Osteochondral tissue regeneration with biofunctional hydrogel scaffolds based on MSCs embedded in an Elastin-Like Recombinamers matrix

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Introduction & Aim of work

Musculoskeletal disorders include a massive number of severe diseases, syndromes and injuries generally painful, that progress with time to degenerative states such as osteoarthritis; moreover articular cartilage is an highly loaded thin layer of tissue with limited regenerative abilities, due to presence of few cells and the lack of vessels or nerves. ELRs play an essential role in developing new therapies to achieve an optimal osteochondral tissue repair, they are able to self-assemble into different structures, of which hydrogels are the most promising for tissue regeneration [1]. Moreover, according to the elastin-like nature of the hydrogel and to the high percentage of elastin present in the native chondral matrix, ELR-based hydrogels, are likely to be similar to hyaline cartilage [2]. In this work we developed a biofunctional hydrogel based on Elastin-Like Recombinamers as injectable scaffold for osteochondral repair.

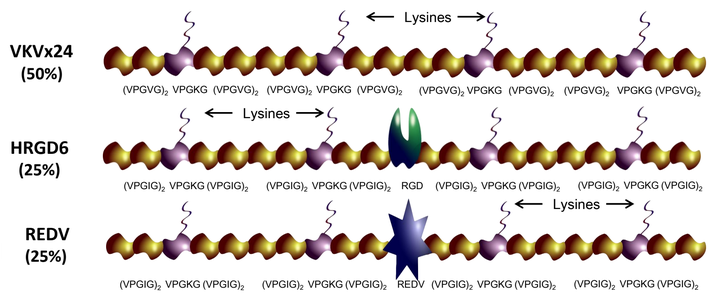
Materials and Methods

Synthesis of the recombinamers containing bioactive sequences was achieved by recombinant DNA techniques, assessed by agarose gel electrophoresis, and expressed in *E. coli*. The produced ELRs have been characterized by standard physicochemical analysis. Chemical modification was performed by catalyst-free click reaction [3] and the modification rate was evaluated by H-NMR. Mechanical features were studied by rheological measurements of the hydrogels and porosity evaluation by Scanning Electron Microscopy (SEM). Cytocompatibility of hMSCs have been evaluated by Cell viability analysis and Dapi/phalloidin staining along 12 days. Human chondrocytes capacity of developing ECM has been evaluated by histological staining after 14 days of 3D hydrogel culture. Finally an *In vivo* study of the hydrogel embedded with hMSCs has been analyzed after 4 months of implantation by macroscopically observation, histological study and the cartilage regeneration rate has been evaluated by ICRS Score (International Cartilage Repair Society).

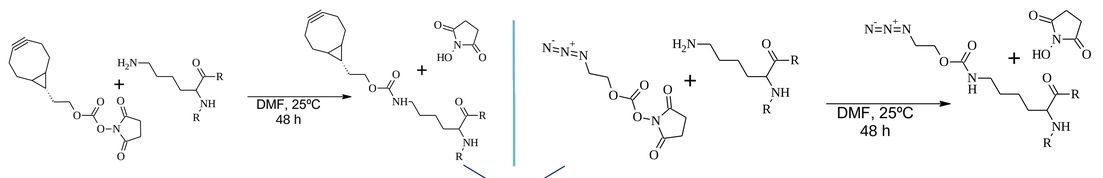
Results and Discussion

ELRs bioactive sequences composition

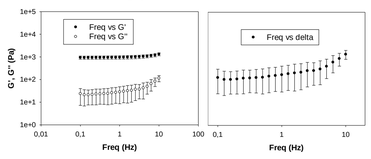
- **VKV:** gives stability to the hydrogel.
- **RGD:** cell adeshion sequence.
- **REDV**: endothelial cell adeshion and sensitiveness to elastase.

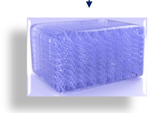


Hydrogel formation and characterization



Rheology

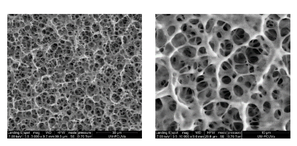




[Hydrogel] 75 mg/ml

S.E.M Analysis

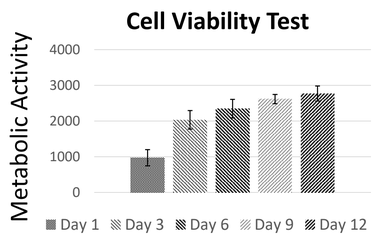
- Pore size: From 3µm to 20µm.
- Interconnected environment.



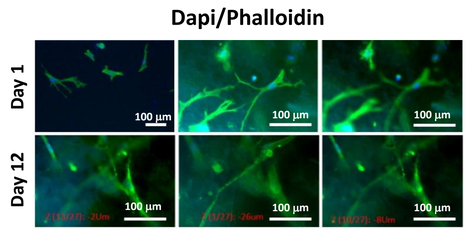
3D hydrogel *In vitro study* with hMSCs and hChondrocyes

[ELR] = 75 mg/ml
Human MSCs (8 millions/ml)

Cell Viability Test

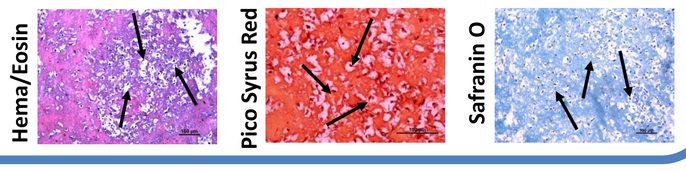


Dapi/Phalloidin



Histology

[ELR] = 75 mg/ml
Human chondrocytes (8 millions/ml)
Incubation time: 14 days



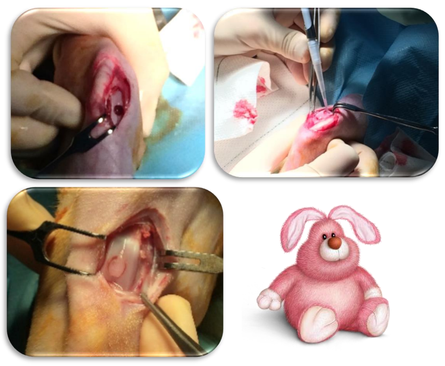
In vivo study: Injectable hydrogel with hMSCs

Components
- 10 New Zealand Rabbits
- hMSCs from Bone Marrow
- ELR hydrogels

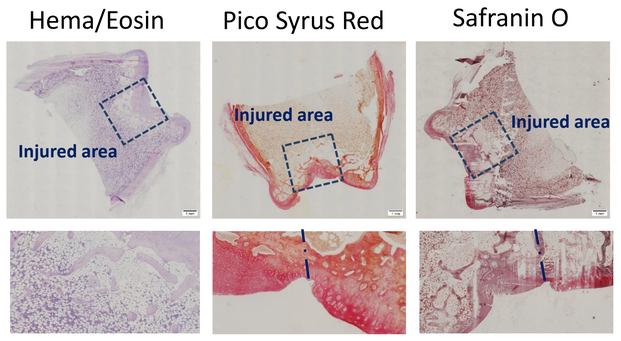
Osteochondral defect
- Subchondral drilling (4x4 mm)
- Femoral condyles of hind legs
- Non-self healing injury

Implants
- Hydrogel & Hydrogel + hMSCs ($8x10^6$/ml)
- Without growth factors and chondrogenic stimulators
- 4 months of implantation

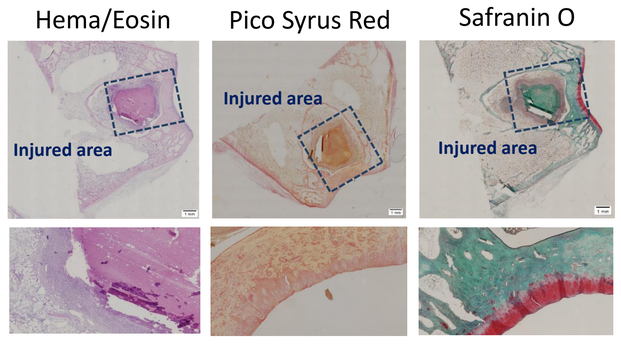
Evaluation methods
- Macroscopically observation
- Histological Study
- ICRS Score



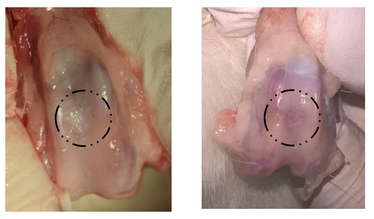
Hydrogel with hMSCs



Hydrogel Itself



Macroscopically observation



I. Degree of defect repair (0-4)
II. Integration to border zone (0-4)
III. Macroscopic apperence (0-4)

Total (0-12)

Histological study

I. Surface (0-3)
II. Matrix (0-3)
III. Cell distribution (0-3)
IV. Cell population viability (0-3)
V. Subchondral bone (0-3)
VI. Cartilage mineralization (0-3)

Total (0-18)

ICRS SCORE*	Macroscopic Observation	Histological Study
Hydrogel:	9,7 ± 1,3	11,4 ± 3,1
Hydrogel + hMSCs:	9,5 ± 1,9	11,3 ± 3,3

* Average of n= 10

Conclusion

To conclude, in this work we obtained a bicomponent system composed by ELRs bioactive hydrogel embedded with hMSCs as injectable scaffold, which have shown adequate composition and biomechanical properties for osteochondral tissue regeneration. *In vitro* studies have been performed with hMSCs (cell viability assay and Dapi/Phalloidin staining) showing a great cytocompatibility of ELRs. Furthermore histological analysis of 3D hydrogel embedded with human chondrocytes show the cells' capacity to develop their own ECM. ICRS Score for *in vivo* study reveals ELRs hydrogel with MSCs a good system for osteochondral regeneration, finally hydrogel itself demonstrates great ability in cells recruitment for an excellent cartilage repair and bone regeneration in time.

References

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[3] I. Gonzalez de Torre et al. Acta Biomaterialia, 10(6), 2495-505 (2014).

Acknowledgements

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