Co-funded by the
Horizon 2020 programme
of the European Union

ONLINE LTP SEMINARS

To educate, inform, and connect the LTP community
During the COVID-19 pandemic and beyond

2 0 2 0

European Research Council
Established by the European Commission
Supporting top researchers
from anywhere in the world

How can we exploit biomaterials for the benefit of plasma medicine?

Cristina Canal

cristina.canal@upc.edu

BIOMATERIALS,
BIOMECHANICS &
TISSUE ENGINEERING

UNIVERSITAT POLITÈCNICA
DE CATALUNYA
BARCELONATECH

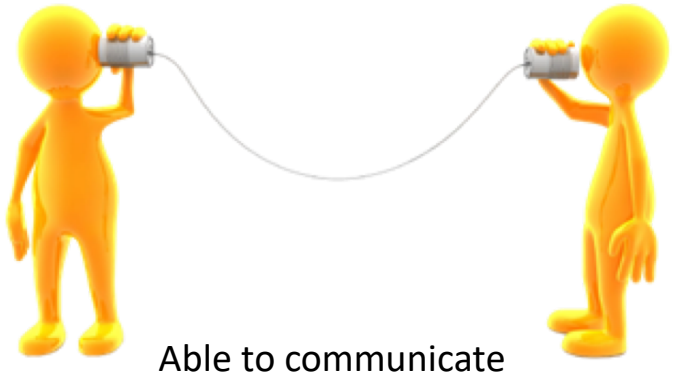
Our AIM

To develop biomaterials for substitution, regeneration and functional repair of tissues and organs

First generation: inert biomaterials

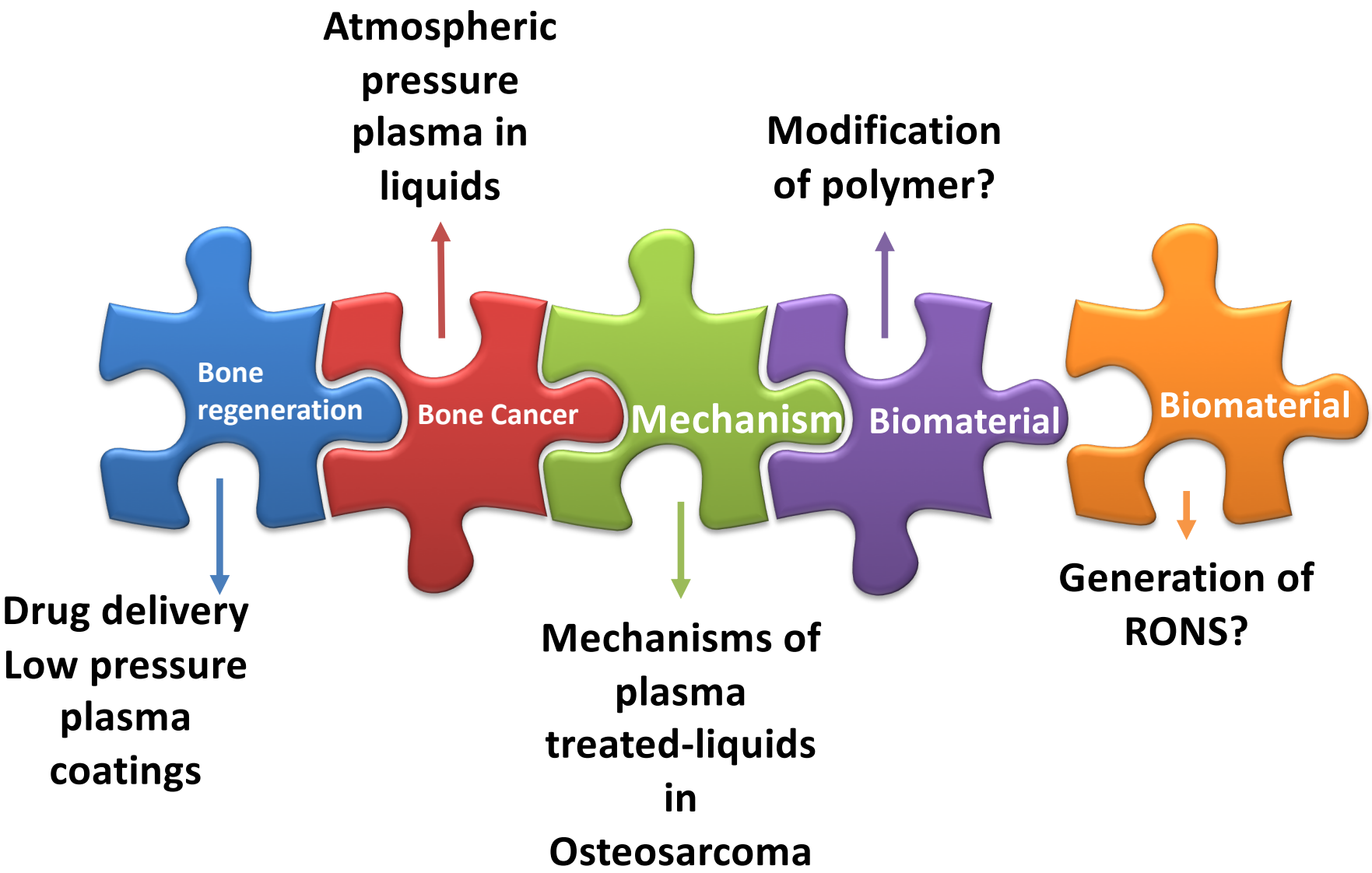
Second generation: resorbable or active biomaterials

Third generation: Bio-instructive materials



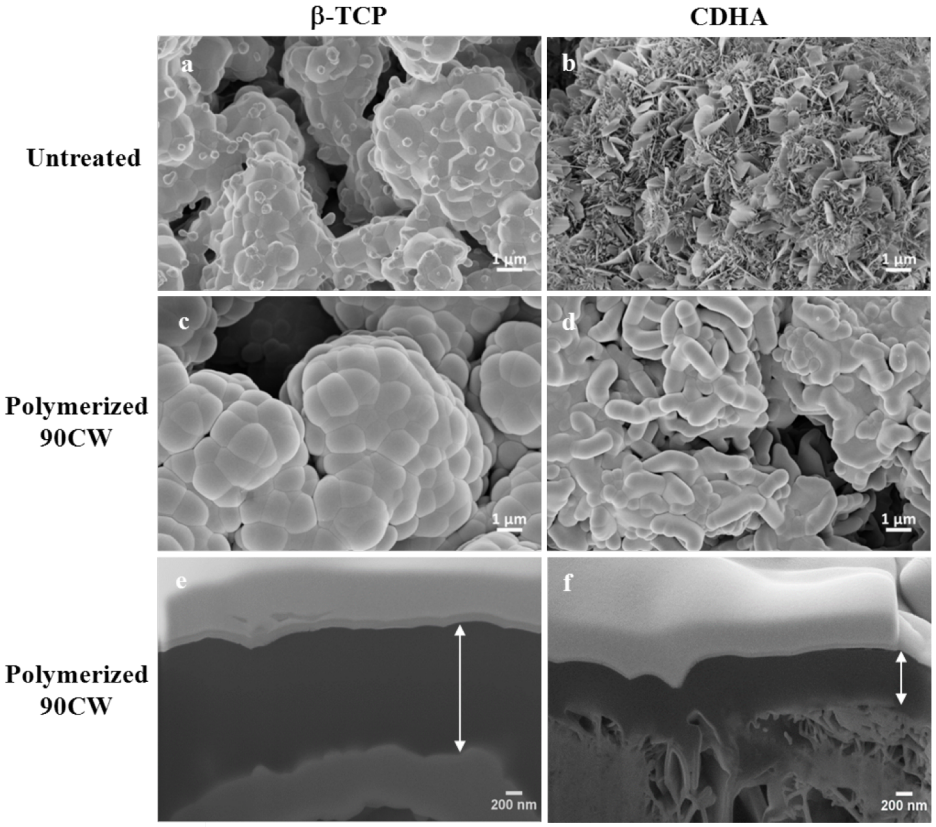
Able to communicate with cells and direct their behaviour to specific responses

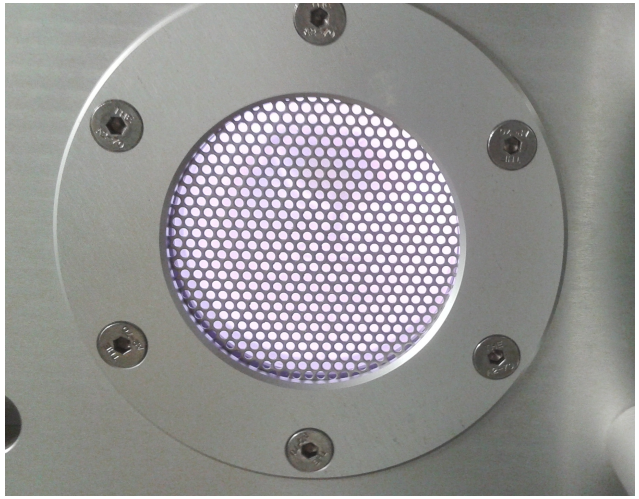
Outline

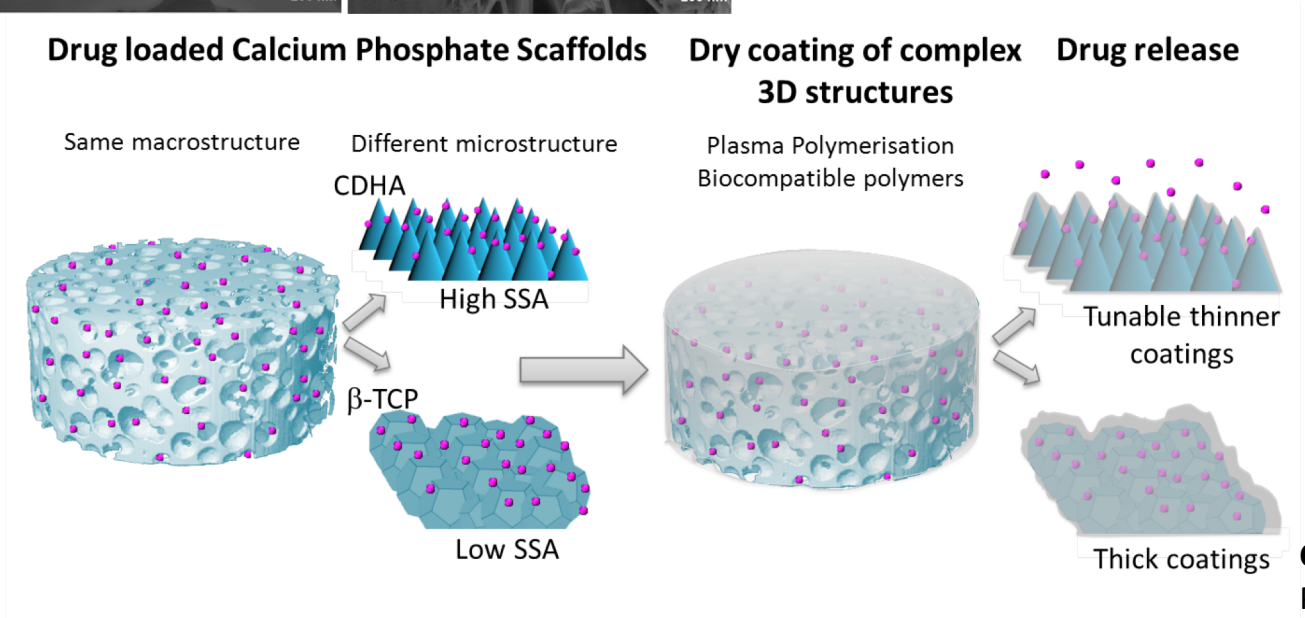

Atmospheric pressure plasma in liquids
Modification of polymer?
Bone regeneration
Bone Cancer
Mechanism
Biomaterial
Biomaterial
Drug delivery
Low pressure plasma coatings
Mechanisms of plasma treated-liquids in Osteosarcoma
Generation of RONS?

Bone regeneration – Drug delivery

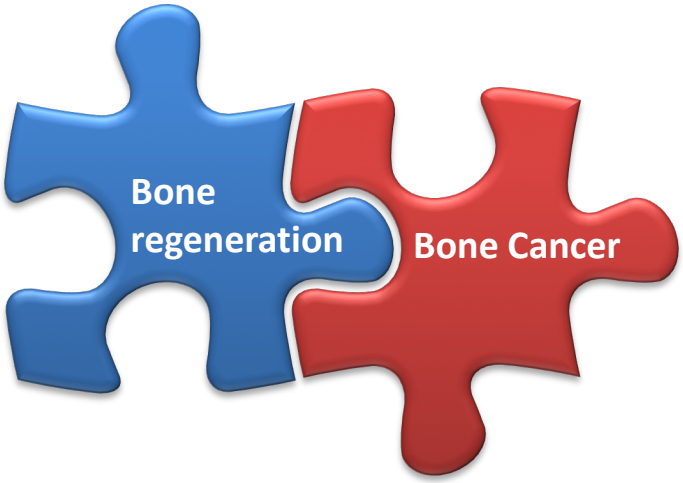
BIOMATERIALS, BIOMECHANICS & TISSUE ENGINEERING







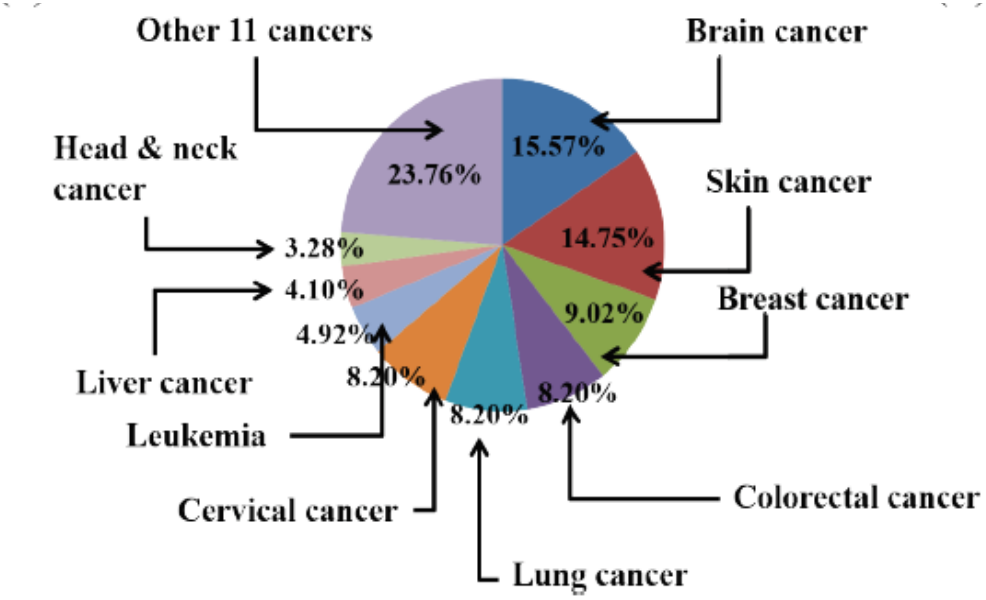
C. Canal, F. Arefi-Khonsari, et al. Polymer. 92, 170 - 178. 2016.


Bone
regeneration
Bone Cancer

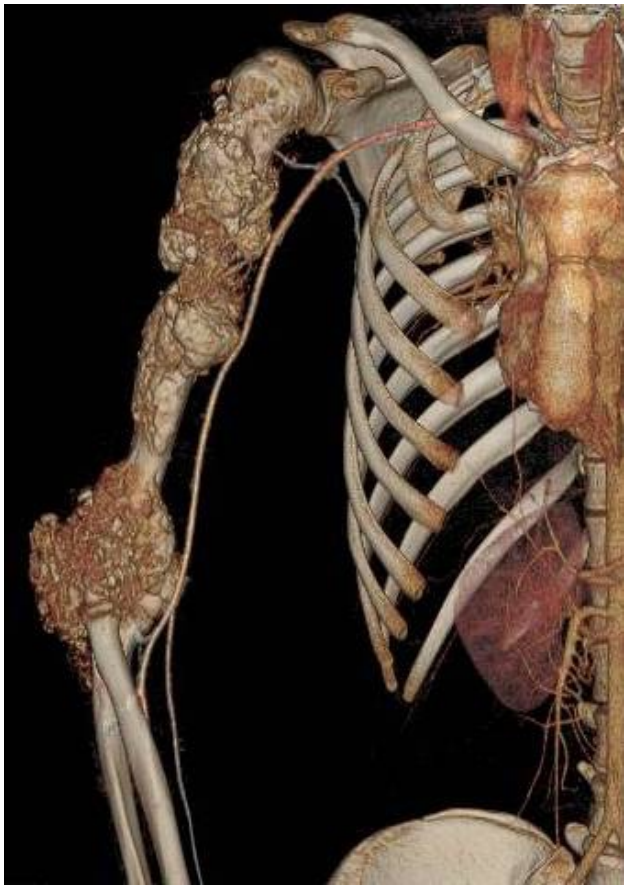
Plasma medicine

BIOMATERIALS, BIOMECHANICS & TISSUE ENGINEERING

Efficacy in a variety of cancers



Osteosarcoma?



E. Robert Clin Plasma Med (2013) / Z. Chen Cancers (2017) / H.R. Mettelman, Clin Plasma med (2015)…

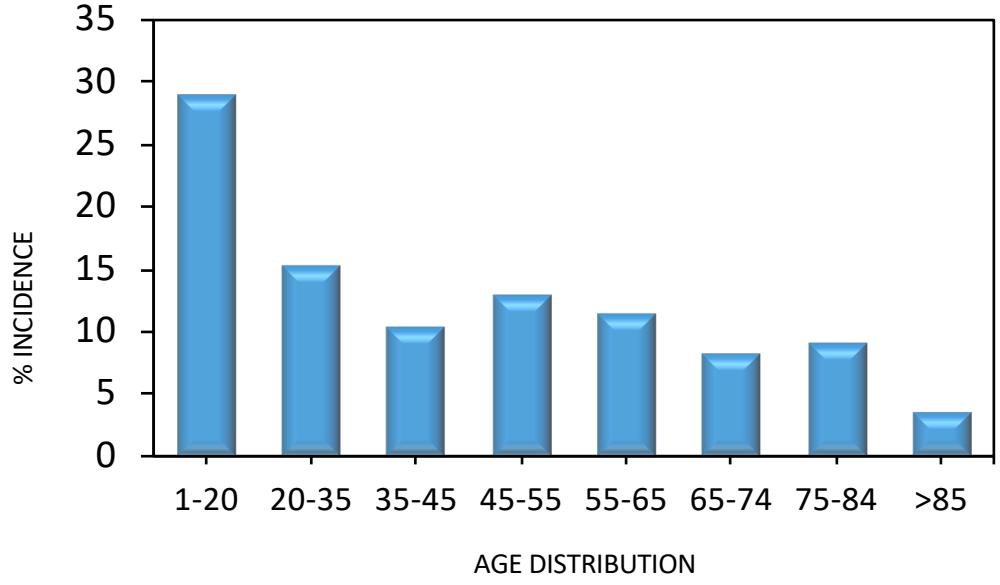
Bone cancer

BIOMATERIALS, BIOMECHANICS & TISSUE ENGINEERING

PRIMARY BONE CANCER

+ 3,600 new cases of bone cancers in the USA

50% mortality rate (2016)



SECONDARY BONE CANCER = METASTATIC

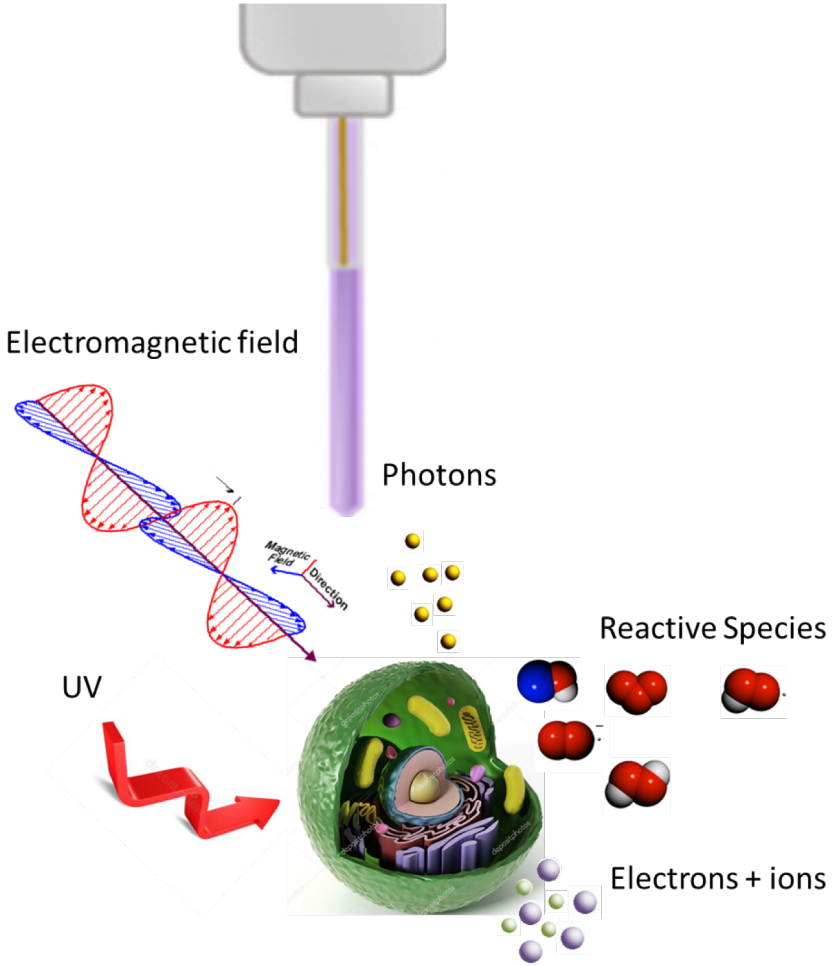
280,000 adults in USA living with metastatic bone disease

68% of cases occurring in patients with primary breast, prostate or lung cancer (2008)

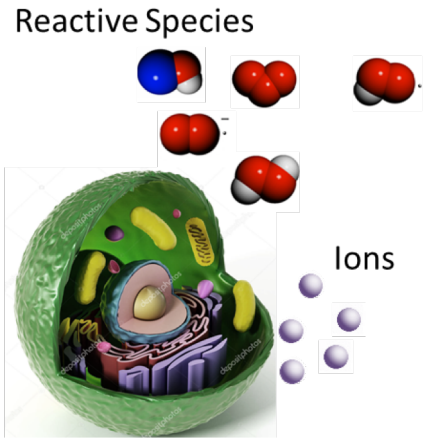
Two possible modes of action

BIOMATERIALS, BIOMECHANICS & TISSUE ENGINEERING

Direct plasma treatment

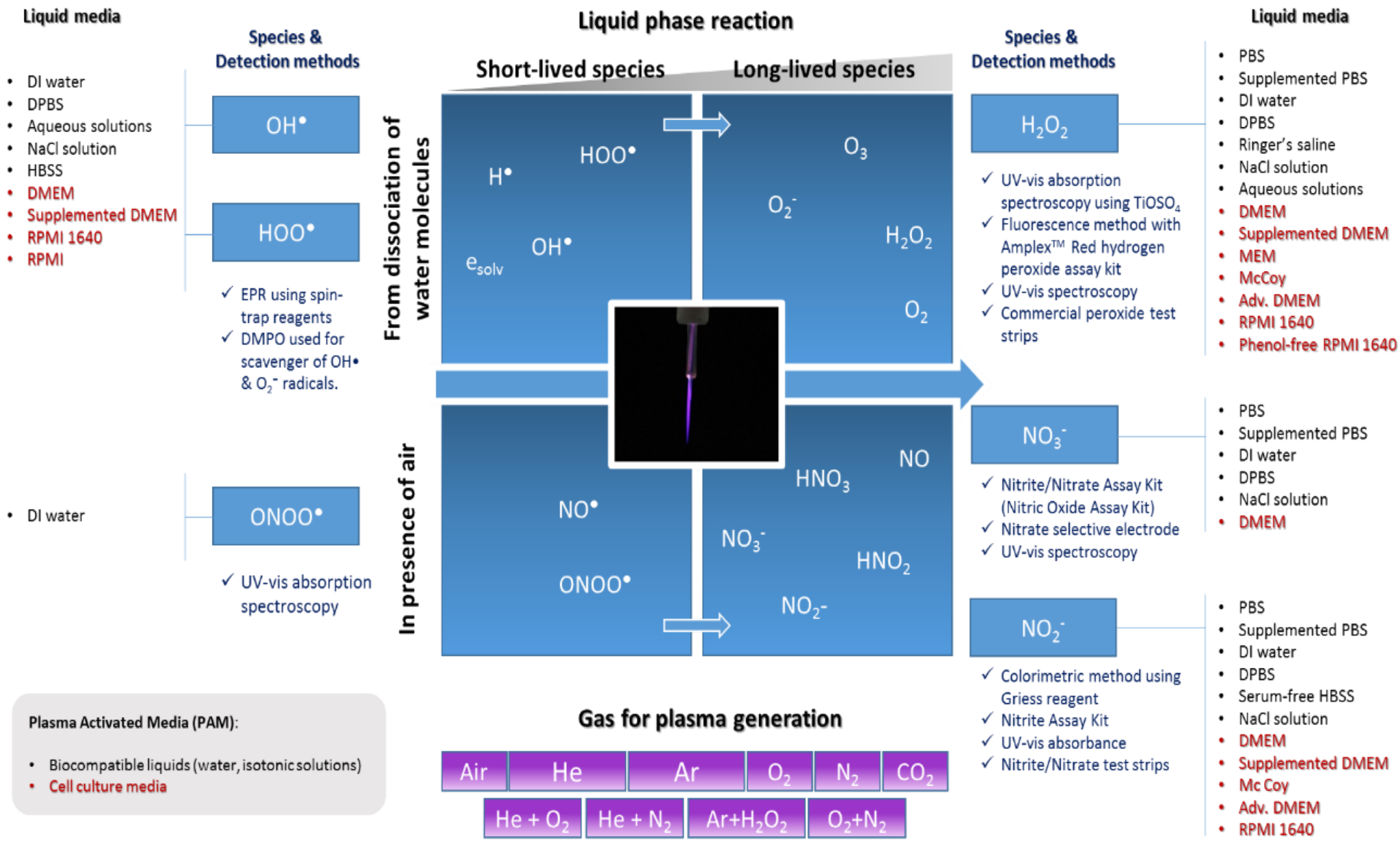


Plasma treated liquids

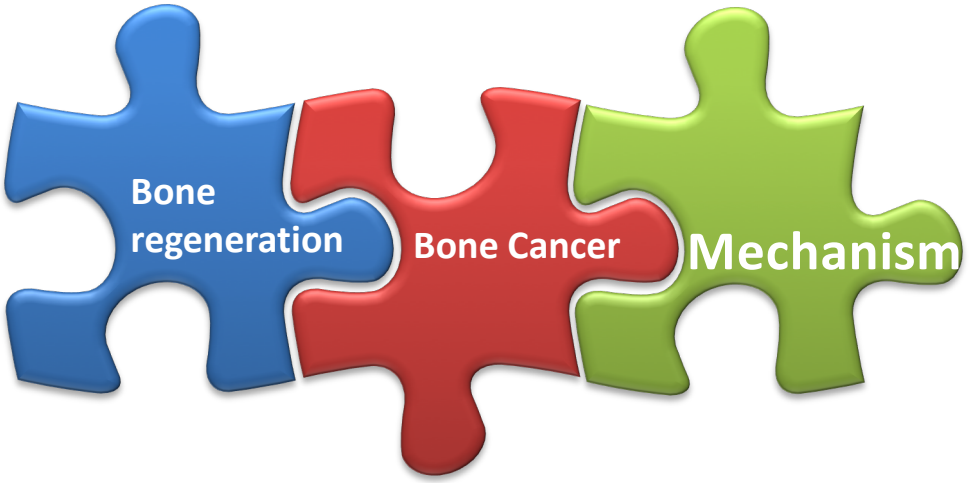


Reactive species in liquids

BIOMATERIALS, BIOMECHANICS & TISSUE ENGINEERING



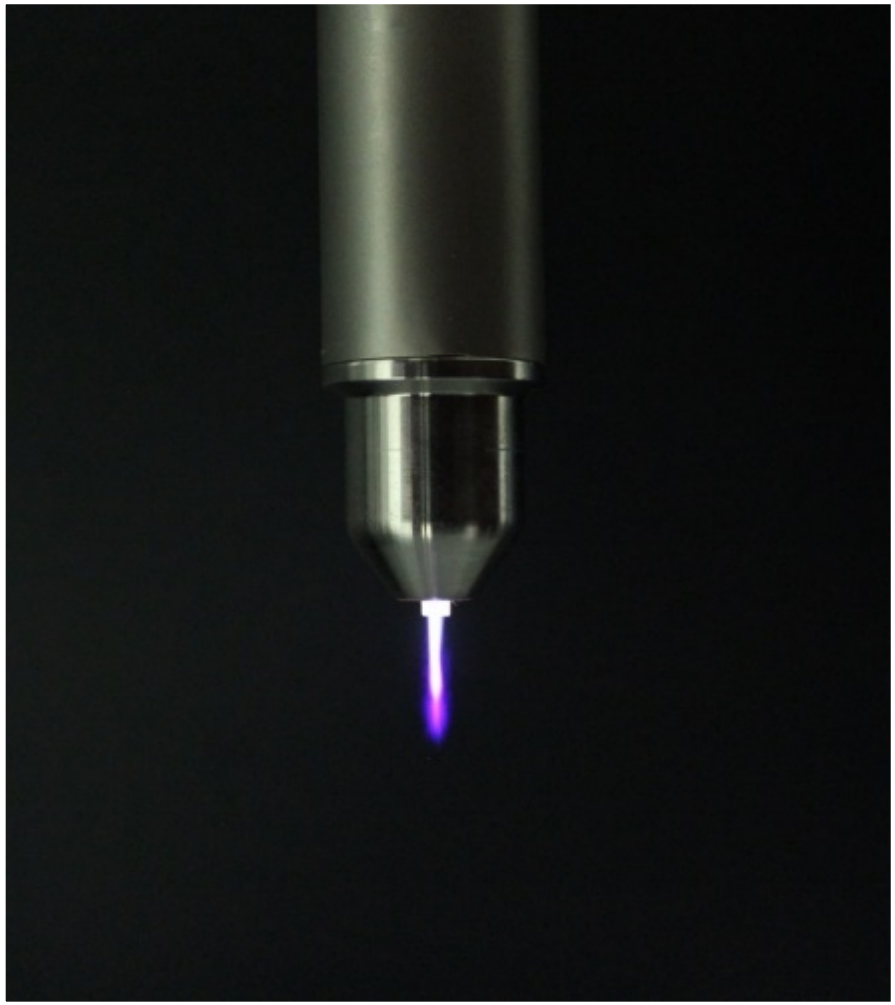
A. Khlyustova, C. Canal et al. Frontiers Chem. Science Eng. 2019


Bone
regeneration
Bone Cancer
Mechanism

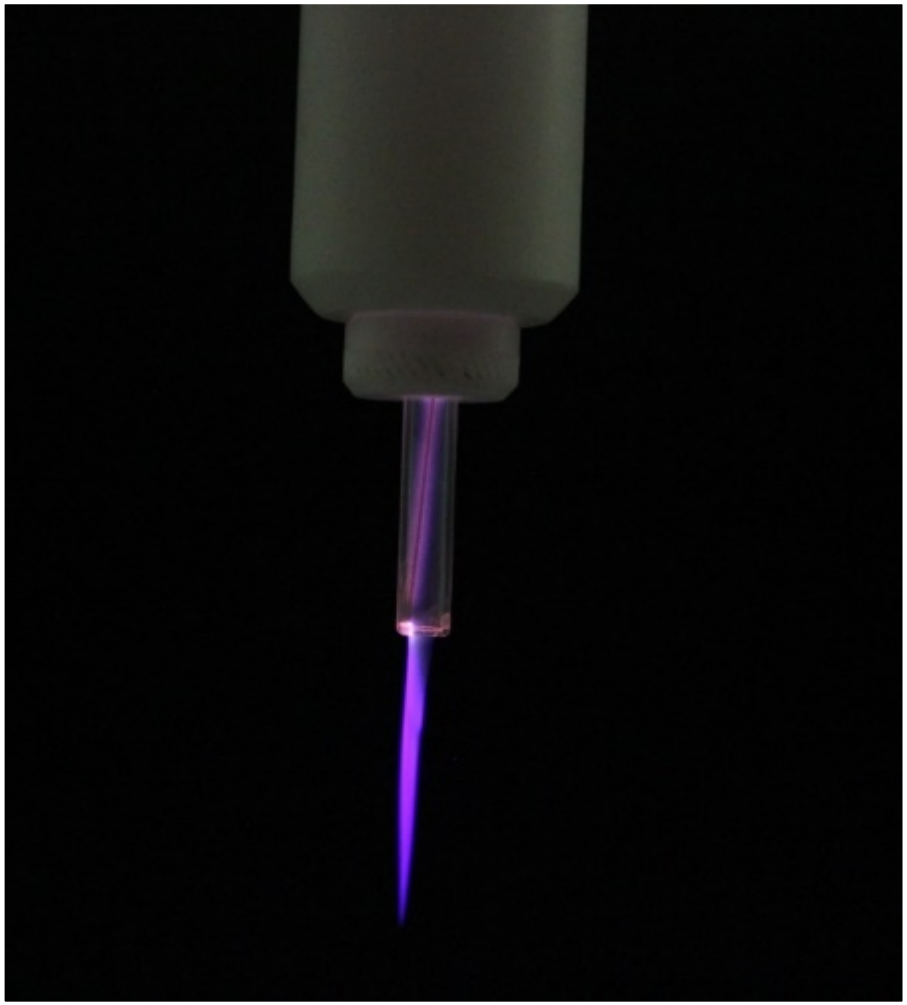
The plasma jets

BIOMATERIALS, BIOMECHANICS & TISSUE ENGINEERING

kINPen



APPJ

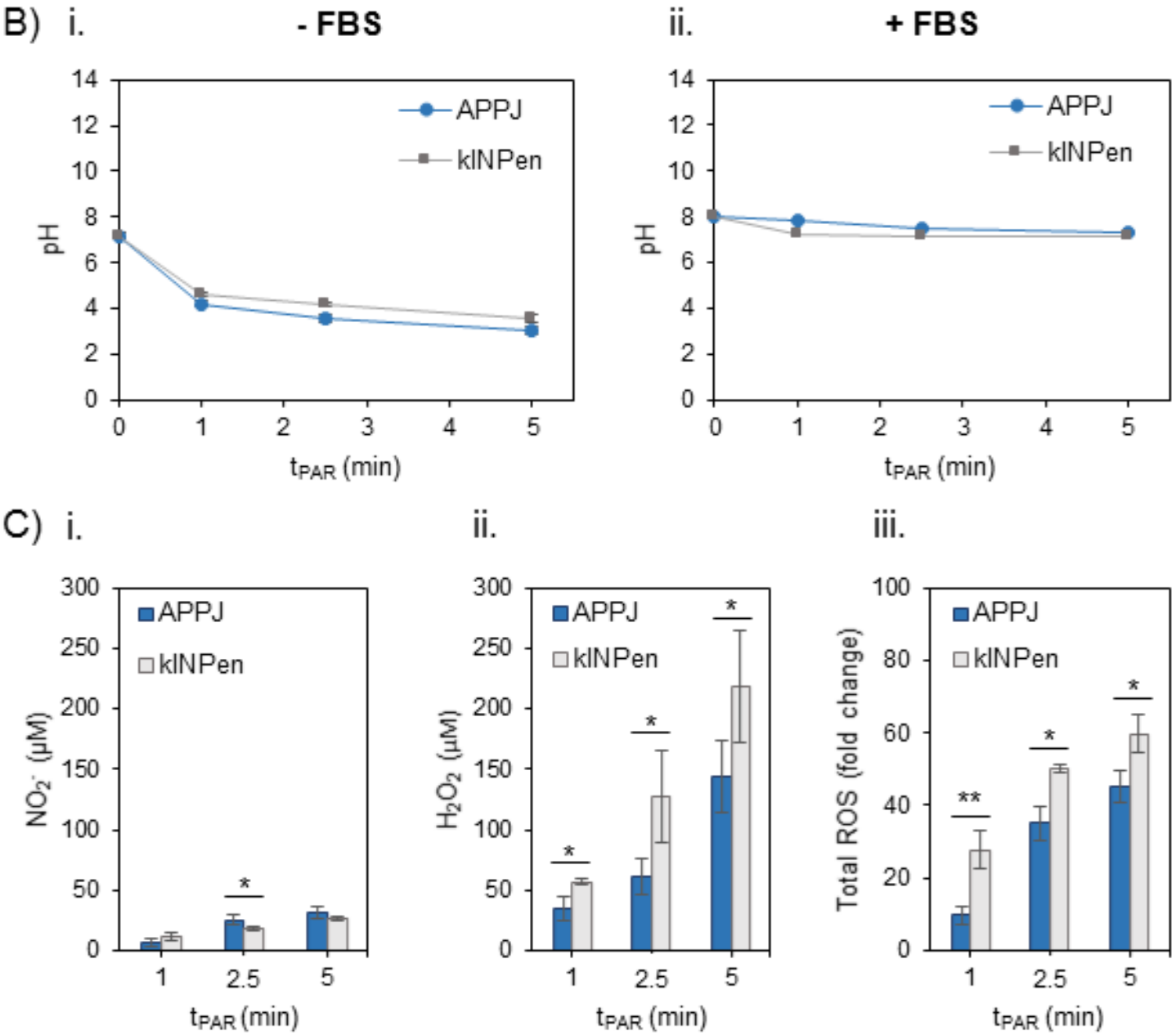


Ar

He

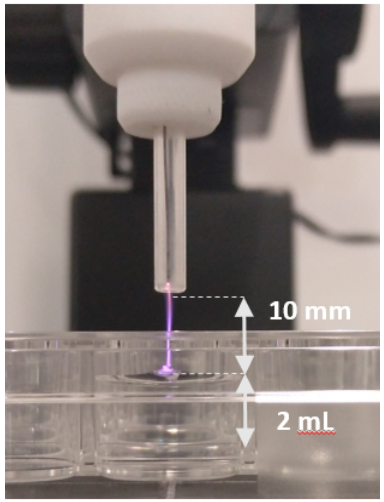
Chemistry in the liquid phase

BIOMATERIALS, BIOMECHANICS & TISSUE ENGINEERING



Ringer's + FBS

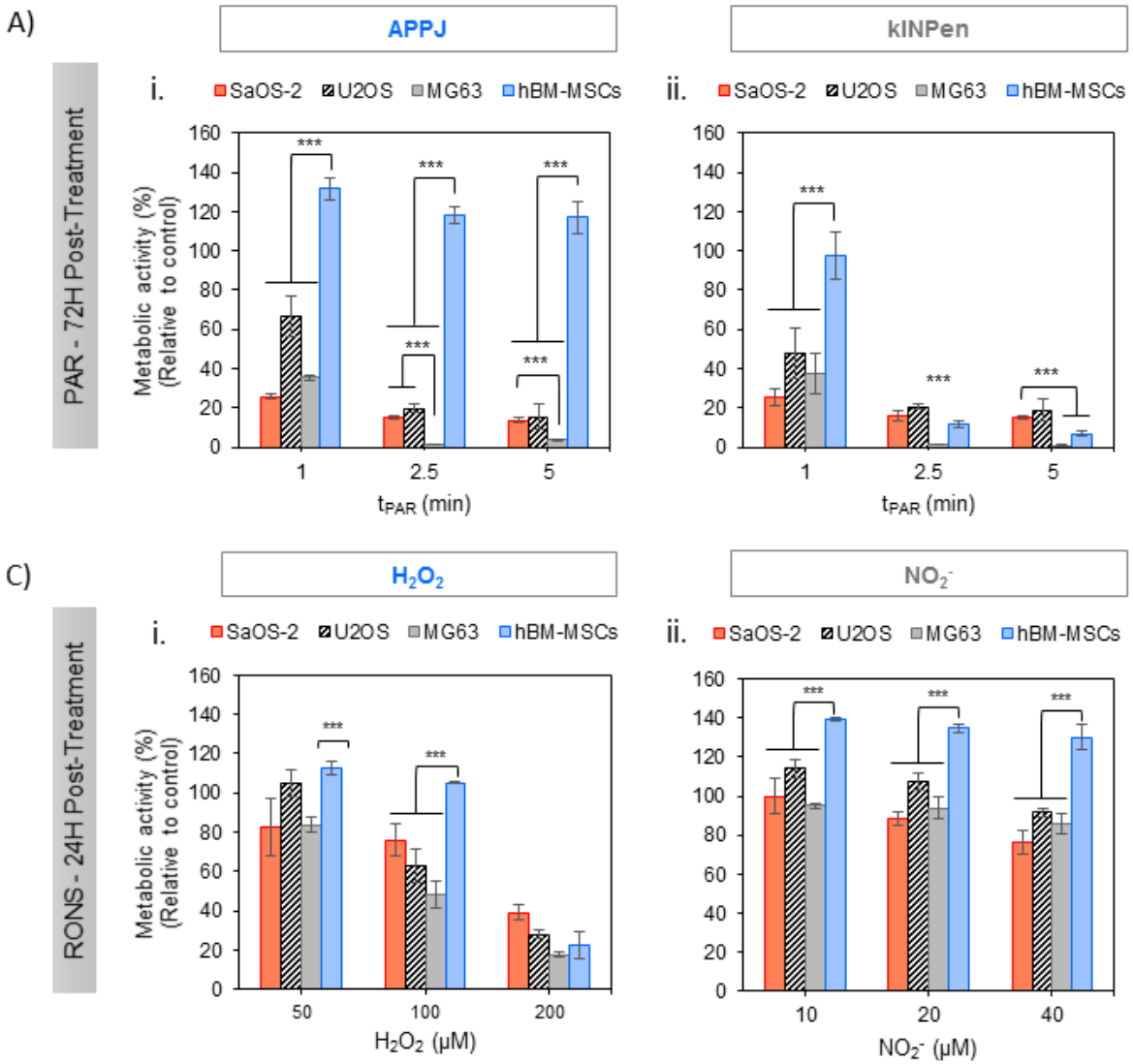
1 L/min
10mm

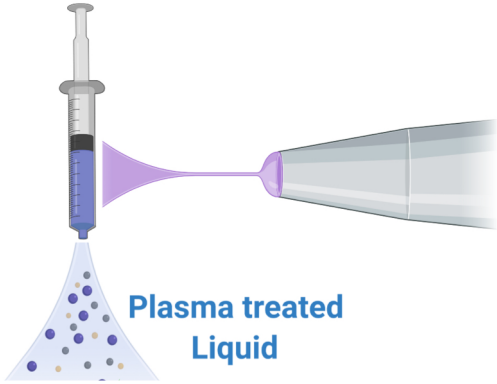


M. Mateu-Sanz, et al. Cancers, vol. 12(1), p. 227. 2020.

2D cell viability

BIOMATERIALS, BIOMECHANICS & TISSUE ENGINEERING





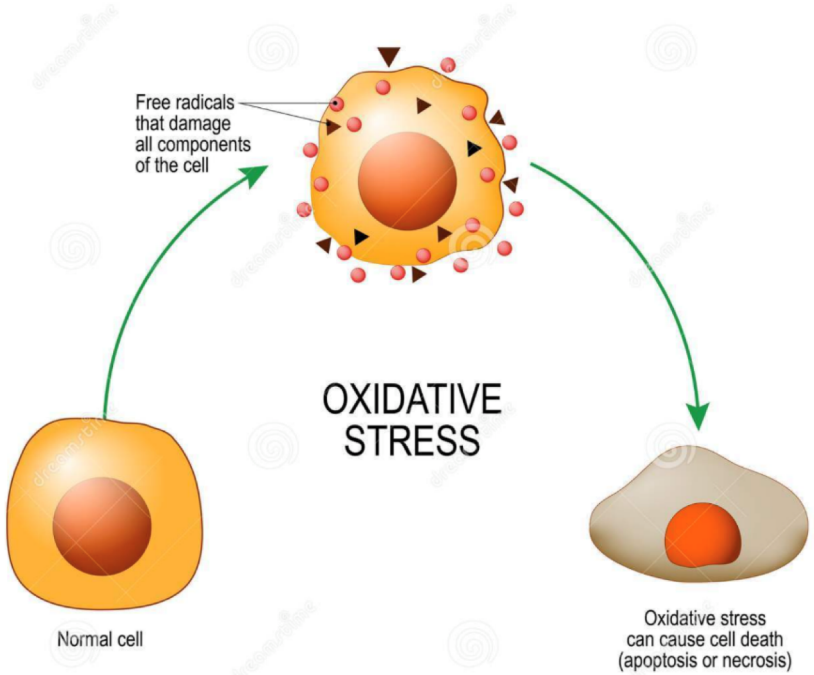
M. Mateu-Sanz, et al. Cancers, vol. 12(1), p. 227. 2020.

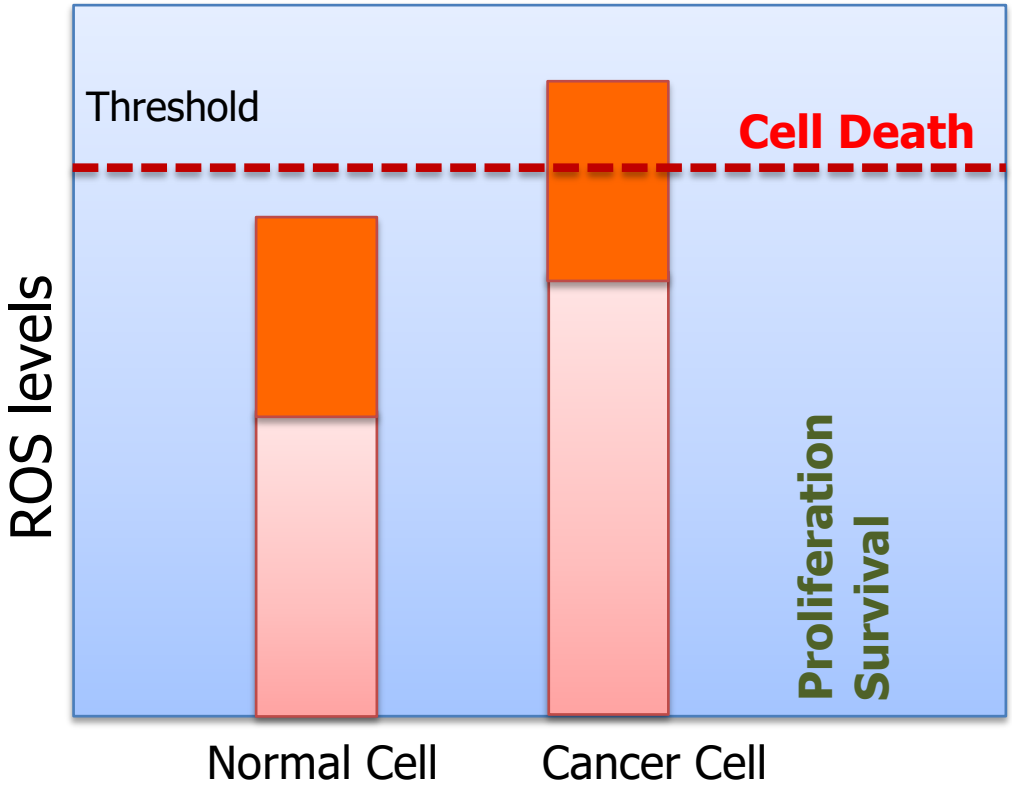
BIOMATERIALS, BIOMECHANICS & TISSUE ENGINEERING

The weapon in plasma treated liquids: Reactive Oxygen and Nitrogen species

Redox Therapies

Reactive Oxygen Species (ROS)



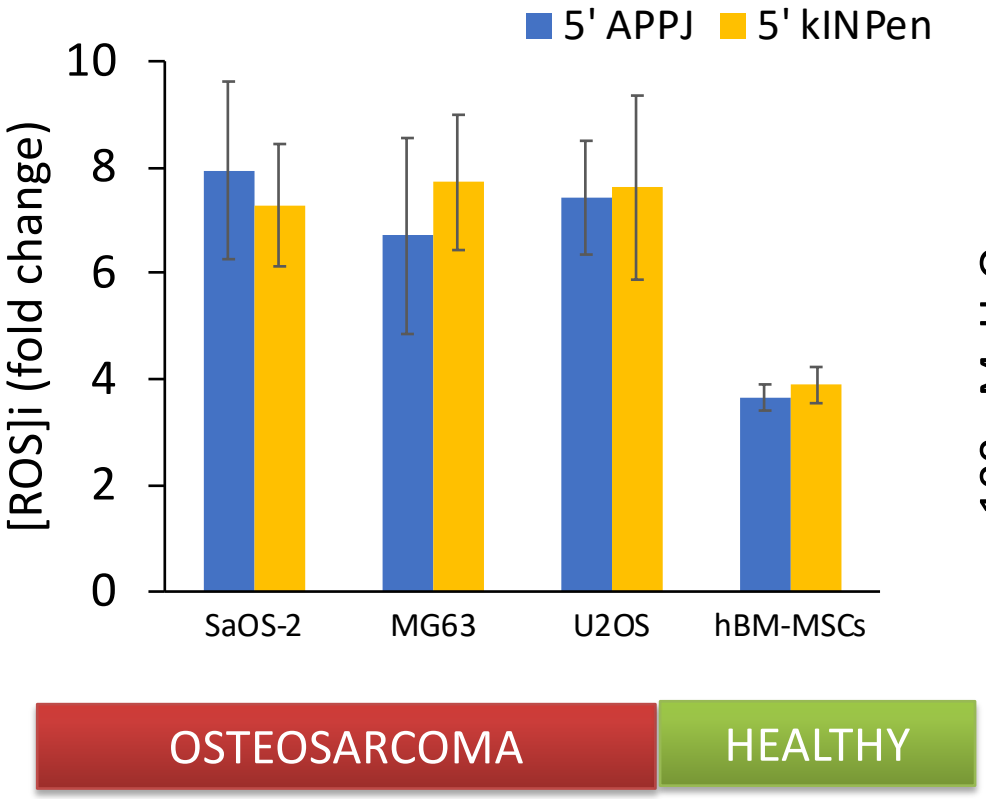


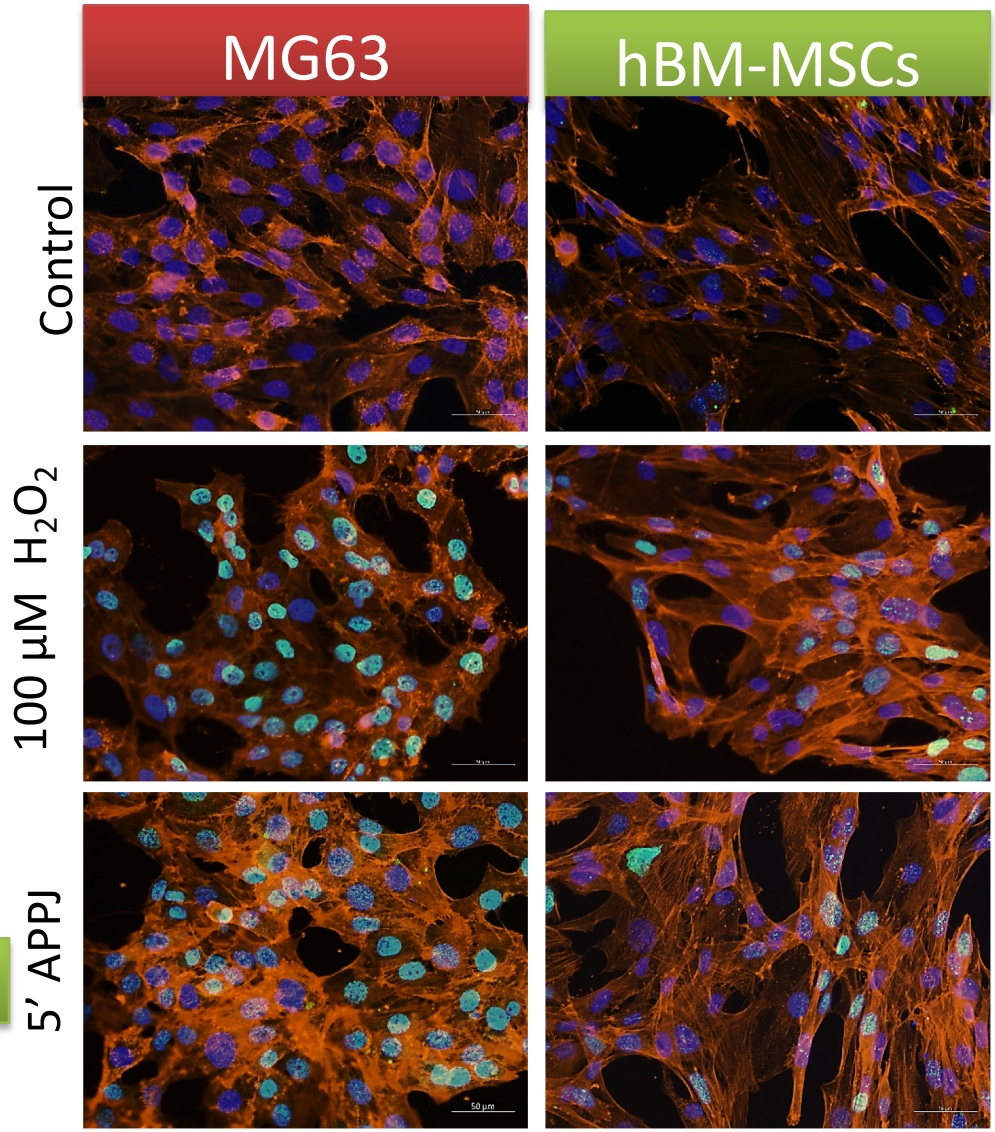
D.B.Graves, Plasma Process. Polymers (2014); G. Bauer, DB Graves , Plasma Process. Polymers (2016)

Mechanisms involved

BIOMATERIALS, BIOMECHANICS & TISSUE ENGINEERING

Intracellular ROS



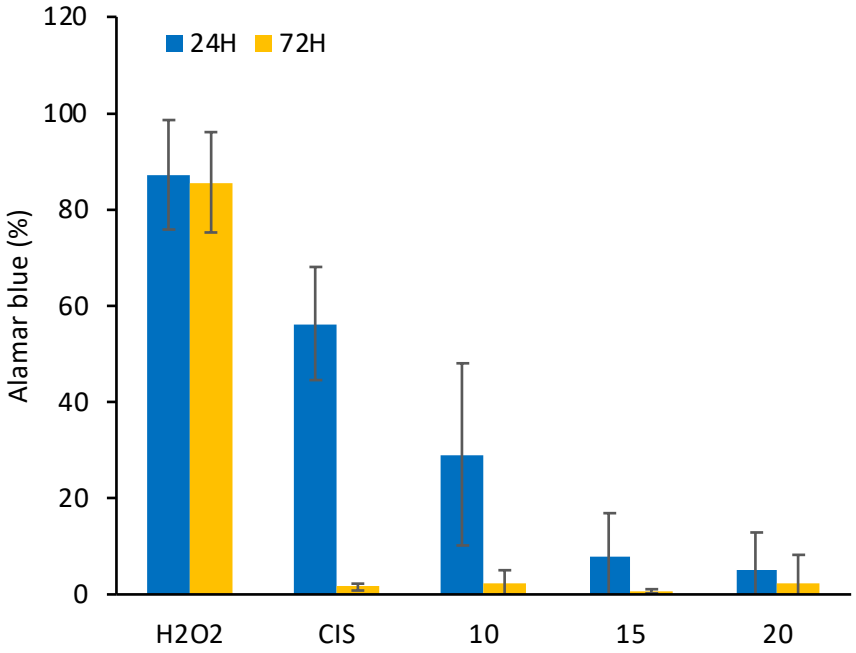


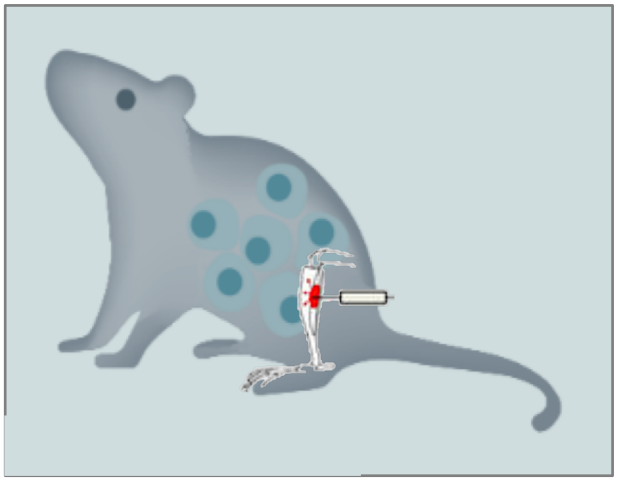
DAPI, pH2AX, Phalloidin

M. Mateu-Sanz, et al. Cancers, vol. 12(1), p. 227. 2020.

3D: Ex-vivo organotypic culture

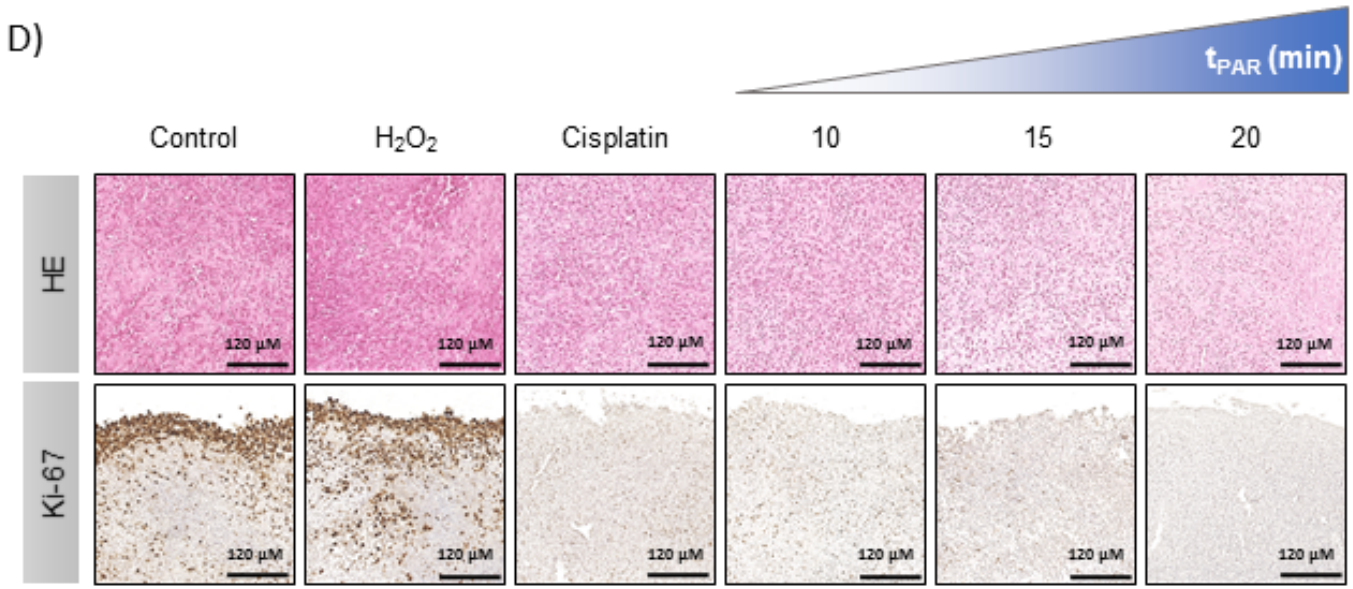
BIOMATERIALS, BIOMECHANICS & TISSUE ENGINEERING





P. Layrolle

Inserm



Dose-dependent reduction of cell viability

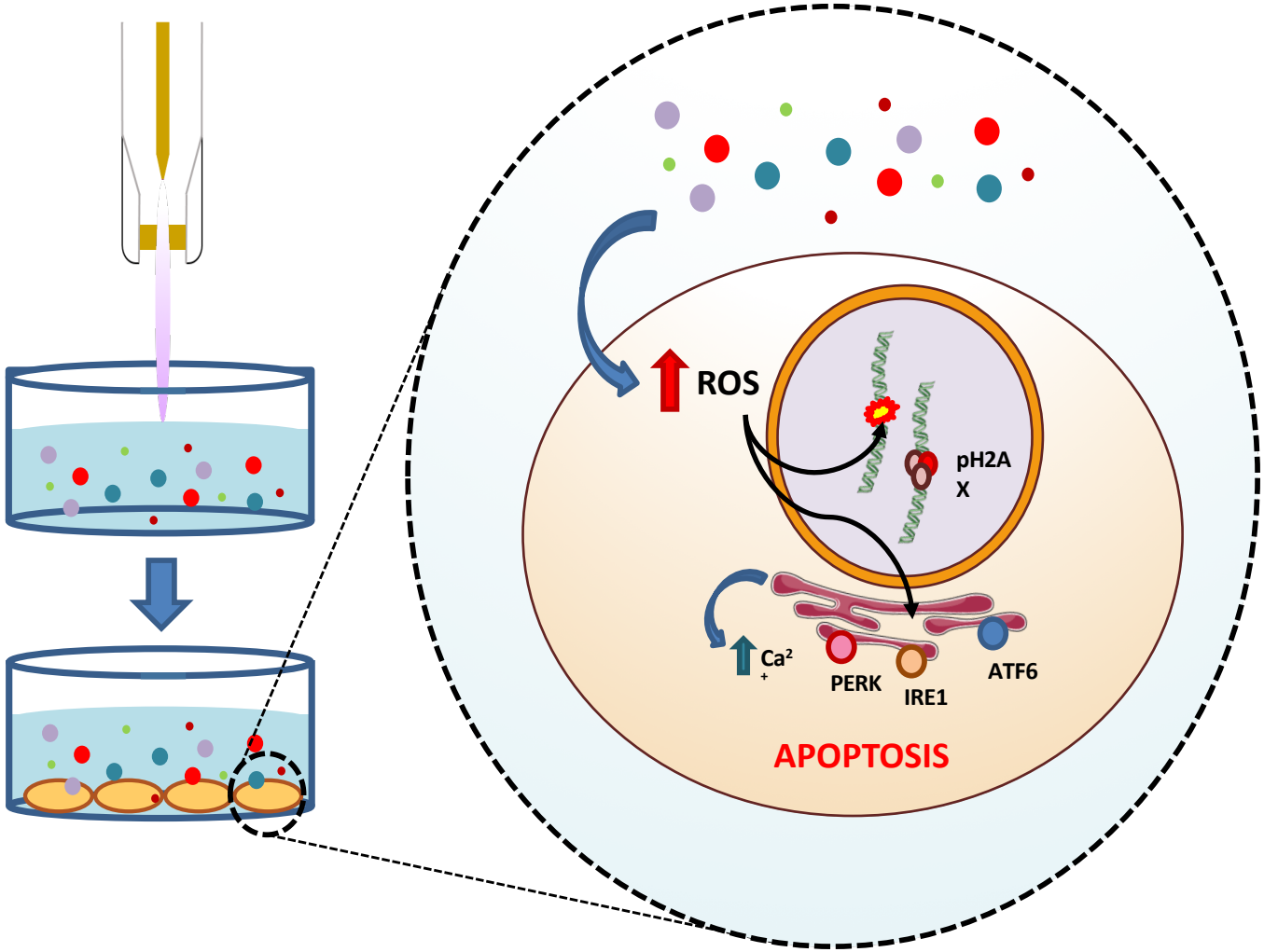
Little toxicity of H_2O_2 in tumor sections

M. Mateu-Sanz, et al. Cancers, vol. 12(1), p. 227. 2020.

Plasma treatment of Ringer's saline generates RONS which, in OS

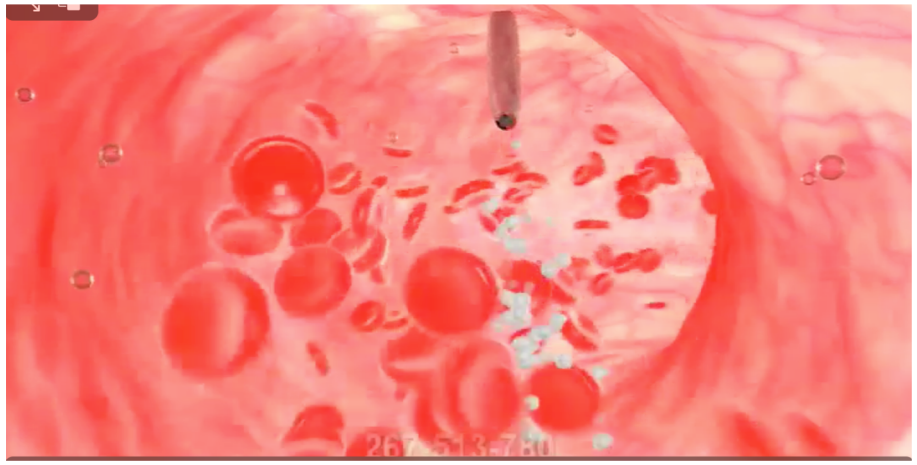
BIOMATERIALS, BIOMECHANICS & TISSUE ENGINEERING

- Induce dose-dependent cell-death in OS cells
- Can be selective towards healthy cells
- A single dose triggers long term effects
- Increase of intracellular RONS leads to higher DNA damage in OS cells



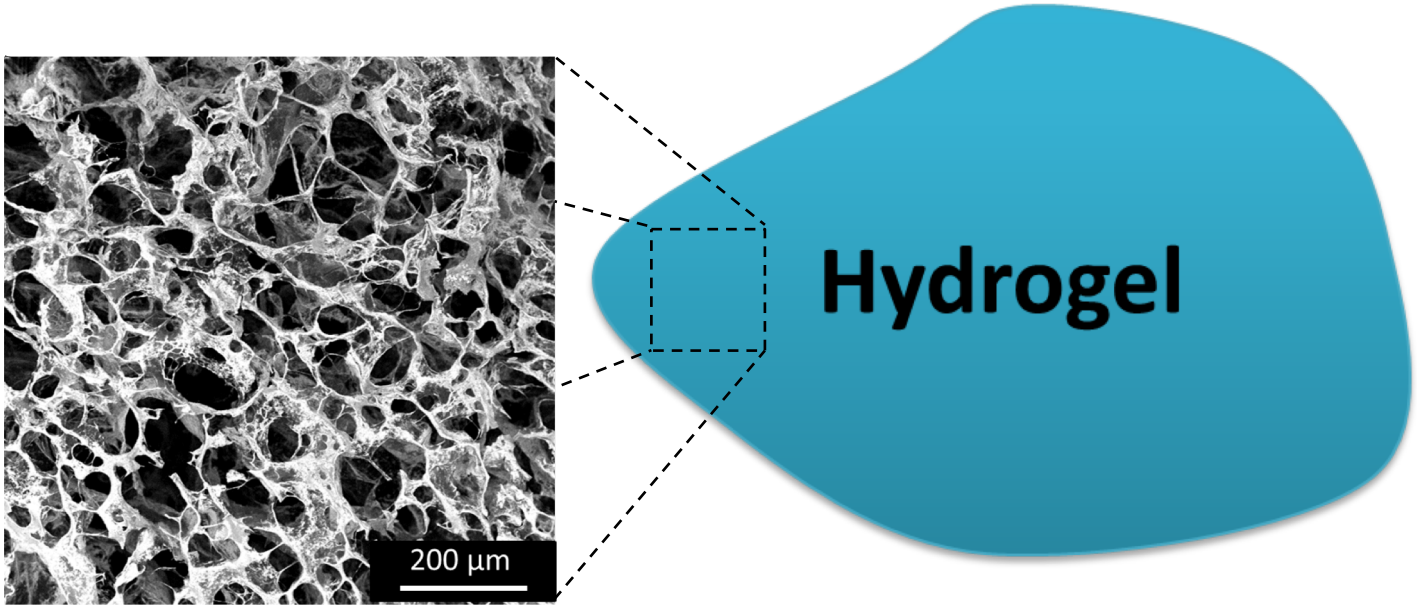
M. Mateu-Sanz, et al. Cancers, vol. 12(1), p. 227. 2020.

Aim



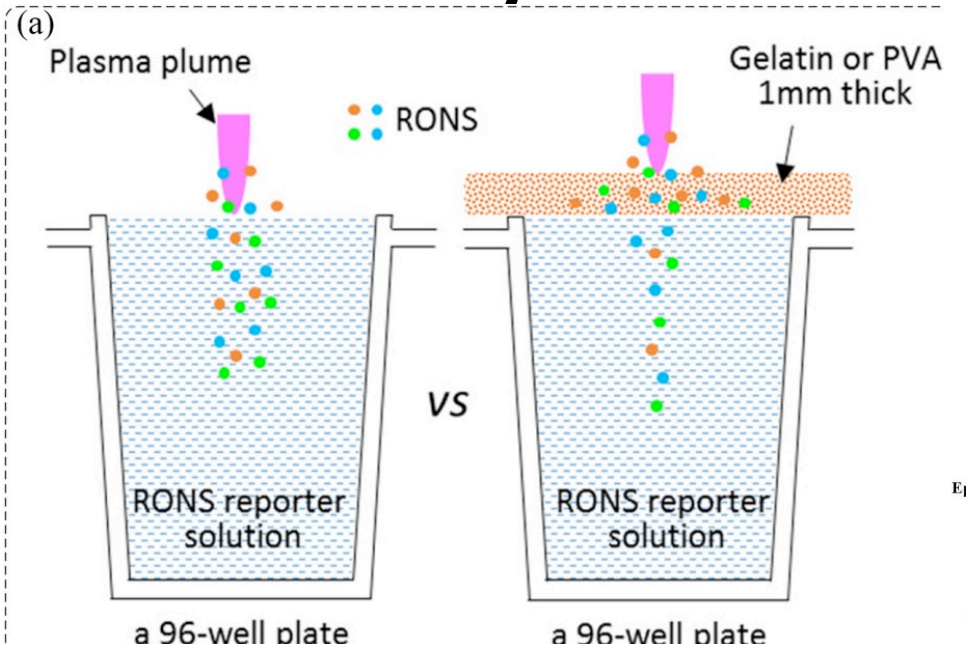
To develop biomaterials that allow the **controlled delivery** of RONS from plasma treated liquids to the tumor site

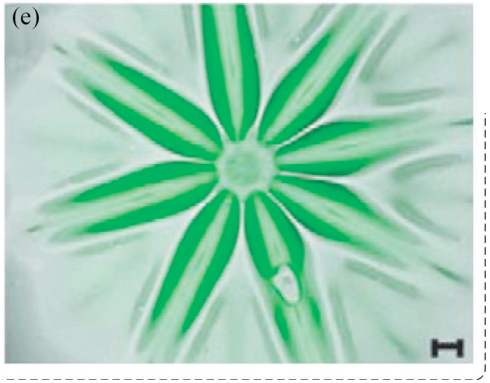
Cross-linked 3D polymer network that, due to their hydrophilic nature, can absorb large quantities of water

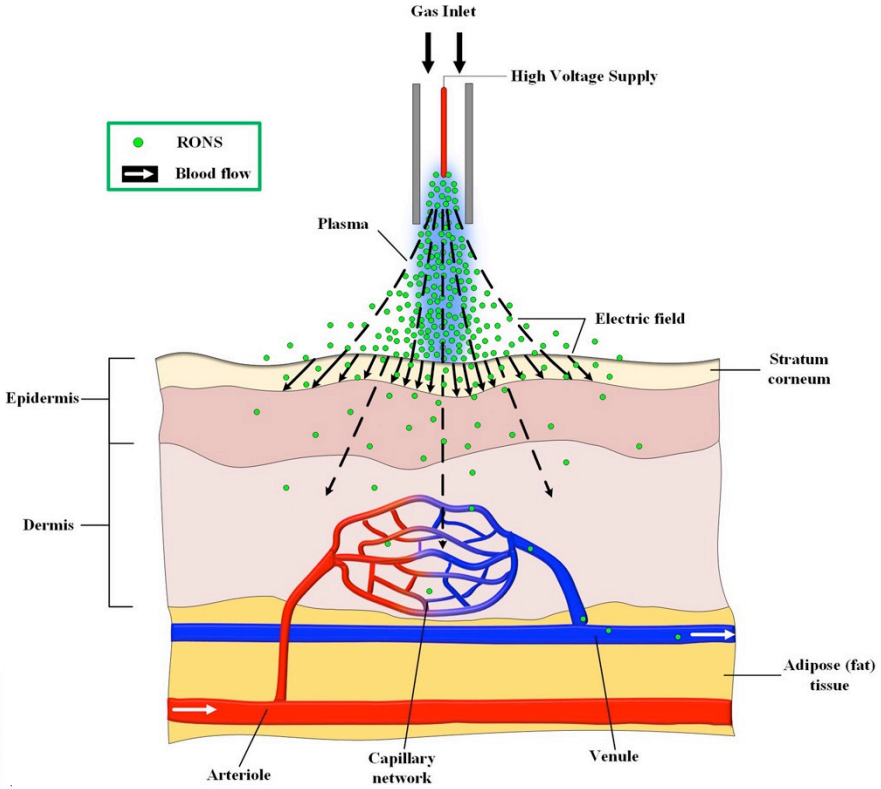


Drug delivery system

Delivery of RONS through skin



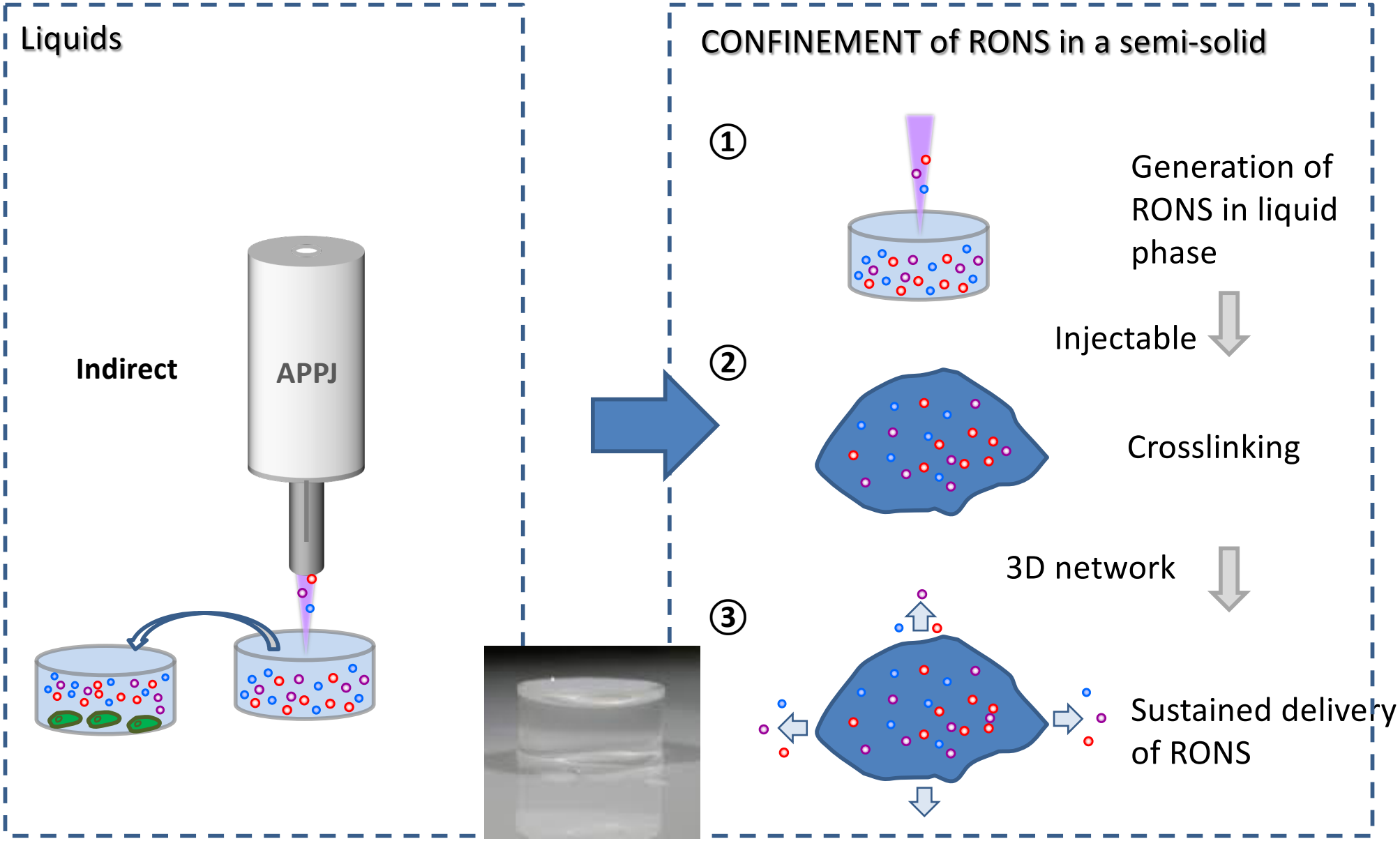




X. Lu, M. Keidar, M. Laroussi, E. Choi, E.J. Szili, K. Ostrikov,Transcutaneous plasma stress: From soft-matter models to living tissues *Materials Science & Engineering R 138 (2019) 36–59*

From liquids to polymer solutions / natural - synthetic

BIOMATERIALS, BIOMECHANICS & TISSUE ENGINEERING

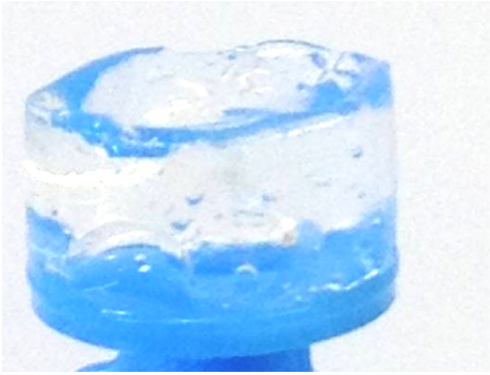


Crosslinking of hydrogels

BIOMATERIALS, BIOMECHANICS & TISSUE ENGINEERING

Chemical Crosslinking

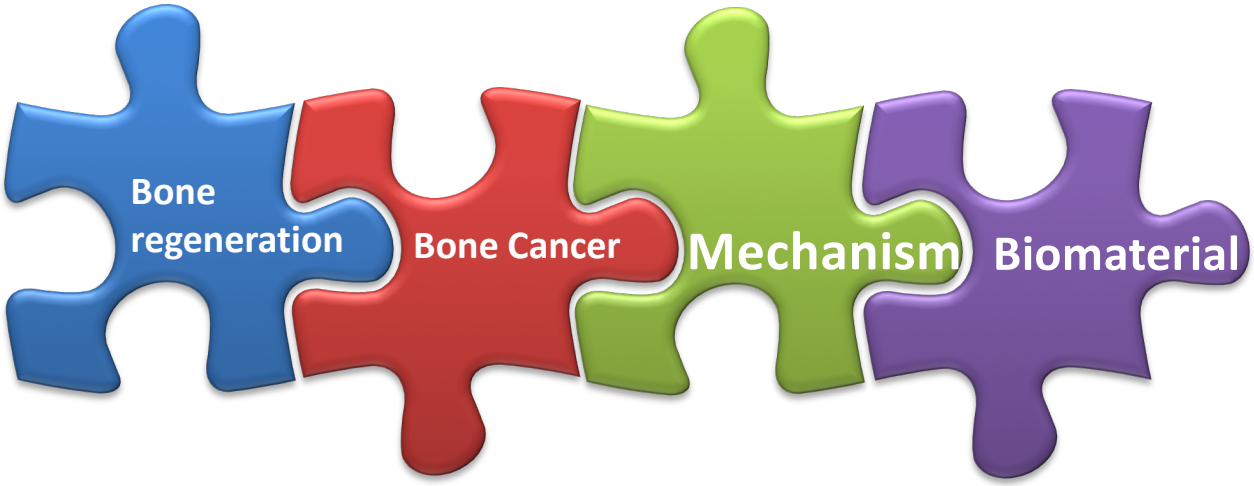
- Photosensitive agents
- Crosslinking molecules
- Polymer-Polymer
- Enzymatic crosslinking



Physical Crosslinking

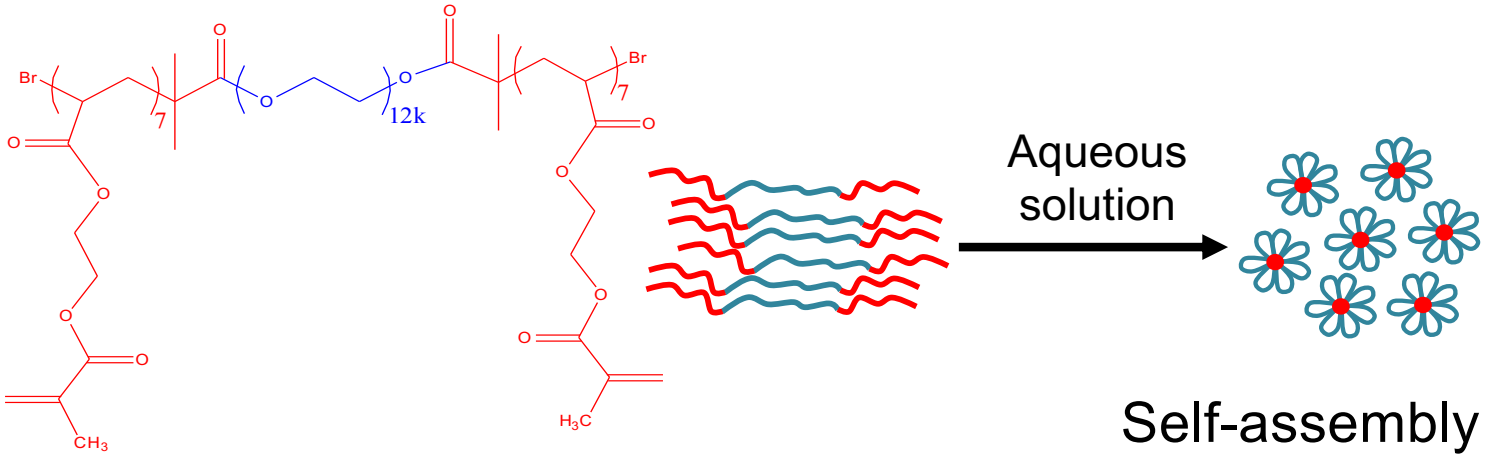
- Ionic interaction
- Hydrophobic interaction
- Hydrogen bonding
- Crystalline formation

Gelation method post-injection


Bone regeneration
Bone Cancer
Mechanism
Biomaterial

Synthetic polymers: UV crosslinking → hydrogels

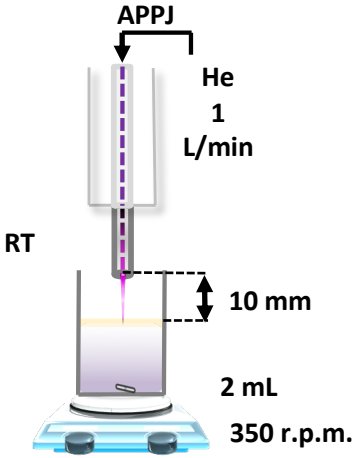
BIOMATERIALS, BIOMECHANICS & TISSUE ENGINEERING

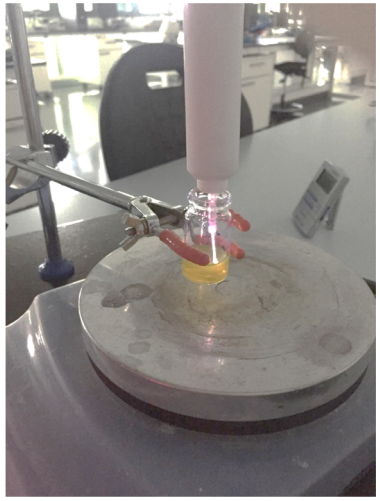


E. Nicol

IMMM Institut des Molécules et Matériaux du Mans

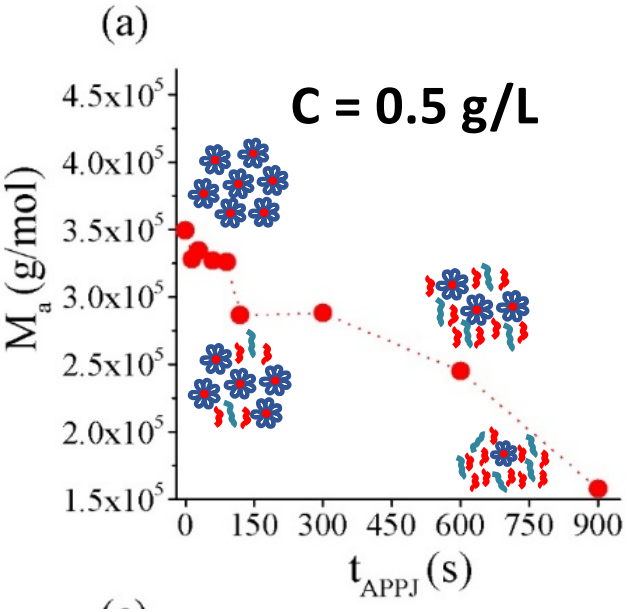
$PMEA_7$-b-POE_{12k}-b-$PMEA_7$

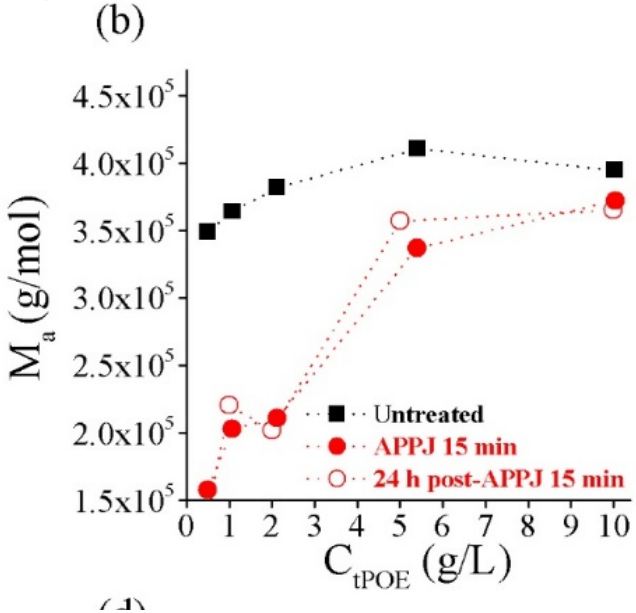




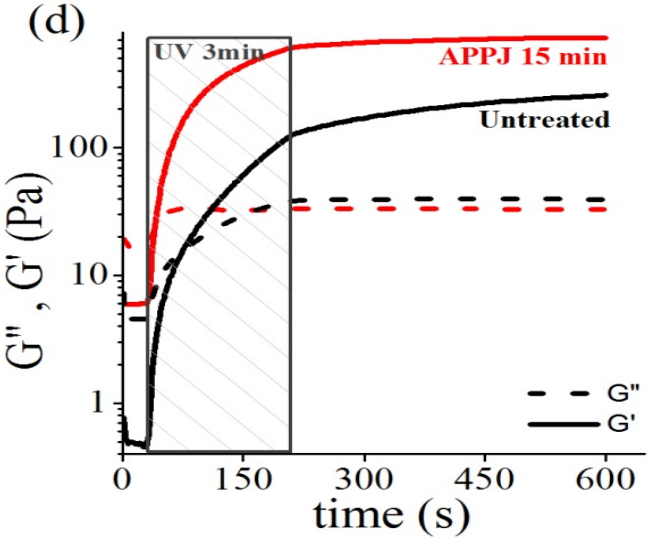
How does plasma affect the polymer ?

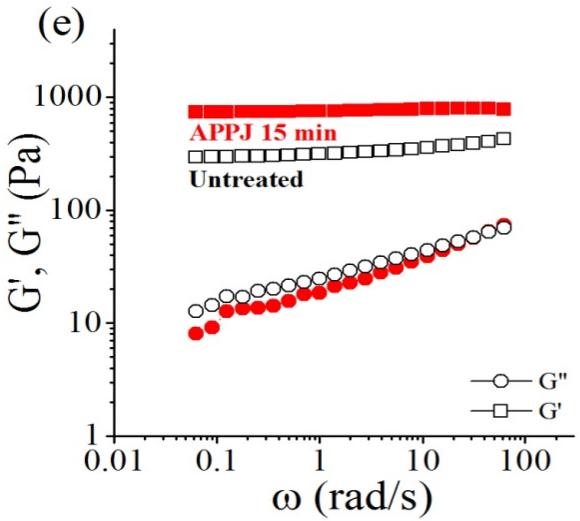
BIOMATERIALS, BIOMECHANICS & TISSUE ENGINEERING

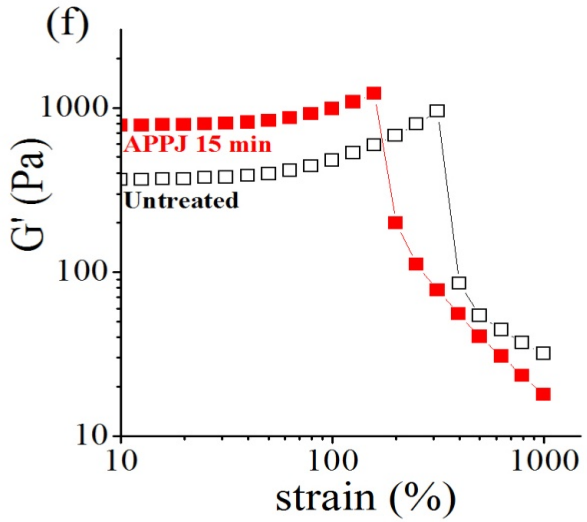




- Chain breaking at low concentrations
- Less differences at high concentrations
- Quicker crosslinking after APPJ

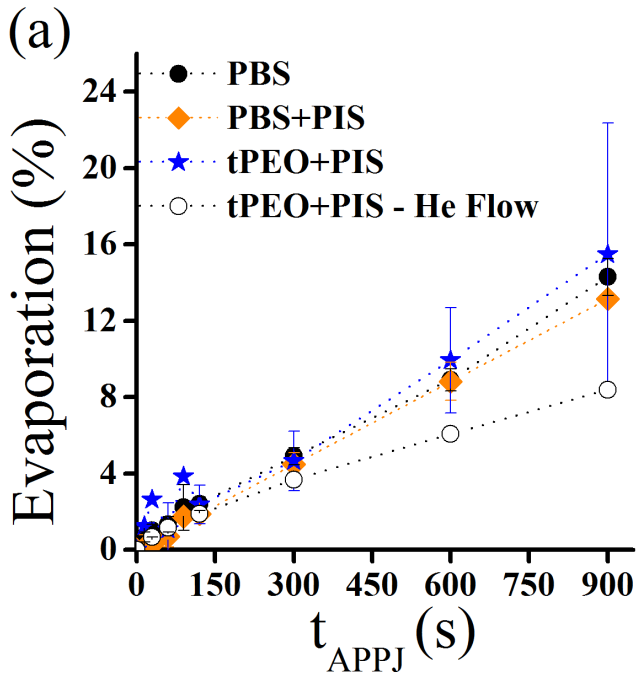


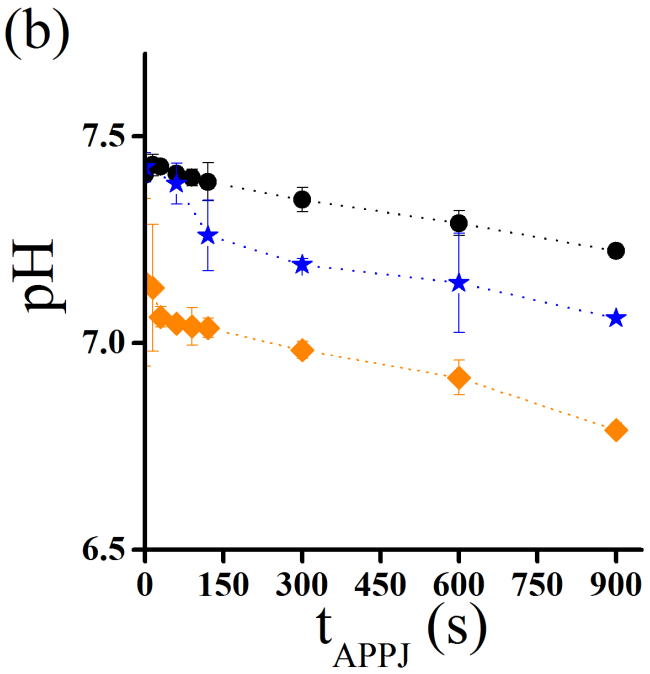


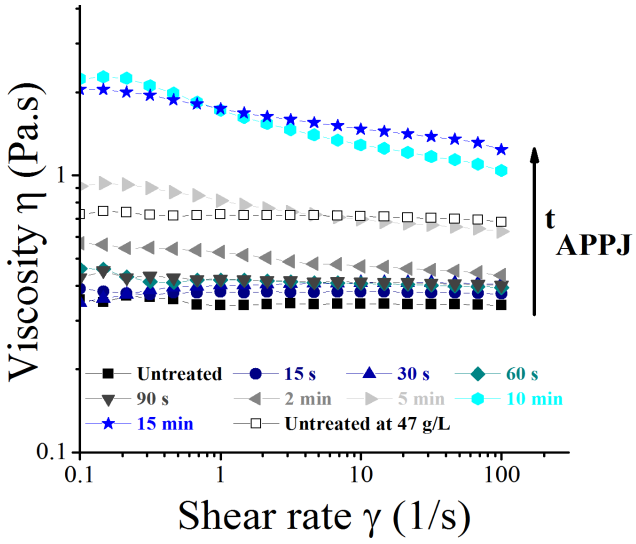


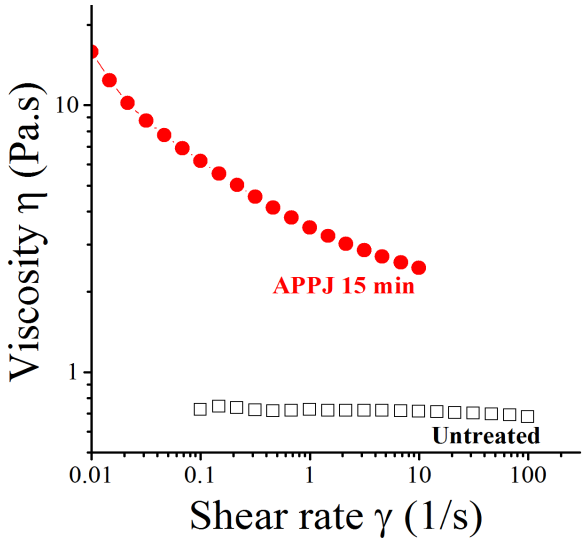
Relevant parameters towards reactivity

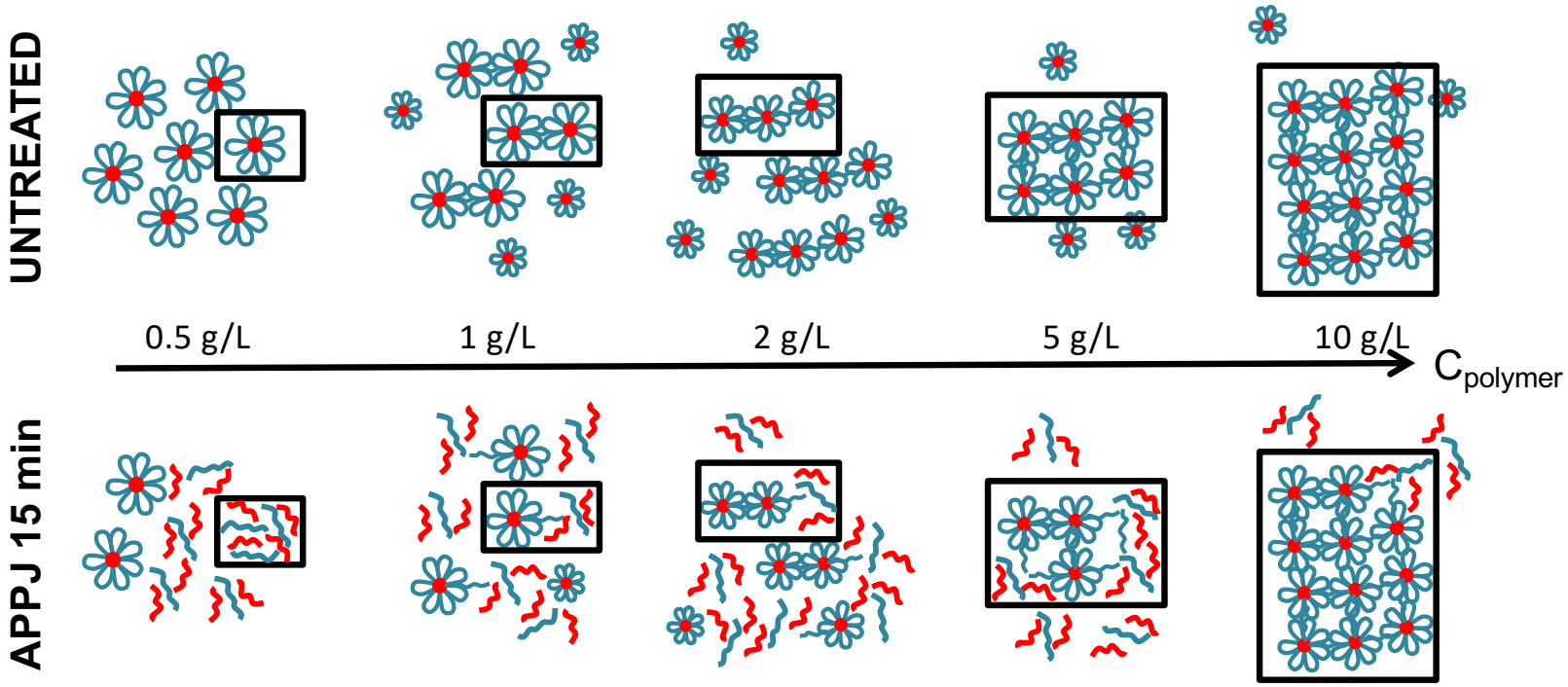
BIOMATERIALS, BIOMECHANICS & TISSUE ENGINEERING



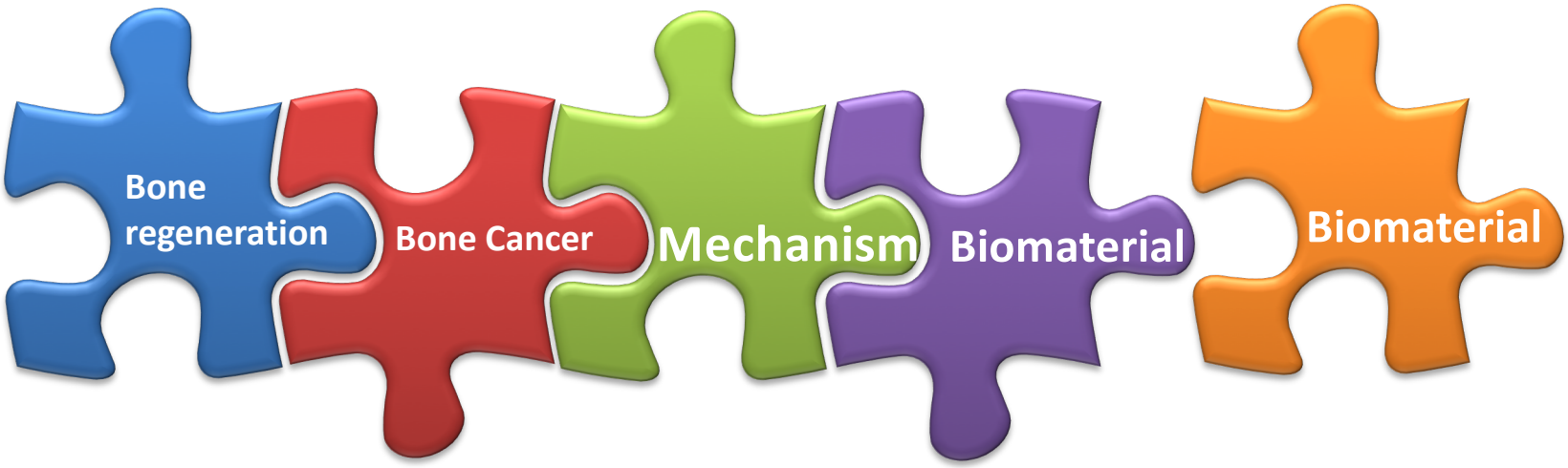






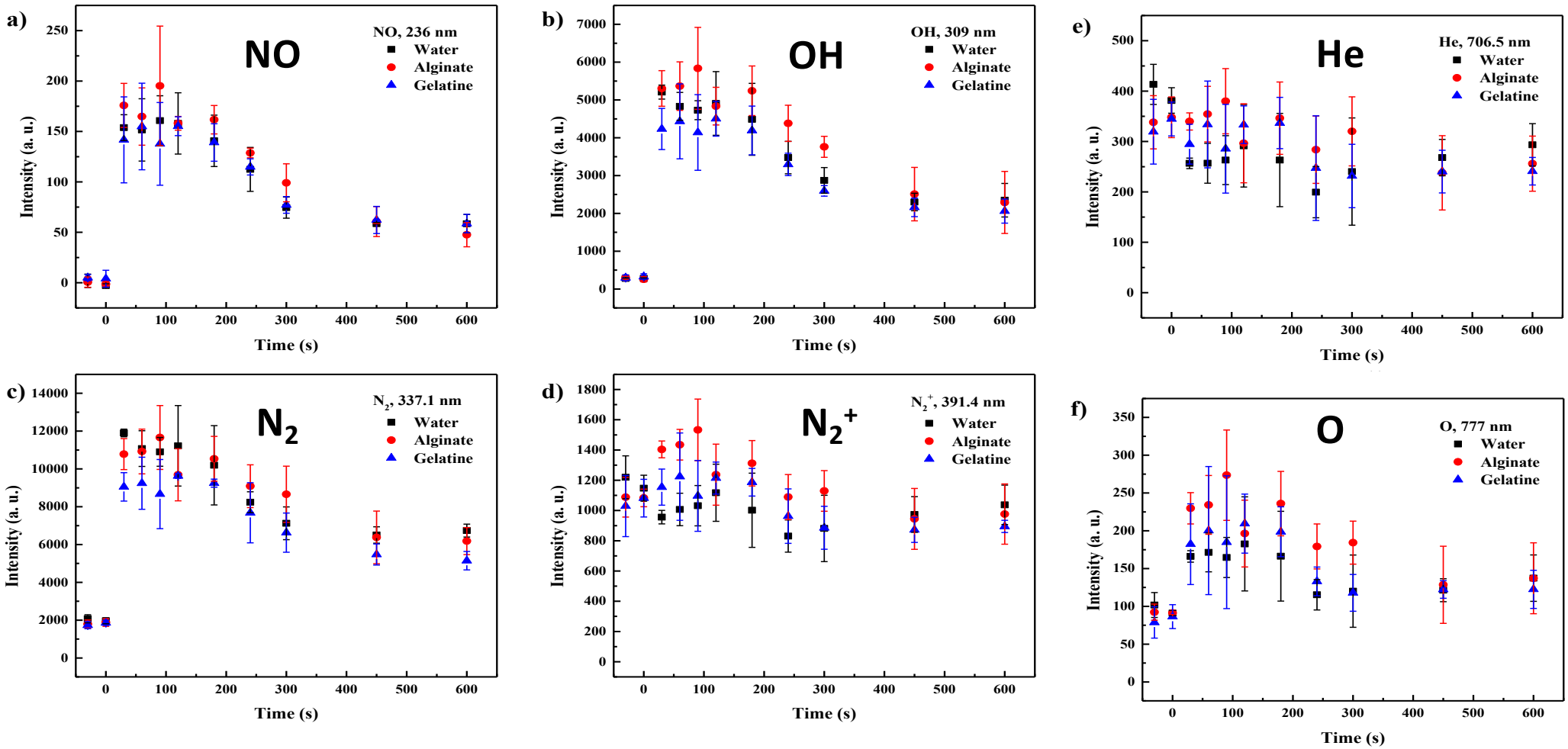


- Irreversible degradation of polymeric flower-like micelles at lower concentration
- Slight prepolymerization of the polymeric network at higher concentration
- Hydrogel-forming capacity kept, stiffer hydrogels

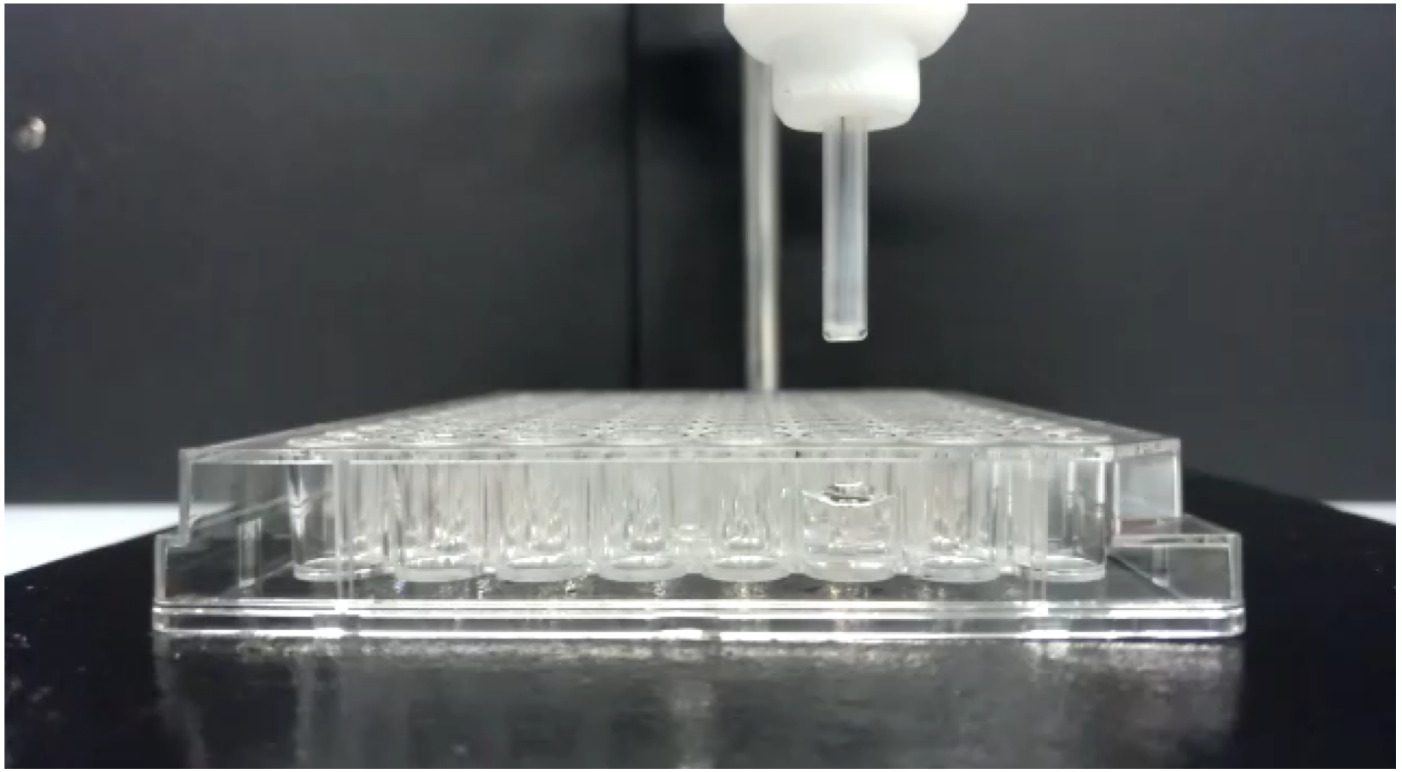

Bone regeneration
Bone Cancer
Mechanism
Biomaterial
Biomaterial

Influence of liquid treated on plasma gas phase species

BIOMATERIALS, BIOMECHANICS & TISSUE ENGINEERING



Diffusion of long-lived species

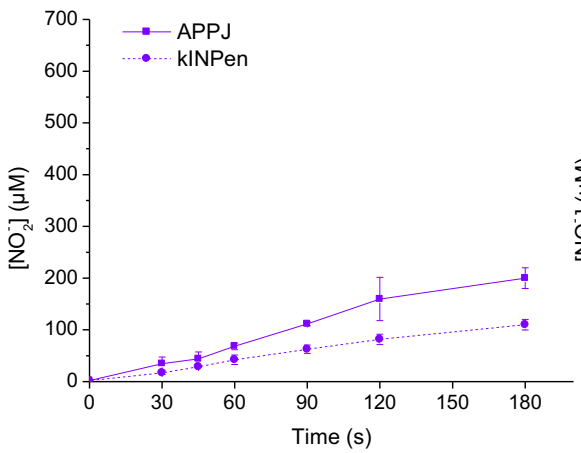


Production of RONS

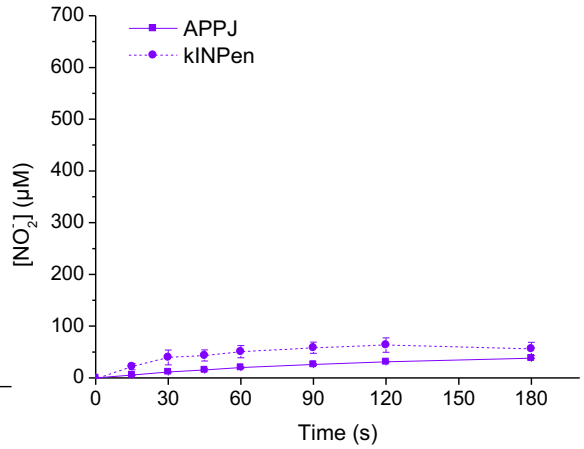
BIOMATERIALS, BIOMECHANICS & TISSUE ENGINEERING

NO_2^-

Alginate

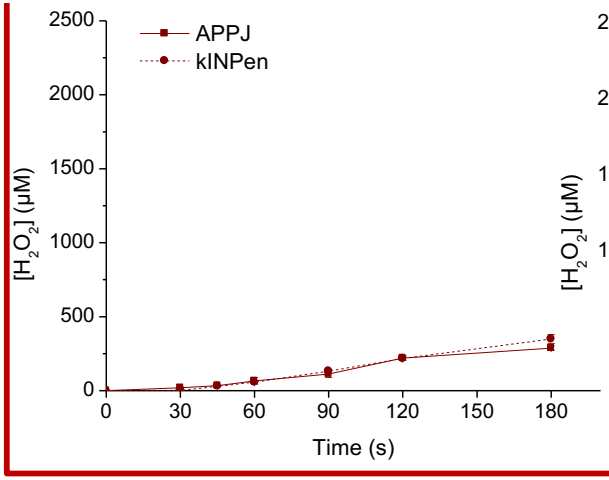


DI water

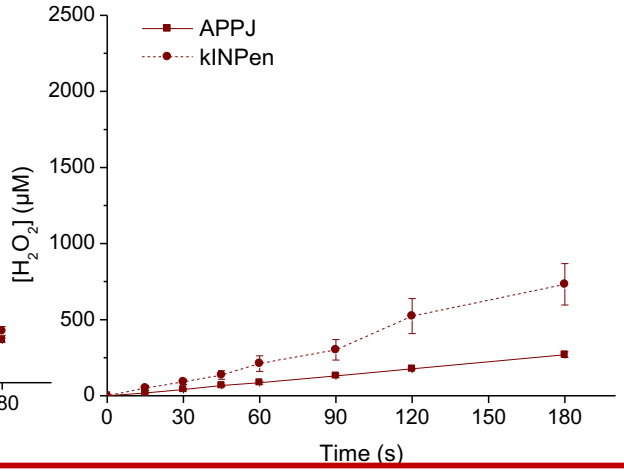


H_2O_2

Alginate



DI water



- Increase of [RONS] with plasma treatment time

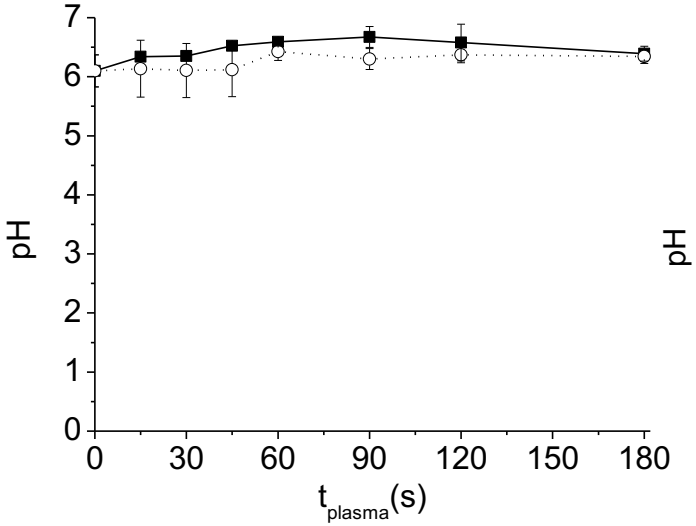
C. Labay, et al. *Sci Reports* 9, 16160 (2019)

Monitoring of pH

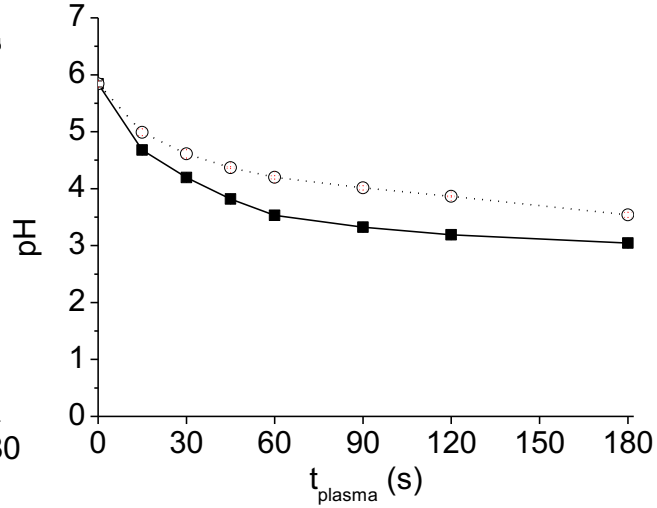
BIOMATERIALS, BIOMECHANICS & TISSUE ENGINEERING

Plasma treatment
1 L/min, 10 mm
2 mL in 24-WP

Alginate



Water



APPJ
kINPen

pH buffering effect of the hydrogels

C. Labay, et al. *Sci Reports* 9, 16160 (2019)

In vitro Osteosarcoma cell viability

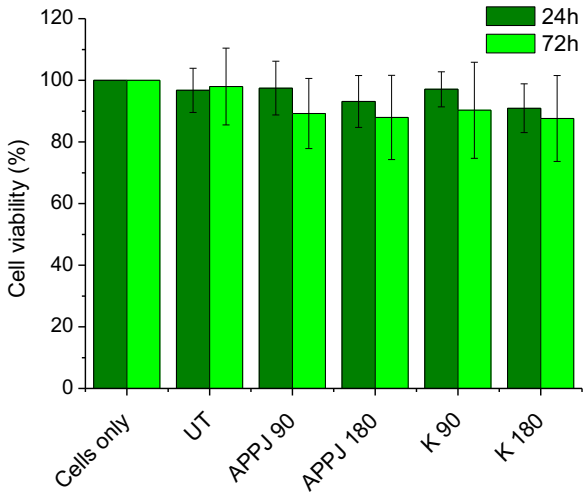
BIOMATERIALS, BIOMECHANICS & TISSUE ENGINEERING

Safety??

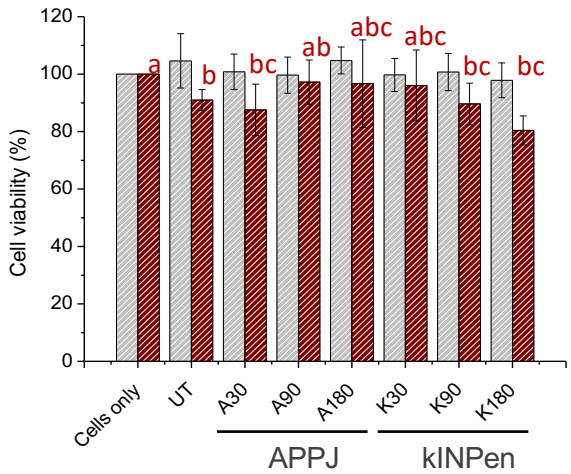
↓

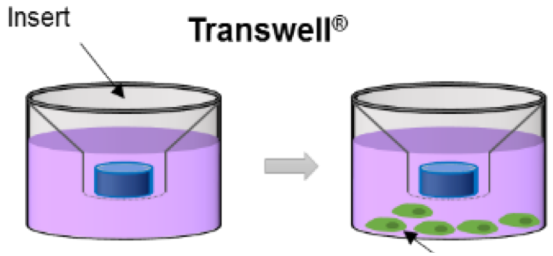
Crosslinking + washing: RONS Released

Alginate



Alginate/gelatin





C. Labay, et al. *Sci Reports* 9, 16160 (2019)

Take home message...

Plasma activated liquids & Osteosarcoma

Selectivity: Osteosarcoma cell lines are more sensitive to PAM than healthy bone cells
Single dose: long term effects
Increase in intracelular RONS
DNA Damage, apoptosis

From 2D to 3D models

Hydrogels & Plasma

Formulation of polymer critical for the amount of RONS generated
Crosslinking ability maintained
Local release of RONS

BIOMATERIALS,
BIOMECHANICS &
TISSUE ENGINEERING

European Research Council
Established by the European Commission
Supporting top researchers from anywhere in the world

Co-funded by the Horizon 2020 programme of the European Union

M. Roldan

E. Nicol
Le Mans Université

U. Cvelbar
Jožef Stefan Institute

R. Rodríguez
SERVICIO DE SALUD DEL PRINCIPADO DE ASTURIAS
Hospital Universitario Central de Asturias

P. Layrolle
Instituts thématiques
Inserm
Institut national de la santé et de la recherche médicale

This presentation reflects only the authors' views and not those of the European Community. The information in this document is provided "as is" and no guarantee or warranty is given that the information is fit for any particular purpose. The user thereof uses the information at its sole risk and neither the European Community nor any member of the Consortium is liable for any use that may be made of the information.

BIOMATERIALS,
BIOMECHANICS &
TISSUE ENGINEERING

cristina.canal@upc.edu

@ERCAPACHE

http://ercapache.upc.edu/