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Microsponges for intra-articular treatment: new evidence

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sponge by nanofaber
u-sponge technology

nanofaber

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DISCLOSURE

Antonio Rinaldi is a co-founder of Nanofaber srl. All results have been produced by third parties funded by public sources

ACKNOWLEDGEMENT & FUNDING

- We acknowledge the support of LAZIO REGION in the scope of funded project "**MIRA - Nuova terapia "microsponge" per il trattamento intra-articolare adiuvante dell'artrite reumatoide e delle malattie reumatiche croniche secondo approccio 3P-medicine**" - bando "Progetti Di Gruppi Di Ricerca 2020" finanziato da Lazio Innova S.p.A. - fondi POR FESR Lazio 2014-2020, domanda n. PROT. A0375-2020- 36793 - Avviso Pubblico "Gruppi di ricerca 2020" - POR FESR Lazio 2014-2020 - Azione 1.2.1 - approvato con Determinazione n. G08487 del 19/07/2020- pubblicato sul BURL N.93 del 23/07/2020 - modificato con Determinazione n. G10624/2020- pubblicato sul BURL n. 116 del 22/09/2020.
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- We acknowledge the support of EIT-HEALTH through ULAB and "LIVING-LAB a TEST BEDS" grants

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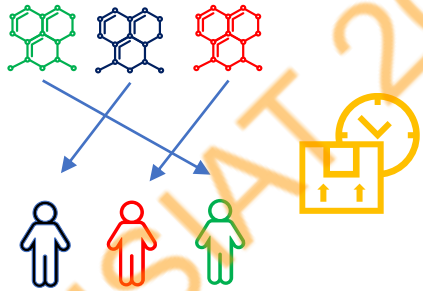
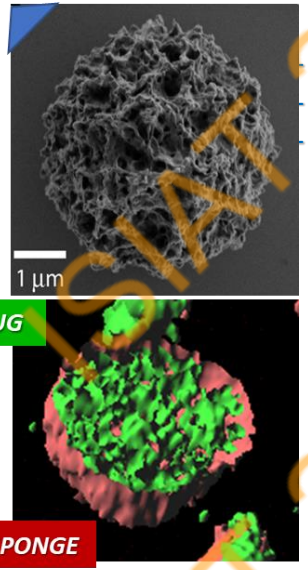
***MICROSPONGE** is our patented <u>universal</u> drug-delivery platform (DDP) to boost the transition to #PrecisionMedicine … starting from slow-delivery therapies for #arthritis*



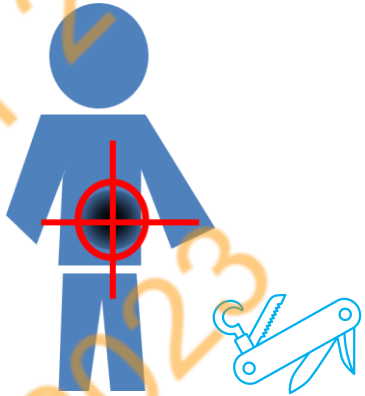
The right DRUG for the right PATIENT at the right TIME



SAFE CARRIER
EFFECTIVE LOADING
UNIVERSAL

Patented Technology
100% Nanofaber

SOON AVAILABLE IN GMP PHARMA GRADE



A MARKET OF:
Hundreds of millions patients
Hundred of billions €/year

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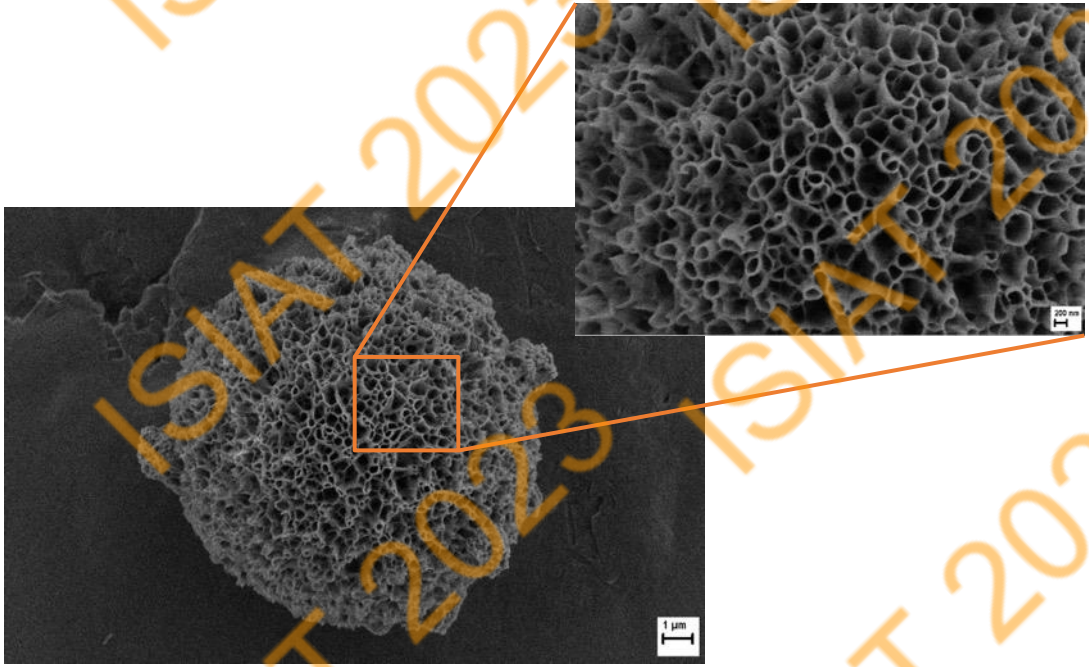
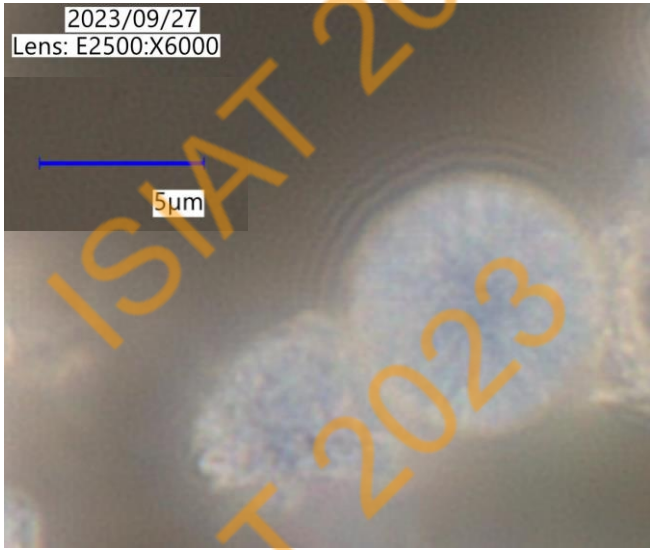
MICROSPONGE DRUG DELIVERY PLATFORM



Scanning electron microscopy (SEM)



High resolution optical microscopy (LOM)

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PRIOR EPISODES ….

Lisbon 3-5 October 2019

Krakow 7-9 October 2021

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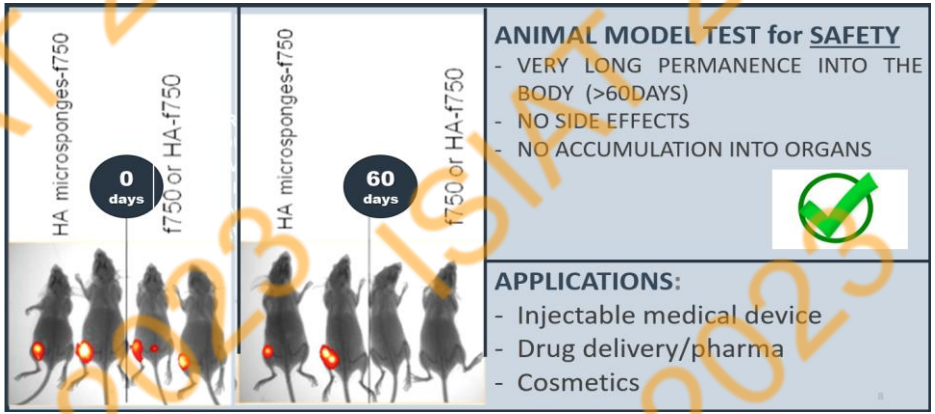
PRIOR EPISODES …. EPISODE 1

Lisbon 3-5 October 2019

FUNDAMENTALS

- LONG RESIDENCY TIME AFTER IA- INJECTION
- SAFETY



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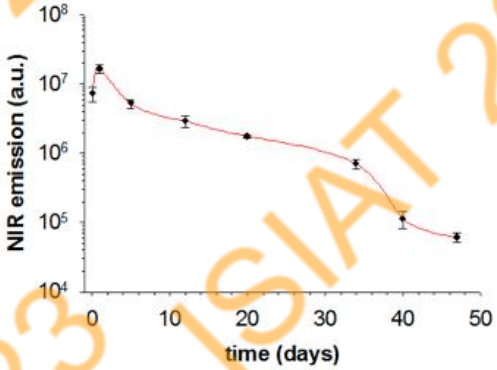
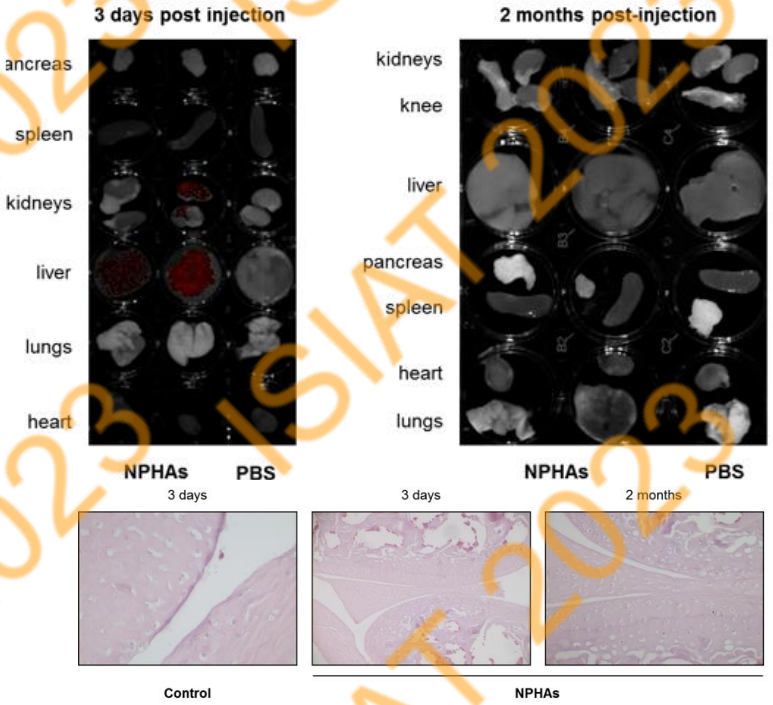
PRIOR EPISODES …. EPISODE 1





SAFETY:

- *No accumulation of sponges in organs*
- *No damage in cartilage*

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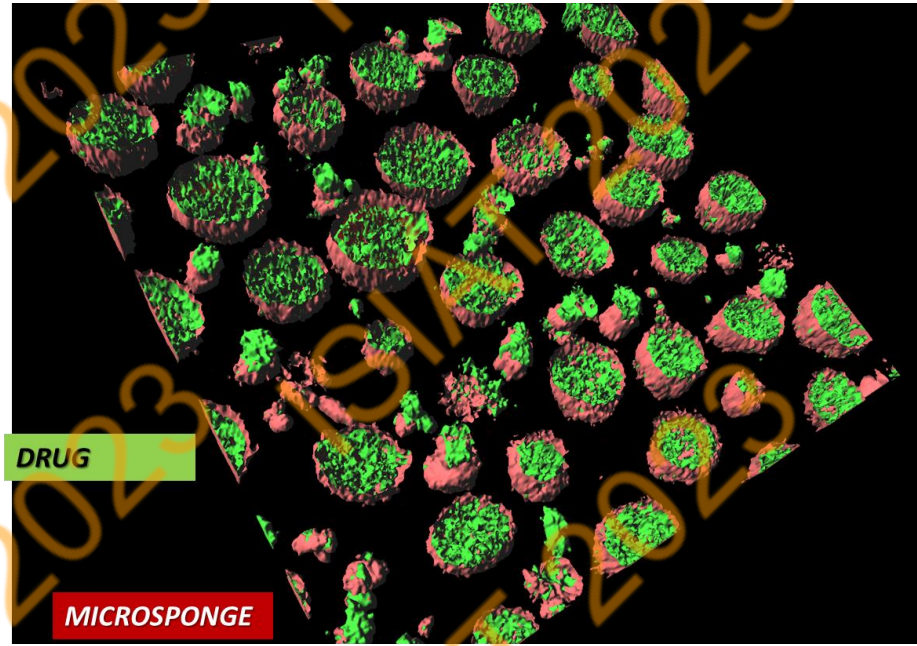
PRIOR EPISODES …. EPISODE 2

Krakow

7-9 October 2021

ADVANCES ON WHAT HAS HAPPENED AND WHERE MICROSPONGE IS HEADING (IN CRD)

- NEW CHEMICAL FORMULATIONS
- DRUG-LOADING PROFILES
- IN-VITRO STUDIES: TOXICOLOGY AND PATHWAYS FOR RHEUMATOID ARTHRITIS (RA)



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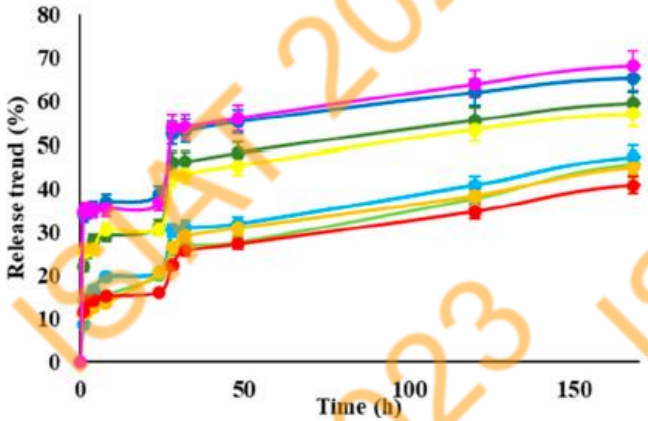
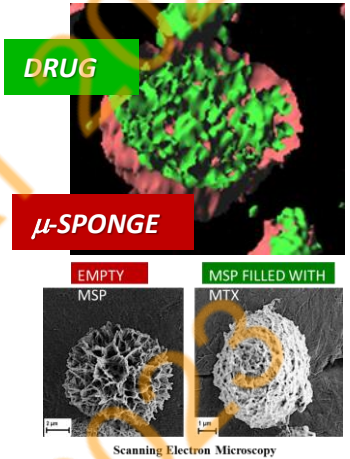
PRIOR EPISODES …. EPISODE 2

Krakow
7-9 October
2021

MICROSPONGE CAN BE LOADED WITH DRUGS

Proof of LOADING (in a few hours) -> RELEASE (in a few days)





Microponge release trend— Protein percent release of ***Lysozyme:*** *CM-dextran in light green, hyaluronic acid in light blue, alginate in orange, dextran in red.*
BSA: *CM-dextran in dark green, hyaluronic acid in blue, alginate in yellow, dextran in pink*

Microsponge can be used for drug slow delivery

Ref. Nanomaterials **2020**, 10, 1075; doi:10.3390/nano10061075

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PRIOR EPISODES …. EPISODE 2

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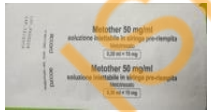
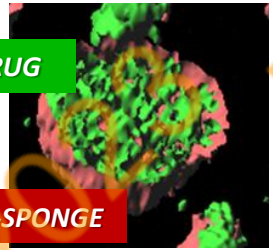
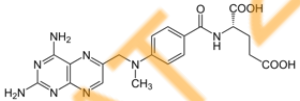
2021

METHOTREXATE -LOADING



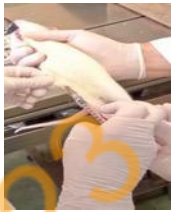


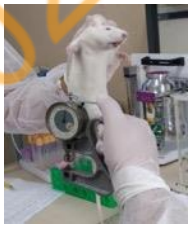


MW : 454.44 g/mol

- ***IN-VITRO* STUDIES**

- ***IN-VIVO* STUDIES**





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THIS PRESENT EPISODE ….

- FOCUS ON RESULTS FOR RA FROM BOTH
  - SUBCUTANEUS TREATMENT
  - INTRA-ARTICULAR TREATMENT
- ABOUT APPLICATION FOR OA
- TECHNOLOGICAL IMPROVEMENT & PERSPECTIVE

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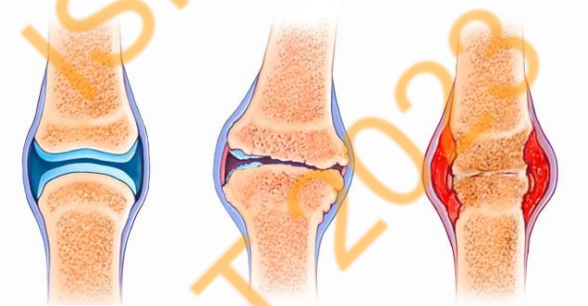

HEALTHY OSTEOARTHRITIS RHEUMATOID ARTHRITIS

The Case of Rheumatoid Arthritis (RA)

PROBLEM

- Chronic SYSTEMIC diseases requiring **lifelong management**
- No universal treatment
- >10k€/year of care … forever
- Severe autoimmune pathology

RA Market worth 27bn yearly by 2027-2030

PAIN: no long-term accessible, sustainable, management solution

For patients & society

- Low Quality of Life
- No long-term management
- Serious Side effects
- Boost Affordability

For Pharma (our customers)

- Innovate and evolve 4 Circular Economy
- Liability & reduction of side effects
- Manage Precision Medicine Transition
- Reduce Costs

-> high willingness to pay for a slow-delivery, effective, safe drug delivery platform

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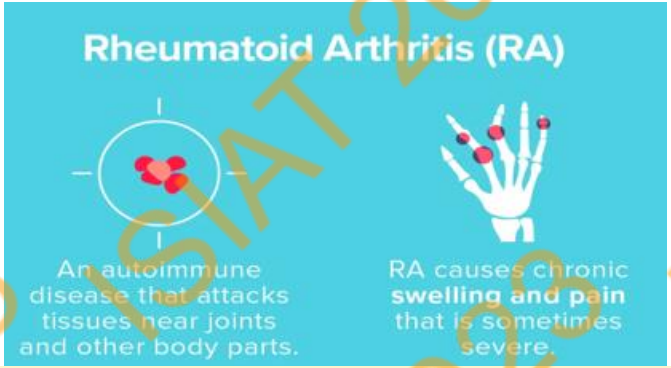
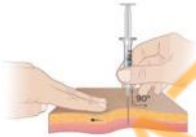
RHEUMATOID ARTHRITIS

is a painful autoimmune and/or inflammatory conditions, rheumatic diseases cause the immune system to attack a person's joints, muscles, bones, connective tissue, or organs





PATIENT PAIN: Chronic condition requiring long-term disease management with disability or limited or no regression. RA uses DMD & oncological drug at system level

NEED: personalized medicine with low dosage and controlled drug release

[1] https://www.healthline.com/health/rheumatoid-arthritis/facts-statistics-infographic#1

[2] https://www.everydayhealth.com/rheumatoid-arthritis/rheumatic-disease-awareness-month/

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1° INVESTIGATION

PRE-Clinical study **Subcutaneous (SB) Treatment**

2 end-points:

- SAFETY
- EFFICACY: non-inferiority or superiority?

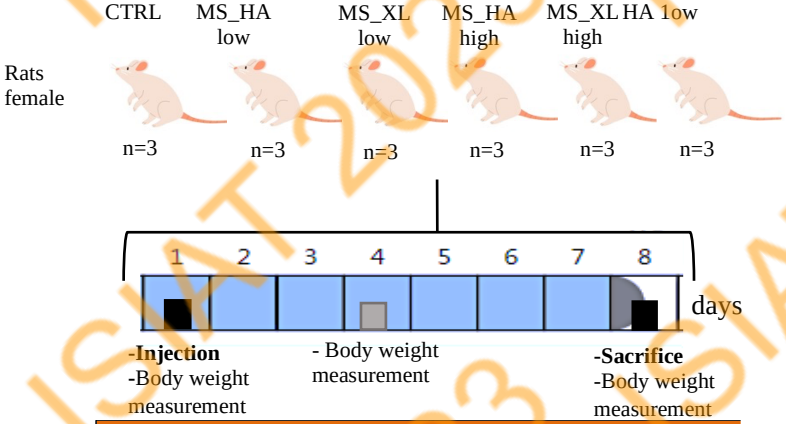
2° INVESTIGATION

PRE-Clinical study **adjuvant Intra-articular (IA) Treatment**

1 end-point:

- EFFICACY: innovative and non-clinical treatment

TOXICOLOGY STUDY: EXPERIMENTAL SCHEME



MICROSPONGE TOXICOLOGY STUDY (Rats)					
EXPERIMENTAL DESIGN					
Groups	*Groups Name*	*Dose Treatment*	*N° Animals*	*male*	*female*
G1	CTRL	-	3	0	3
G2	MS_HA low	1 mg	3	0	3
G3	MS-XL low	0,75 mg	3	0	3
G4	MS_HA high	5 mg	3	0	3
G5	MS-XL high	3,75 mg	3	0	3
G6	HA low	0,25 mg	3	0	3
			18	0	*18*

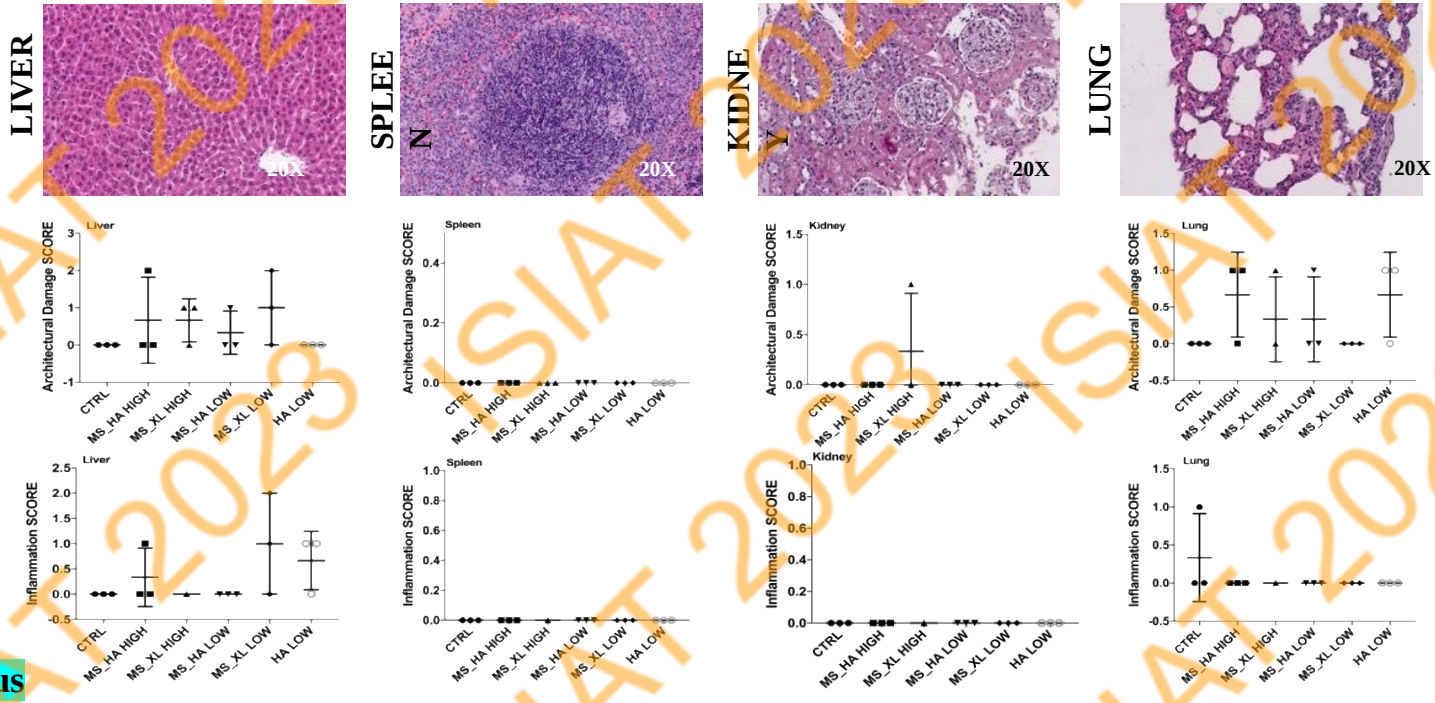
subcutaneous

RESULTS:

- No significant alteration in Hematological Analysis (data not shown)
- No significant alteration in Biochemical Analysis (data not shown)

HISTOPATHOLOGICAL EXAMINATION

RESULTS: No significant damage in histology



subcutaneous

***IN-VIVO* STUDIES**

1° INVESTIGATION

PRE-Clinical study **Subcutaneous (SB) Treatment**

2 end-points:

- SAFETY ✅
- EFFICACY: non-inferiority or superiority?

2° INVESTIGATION

PRE-Clinical study **adjuvant Intra-articular (IA) Treatment**

1 end-point:

- EFFICACY: innovative and non-clinical treatment

IN VIVO MICROSPONGE SUBCUTANEUS THERAPY FOR RA : EXPERIMENTAL SCHEME

SUBCUTANEOUS INJECTION OF MICROSPONGES

SUBCUTANEOUS MICROSPONGE & RHEUMATOID ARTHRITIS (Rats)						
EXPERIMENTAL DESIGN						
Groups	*Groups Name*	*MS Dose*	*METO Dose*	*N° Animals*	*male*	*female*
G1	CTRL	-	-	4	2	2
G2	MS	13 mg	-	4	2	2
G3	METO	-	0,125 mg	4	2	2
G4	ME+METO	13 mg	0,125 mg	4	2	2
G5	MSP+METO_LIOF	13 mg	0,125 mg	4	2	2
				20	10	10

RA Induction T0: Collagen II IFA

Boosting T7: Collagen II IFA

T14: MSP_SB treatment

T21: MSP_SB treatment

T28: MSP_SB treatment

T35: Sacrifice by CO_2

[Thimus, Spleen and Ankle Joints were collected]

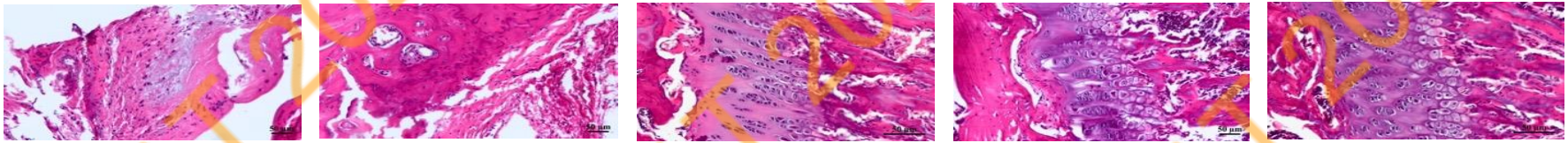
subcutaneous

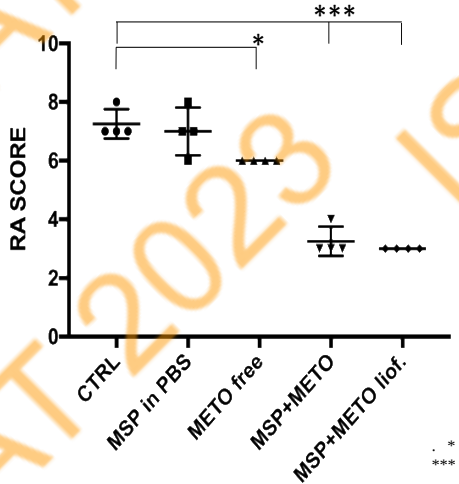
IN VIVO MICROSPONGE SUBCUTANEUS THERAPY FOR RA: EXPERIMENTAL SCHEME

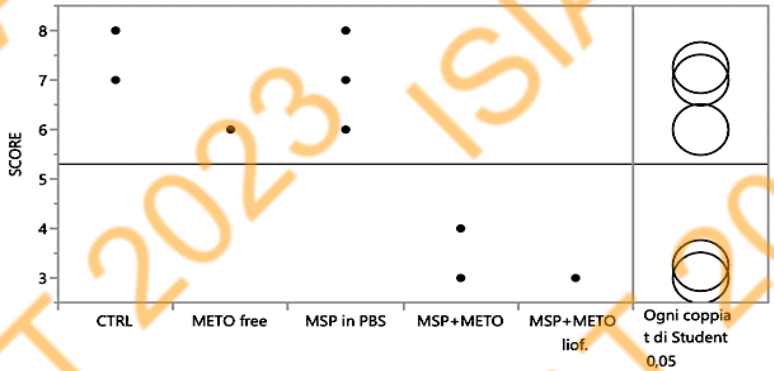
Histological differences between the groups: MSP+METO improved clinical arthritic conditions in rats

knee joint

CTRL | MSP in PBS | METO free | MSP+METO | MSP+METO liof.







subcutaneous

. * p < 0,05
*** p < 0,001

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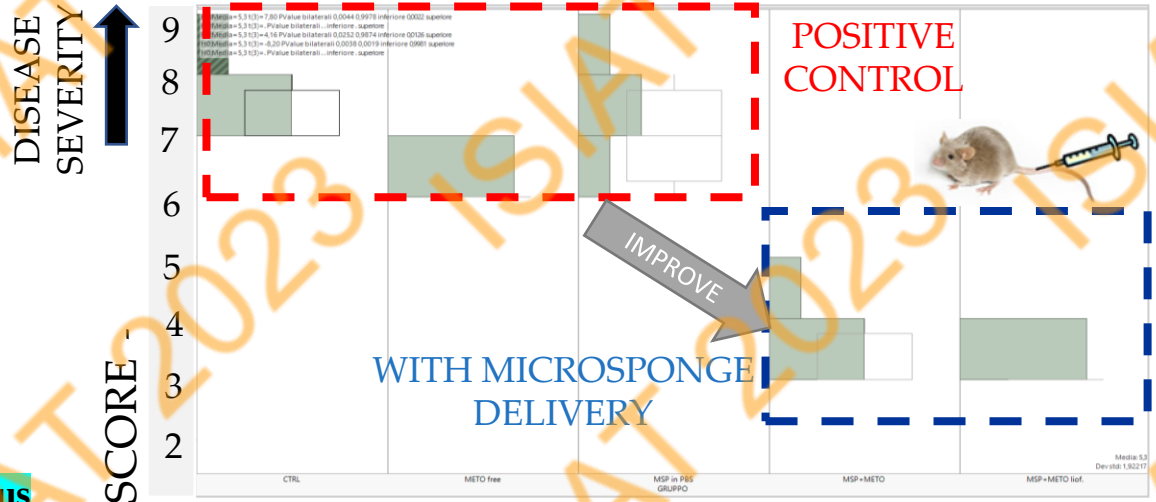
RESULTS

	Score
Control : Rat with severe RA & no therapy	8
Free DRUG therapy	6
DRUG in MICROPONGE therapy	3

>100% improvement
in therapeutic efficacy
(histology scoring)

REPLICATED 3 TIMES



subcutaneous

***IN-VIVO* STUDIES**

1° INVESTIGATION

PRE-Clinical study **Subcutaneous (SB) Treatment**

2 end-points:

- SAFETY ✓
- EFFICACY: superiority ✓

2° INVESTIGATION

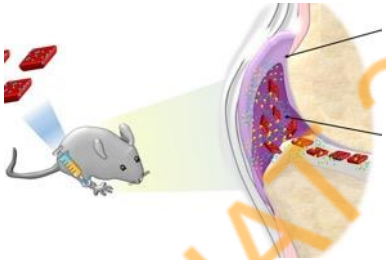
PRE-Clinical study **adjuvant Intra-articular (IA) Treatment**

1 end-point:

- EFFICACY: innovative and non-clinical treatment

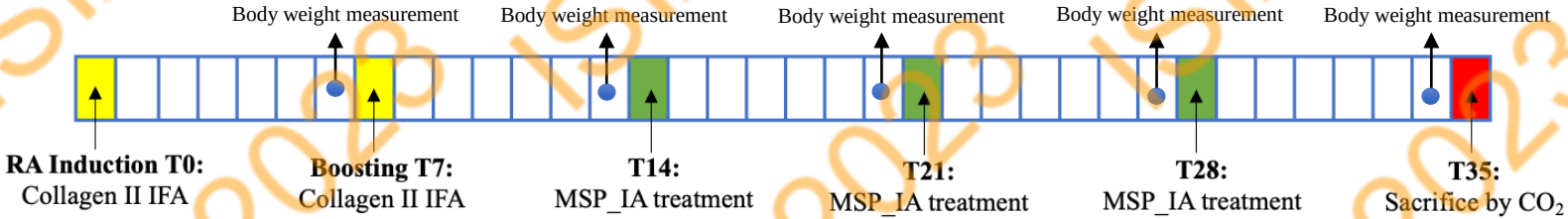
IN VIVO MICROSPONGE IA THERAPY FOR RA: EXPERIMENTAL SCHEME

Left back knee joint



INTRA-ARTICULAR INJECTION OF MICROSPONGES

INTRA-ARTICULAR MICROSPONGE & RHEUMATOID ARTHRITIS (Rats)						
EXPERIMENTAL DESIGN						
Groups	*Groups Name*	*MS Dose*	*METO Dose*	*N° Animals*	*male*	*female*
G1	CTRL -	-	-	3	3	0
G2	CTRL +	-	-	4	4	0
G3	MS-HA_IA	1 mg	-	4	4	0
G4	FREE METO_IA	-	0,125 mg	4	4	0
G5	MS-HA+METO_IA	1 mg	0,125 mg	4	4	0
G6	MS-HA + METO LIOF._IA	1 mg	0,125 mg	4	4	0

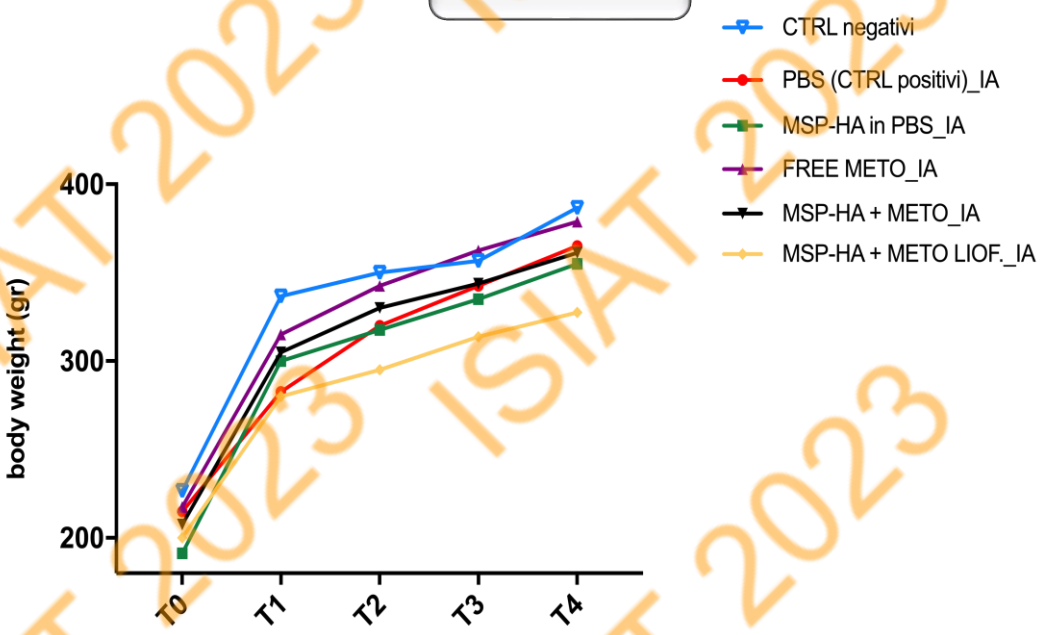


[Serum, Fecal Samples, Thimus, Spleen, Ankle Joints, Liver, Gut, Kidney, Lung, Stomach and Bladder were collected]

intra-articular

IN VIVO MICROSPONGE IA THERAPY FOR RA: RESULTS

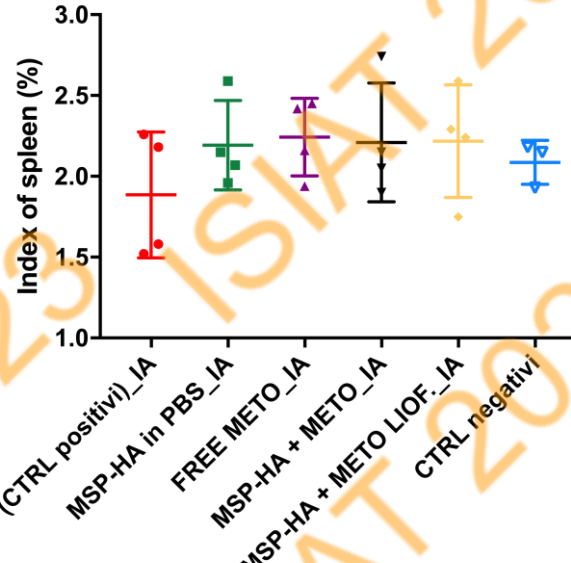
- No significant difference was observed in the **body weight** →
- No significant alteration in **Hematological Analysis** (data not shown)
- No significant alteration in **Biochemical Analysis** (data not shown)

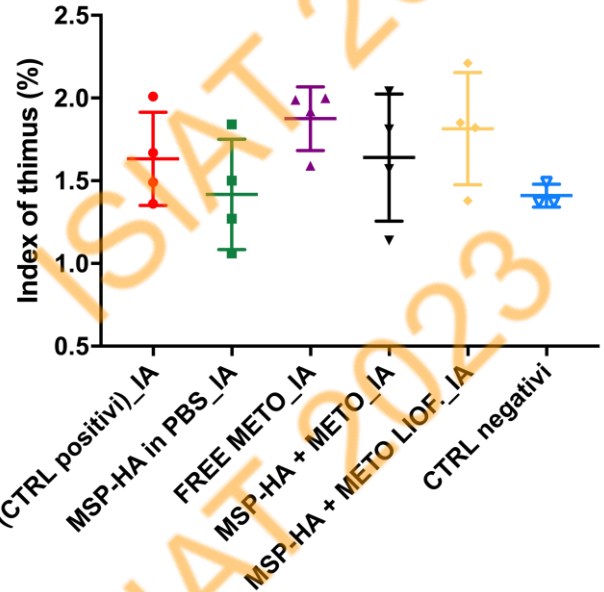


intra-articular

IN VIVO MICROSPONGE IA THERAPY FOR RA: RESULTS

No Modification of the Spleen and Thymus Index of Rats





intra-articular

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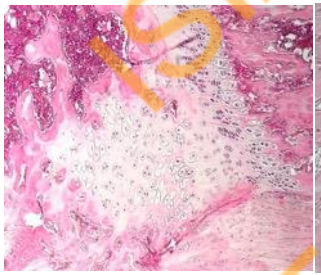
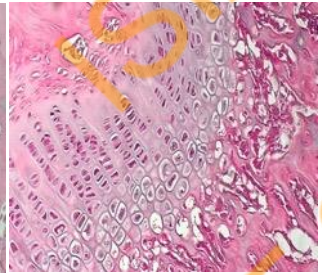
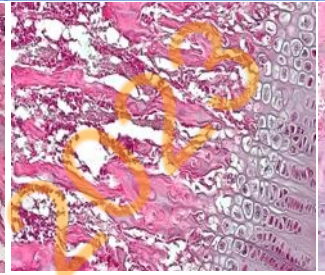
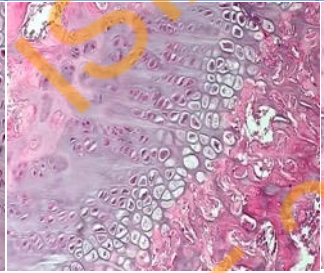
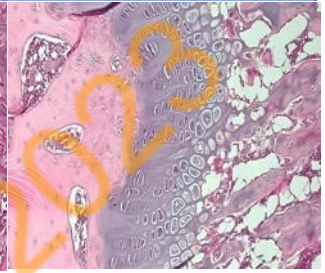
IN VIVO MICROSPONGE IA THERAPY FOR RA:

knee joint

RESULTS

Histological differences between the groups were found

We also found BILATERAL treatment from single sided IA therapy

CTRL +	CTRL -	MSP_IA	FREE METO_IA	MSP+METO_IA	MSP+METO liof._IA
					

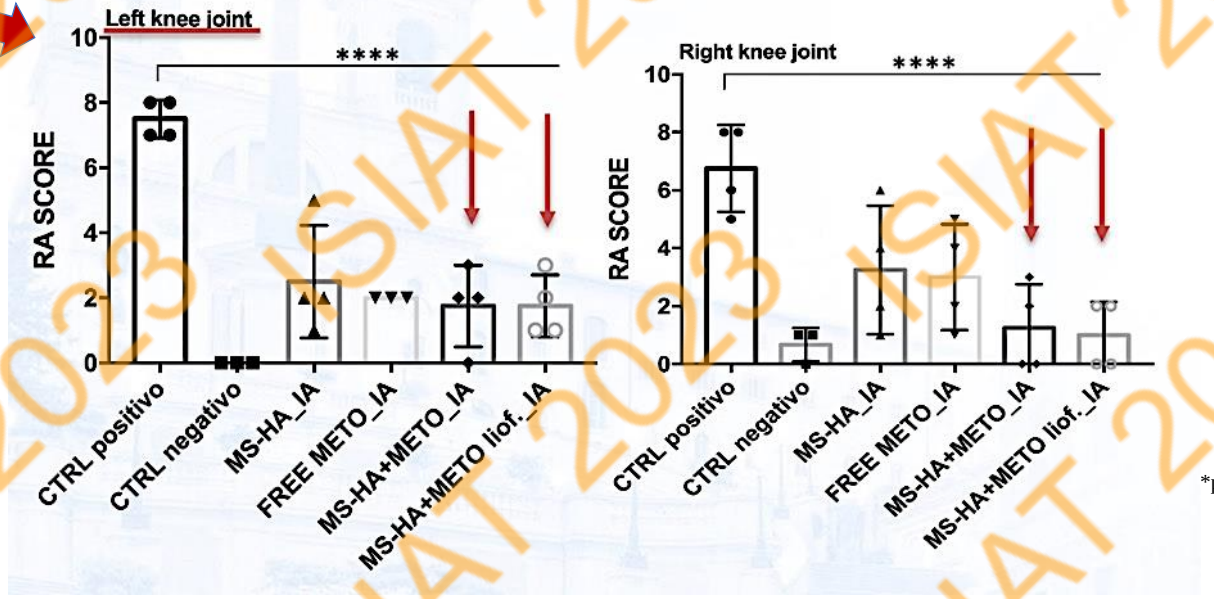
intra-articular

IN VIVO MICROSPONGE IA THERAPY FOR RA:

RESULTS

More significant reduction of RA score was observed in groups treated with MSP+METO liof.

Treated knee



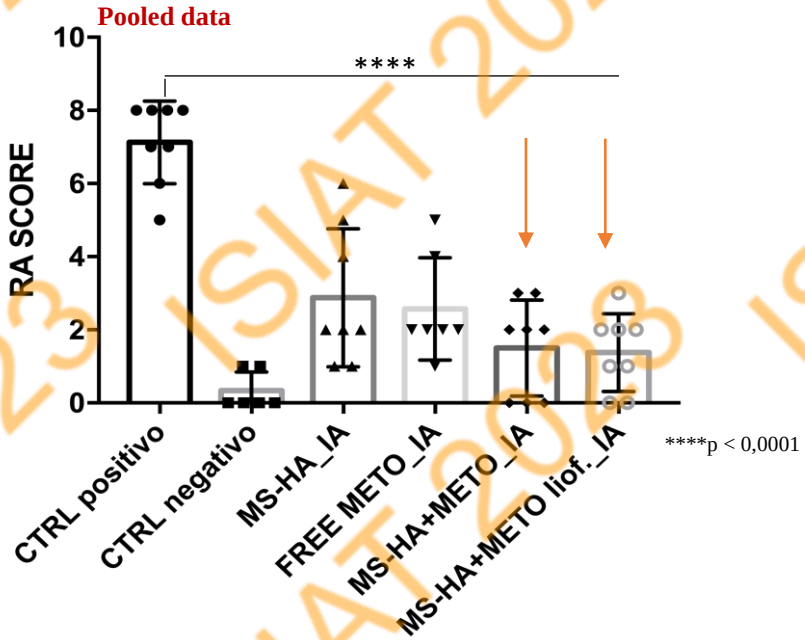
*p < 0,0001

intra-articular

IN VIVO MICROSPONGE IA THERAPY FOR RA:

RESULTS

We obtain a trend very similar to subcutaneous



Where does this make sense?

It provides a basis for ADJUVANT IA Therapy of RA in large joints resistant to drug or juvenile RA

Athens

5-7 October 2023

***IN-VIVO* STUDIES**

1° INVESTIGATION

PRE-Clinical study **Subcutaneous (SB) Treatment**

2 end-points:

- SAFETY ✓
- EFFICACY: superiority ✓

2° INVESTIGATION

PRE-Clinical study **adjuvant Intra-articular (IA) Treatment**

1 end-point:

- EFFICACY: innovative and non-clinical treatment ✓

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CONCLUSIONS FOR RA: NOW WE KNOW…

- **MSP have proved to be safe and non-toxic *in vivo studies. MSP* do not accumulate or alter the functioning of the organs;**

- **Slow delivery of DISEASE MODIFYING DRUGS can be very effective to reduce dose or frequency → higher quality of life**

- **Using our platform to administer MTX (SuBcutaneously and IntraArticular) decreases significantly RA score compared to the drug alone. This is allowed by a prolonged, but slower release of the drug from our system.**

IA -adjuvant therapy for RA

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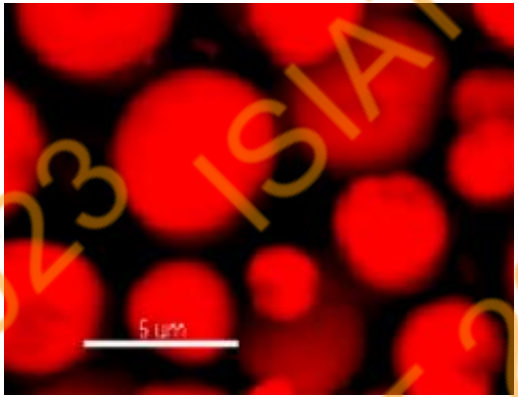
OSTEOARTHRITIS

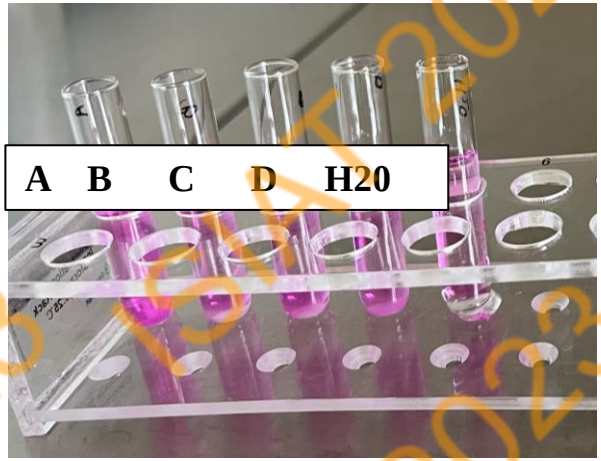
ONGOING PRECLINICAL TRIALS

- **Focus on slow-delivery of a HMW linear hyaluronic acid (800KDa), as well as API and biomolecules such as peptides**



TECHNOLOGICAL WORK

- **MSP samples: Enodtoxin free**



- **GMP Batches available in 6-9 months**

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THANK YOU

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Italian National Agency for New Technologies, Energy and Sustainable Economic Development

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Alginate Microsponges as a Scaffold for Delivery of a Therapeutic Peptide against Rheumatoid Arthritis

by Daniela Ariaudo [1], Francesca Cavalieri [1], Antonio Rinaldi [2,3], Ana Aguilera [4], Matilde Lopez [4], Hilda Garay Perez [5], Ariel Felipe [5], Maria del Carmen Dominguez [5], Odalys Ruiz [4], Gillian Martinez [6] and Mariano Venanzi [1,*]

FULL PAPER
Drug Delivery

Particle & Particle Systems Characterization
www.particle-journal.com

Hyaluronic Acid Nanoporous Microparticles with Long In Vivo Joint Residence Time and Sustained Release

Graziana Palmieri, Antonio Rinaldi, Luisa Campagnolo, Mariarosaria Tortora, Maria Federica Caso, Maurizio Mattei, Andrea Notargiacomo, Nicola Rosato, Massimo Bottini, and Francesca Cavalieri**

nanomaterials

Article

Nanoporous Microsponge Particles (NMP) of Polysaccharides as Universal Carriers for Biomolecules Delivery

Maria Federica Caso [1,†], Felicia Carotenuto [2,3,†], Paolo Di Nardo [2,3,4], Alberto Migliore [5], Ana Aguilera [6], Cruz Matilde Lopez [6], Mariano Venanzi [7], Francesca Cavalieri [7,*] and Antonio Rinaldi [1,8,*]