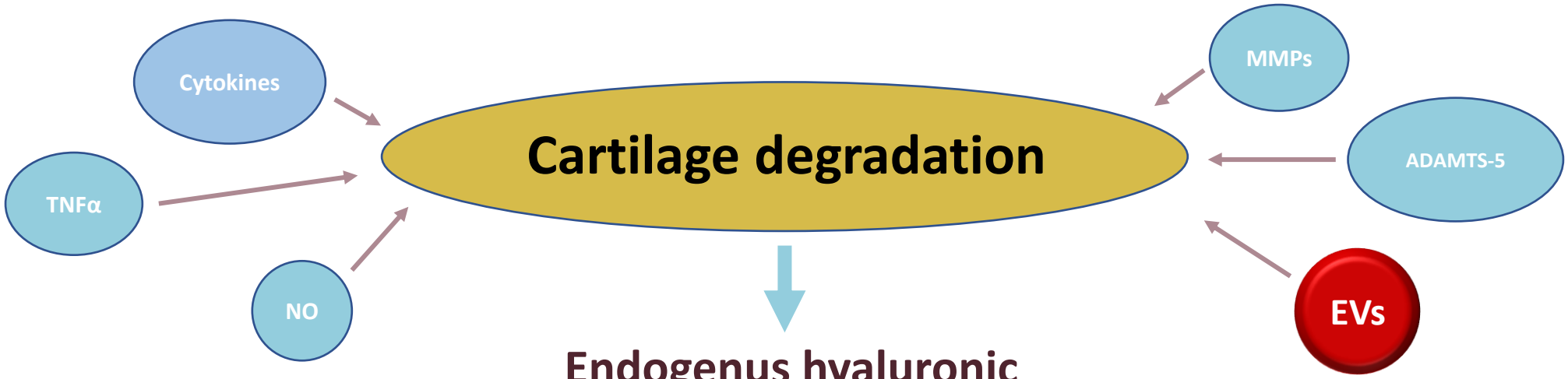
EXTRACELLULAR VESICLES

- Extracellular vesicles (EVs) are emerging as **important players in OA**
- EVs are involved in **cell-to-cell communication** and transfer of biologically active meterials

EVs **contribute to inflammation in activated OA** promoting:

- Synovial Inflammation
- Subchondral bone remodeling
- Chondrocyte catabolism and cartilage destruction

INFLAMMATION



Endogenus hyaluronic acid degradation

Low molecular weight hyaluronic acid fragments

Reduction of viscoelastic properties of hyaluronic acid

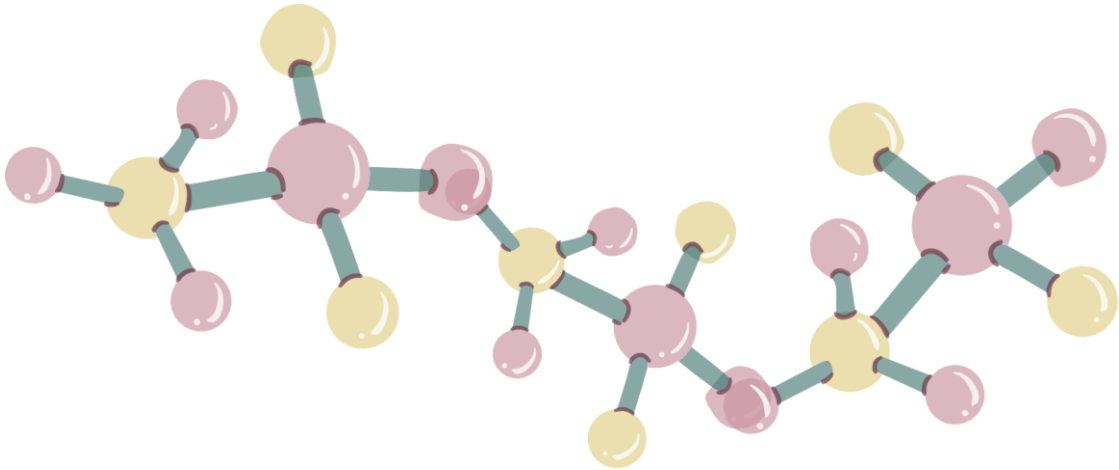
HYALURONIC ACID

- A linear polymer consisting of anionic, nonsulfated **glycosaminoglycan** repeats distributed widely throughout connective, epithelial, and neural tissues
- **The main component of the synovial fluid**
- Principally synthetized by chondrocytes, synoviocytes and fibroblasts

THE IMPORTANCE OF CONCENTRATION AND MOLECULAR WEIGHT OF ENDOGENOUS HA

HIGH MOLECULAR WEIGHT (>3 x 10^6 DA)

- Optimal rheological properties
- Anti-inflammatory effect
- Promotes repair of tissue damage



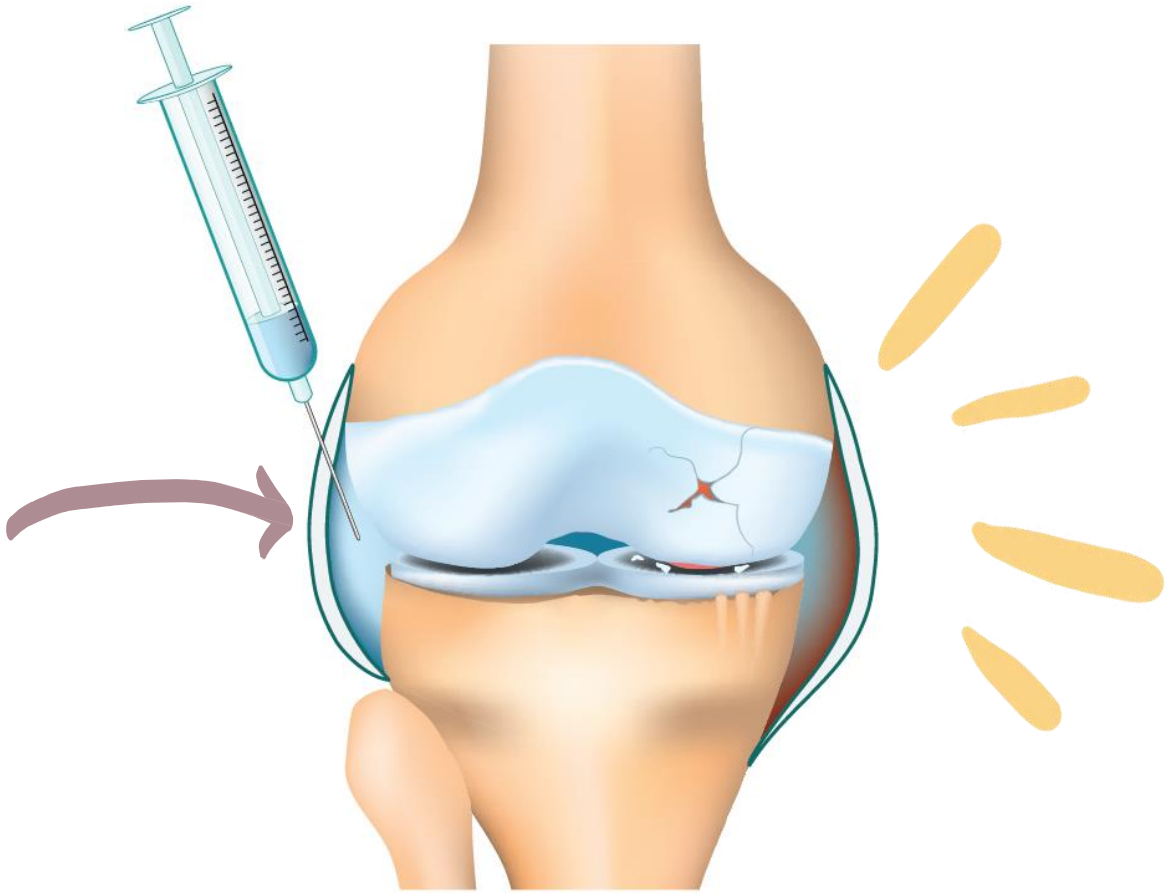
VS

LOW MOLECULAR WEIGHT (<1.5 x 10^6 DA)

- Decreased rheological properties

TREATMENT OF OA

- NSAIDs, SYSADOA
- HMW IA HA INJECTIONS
- SURGERY

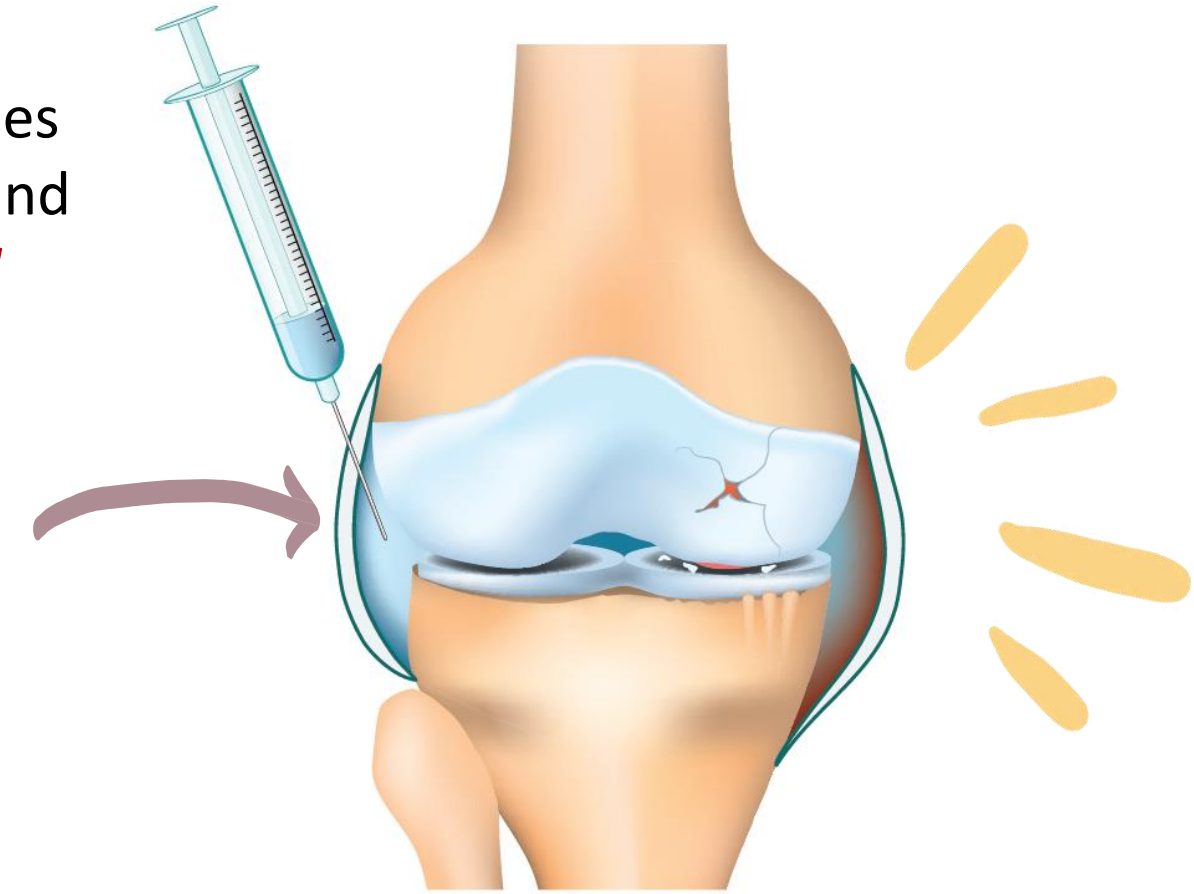


TREATMENT OF OA

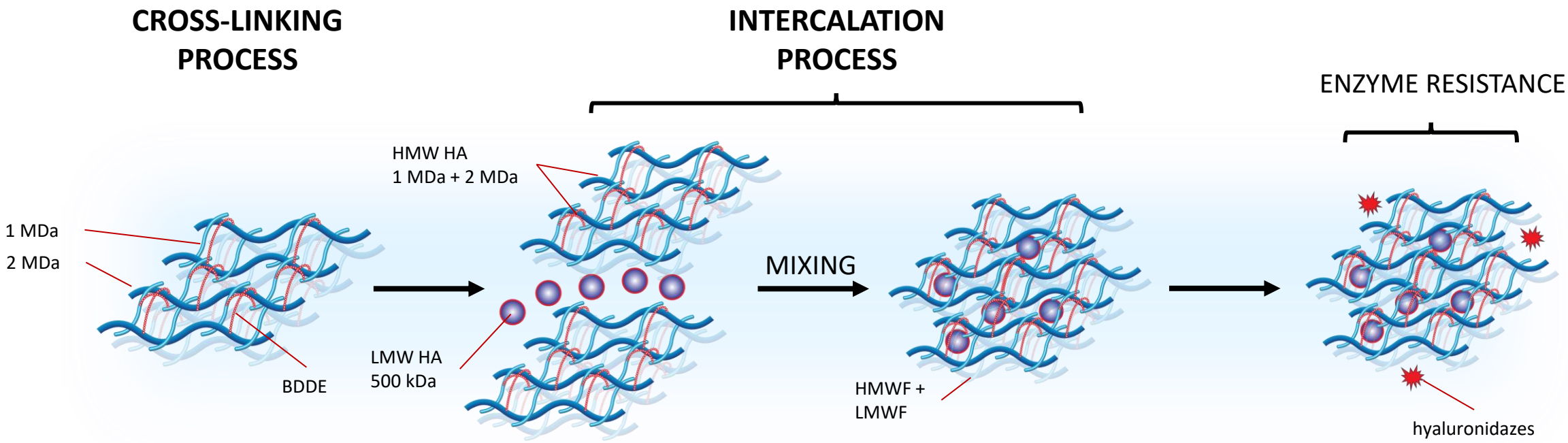
Exogenous HA for infiltrative purposes range in size from 500 to 7000KDa, and may be **linear** and/or ***cross-linked***

Linear HA

Cross-linked HA with BDDE



The blend of Cross-linked And Linear Hyaluronic Acid (CLHA)



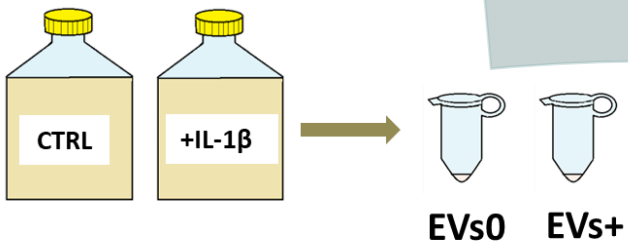
CLHA consists of:

- ***Cross-linked*** **high molecular weight fraction** of HA (HMW HA) of 1 and 2 MDa
- **Linear lower molecular weight fraction** of HA (LMW HA) of 500 kDa

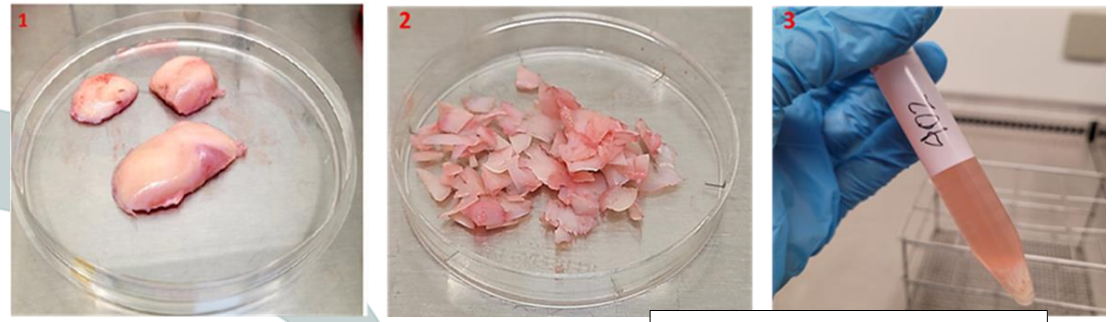
AIM OF THE STUDY

The aim of this study is
to see wether and how **HA is capable**
to interact with EVs in an experimental model
representative of OA.

MATERIALS AND METHODS



Collection of EVs from THP-1 cells
EVs-: EVs from non-activated THP-1
EVs+: Evs from «inflamed» THP-1



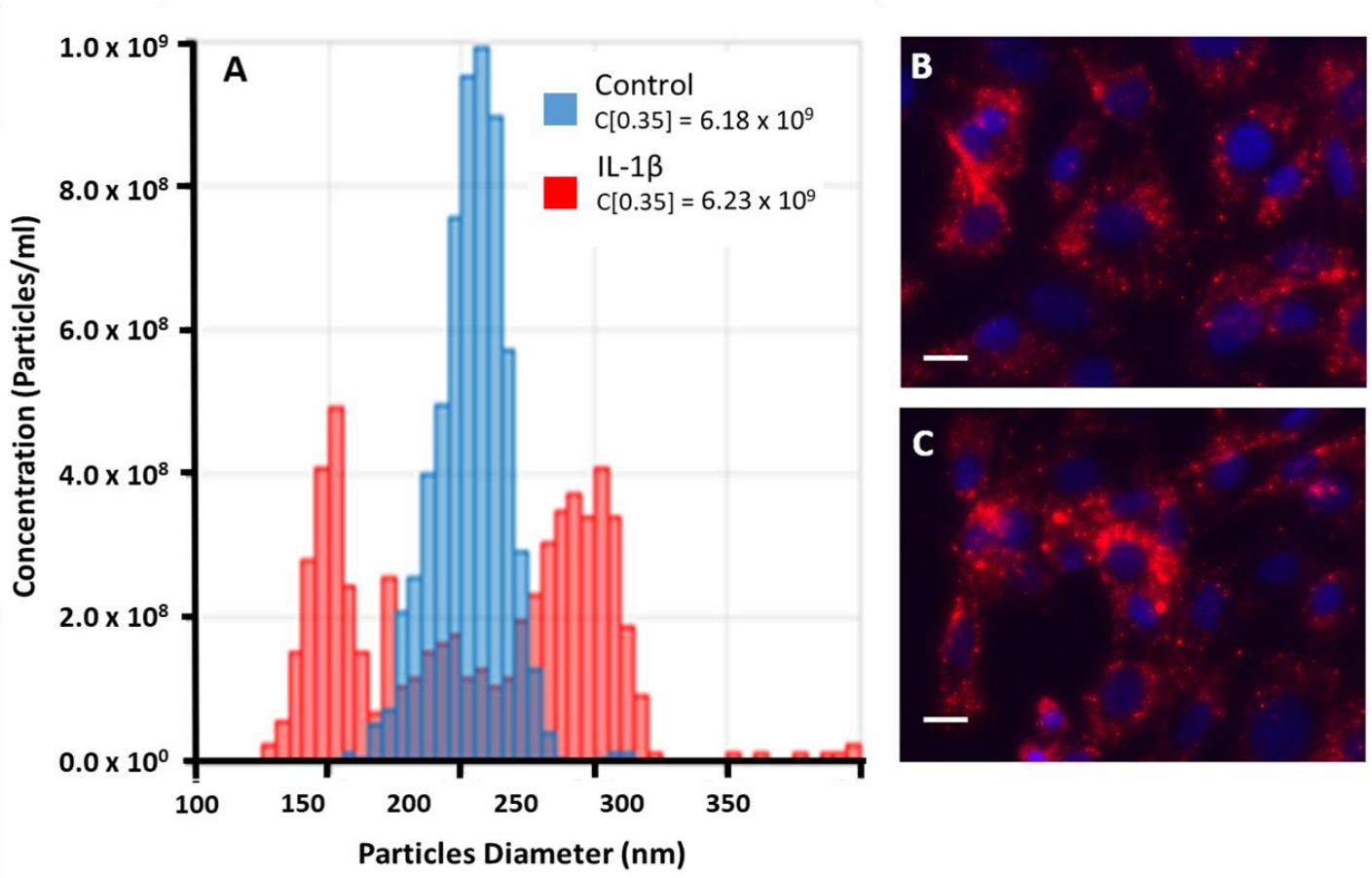
*Auth. E.C. H1512058528966

Chondrocytes isolation
from OA patients (**COA**)

COA treatment with EVs- or EVs+
w/wo HMW hyaluronic acid

Analysis of
inflammation markers

ANALYSIS OF EVs- AND EVs+ AND COA INTERNALIZATION

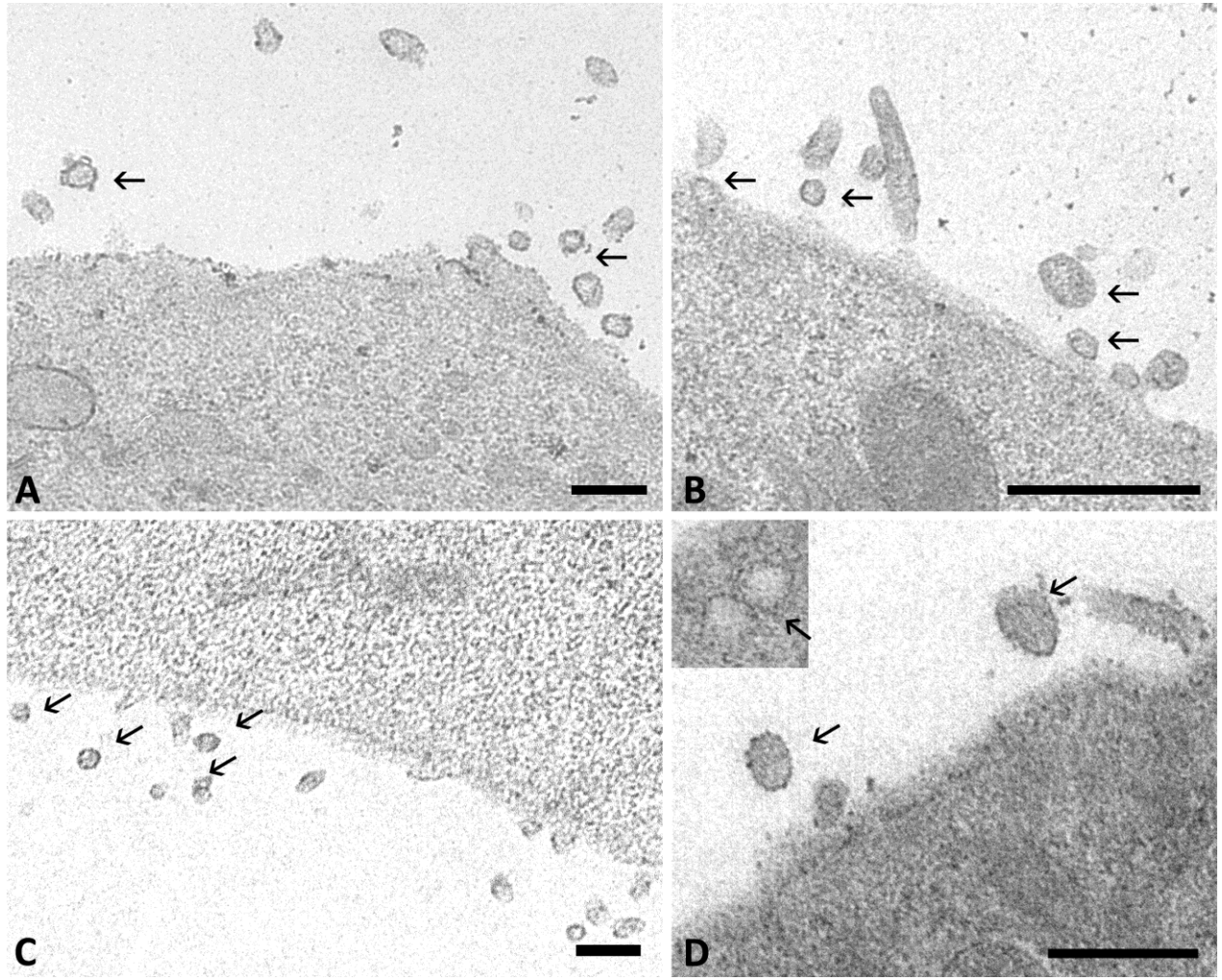


Size-distribution analysis of EVs.

(**A)** Red and blue columns refer to EVs- and EVs+, respectively; particle concentrations are shown in the upper left corner.

(B) and **(C)** show EVs- and EVs+ extracellular vesicles (stained in red with PKH26) internalized within COA cells (nuclei stained with DAPI).

TEM MICROGRAPHS OF EVs- AND EVs+ IN/OUT HC CELLS



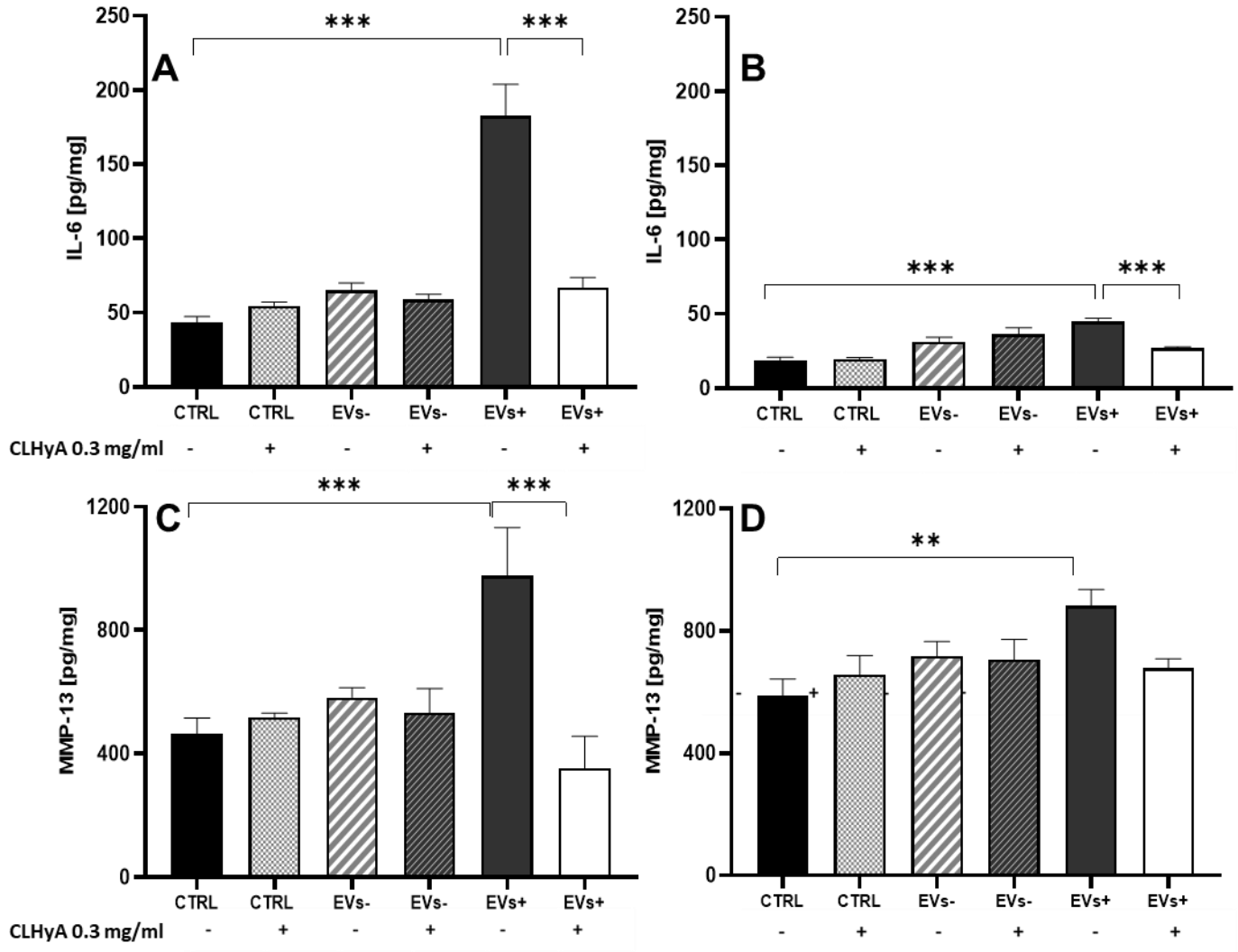
EVs- (A, B) and EVs+ (C, D) are rounded, with conserved membrane, and appear inside and outside the cells.

EVs+ showed diameters between 120 - 180 nm, while EVs+ 170 - 210 nm.

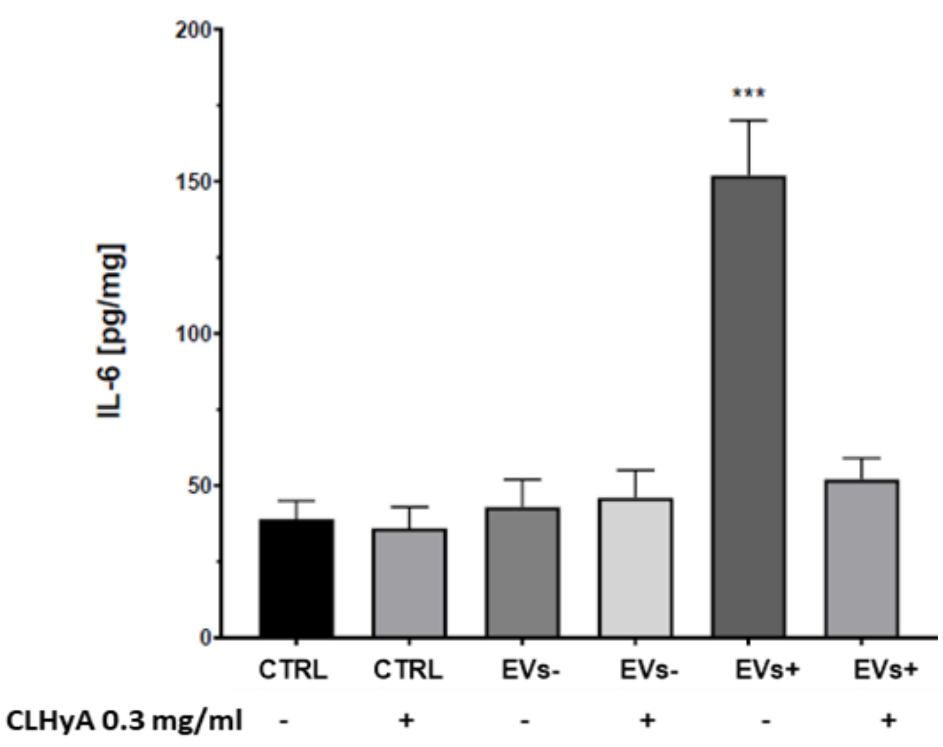
Arrows (→) indicate EVs

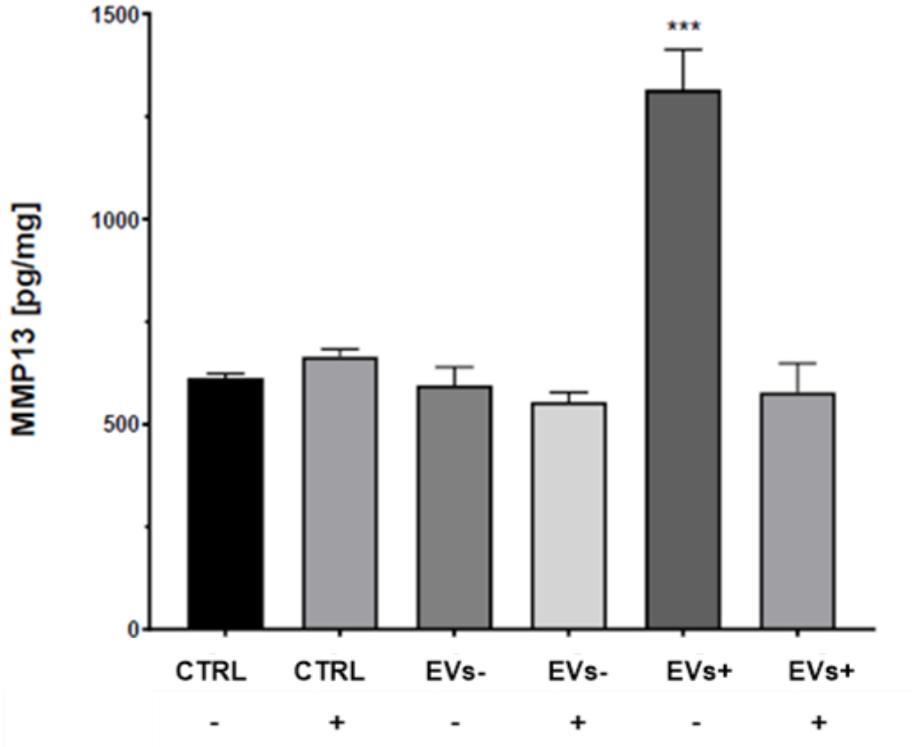
A, B, C, D, Bar = 500 nm

EFFECT OF CLHA ON THE LEVELS OF IL-6 and MMP-13 IN COA (A and HC cells EXPOSED TO EVs- OR EVs+)



EFFECT OF CLHA ON THE LEVELS OF IL-6 and MMP-13 IN HC cells EXPOSED TO EVs- OR EVs+





THE STUDY OF MICROVESICLES/HA INTERACTIONS: HOW TO…

1) CLHA is bound to Biotyn to obtain biotinylated-CLHA

2) Incubate biotynilated-CLHA with Evs+

3) Prepare streptavidin magnetic microbeads*

* Streptavidin tightly binds to biotyn residues

4) Mix 2 and 3 and expose to a magnet**

** Magnets attract microbeads bound to biotyinilated HA

5) Centrifuge to precipitate microbeads and search for Evs in the pellet***

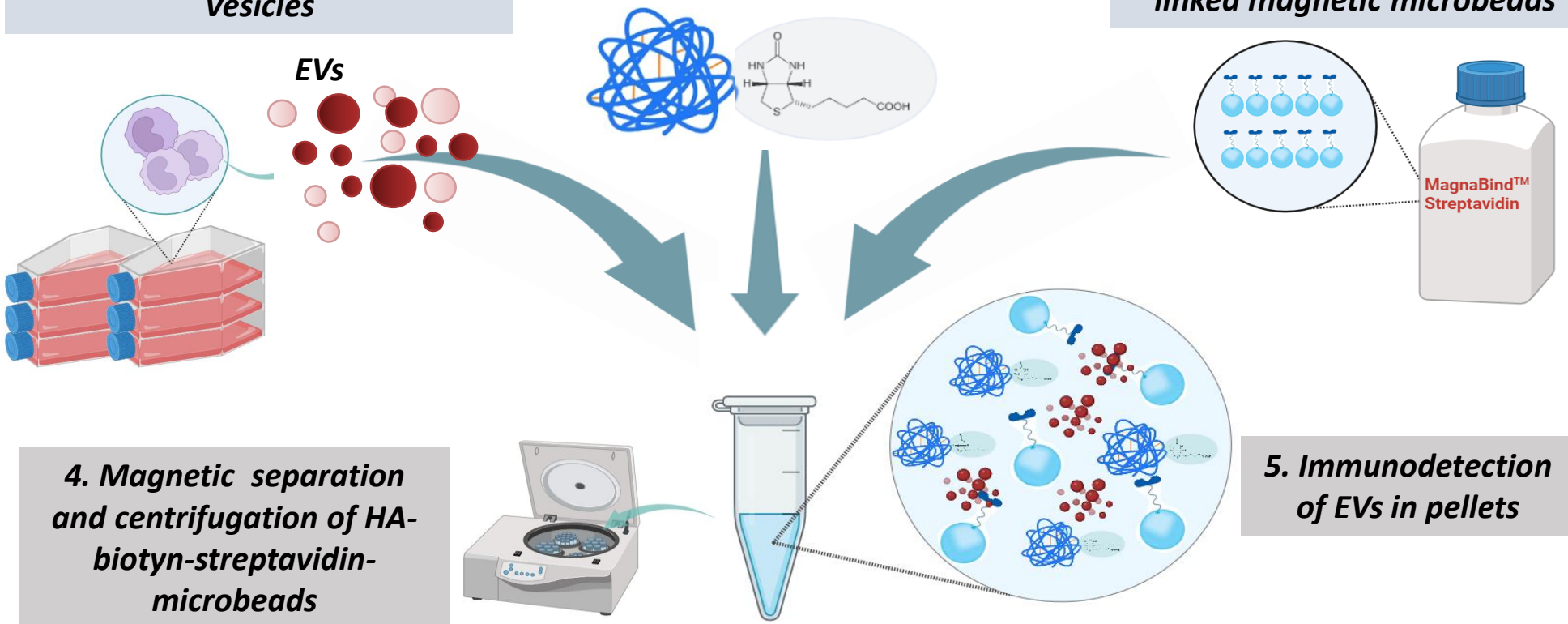
*** if EVs are found, it means that they had been «captured» by CLHA

INFLUENCE OF BIOTINYLATED CLHA ON EVS

1. Isolation of Extracellular Vesicles

***2.* Biotinylation of HA or CLHA**

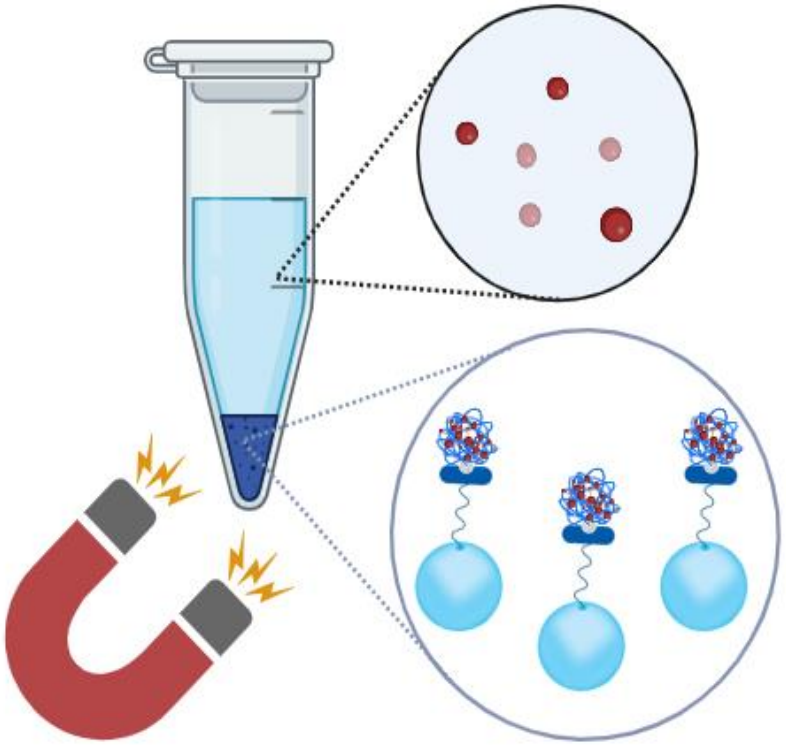
3. Addition of streptavidin-linked magnetic microbeads



4. Magnetic separation and centrifugation of HA-biotyn-streptavidin-microbeads aggregates

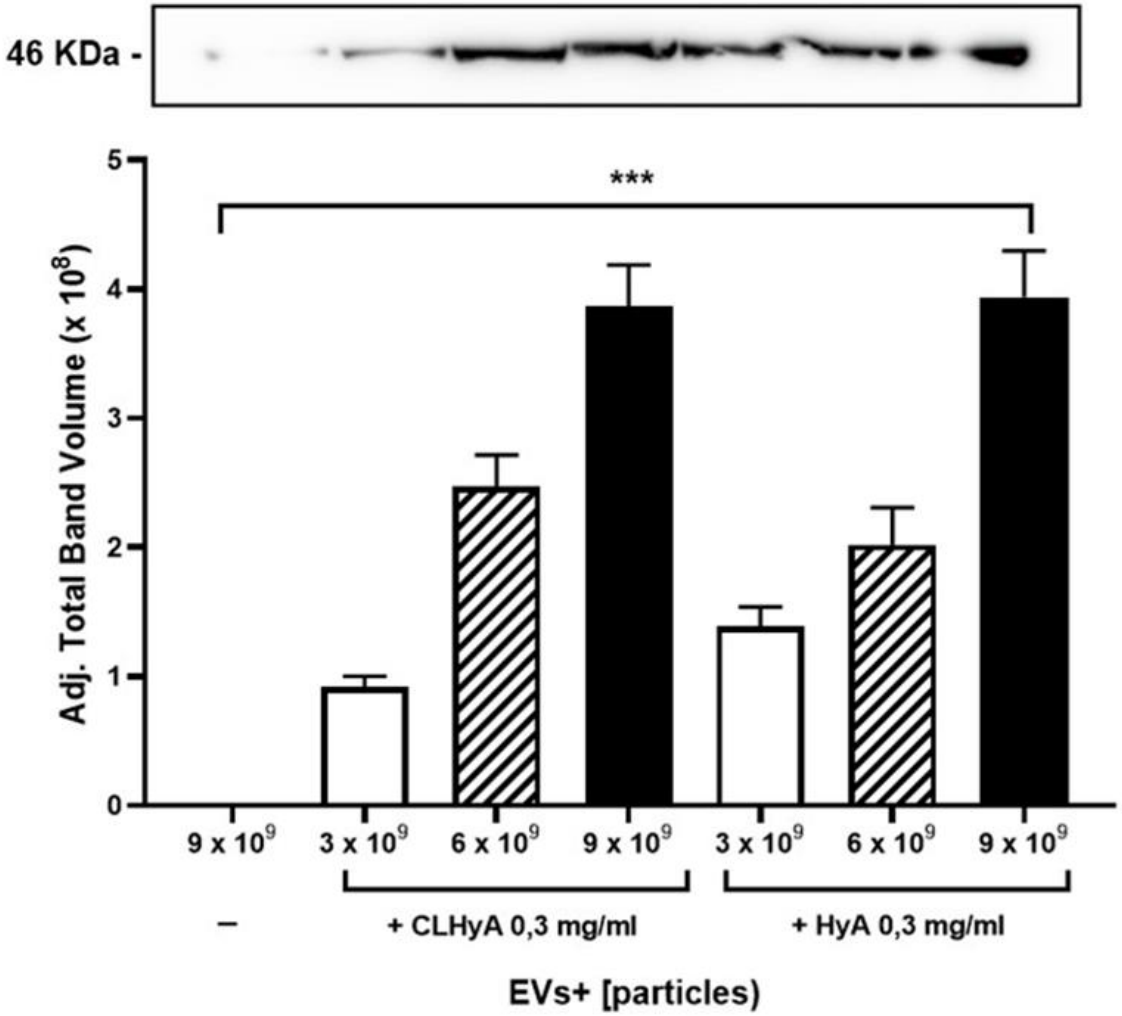
5. Immunodetection of EVs in pellets

SEPARATION OF HYALURONIC ACID BOUND TO MICROVESICLES



- Biotinylated-HA binds to streptavidin-coated magnetic beads through specific biotin-streptavidin binding;
- Biotinylated-HA bound to beads is then separated with a magnetic field
- HA bound microvesicles, upon magnetic separation HA-microvesicles aggregates will be scored.

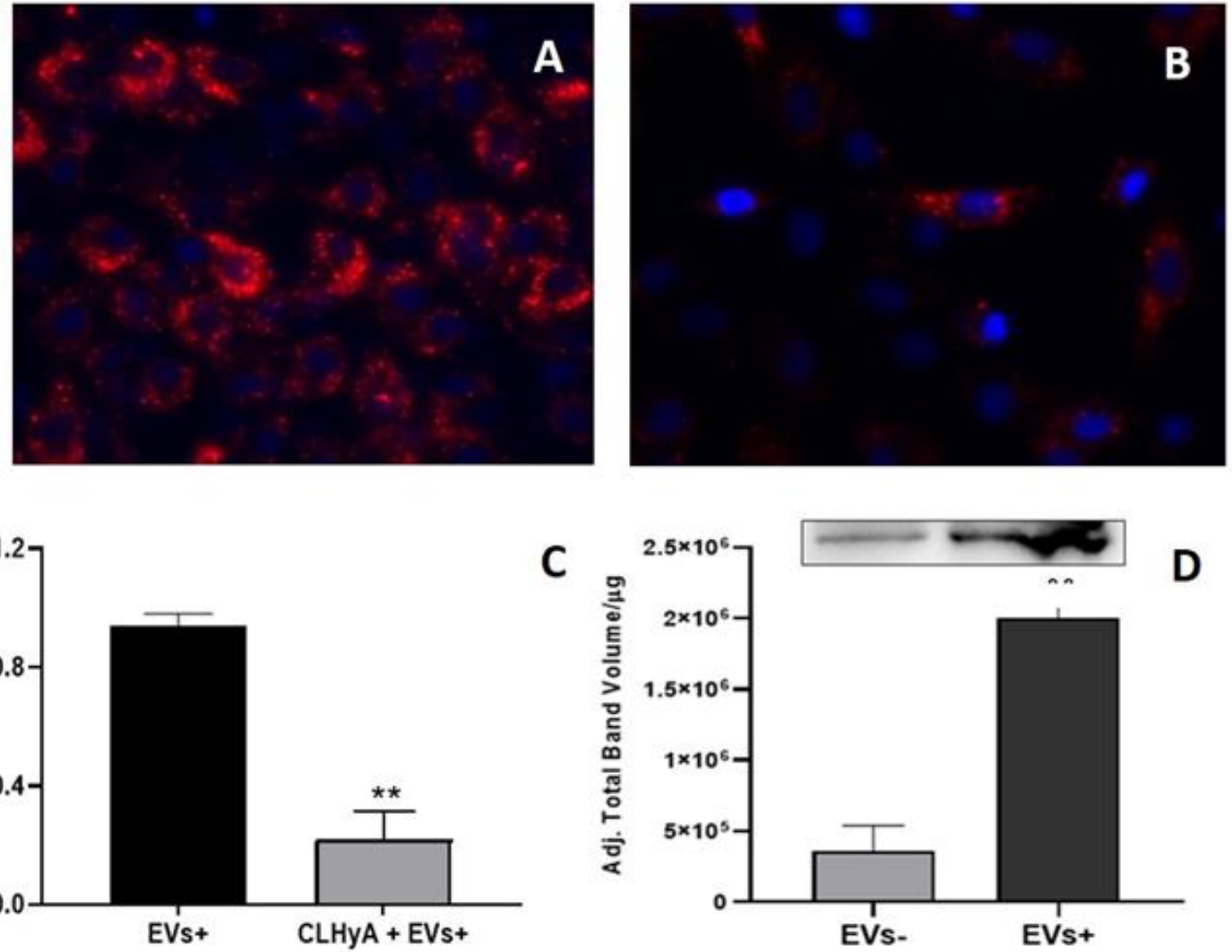
Western Blot Analysis of Tsg101 associated to hyaluronate coated microbeads



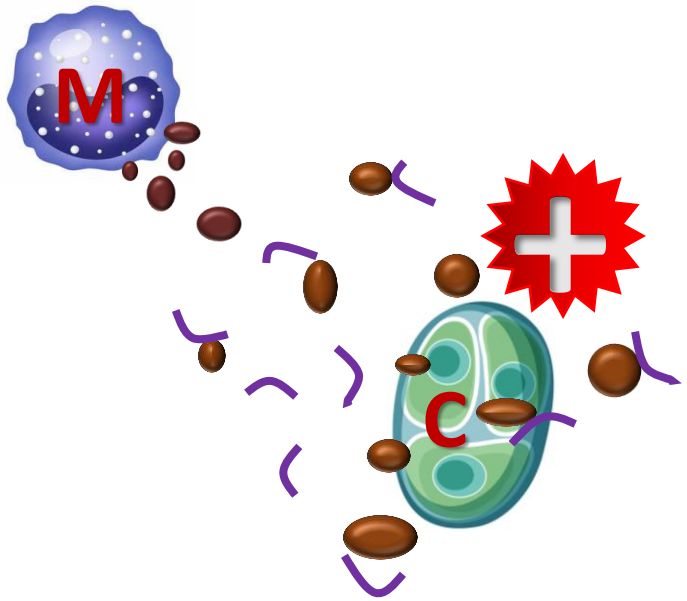
The presence of Tsg101 in microbeads fraction is indicative of the presence of EVs.

Cellular internalization of EVs+ in HC cells

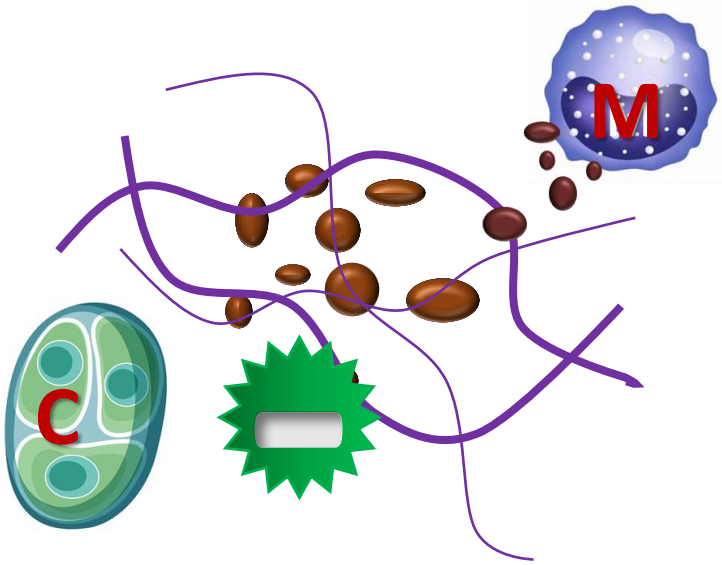
(A) and **(B)** representative micro-graphs of EVs+ internalization in HC cells in the absence (**A**) or presence (**B**) of 0,3 mg/ml CLHyA: red extracellular vesicles (stained with PKH26) surround blue HC cells nuclei (stained with DAPI);
(C) quantitative cytofluorimetric determination of EVs+ internalization in the absence or presence of 0,3 mg/ml CLHyA;
(D) expression level of CD44 in EVs- and EVs+ as assayed with Western blotting (the corresponding bands are shown in the inset).



Differential interactions of Evs with HMW HA VS fragmented HA



In untreated active OA joints, **HA** (violet) is degraded into small LMW fragments that do not limit the diffusion of the proinflammatory **EVs** (red) released by activated **macrophages** (M), so that they can freely diffuse and deliver their noxious cargo to **chondrocytes** (C).



Replenishing joints with intact HMW HA, entraps **EVs** thus reducing their capacity to freely diffuse through synovial fluid and deliver noxious signals to target **chondrocytes** (C).

CONCLUSIVE REMARKS

- EVs derived from IL-1β stimulated human monocytic cells promote the onset of relevant pro-inflammatory events in COA, a finding in keeping with observation from other groups indicating that EVs released within the joint contribute to the progression of GA conditions.
- Indeed EVs+ isolated from IL-1β-stimulated-THP-1 **promote pro-inflammatory events** in COAs, probably caused by their cargo.
- CLHA was found to prevent and/or affect the cellular responses caused by the signals delivered by EVs+: in particular the increase of MMP-13 and IL-6 levels was virtually abrogated.
- To our best knowledge, this study is the **first report** indicating that HA - a blend of linear and cross-linked formulation - interacts with EVs+ ameliorating the status of targeted chondrocytes.

CONCLUSIVE REMARKS

- A better characterization and comprehension of CLHA-EVs reciprocal interactions under disease conditions may pave the way to the exploitation of targeting EVs with HA, along with the identification of the fundamental structure-activity relationships responsible for EVs-HA interplay.

THANKS FOR YOUR ATTENTION

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