UNIVERSITÀ DEGLI STUDI DI BRESCIA

ARTIFICIAL INTELLIGENCE IN MEDICINE AND INNOVATION IN CLINICAL AND METHODOLOGICAL RESEARCH

XXXIX CYCLE

Coordinatore: Prof. Domenico Russo

DEVELOPMENT OF A 3D MODEL BASED ON BIOCOMPATIBLE POLYMERIC SCAFFOLDS ENGINEERED WITH HUMAN MESENCHYMAL STROMAL CELLS FOR SKIN REGENERATIVE MEDICINE

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Supervisor: Prof. Domenico Russo

Co-Supervisor: Dott.ssa Federica Re

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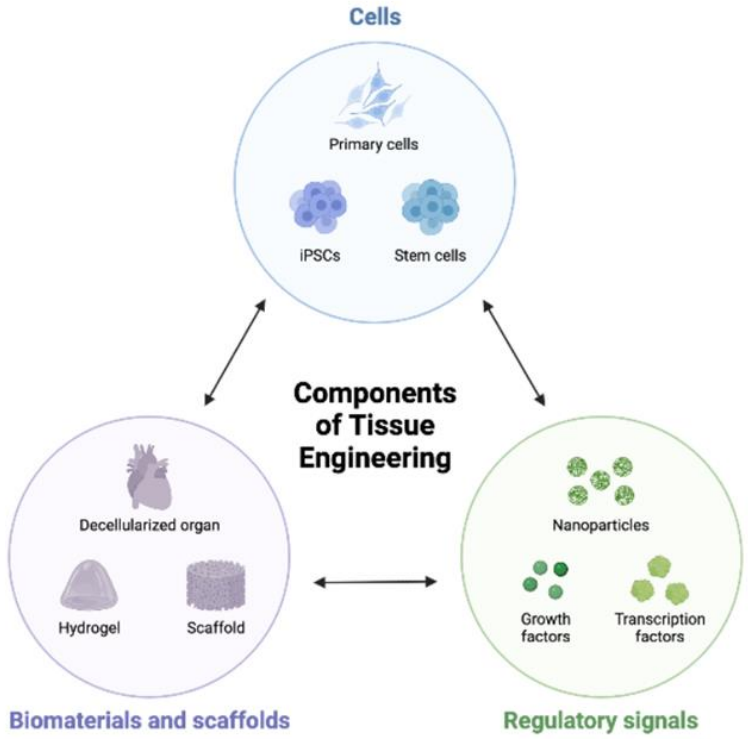
BACKGROUND

Regenerative medicine is an interdisciplinary branch of medicine focused on the repair, regeneration and replacement of damaged cells, tissues or organs in order to restore normal physiological function.

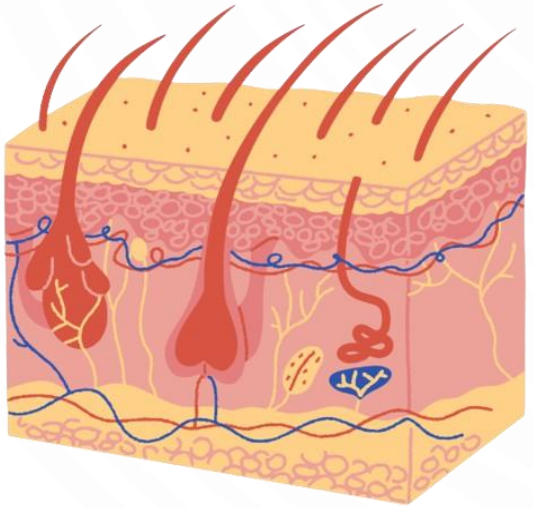
1. Stem cells
2. Scaffold/biomaterials
3. Growth factors



A. J. Salgado et al., "Tissue engineering and regenerative medicine: Past, present, and future," in International Review of Neurobiology, vol. 108, 2013 doi: 10.1016/B978-0-12-410499-0.00001-0

BACKGROUND

The **skin** belongs to the integumentary system and is the largest organ in the body (it corresponds to about 16% of our weight).
It is the first barrier against the external environment and is able to repair itself thanks to the stem cells present into the layers.



LAYER	CONSTITUENT CELLS
EPIDERMIS	Keratinocytes, stem cells, melanocytes
DERMIS	Fibroblasts
HYPODERMIS	Adipocytes

BACKGROUND

Skin wounds are a common complication of diseases (such as diabetes, vasculopathies, surgery, and burns) and the healing process requires a set of molecular and biological events involving numerous cells types.

SKIN SUBSTITUTES IN CLINIC

- Dermal, epidermal and dermo-epidermal equivalents
- Autologous, allogeneic, xenogeneic grafts
- Permanent, semi-permanent, temporary
- Cellular or acellular

The **current therapeutic strategy** involves the use of SKIN GRAFT of different derivation and composition.

MatriDerm®

BACKGROUND

PRO	CONS
Excellent in vitro and in vivo integration	Risk of infection or rejection
Adequate elasticity and structure to the skin	High cost
Hemostatic properties	Limited availability of autologous skin grafts

Nowadays, **THERE IS NO** an equivalent cutaneous able to satisfactorily mimic the native skin, nor a bio-engineered model that is also capable of facilitating the process of skin repair, especially if characterized by a profound loss of substance.

PROJECT IDEA AND OBJECTIVES

Develop a three-dimensional (3D) model based on the use of bioengineered polymeric scaffolds and human mesenchymal stem cells to study skin repair mechanisms and create an innovative strategy to regenerate damaged skin tissue.

1

2D MODEL: Develop and standardize a protocol for differentiating stem cells into fibroblasts and keratinocytes.

2

Create new **SCAFFOLD** and improve existing ones to find the best structure to reply to the clinical needing.

3

3D MODEL: Combine the first two objectives and apply the differentiation protocol to stem cells seeded into scaffolds.

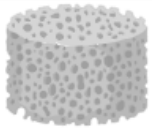
EXPERIMENTAL DESIGN

1

2D MODEL: Develop and standardize a protocol for differentiating stem cells into fibroblasts and keratinocytes.

- ✓ Bibliographic search of markers and differentiation factors;
- ✓ Evaluation of markers expression at RNA and protein levels at various time points.

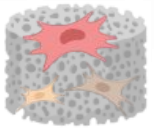
2



Create new **SCAFFOLD** and improve existing ones to find the best structure to meet clinical needs.

- ✓ Morphology and porosity;
- ✓ Composition;
- ✓ Chemical-physical and mechanical properties;
- ✓ Swelling ratio and mass loss.

3



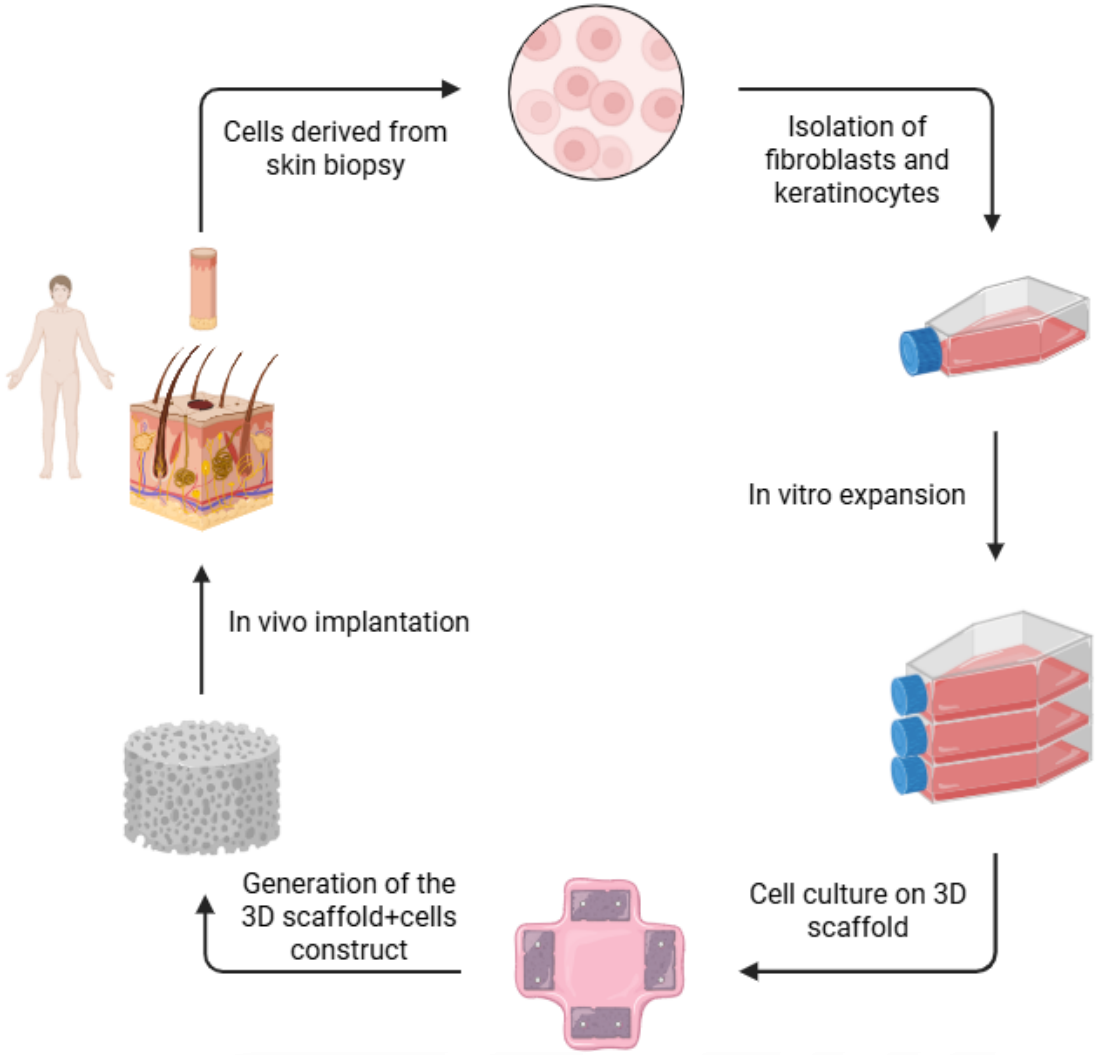
3D MODEL: Combine the first two objectives and apply the differentiation protocol to stem cells seeded into scaffolds.

- ✓ Scaffold biocompatibility testing (cell viability and proliferation);
- ✓ Application of differentiation protocol to stem cells inside the scaffolds and markers evaluation.

Cells will be grown and functionally characterized by comparing the effects of **human Platelet Lysate** (hPL) versus **Fetal Bovine Serum** (FBS) as source of growth factors into the culture media for skin regeneration.

EXPERIMENTAL DESIGN

Isolation, expansion and scaffold seeding of fibroblasts and keratinocytes derived from **skin biopsies** taken from healthy patients.



RESULTS: Improvement of scaffolds for cutaneous applications

gels

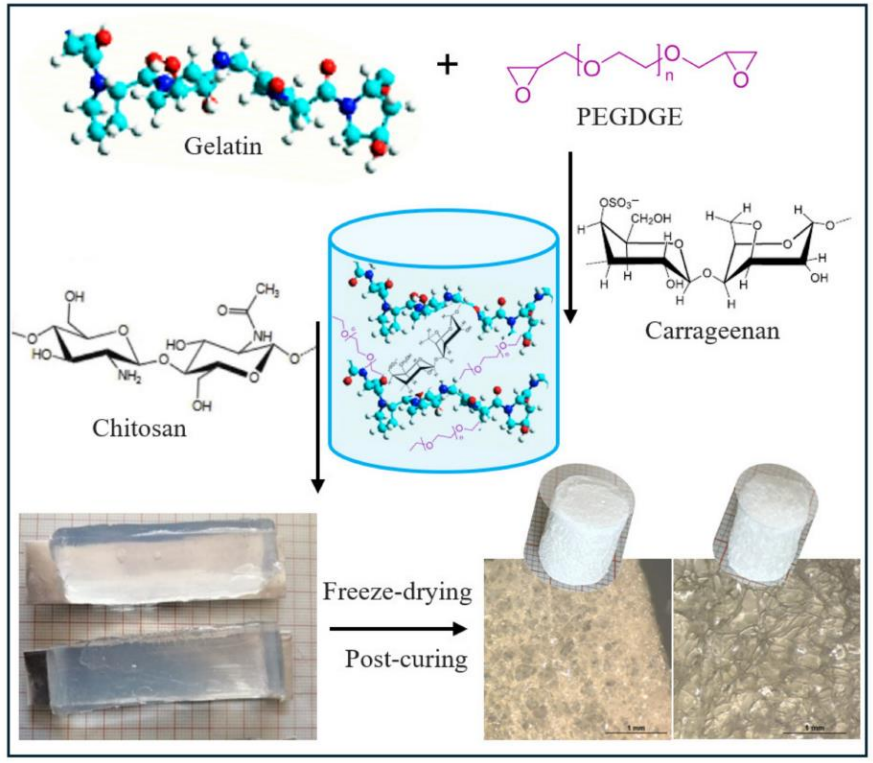
MDPI

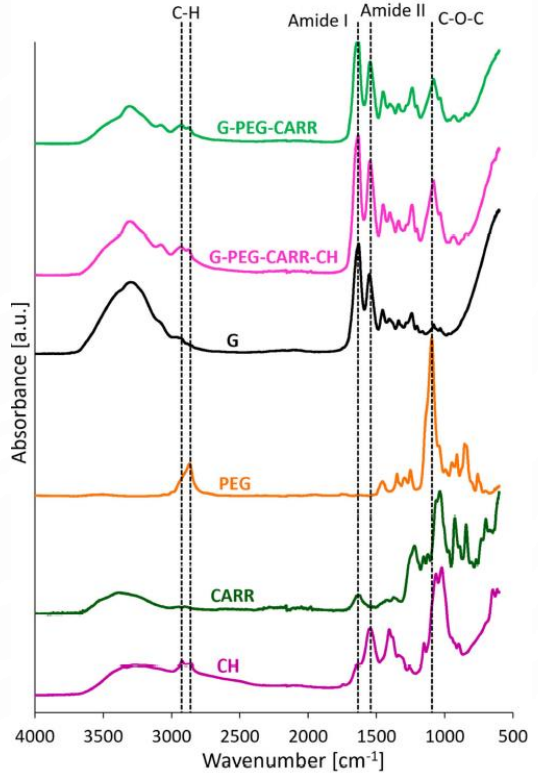
Article

Gelatin-Based Scaffolds with Carrageenan and Chitosan for Soft Tissue Regeneration

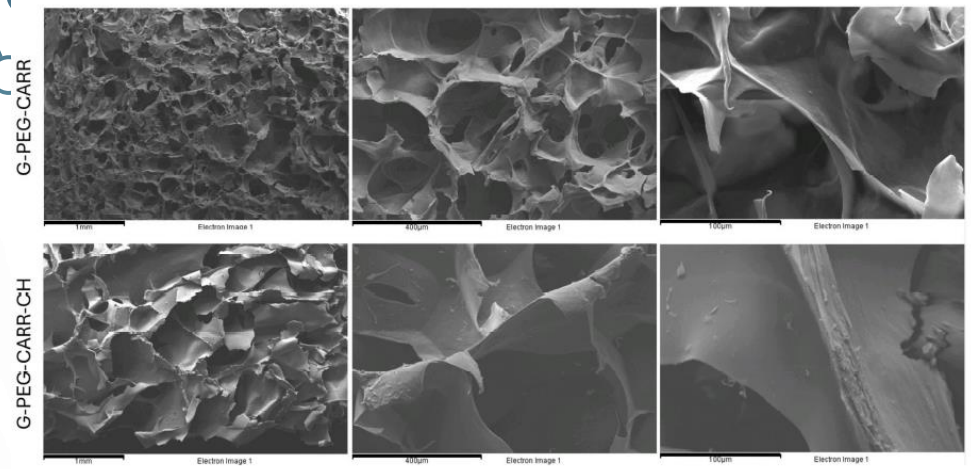
Chiara Pasini [1,†], Federica Re [2,3,†], Federica Trenta [2,3], Domenico Russo [2] and Luciana Sartore [1,*]

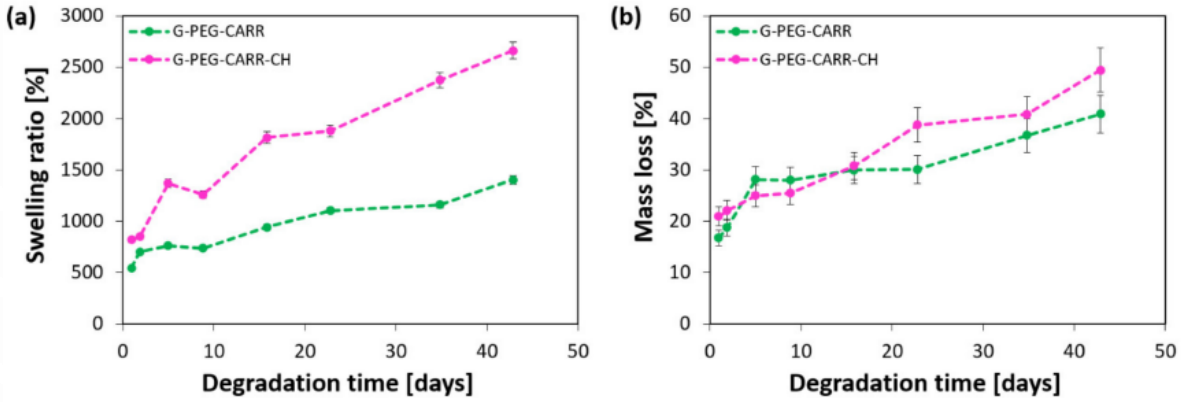
Hydrogel	Composition				Physical properties		
	G [wt%]	PEG [wt%]	CARR [wt%]	CH [wt%]	Apparent density [g/cm³]	Porosity [%]	Swelling ratio (24 h) [%]
G-PEG-CARR	75	17.5	7.5	-	0.074 ± 0.01	80 ± 5	550 ± 15
G-PEG-CARR-CH	69	17	6	8	0.080 ± 0.01	78 ± 7	830 ± 25

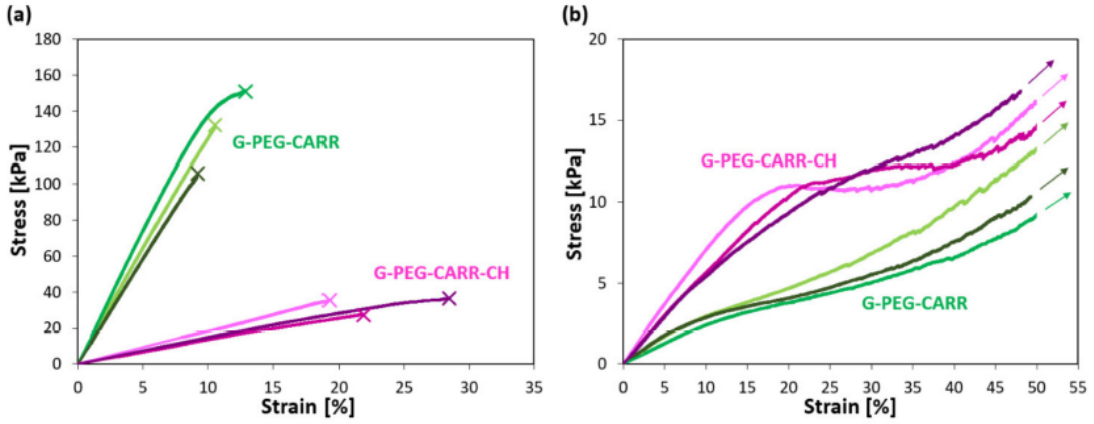


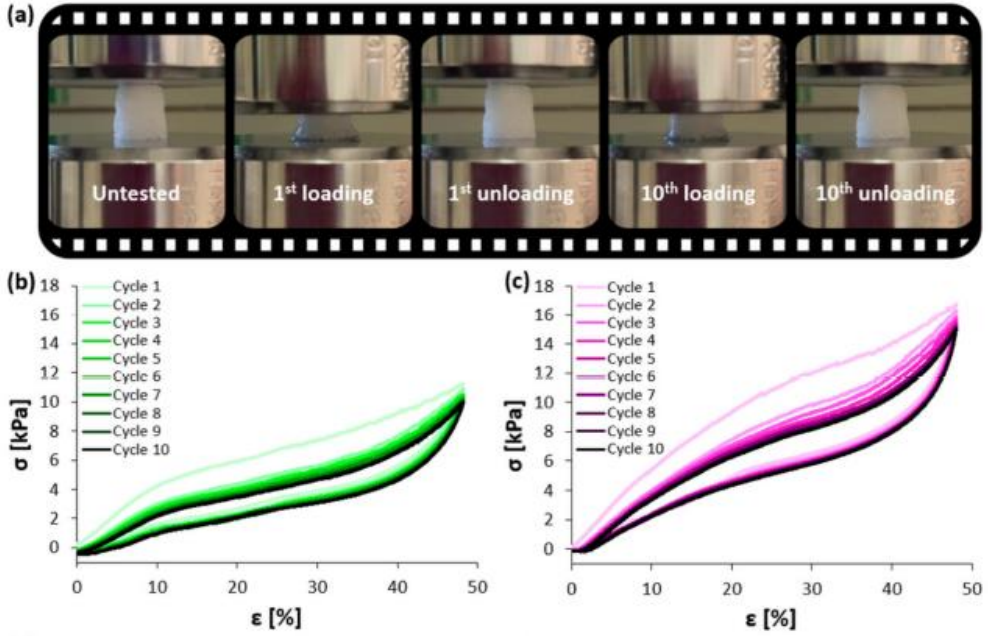


RESULTS: Improvement of scaffolds for cutaneous applications



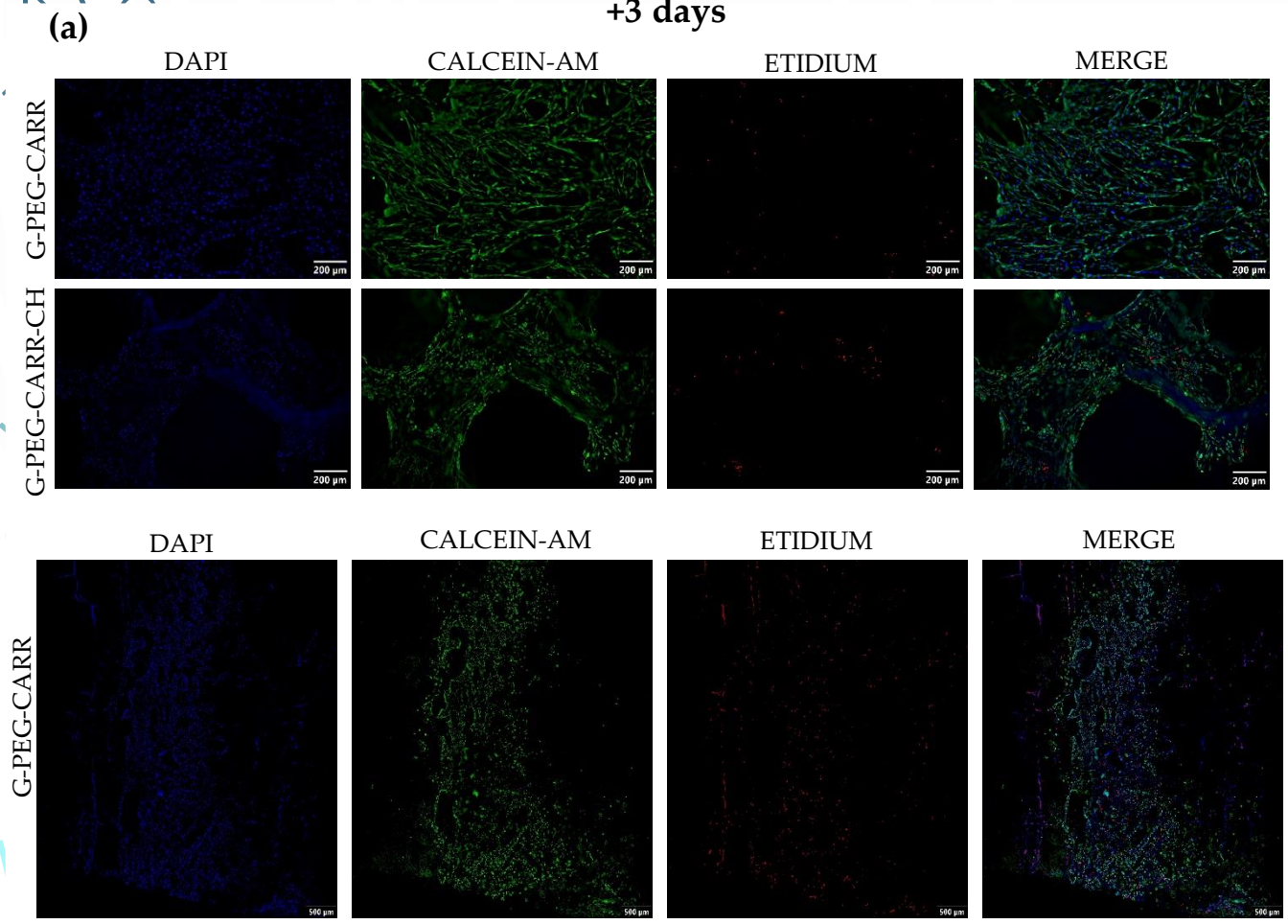


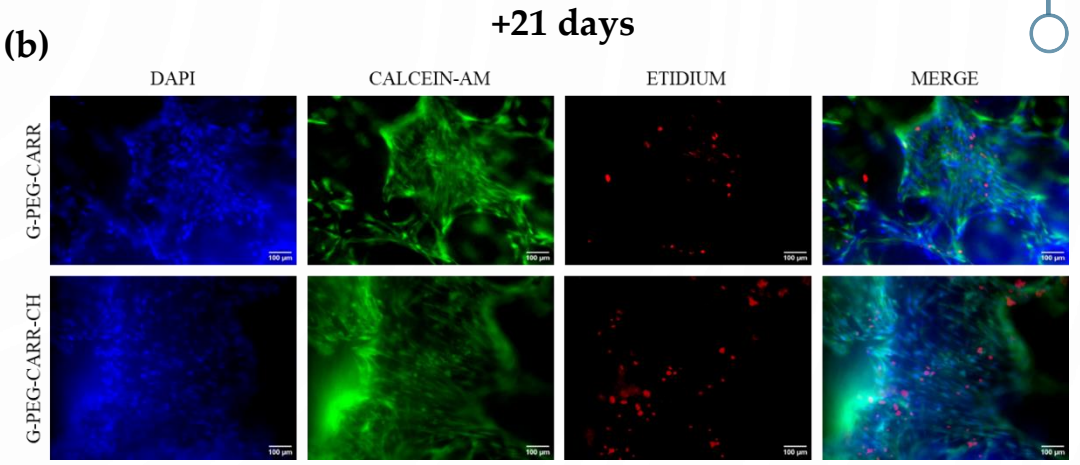


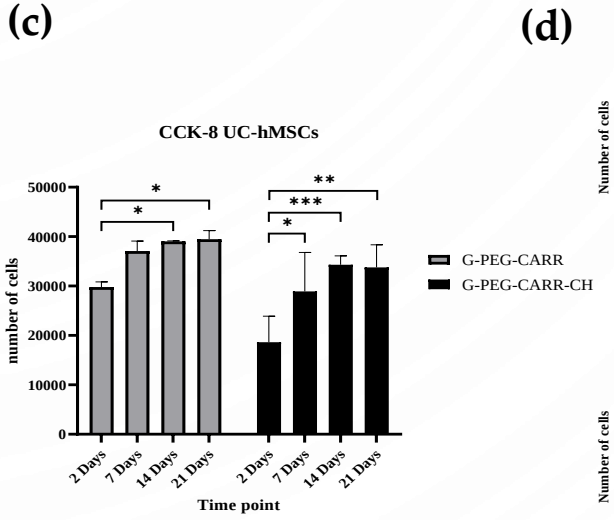


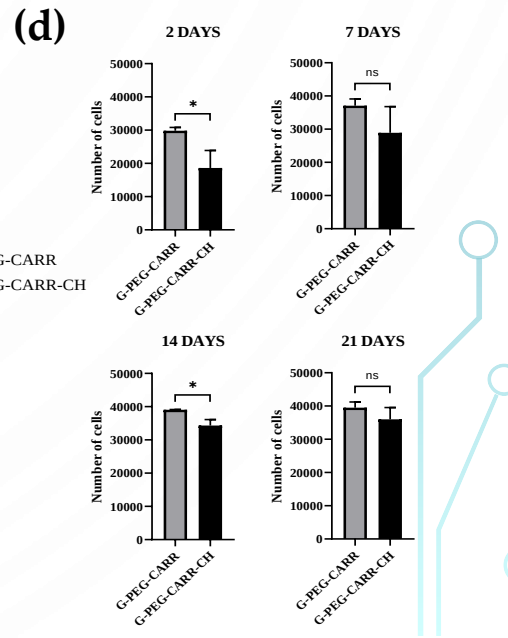
Pasini C, Re F, **Trenta F**, Russo D, Sartore L. Gelatin-Based Scaffolds with Carrageenan and Chitosan for Soft Tissue Regeneration. Gels. 2024 Jun 28;10(7):426. doi: 10.3390/gels10070426.

RESULTS: Improvement of scaffolds for cutaneous applications









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RESULTS: Optimization and standardization of cell culture procedures for skin regeneration

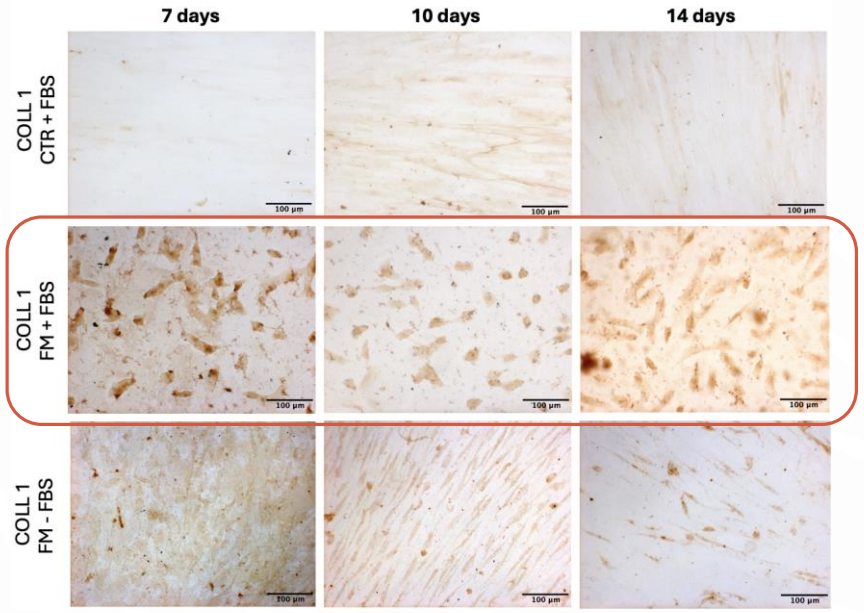
FIBROBLASTIC DIFFERENTIATION →

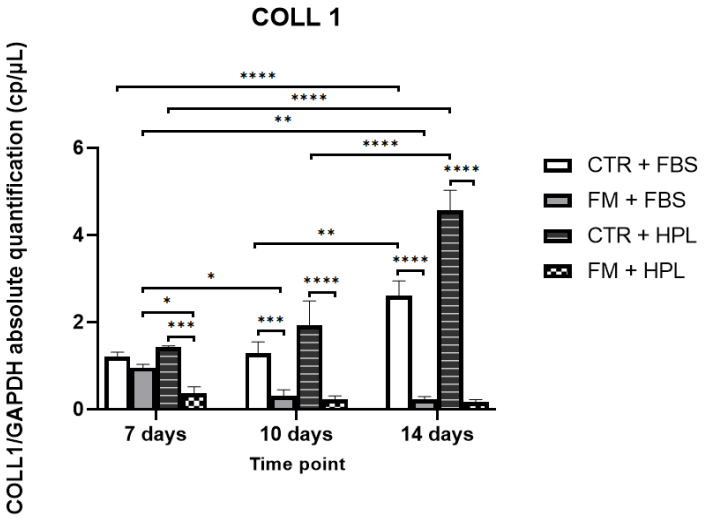
Medium+growth factors	Markers	Time points
ADMEM + 5%FBS + 50 mM ascorbic acid, TGF-P, IGF-I, EGF, insulin and bFGF.	COL I, COL III, COL IV	2, 7, 10, 14, 21, 28 days
DMEM + 100 ng/ml di CTGF + 50 ug/ml ascorbic acid	Tenascin – C, Fibronectin	
DMEM + 100 ng/ml di CTGF	Vimentin, Elastin	
DMEM + 2 mM L-Glutammina + 20%FBS + 100 ng/mL p/s	CD44	

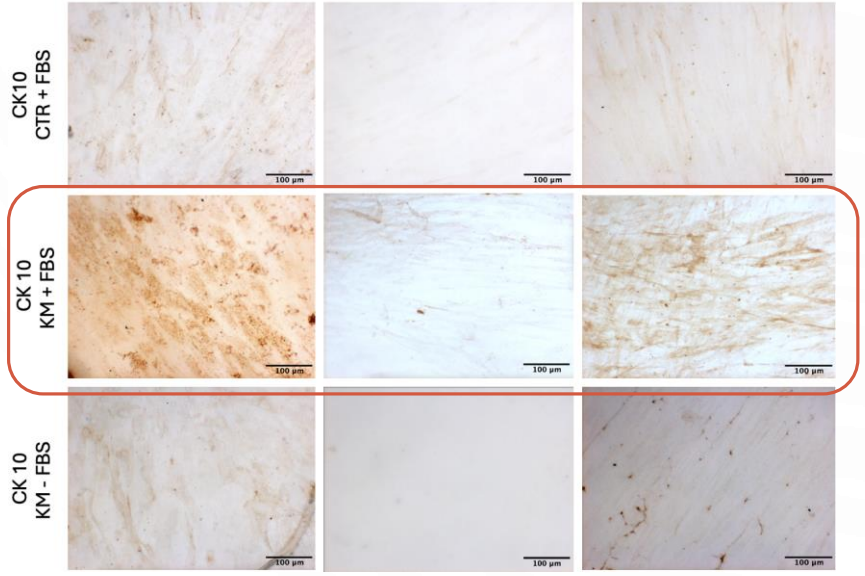
KERATINOCYTIC DIFFERENTIATION →

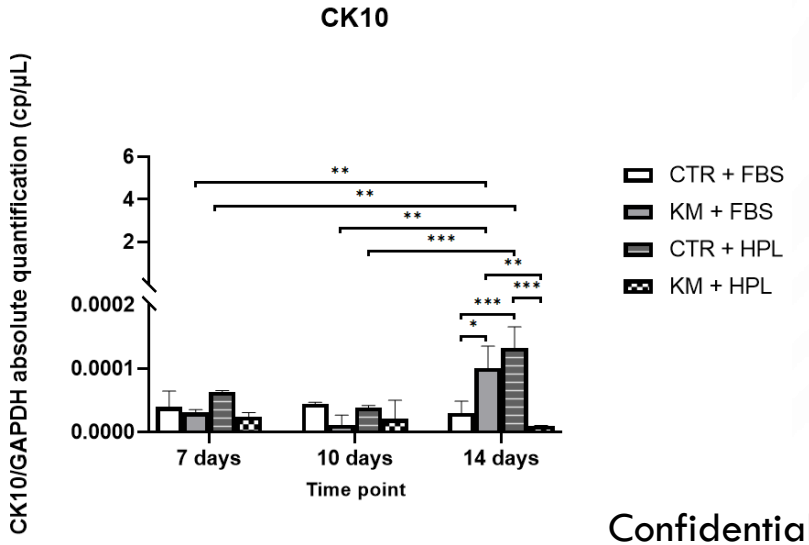
Medium+growth factors	Markers	Time points
KSFM + 1,8 mM CaCl2 + 5 ng/mL EGF	p63	1, 4, 7, 11, 14, 17, 21 days
KSFM + 1µM acido retinoico + coated plate with CollV + 25ng mouse BMP4	CK1 – CK5 – CK10 – CK14	
DMEM +1 µM All-trans retinoic acid, 25 ng/ml BMP4	Involucrin, E – caderin	
DMEM +1 µM All-trans retinoic acid, 25 ng/ml BMP4	Fibronectin	

RESULTS: Optimization and standardization of cell culture procedures for skin regeneration









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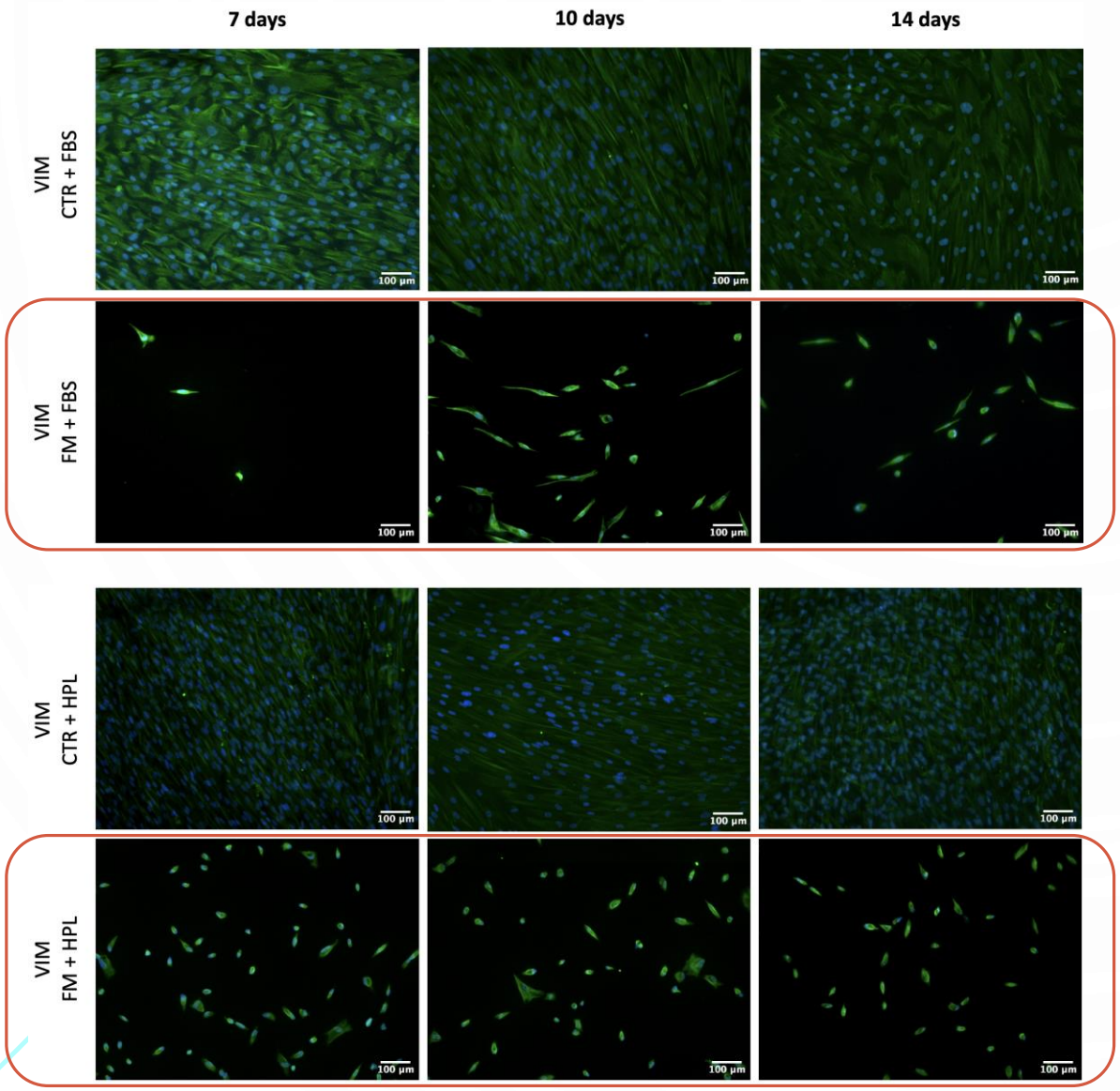

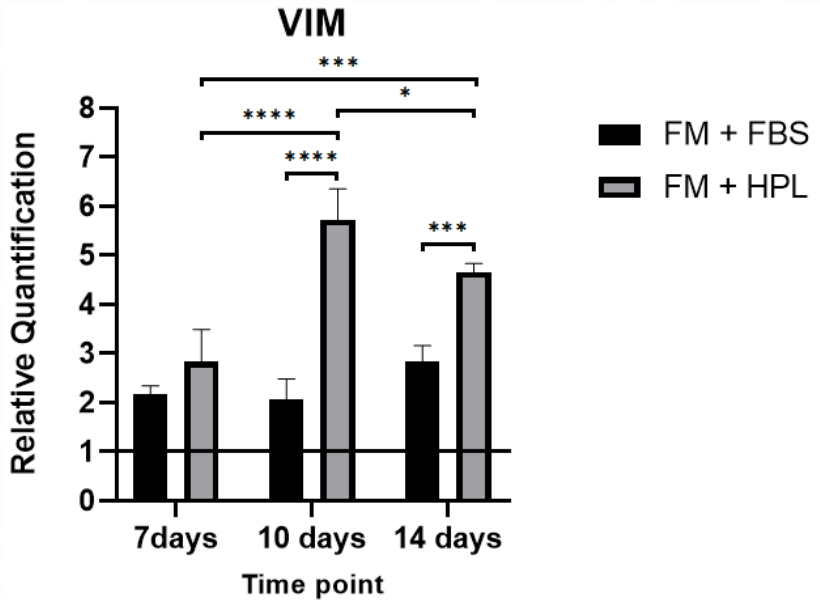

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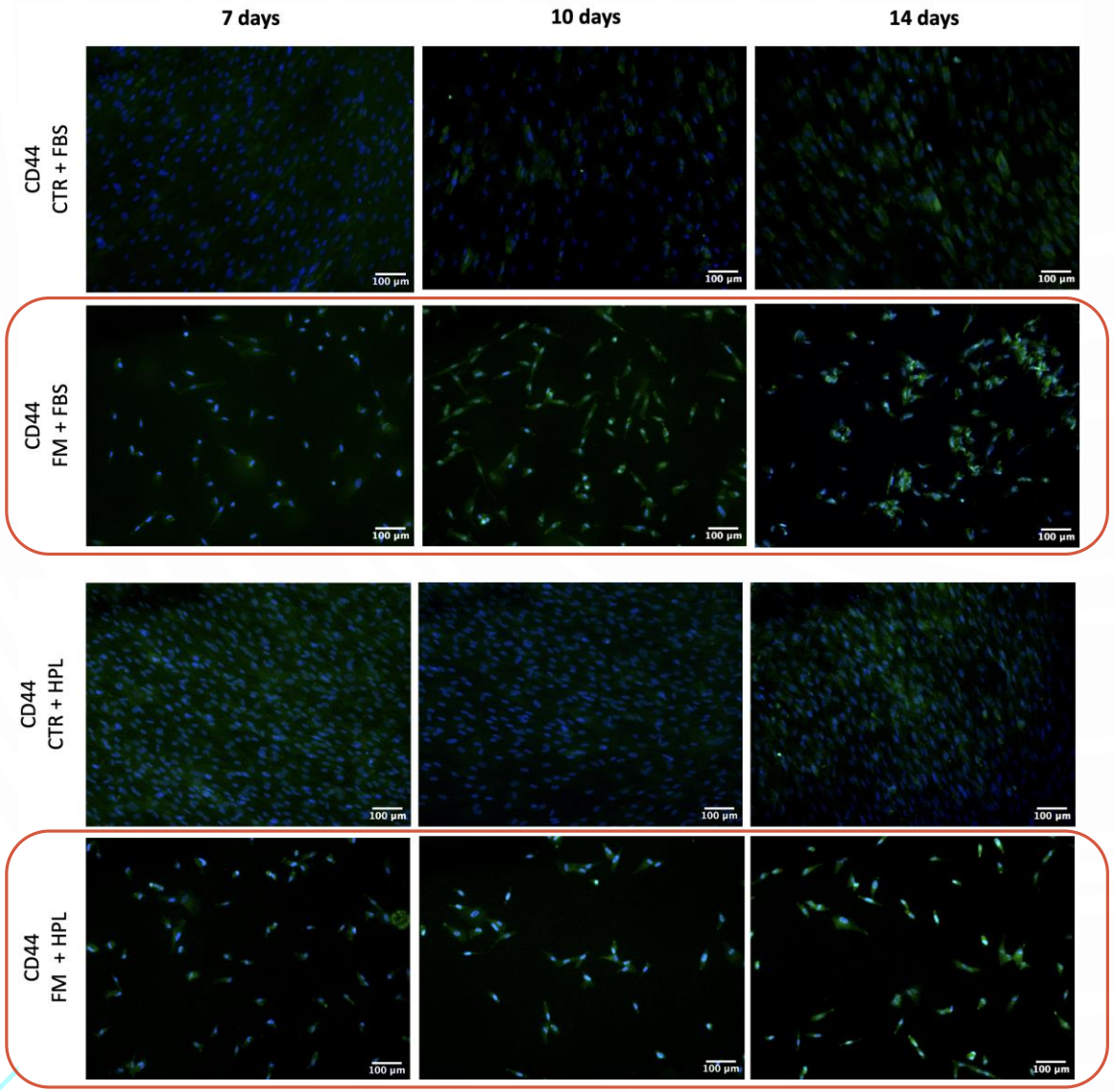

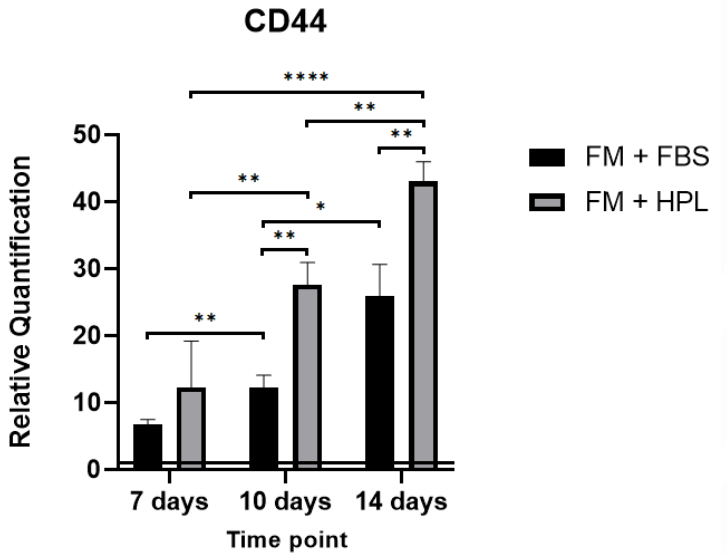

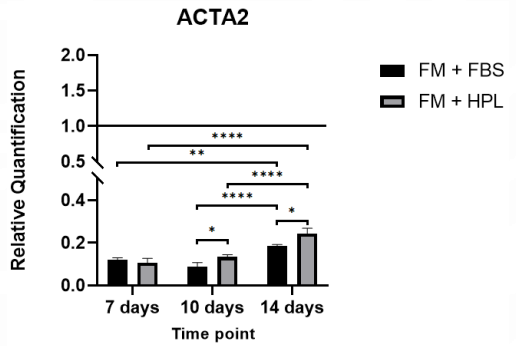

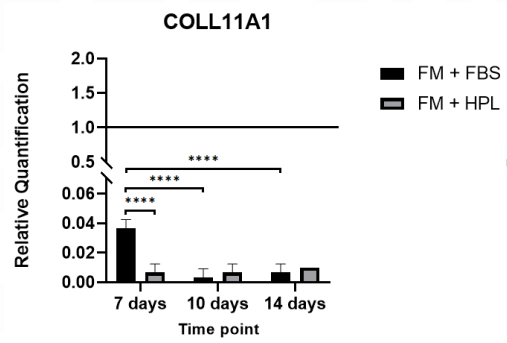

CONCLUSIONS AND FUTURE PERSPECTIVES

It will remain to investigate:

- Carrageenan-based hydrogels with other formulations (in terms of shape, porosity, interconnection and composition) and test other scaffolds with the aim of getting ever closer to clinical needs;
- Which growth factors and pathways are involved in the fibroblast differentiation and try to test different starting cell densities and understand if the cells are metabolically active;
- Apply the protocol for keratinocyte differentiation and investigate the protein and molecular expression of markers;
- Carry out a comparison with positive controls, isolating fibroblasts and keratinocytes directly from skin biopsies.