Marie Skłodowska-Curie Actions (MSCA)
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Hyaluronic Acid Based Hydrogels

Evgenia Tsanaktsidou, CPERI/CERTH

"Engineering Responsive and Biomimetic Hydrogels for Biomedical Therapeutic and Diagnostic Applications"

3rd BIOGEL Network Meeting

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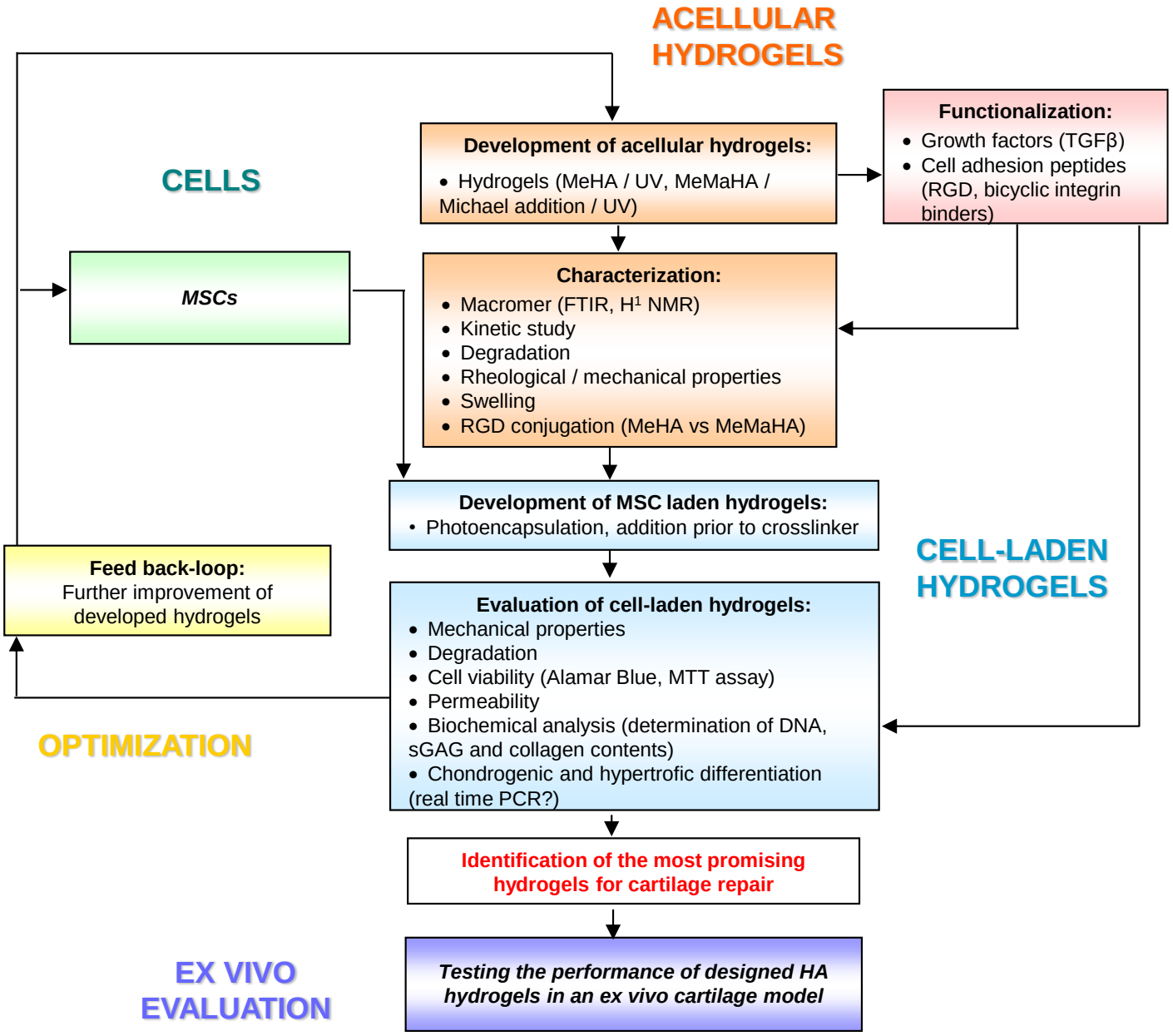
Position ESR2

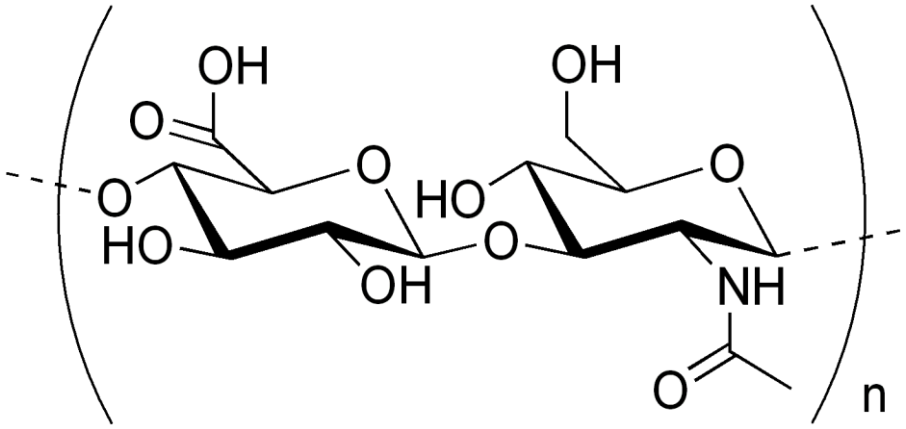
The main goal is to develop functionalized HA based hydrogels that will promote chondrogenesis of the encapsulated MSCs and result in an overall increase in cartilage matrix content and matrix spatial distribution, as well as restrict hypertrophic differentiation of the chondrogenically induced MSCs resulting in less matrix calcification.

Workplan


ACELLULAR HYDROGELS
Development of acellular hydrogels:
• Hydrogels (MeHA / UV, MeMaHA / Michael addition / UV)
Functionalization:
• Growth factors (TGFβ)
• Cell adhesion peptides (RGD, bicyclic integrin binders)
CELLS
MSCs
Characterization:
• Macromer (FTIR, H1 NMR)
• Kinetic study
• Degradation
• Rheological / mechanical properties
• Swelling
• RGD conjugation (MeHA vs MeMaHA)
Development of MSC laden hydrogels:
• Photoencapsulation, addition prior to crosslinker
CELL-LADEN HYDROGELS
Feed back-loop:
Further improvement of developed hydrogels
Evaluation of cell-laden hydrogels:
• Mechanical properties
• Degradation
• Cell viability (Alamar Blue, MTT assay)
• Permeability
• Biochemical analysis (determination of DNA, sGAG and collagen contents)
• Chondrogenic and hypertrofic differentiation (real time PCR?)
OPTIMIZATION
Identification of the most promising hydrogels for cartilage repair
EX VIVO EVALUATION
Testing the performance of designed HA hydrogels in an ex vivo cartilage model

Hyaluronic acid (HA)

- Linear polysaccharide of alternating D-glucuronic acid and N-acetyl-D-glucosamine.
- Can be modified through its carboxyl and hydroxyl groups (esterification, methacrylation) and subsequently crosslinked into hydrogels.



Can be easily and controllably produced in large quantities through microbial fermentation, enabling the scale-up of HA-derived products and avoiding the risk of animal-derived pathogens.

HA Hydrogels

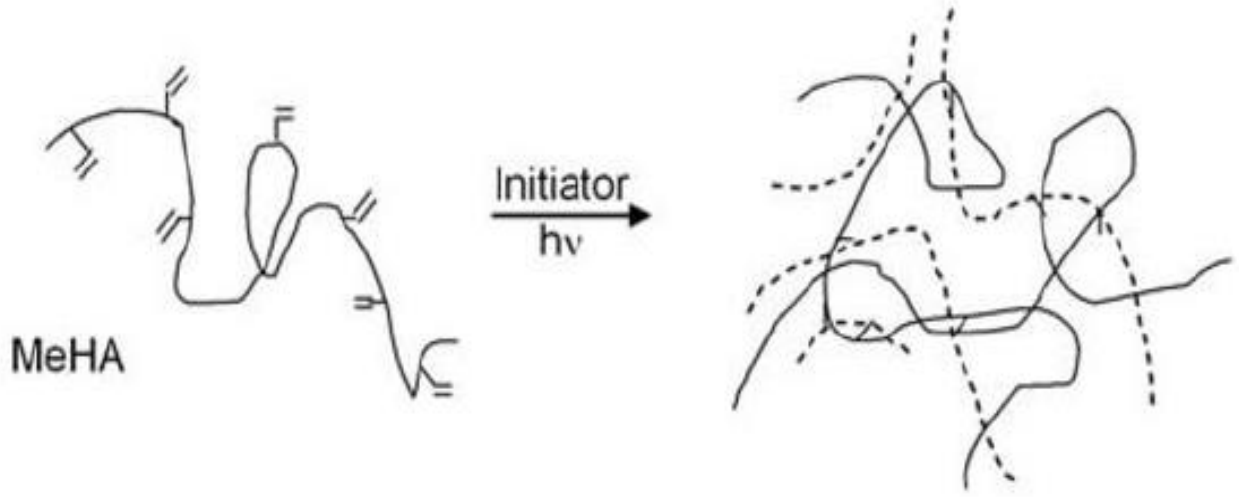
- HA hydrogels are formed by methacrylation or esterification and subsequent crosslinking.
- Their properties (e.g., volumetric swelling ratios, mechanical properties) can be tuned by varying the degree of methacrylation, macromer MW, macromer concentration, etc.
- Undergo enzymatic degradation via hyaluronidases.

For applications in cartilage repair, it has been found that the HA hydrogels not only support and maintain chondrocyte viability and phenotype when cultured in vitro and in vivo, but also that HA hydrogel chemistry supports and promotes the chondrogenic differentiation of MSCs.

MeHA Hydrogels

- Photopolymerized MeHA hydrogels functionalized or not with adhesion peptides will be developed as controls.

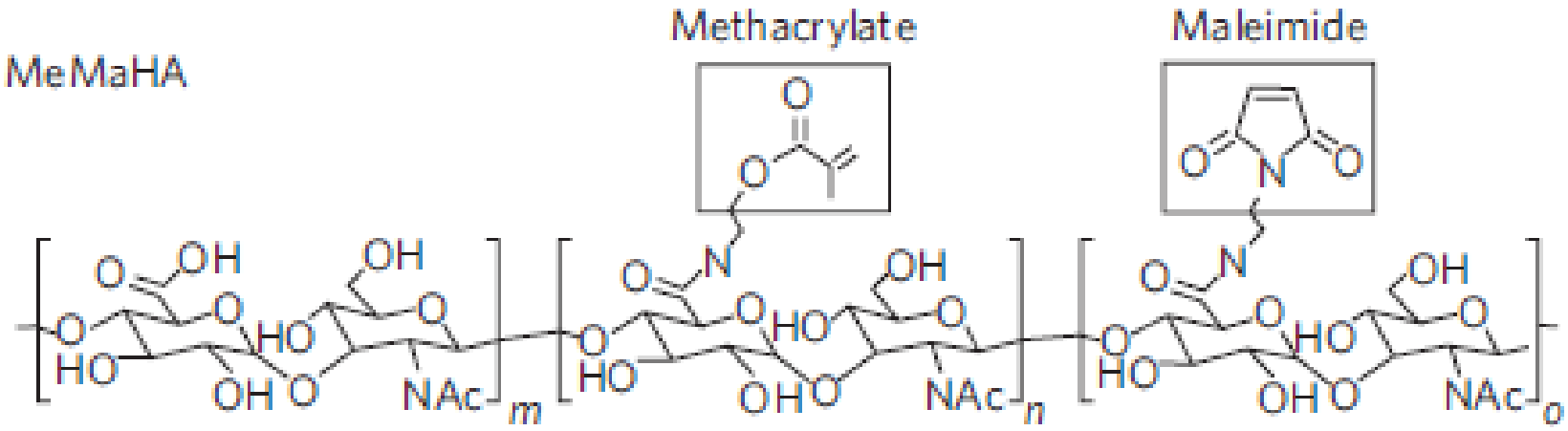
Photopolymerization of MeHA



The macromer solution is injected into a mold or between glass slides and exposed to UV light

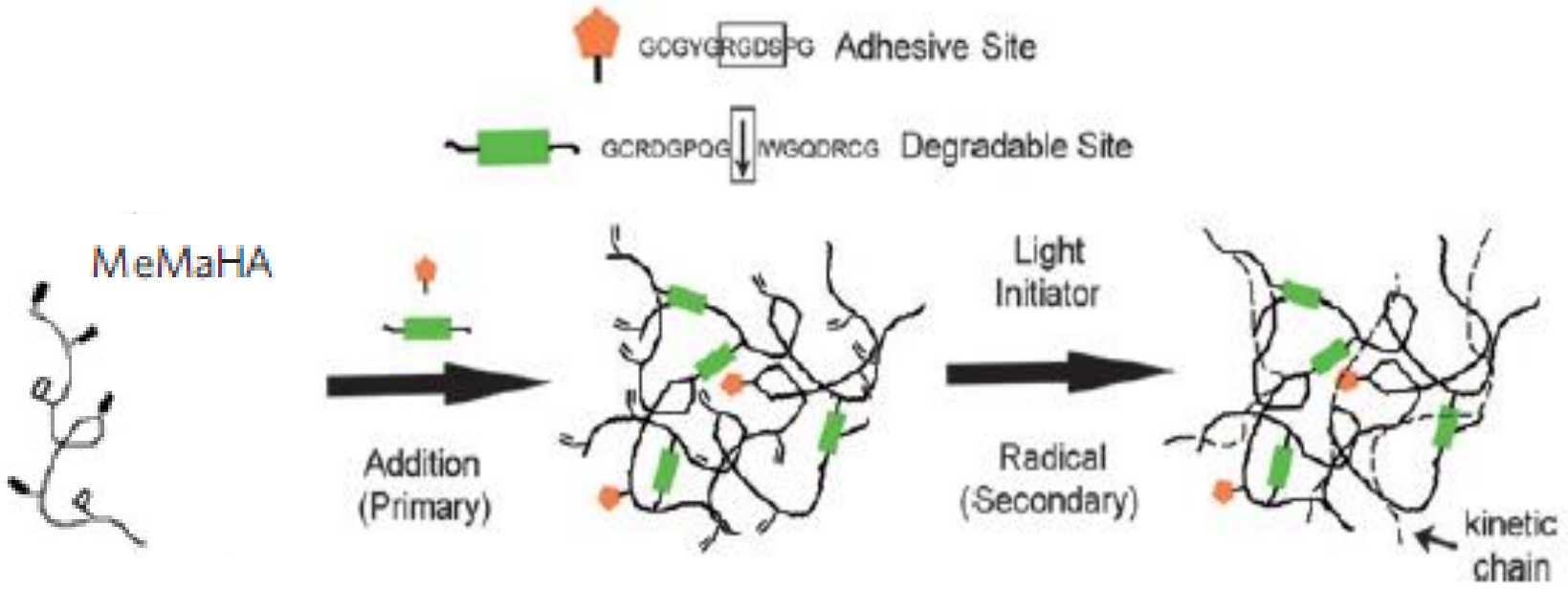
MeMaHA Macromer

- HA based hydrogels containing both adhesive sites and bifunctional matrix metalloproteinase (MMP)-degradable peptides will be formed from HA functionalized with both methacrylate and maleimide groups (MeMaHA).



MeMaHA Hydrogels

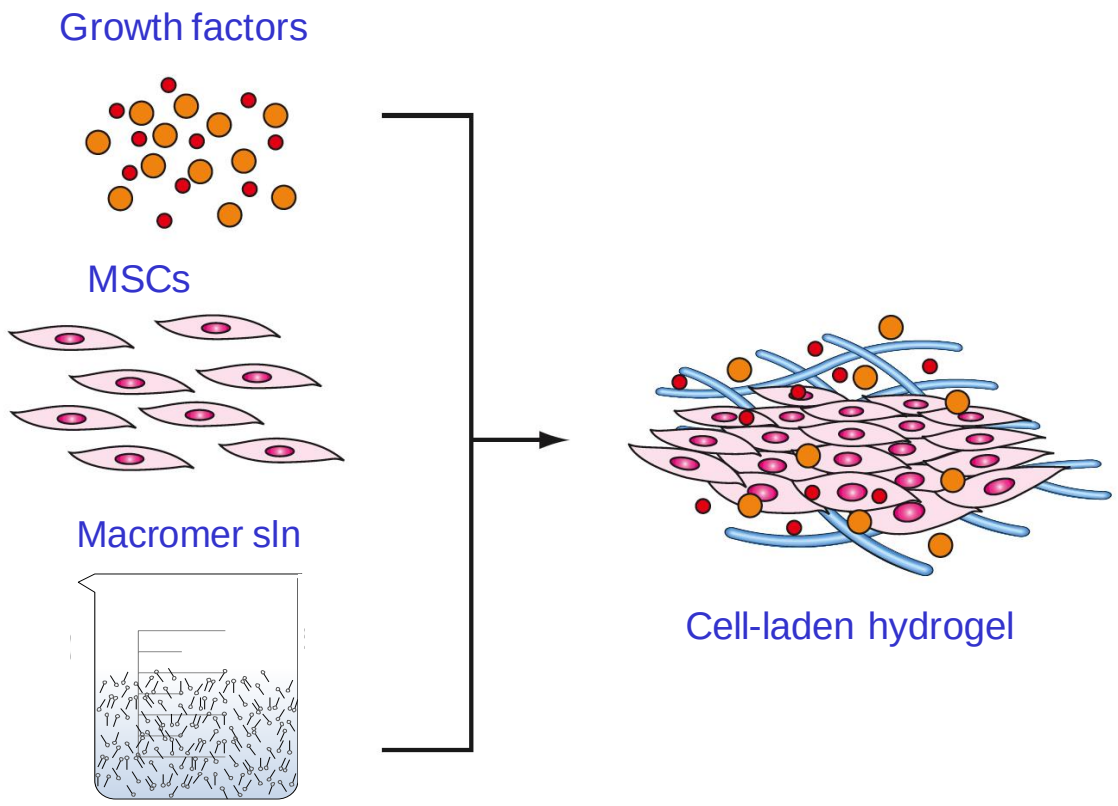
- Cys-containing RGD peptides as well as specifically designed bicyclic integrin binders will be conjugated to MeMaHA via a Michael-type addition reaction.
- Maleimide groups will react via addition reactions with thiols from cysteines on selected MMP-degradable peptides resulting in the formation of covalently crosslinked hydrogels.
- The formed hydrogels will be further photopolymerized to introduce kinetic chains that impede proteolytic degradation.



Biofunctional Cell-laden Hydrogels

- MSCs will be suspended in macromer solutions.
- Growth factors (e.g., TGFs) will be also added to the macromer solution.

Development of Biofunctional Cell-laden Hydrogels



Adhesion peptides will have been coupled to the produced macromer

Optimization - Evaluation

- The produced HA hydrogels will be tuned with respect to degree of crosslinking, mechanical/rheological properties, morphology, degradation and release of growth factors.
- The performance of the hydrogels and their ability to control MSCs differentiation and cartilage repair will be tested in in vitro models and an ex vivo cartilage model, respectively.

Deliverables

D1.1: MeHA and MeMaHA: HA with different chemical modifications and a range of molecular weight

D2.1: MeHA and MeMaHA hydrogels with optimal mechanical properties: MeHA and MeMaHA hydrogels with optimal mechanical properties for MSC differentiation

D3.1: MeHA and MeMaHA hydrogels with defined biological properties: Modified HA hydrogel for promoting MSC differentiation

D3.6: In vitro and ex vivo testing of designed hydrogels: Performance of biomimetic hydrogels in in vitro and ex vivo models

Long-Term Career Objectives

- Increase my knowledge in the biomaterials field and its use in regenerative medicine, particularly in cartilage repair.
- Gain specialized skills in experimental design, in conducting experiments and analyzing results.
- Get a high-level training in hydrogel and peptide synthesis and biomedical techniques.
- Develop other expertise essential to become a proficient researcher like enhancing my communication and presentation skills.

Short-Term Objectives

- Fabricating 3D hydrogels promoting chondrogenesis and restricting hypertrophy of the encapsulated MSCs.
- Effect of hydrogel synthesis parameters on the degree of crosslinking, mechanical/rheological properties, morphology, degradation, release of growth factors, MSCs chondrogenesis, etc.
- Experimental evaluation of linear and specifically designed bicyclic adhesion peptides.
- Comparison of MMP-sensitive with MMP-insensitive hydrogels with respect to cartilage matrix content and matrix spatial distribution.
- Evaluation of the performance of optimized hydrogels and their ability to control MSCs differentiation and cartilage repair in in vitro models and ex vivo cartilage models.

Secondments

PEPSCAN: March 1, 2016 – June 30, 2016: Fabrication of peptides to promote MSCs differentiation

RU: August 2016: Testing of mechanical properties of HA hydrogels

LTG: June 1, 2017 - October 31, 2017: Testing the performance of designed HA hydrogels in ex vivo cartilage model