UNIVERSITÀ DI PAVIA
Department of Drug Sciences

From mesenchymal stem/stromal cells to cell products: pharmaceutical development of secretome as new biological medicinal product

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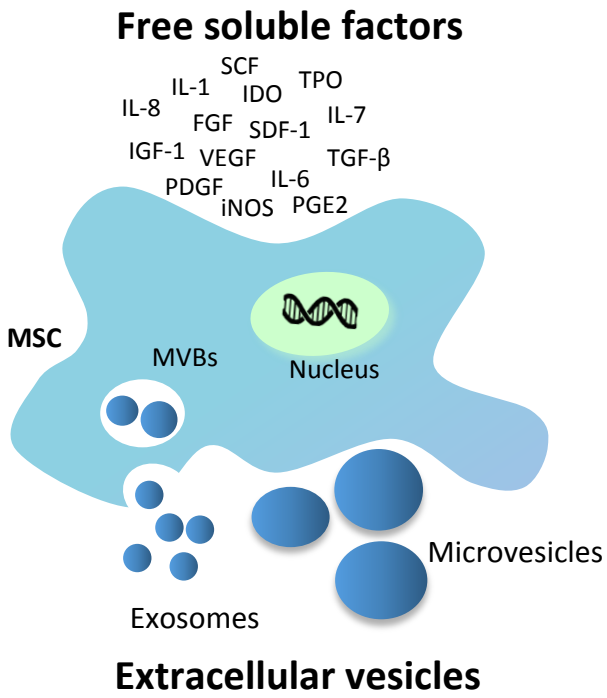
NanoInnovation 2020
Rome, September 17th, 2020

The challenge

To formulate **secretome** as a pure, solid, stable, well storable and standardized **nanostructured medicinal product**, to be used as a safe and effective substitute of MSCs in therapy



Quality

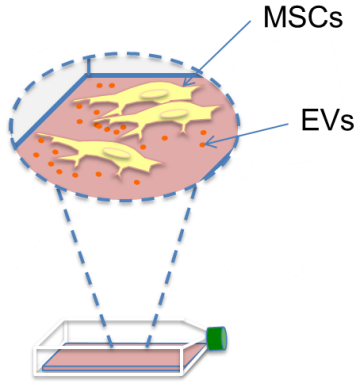
Safety

Efficacy

GOOD MANUFACTURING PRACTICE GMP

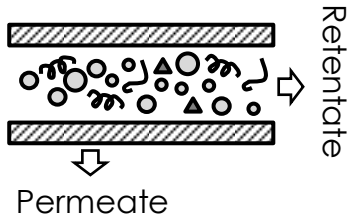
Industrial scale-up

Lyo-secretome production



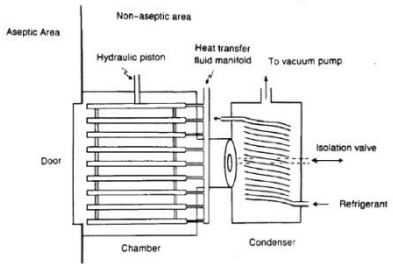
Serum starvation

Supernatant recovery



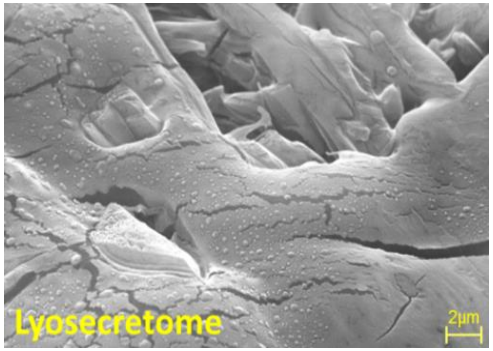
Ultrafiltration

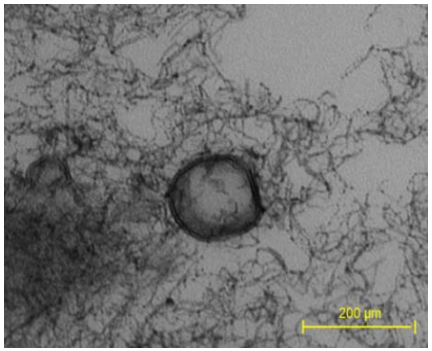
Formulation

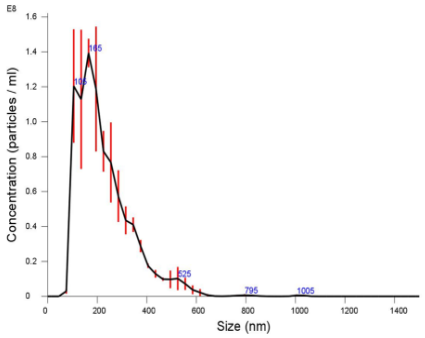


Freeze-drying

Total protein and lipid		Morphology and ultrastructure
Proteomic		Physical-chemical characterization
EVs sizing and quantification		Microbiological control

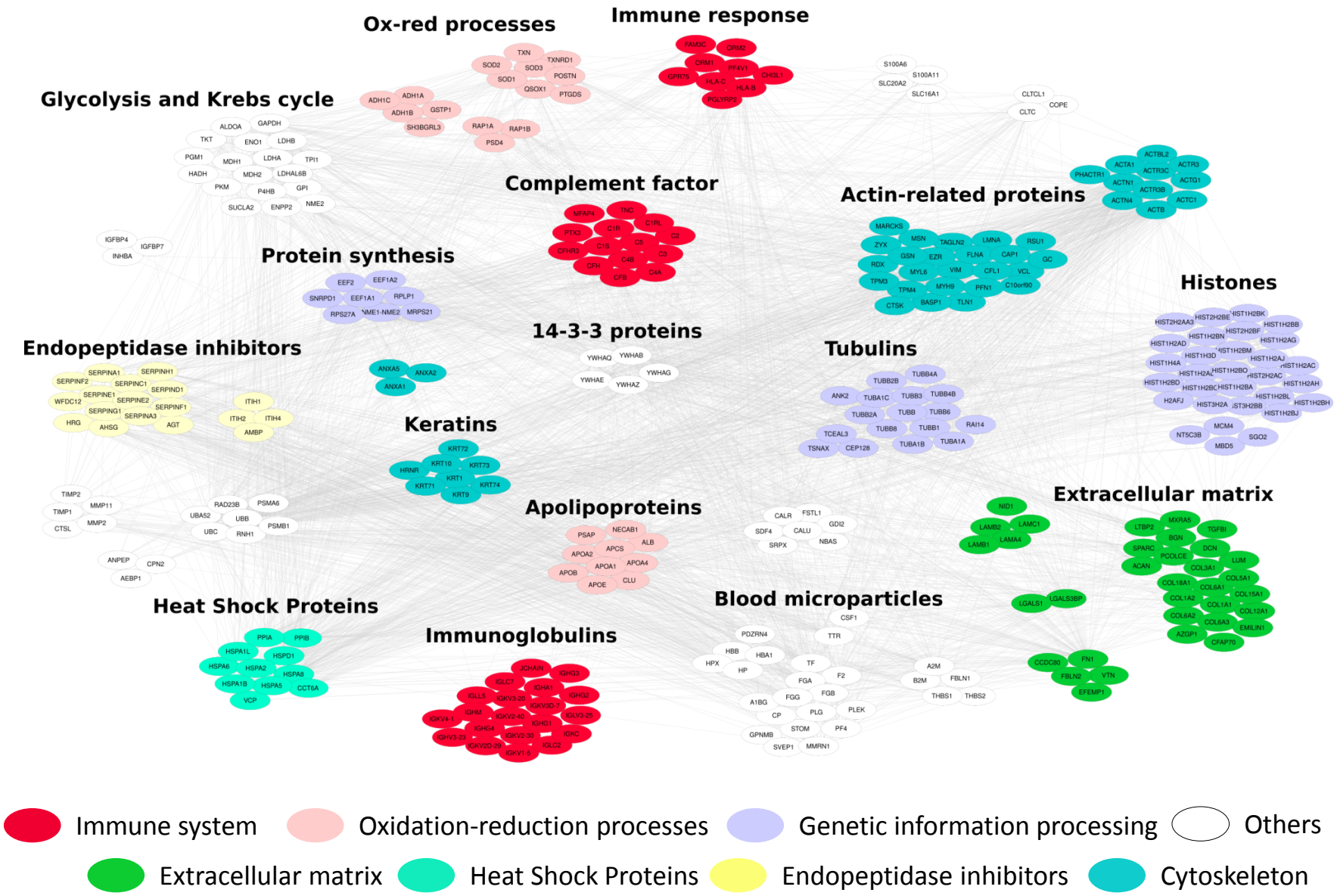






Bari et al. Cells 2018, 7, 190.
Perteghella, Bari et al. WO2018078524 (A1).

What are the Active Ingredients in lyo-secretome?



Protein-protein interaction network and functional classification of proteins identified by LC-MS/MS, Bari et al. Cells 2018, 7, 190.

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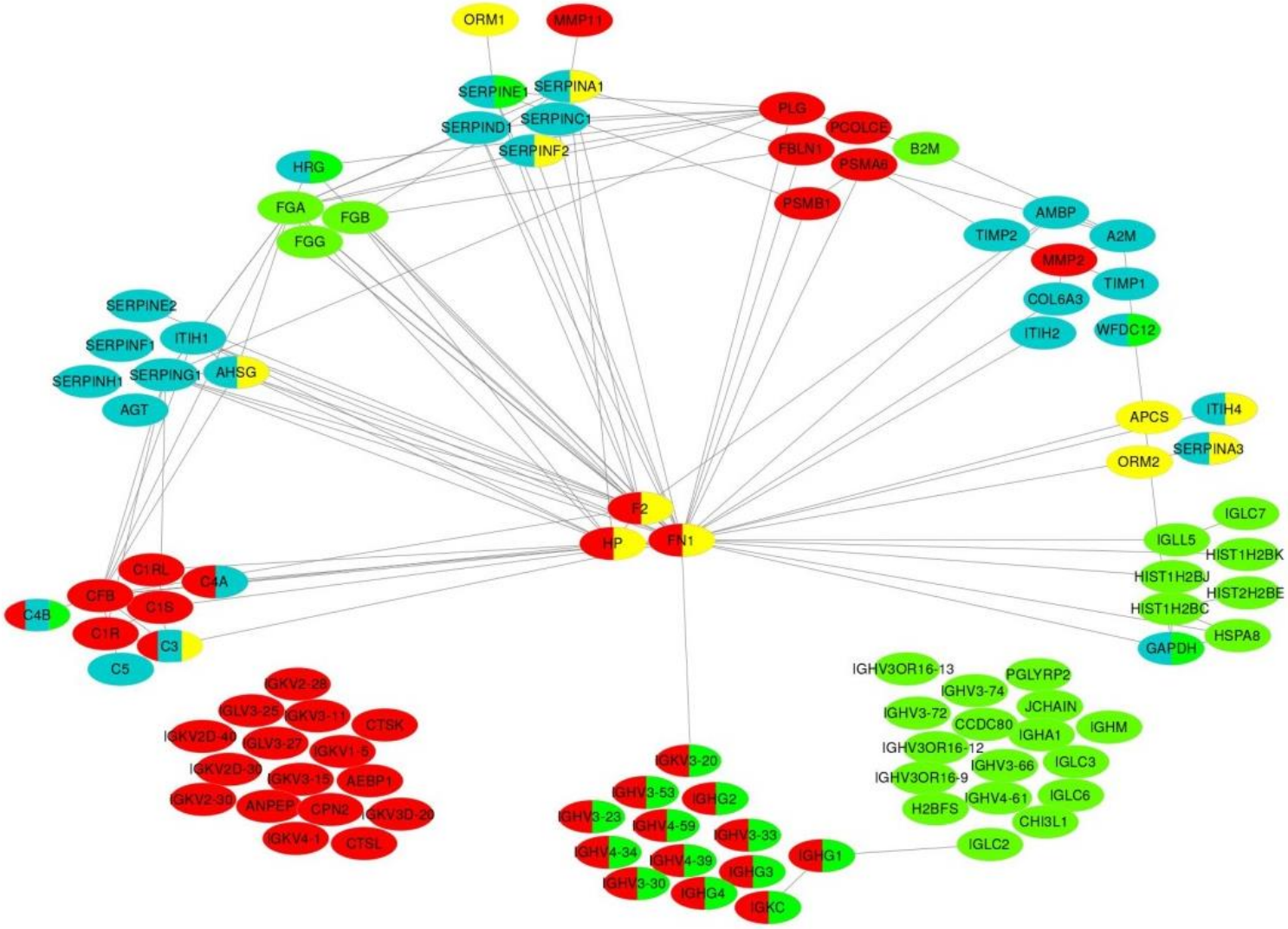
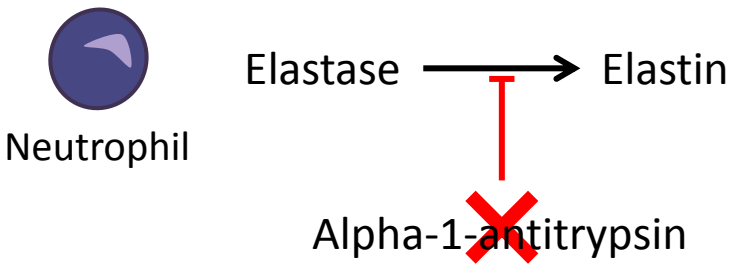
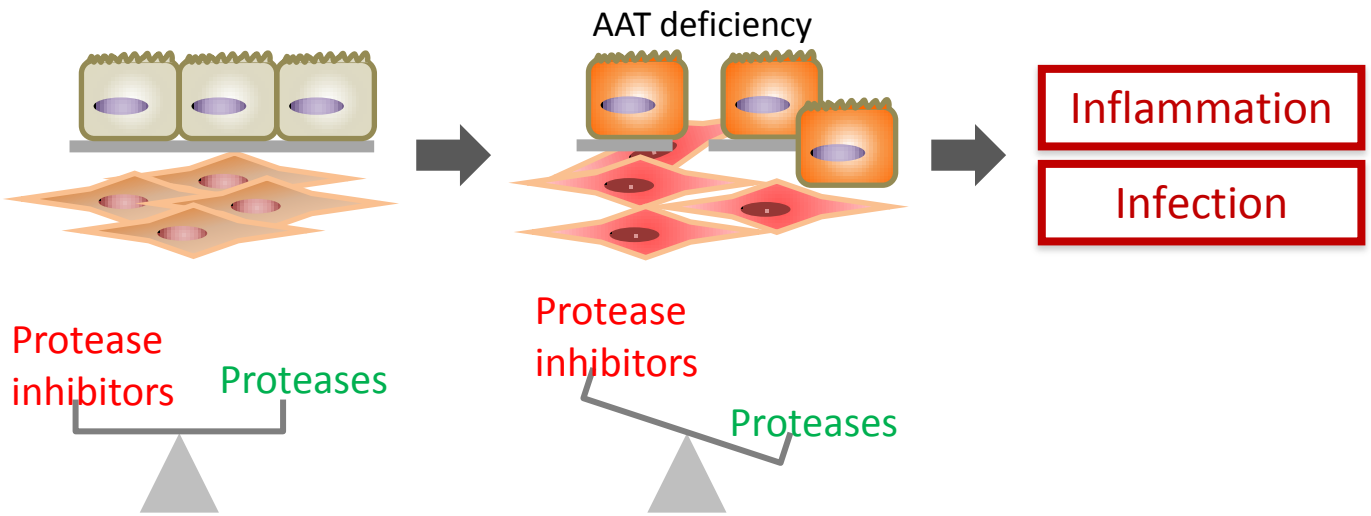


Protease activity Response to bacteria Acute-phase response Protease inhibitor

Protein-protein interaction network and functional classification of proteins identified by LC-MS/MS, Bari et al. Cells 2019, 8, 965.

Alpha-1-antitrypsin (AAT) deficiency



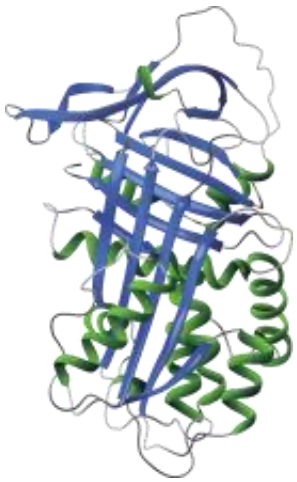


Therapeutic goals:

Protease inhibition | Antimicrobial action | Immunomodulation

Taggart et al. Eur. Respir. J. 2017, 49:1501200.
Bari et al. J. Control. Release 2019, 309, 11-24.

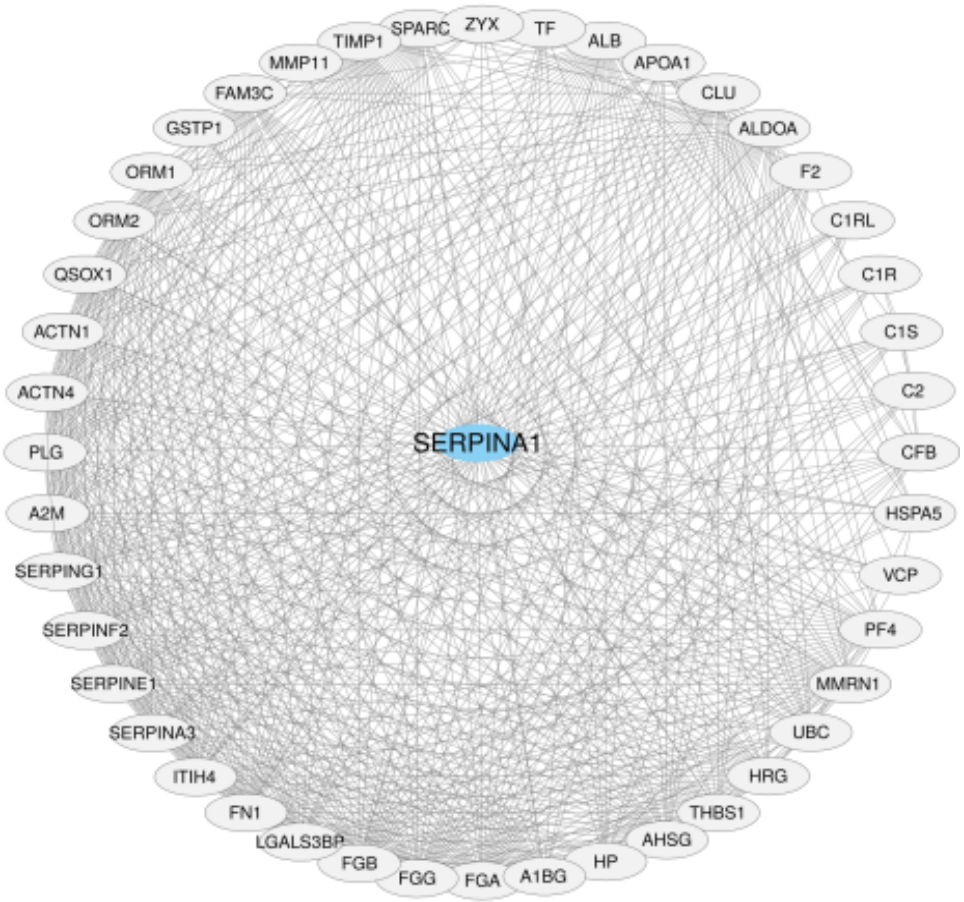
Alpha-1-antitrypsin (AAT) content in lyo-secretome



Alpha-1-antitrypsin (AAT), 52 kDa

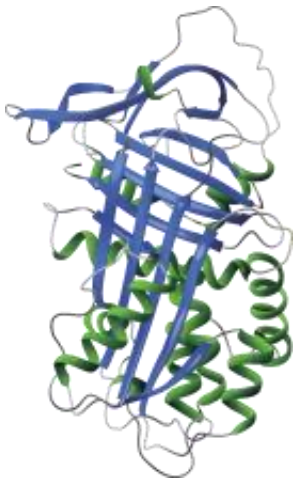
Lyo-secretome batch	AAT (µg/mg)
1	0.78 ± 0.06
2	0.83 ± 0.08

Mean ± St. Dev, n=3



Bari et al. Cells 2019, 8, 965.

Alpha-1-antitrypsin (AAT) content in lyo-secretome



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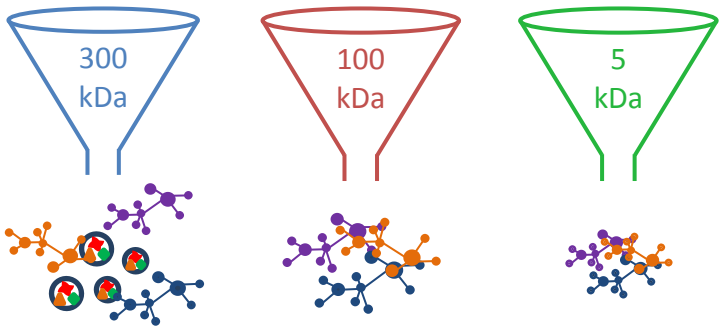
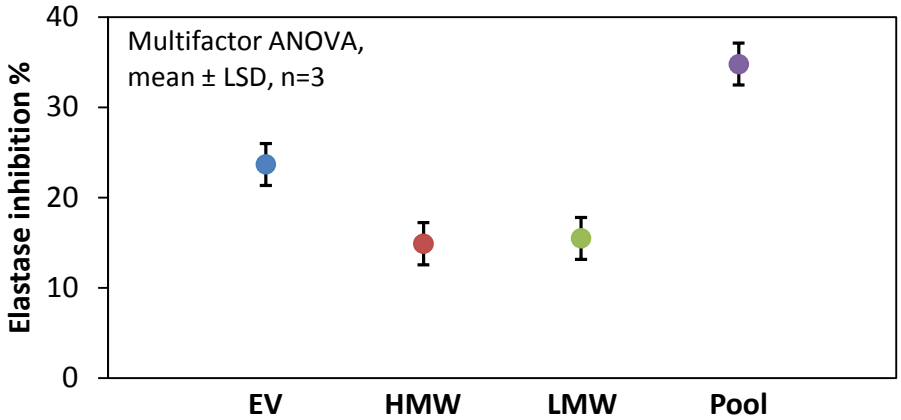
Mean ± St. Dev, n=3

MSC-secretome



	EV	HMW	LMW
Molecular weight (kDa)	> 300	100-300	5-100
AAT (µg/mg)	1.37 ± 0.25	0.16 ± 0.05	1.38 ± 0.64

Mean ± St. Dev, n=3



Bari et al. Cells 2019, 8, 965.

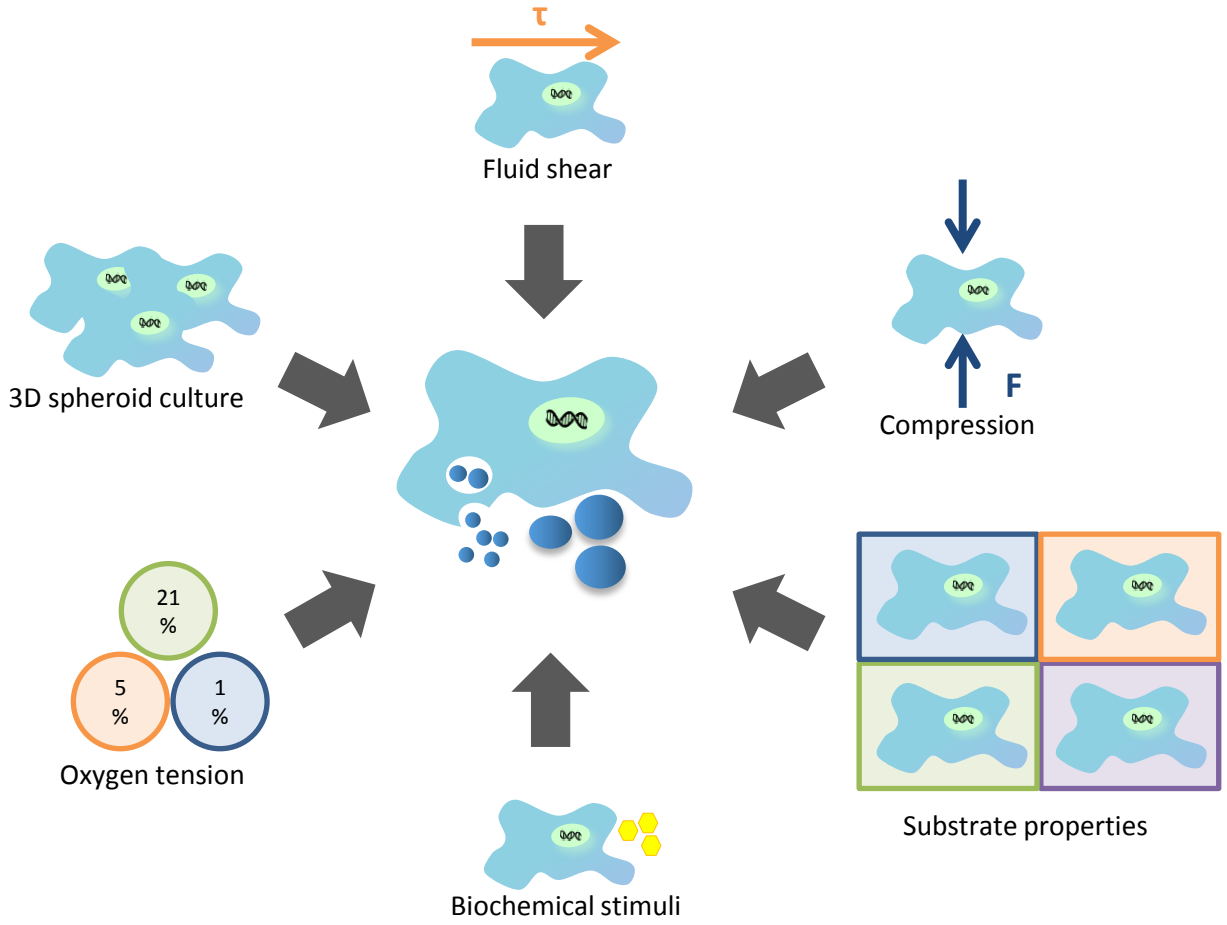
Can MSCs be “educated”?

Donor’s conditions

Cell culture conditions

Cell confluence level

Microenvironment

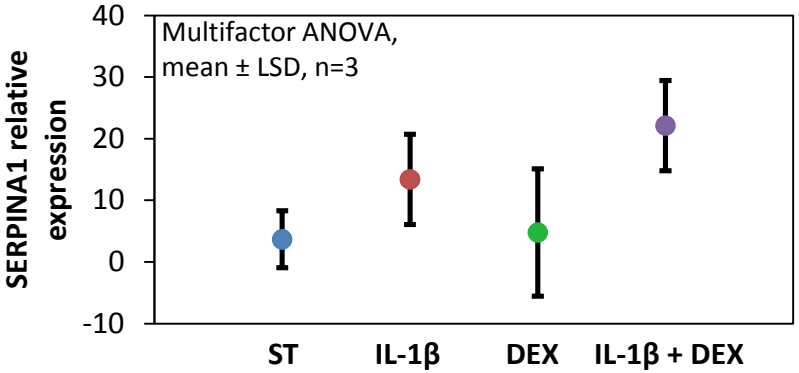


Bari et al. J. Control. Release 2019, 309, 11-24.

AAT gene expression and protease inhibition

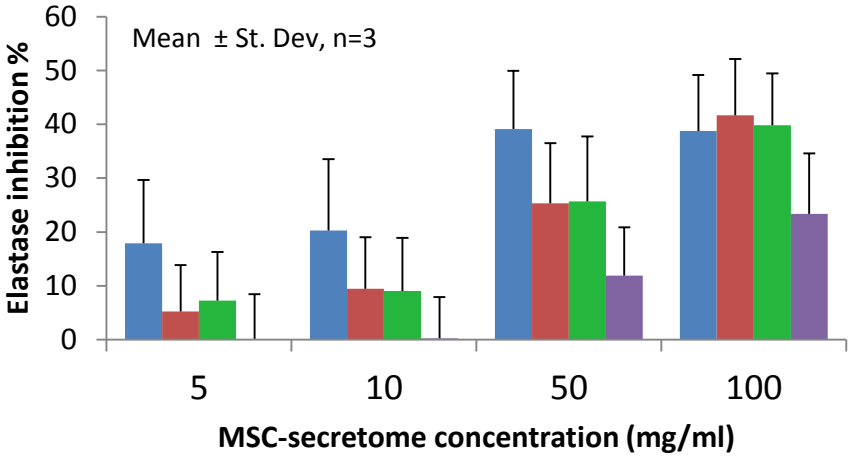
Biochemical stimuli

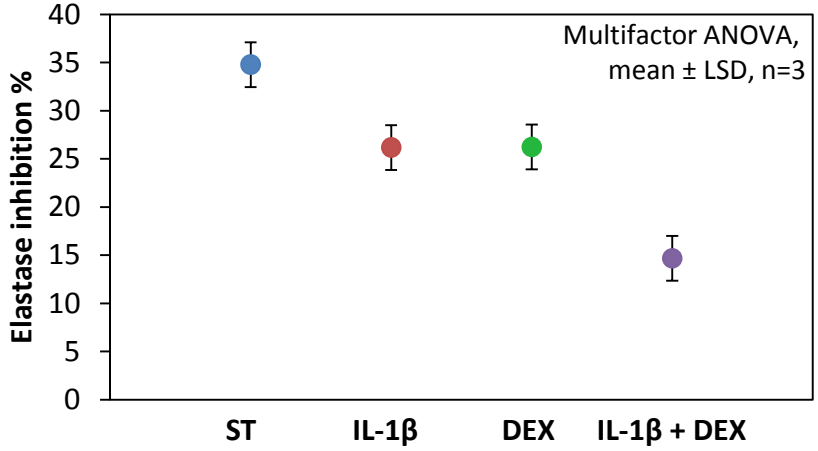
Serum starvation (ST) and IL-1β, Dexamethasone (DEX), DEX + IL-1β



Stimulating condition	Proteins (µg/mg)
ST	0.82 ± 0.09
IL-1β	0.93 ± 0.08
DEX	1.48 ± 0.31
IL-1β + DEX	0.86 ± 0.06

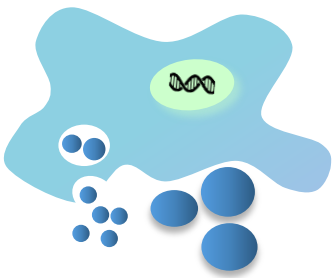
Mean ± St. Dev, n=3





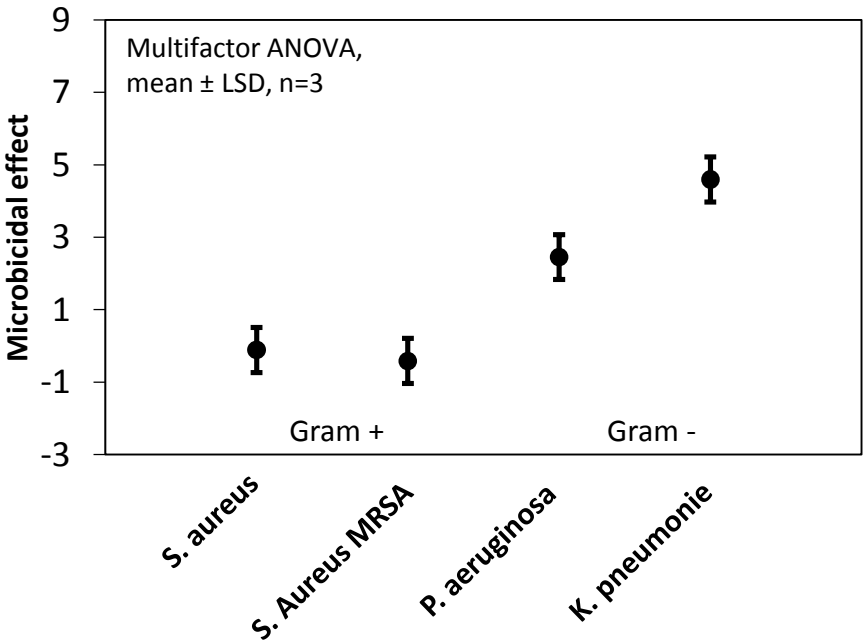
Bari et al. Cells 2019, 8, 965.

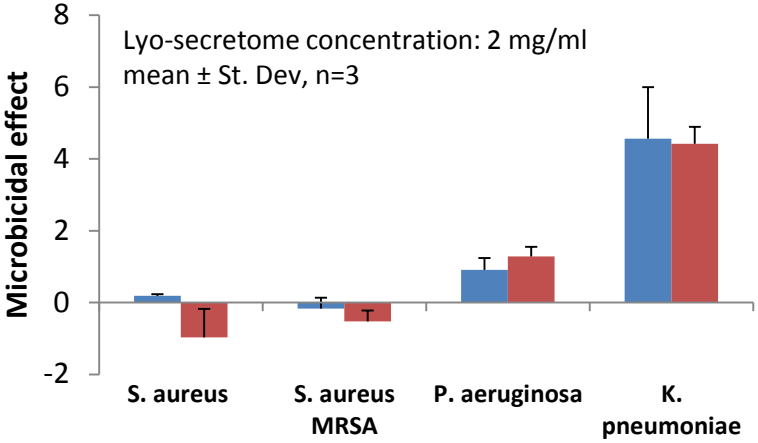
Lyo-secretome potency: antimicrobial activity

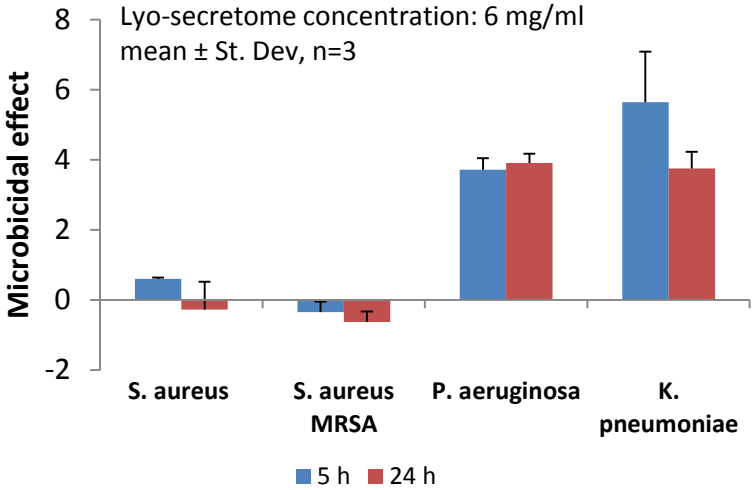


Immune-mediators
IL-6, IL-8, CSF

Antimicrobial peptides
β-defensin 2, Hepcidin, etc

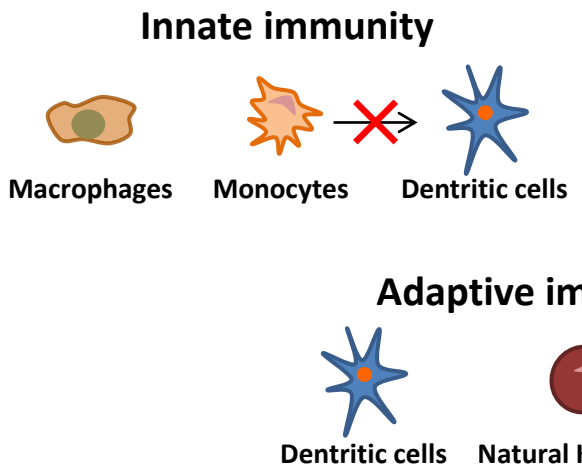






Bari et al. Cells 2019, 8, 965.

Lyo-secretome potency: immunomodulation



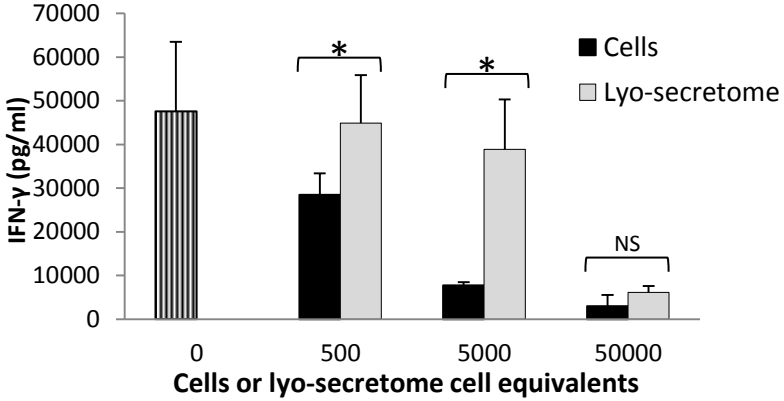
	IL-10 (µg/mg)	IFN-γ (µg/mg)	IL-6 (µg/mg)
Lyo-secretome	0.25 ± 0.118	<0.0012	>60

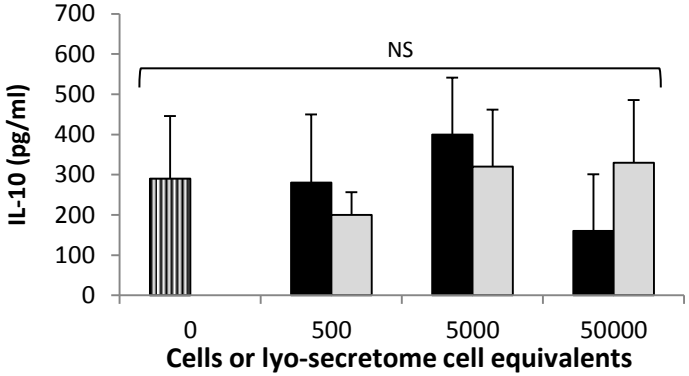
Mean ± St. Dev, n=3

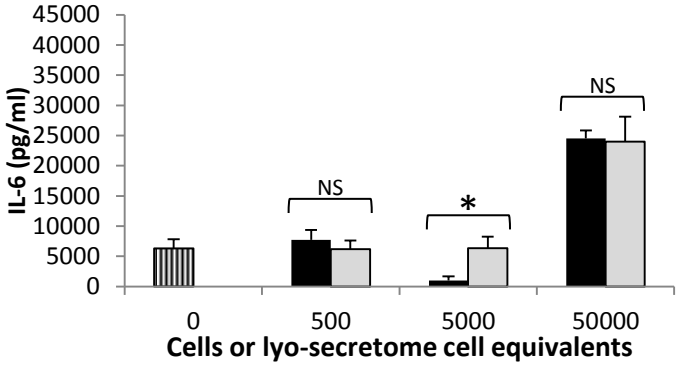
Nanomedicine

Freeze-dried and GMP-compliant pharmaceuticals containing exosomes for acellular mesenchymal stromal cell immunomodulant therapy

Elia Bari[1], Sara Perteghella*[,1,2], Laura Catenacci[1], Marzio Sorlini[2,3], Stefania Croce[4], Melissa Mantelli[5], Maria A Avanzini[5], Milena Sorrenti[1] & Maria L Torre[1,2]







Bari et al. Nanomedicine 2019.

Lyo-secretome for COVID-19

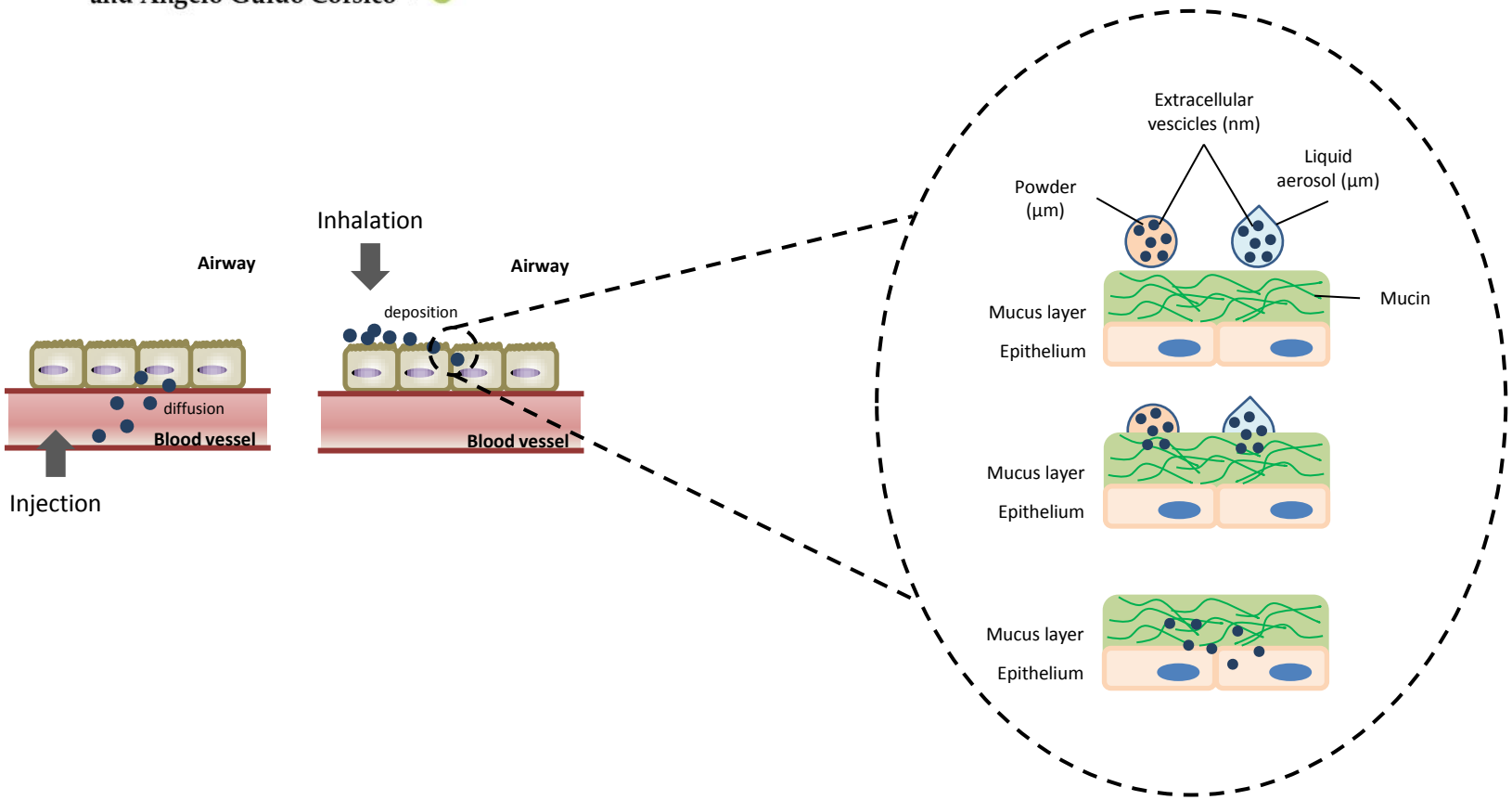
cells

MDPI

Perspective

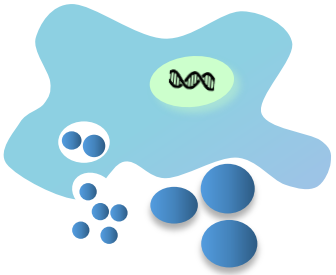
Mesenchymal Stromal Cell Secretome for Severe COVID-19 Infections: Premises for the Therapeutic Use

Elia Bari [1], Ilaria Ferrarotti [2], Laura Saracino [3], Sara Perteghella [1,4], Maria Luisa Torre [1,4,*] and Angelo Guido Corsico [2,4]



Bari et al. J. Control. Release 2019, 309, 11-24.

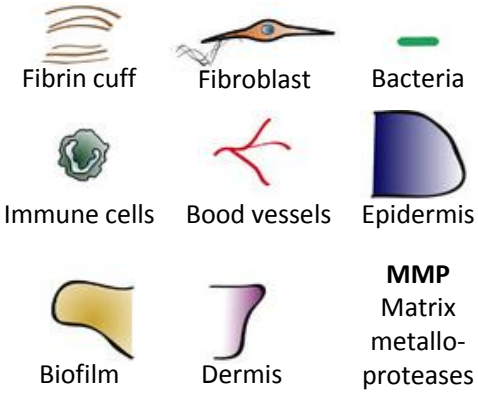
Lyo-secretome mediated regeneration

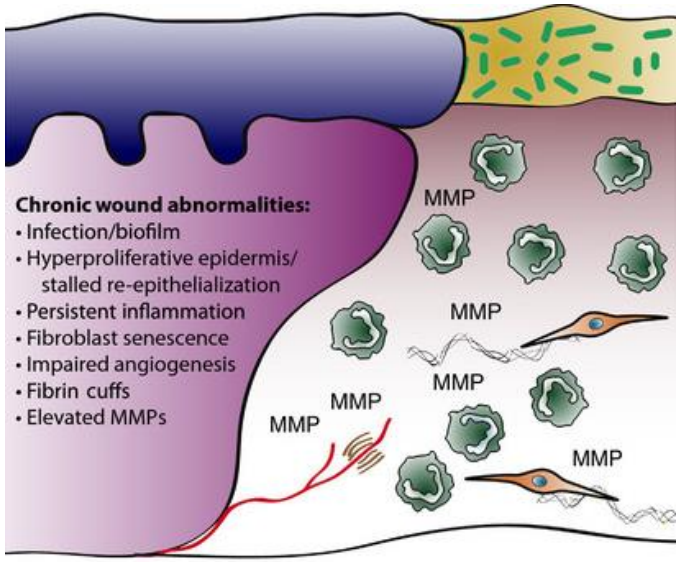


Protease inhibition

Antimicrobial action

Immunomodulation





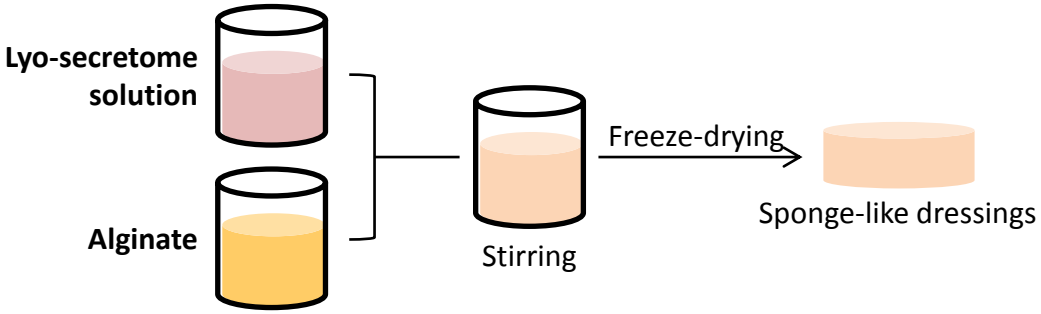
Inflammation

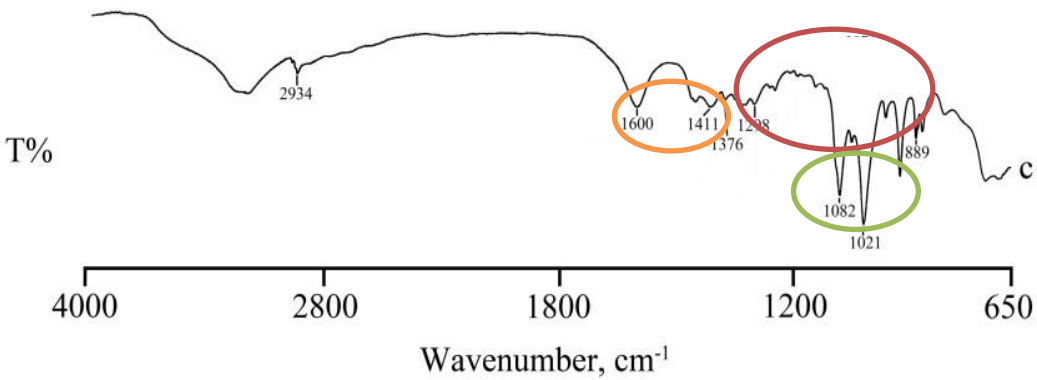
Infection

Protease excess

Bari et al. J. Control. Release 2019, 309, 11-24.
Martin et al. Brit. J. Dermatol. 2015, 173(2).

Sponge-like dressing preparation and characterization

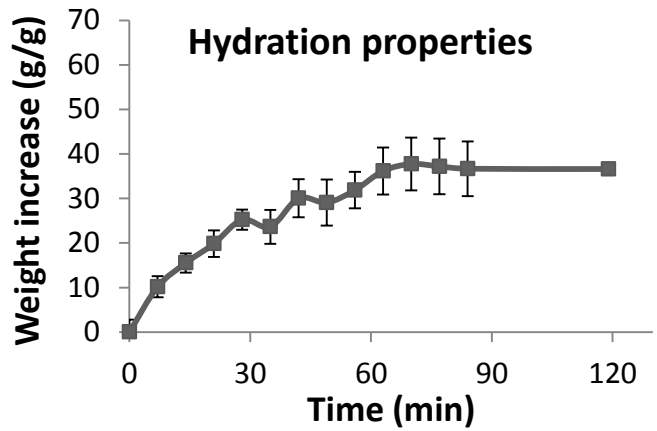


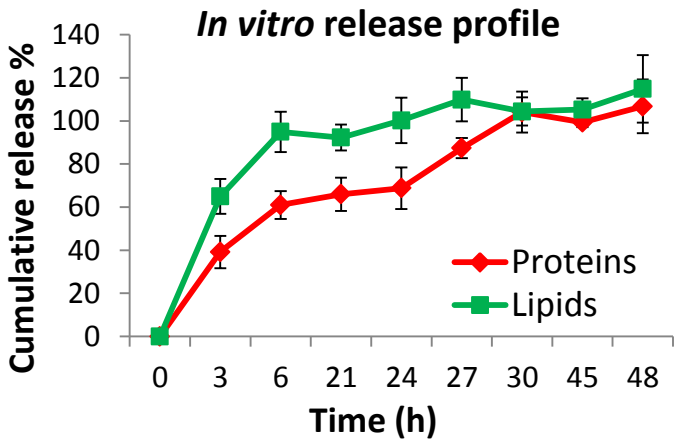


Lipid esters stretching vibrations

COOH stretch vibrations

C-O-C stretch vibration

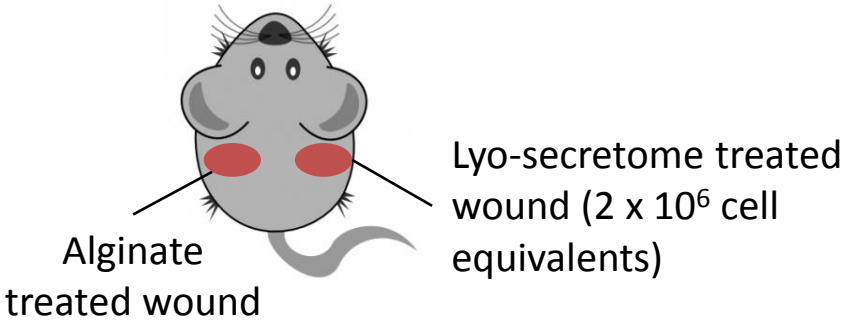




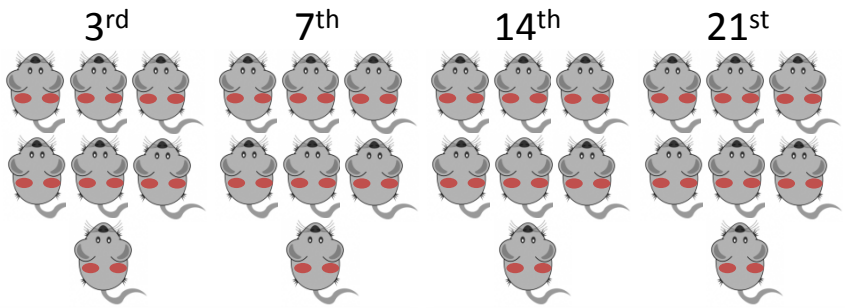
Bari et al. Eur. J. Pharm. Biopharm, 2020, 37-48.

In vivo efficacy

Mouse skin wound model

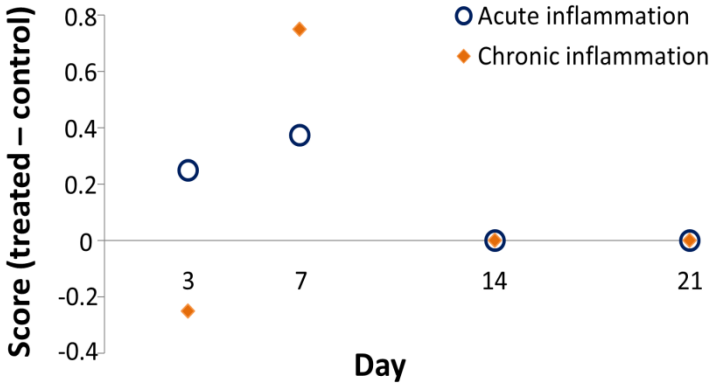


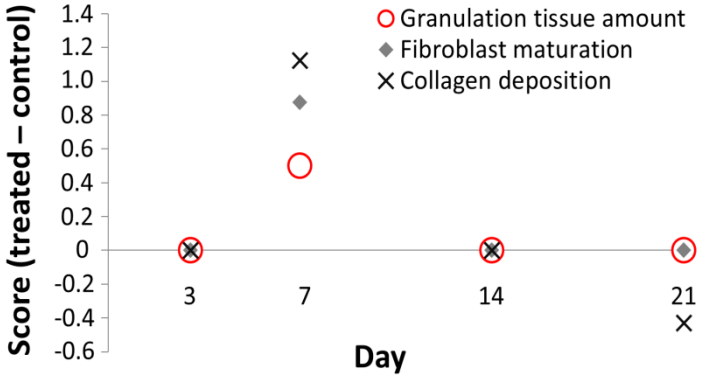
Treatment day

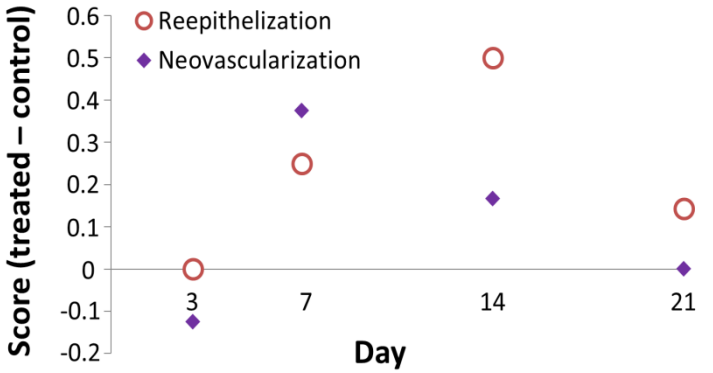


Acute and chronic inflammation	Granulation tissue amount
Fibroblast maturation	Collagen deposition
Reepithelization	Neovascularization

Score = treated – control







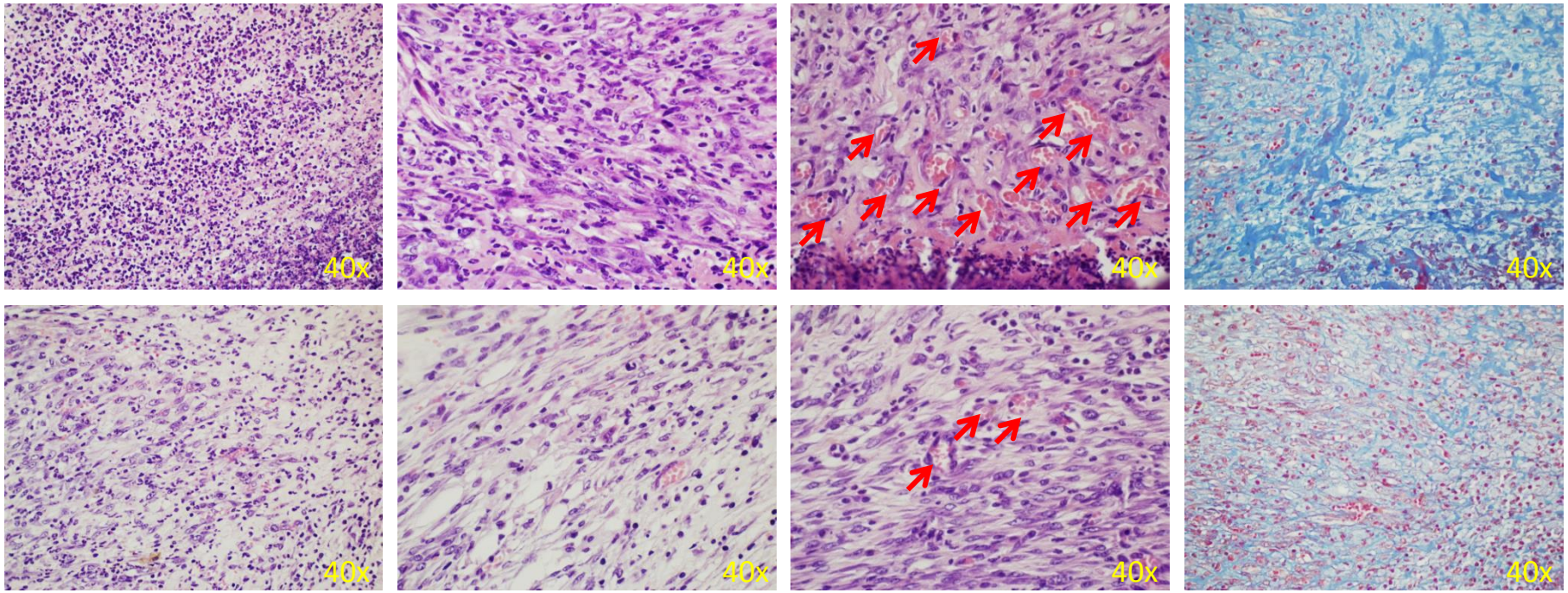
Kruskal-Wallis and Mann-Whitney tests, $p<0.05$, Bari et al. Eur. J. Pharm. Biopharm, 2020, 37-48.

Histological investigation

7th day

Lyo-secretome

CTR



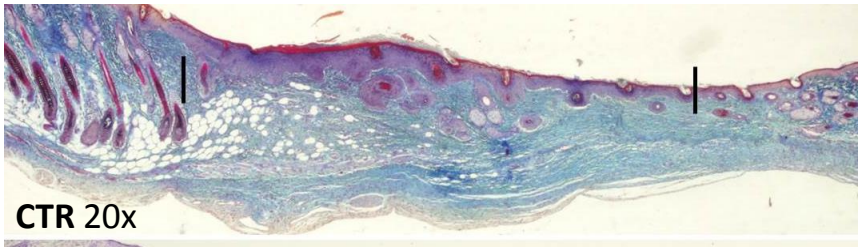
Intense acute inflammatory reaction

Larger amounts of fibroblasts

More new blood vessels

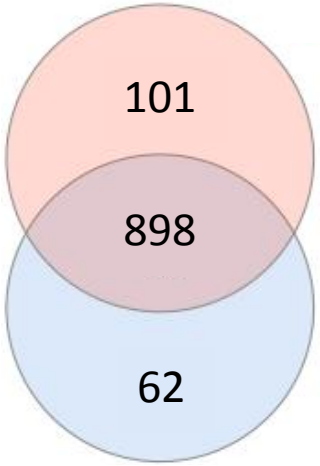
More collagen deposition

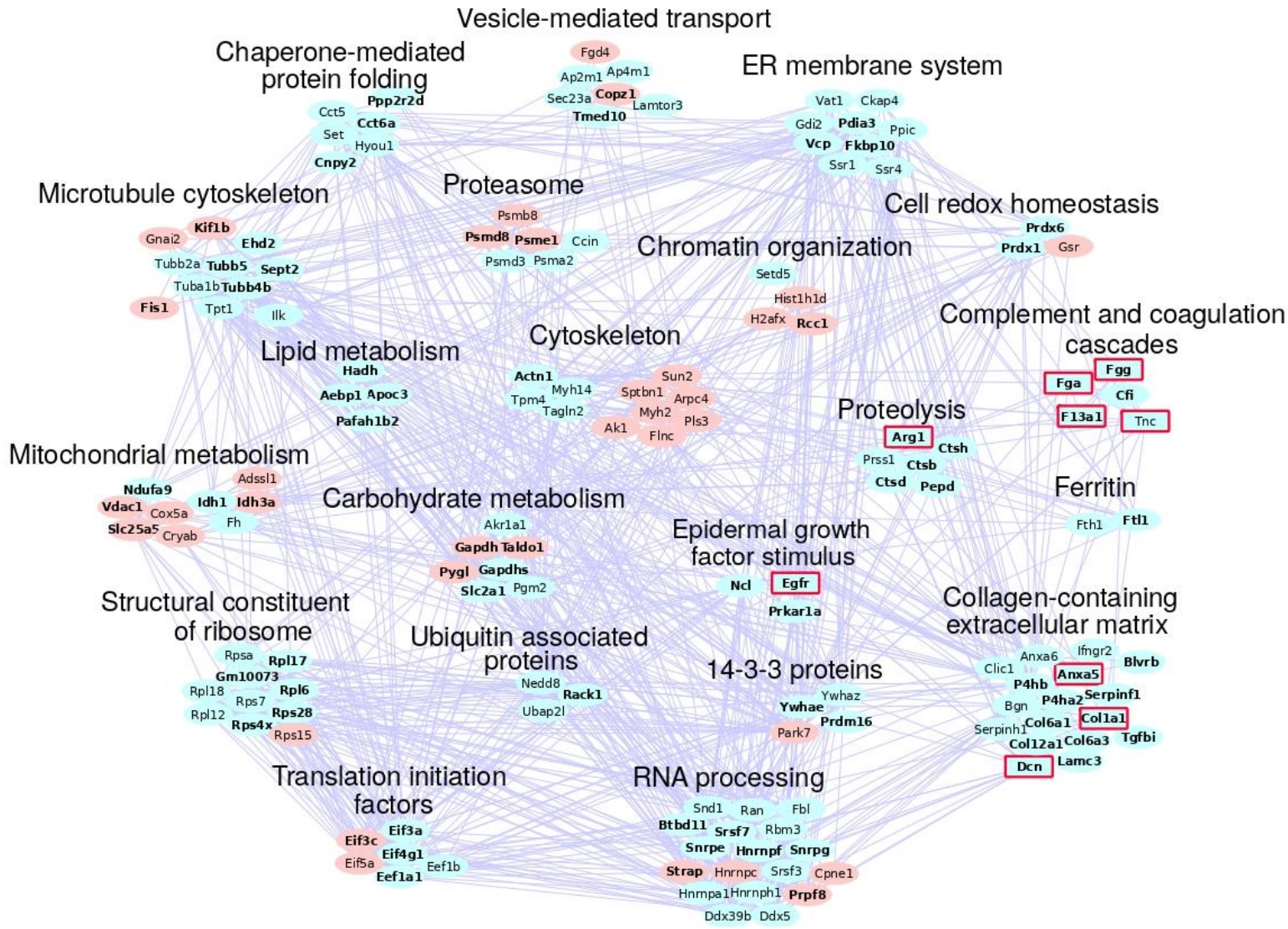
14th day



Bari et al. Eur. J. Pharm. Biopharm, 2020, 37-48.

Lyo-secretome proteomic network of healing





Proteins up-regulated in Treated Proteins up-regulated in CTR Proteins involved in wound healing

Protein-protein interaction network and functional classification of proteins identified by LC-MS/MS, Bari et al. Eur. J. Pharm. Biopharm, 2020, 37-48.

Thanks to…

Interreg
Fondo Europeo di Sviluppo Regionale
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