

Platelet rich plasma enhancement of ex-vivo human skin cultures



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ITALIA



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**20-21 Giugno 2019
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NINTH INTERNATIONAL
CONFERENCE



REGENERATIVE
SURGERY

Rome, Italy
December 15/16, 2017



Regenerative Surgery *The Cutting Edge*

Program sessions include:

- Engineering Regeneration
- Smart Therapies and Technologies
- Reconstructive Transplantation

Cell, Tissue and Organ Technologies



ISPRES



7th International Conference on
**Tissue Engineering &
Regenerative Medicine**
October 02-04, 2017 Barcelona, Spain
Theme: Transforming Repairs into Regeneration

Medicina Rigenerativa

Trasformare la riparazione in rigenerazione



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Liang J, Kang D, Wang Y, Yu Y, Fan J,
Takashi E (2015) *Carbonate Ion-Enriched Hot Spring
Water Promotes **Skin Wound Healing** in Nude Rats.*
PLoS ONE 10(2): e0117106.

«the molecular mechanism underlying beneficial effects of carbonate
ion-enriched spring water is currently unknown”

The Ancient **Healing Powers of Natural Hot Springs**

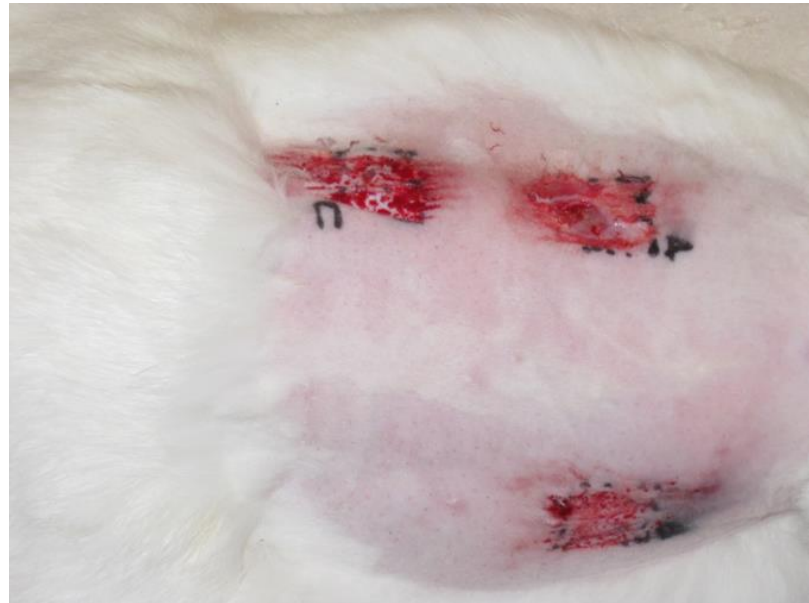
By [Susan Hartzler](#), Public Relations Executive, Mental Marketing

Hideo TSUJI The Journal of The Japanese Society of
Balneology, Climatology and Physical Medicine
Vol. 30 (1966-1967) No. 1-2 P 14-18 *Effects of Thermal
Spring Bathing on **Wound Healing***

Modello sperimentale animale

Perdita sostanza superficiale dermo-epidermica

Coniglio New Zealand



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A: Medicazione asciutta convenzionale

B: Imbibizione quotidiana con soluzione fisiologica

C: Imbibizione quotidiana con acqua termale di Comano



Impatto etico studio

STEP 1

- 6 animali
- 1 biopsia con sacrificio animale/giorno per 6 giorni

STEP 2

- 16 animali
- 1 biopsia con sacrificio di 8 animali/giorno 3
- 1 biopsia con sacrificio di 8 animali/giorno 4

Totale animali esperimento = 22



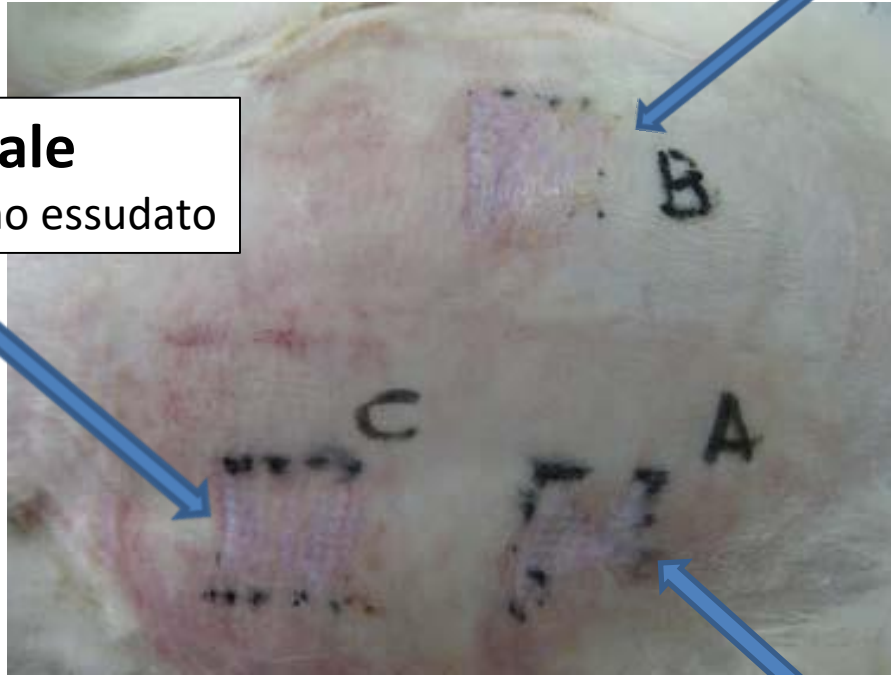
Giorno 4

Soluzione fisiologica

Riepitelizzazione diffusa parcellare, modico essudato

Acqua termale

Guarigione completa, no essudato



Medicazione asciutta

Riepitelizzazione < 50%, essudato fibrinoso



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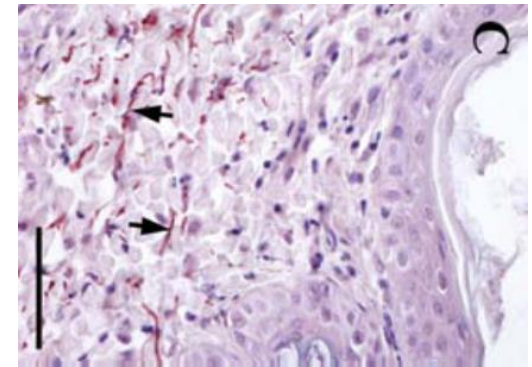
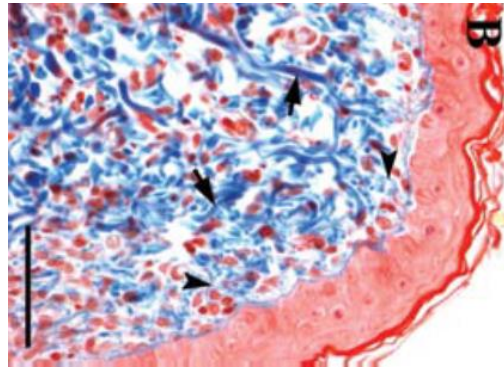
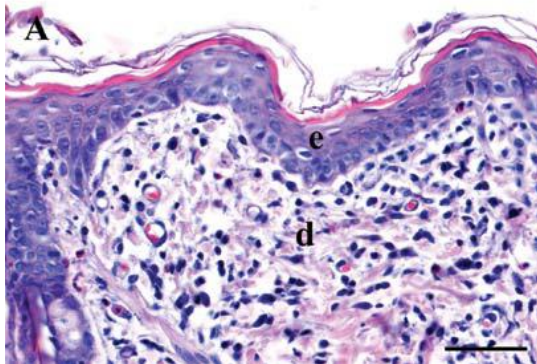
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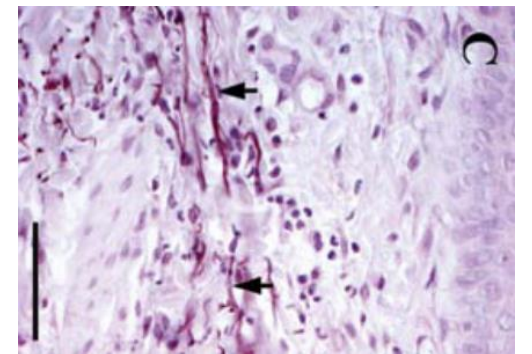
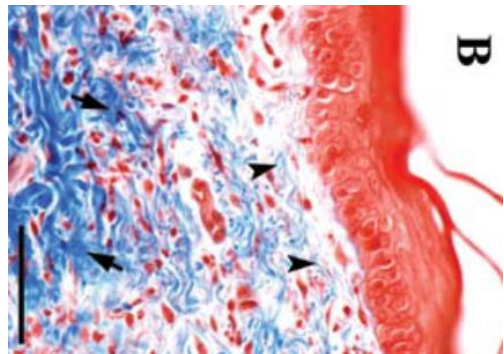
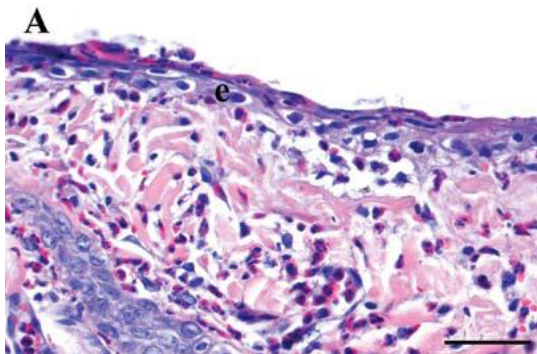
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Giorno 4

Cute sana

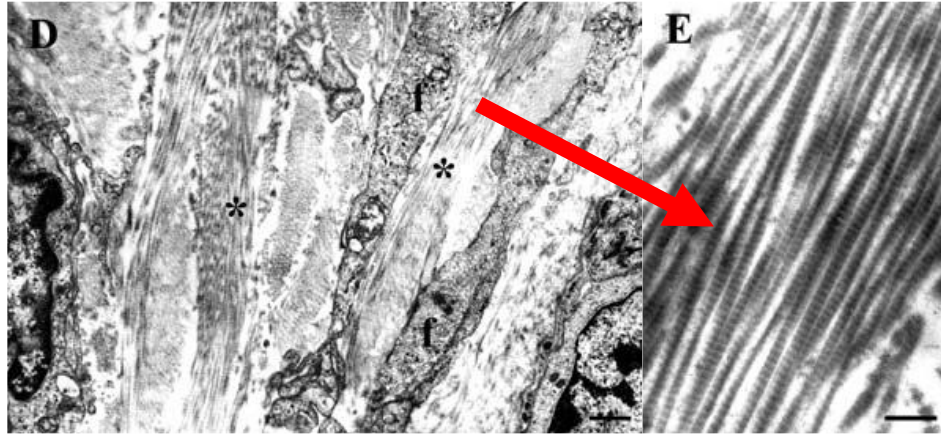


Acqua termale

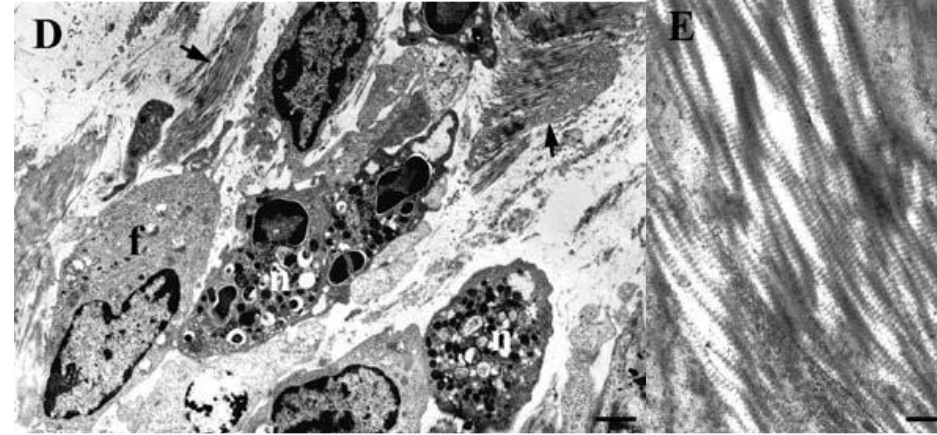


Microscopia elettronica

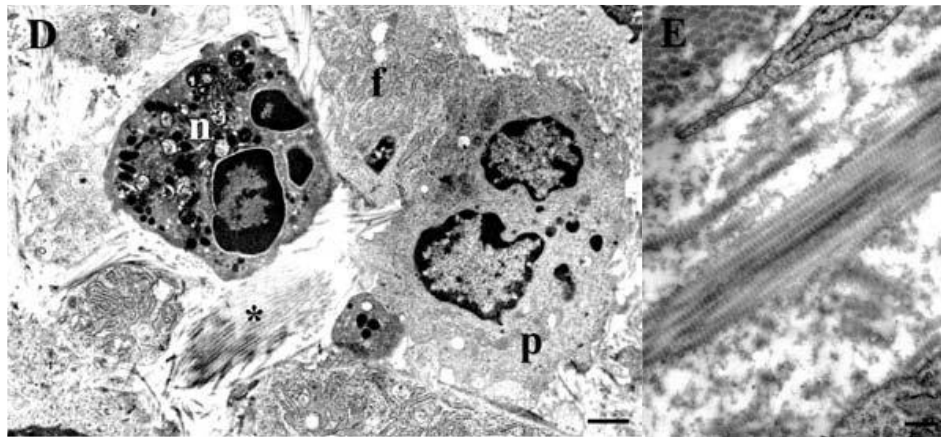
Cute sana



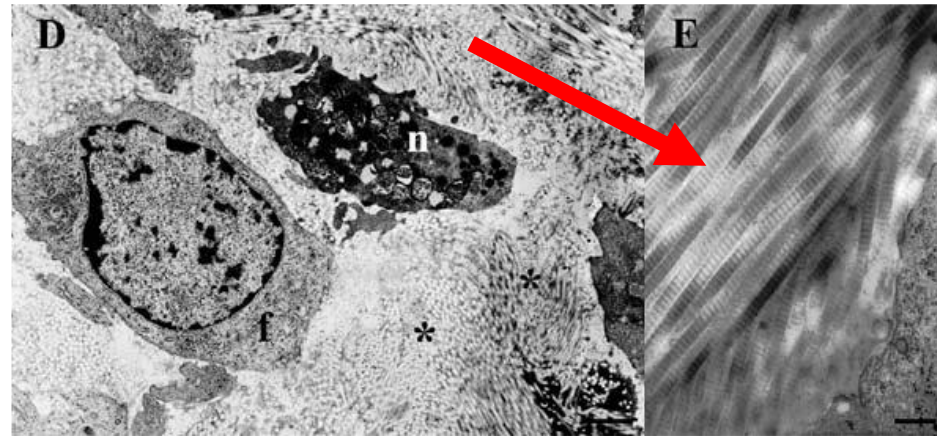
Medicazione asciutta



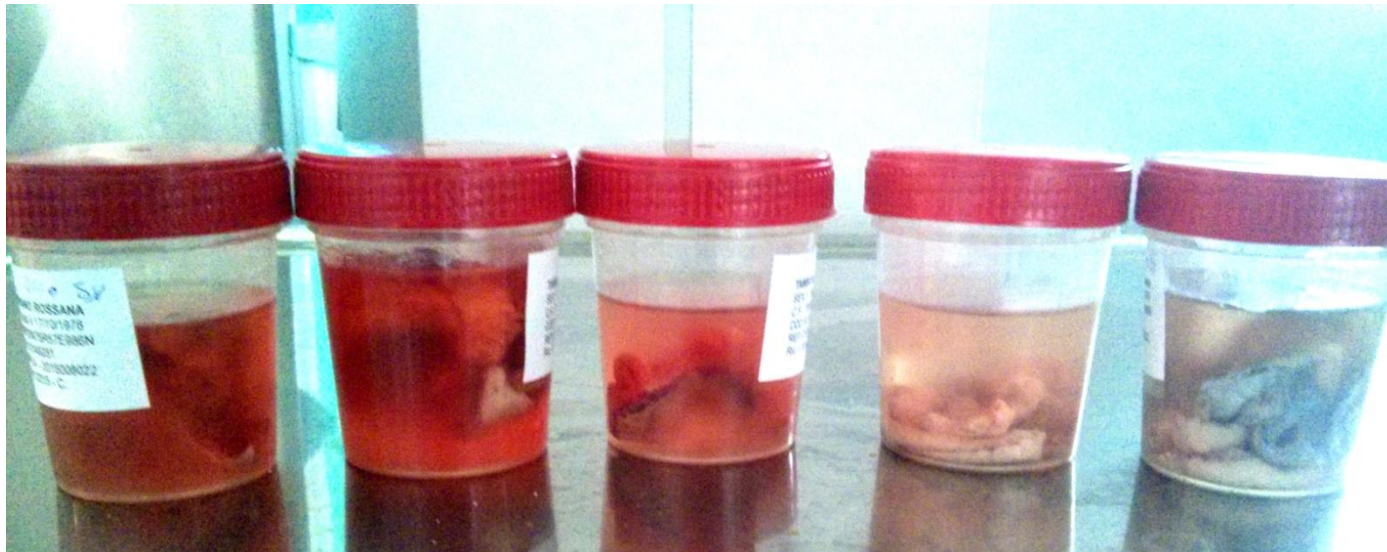
Soluzione fisiologica



Acqua termale



CAMPIONI DI CUTE UMANA



IN VITRO



EX VIVO



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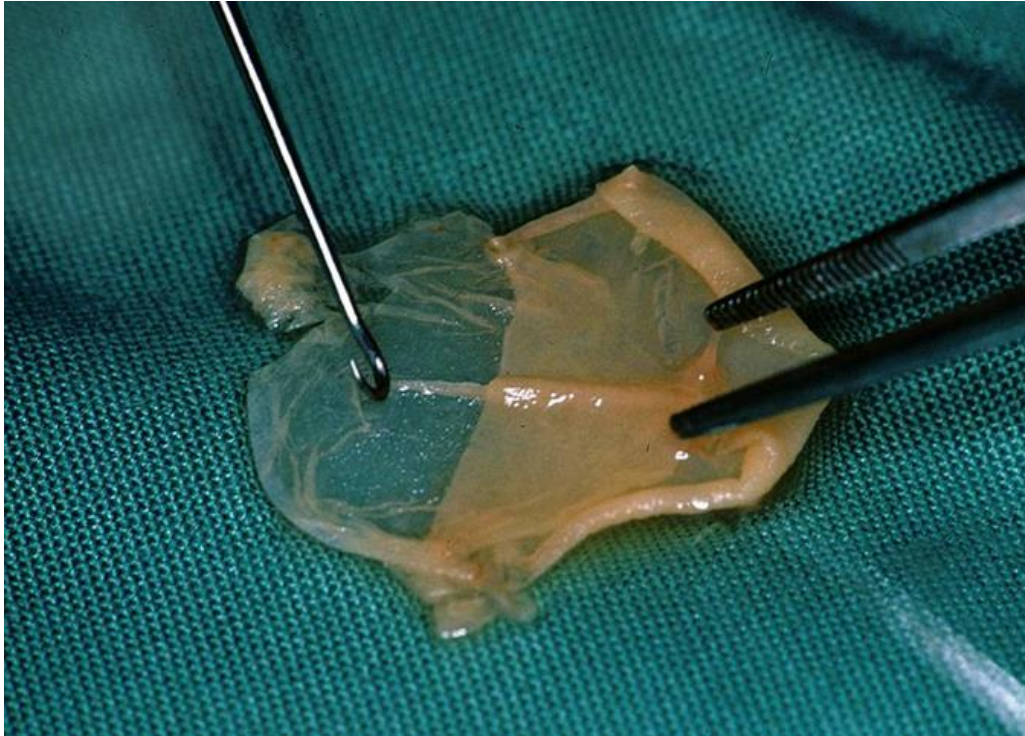
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Modello umano *in-vitro*



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FIBROBLASTI COLTIVATI IN DIFFERENTI MEZZI DI COLTURA

VALUTAZIONE VITALITÀ IN 6 DIVERSE CONDIZIONI

GIORNI 1, 2, 3, 7

| | 100% | 90% | 80% | 70% | 60% | 50% |
|---|--------------|-------------|-------------|-------------|-------------|-------------|
| <u>eDMEM</u> | 1000 μ L | 900 μ L | 800 μ L | 700 μ L | 600 μ L | 500 μ L |
| Acqua termale  | 0 μ L | 100 μ L | 200 μ L | 300 μ L | 400 μ L | 500 μ L |



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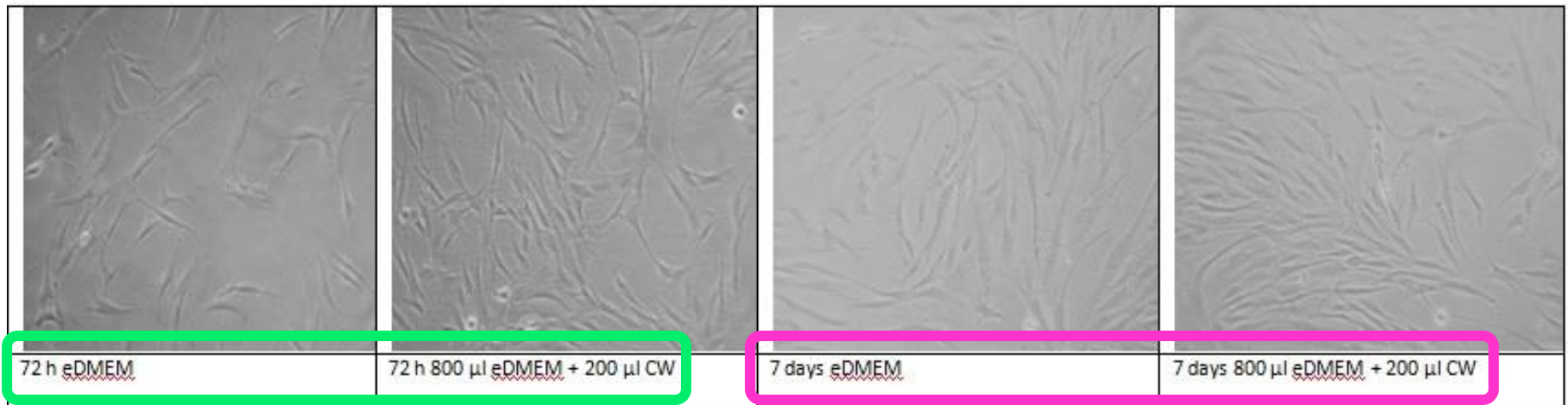
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Effects of a spring water on human skin fibroblast *in vitro* cultures: preliminary results

Giovanni NICOLETTI^{1,2,3*}, Marco SALER¹, Tommaso PELLEGGATTA^{1,2},
Alberto MALOVINI⁴, Angela FAGA^{1,2,3}, Alessandro SCALISE⁵, Federica RIVA⁶



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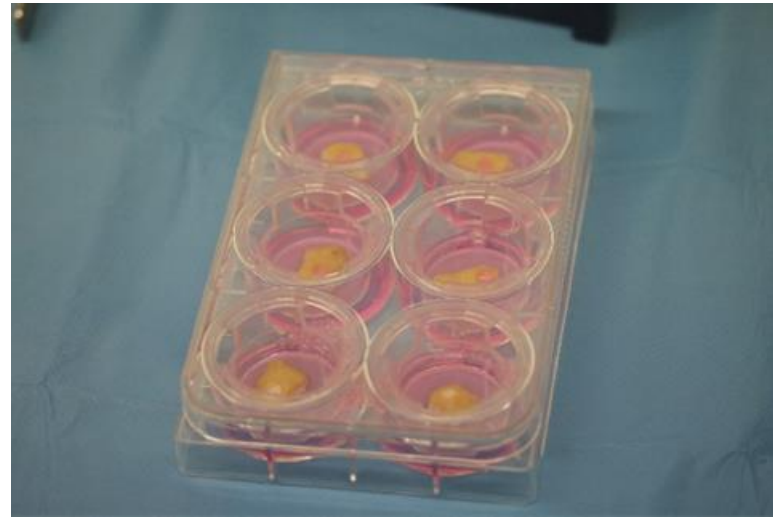
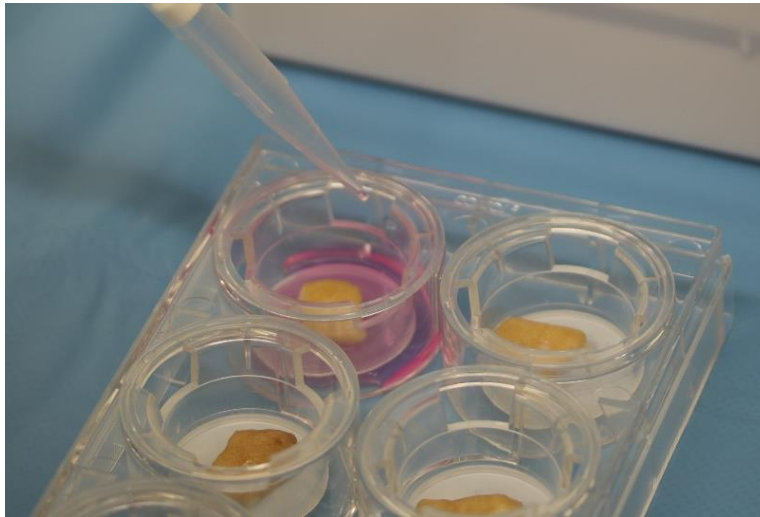
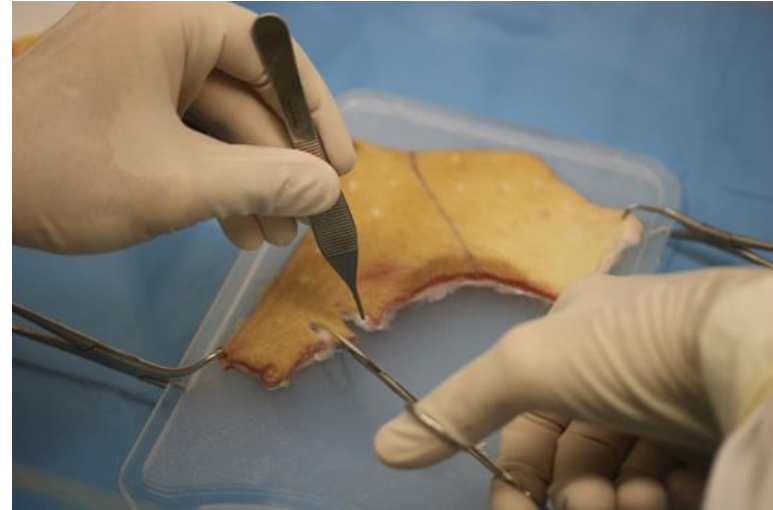
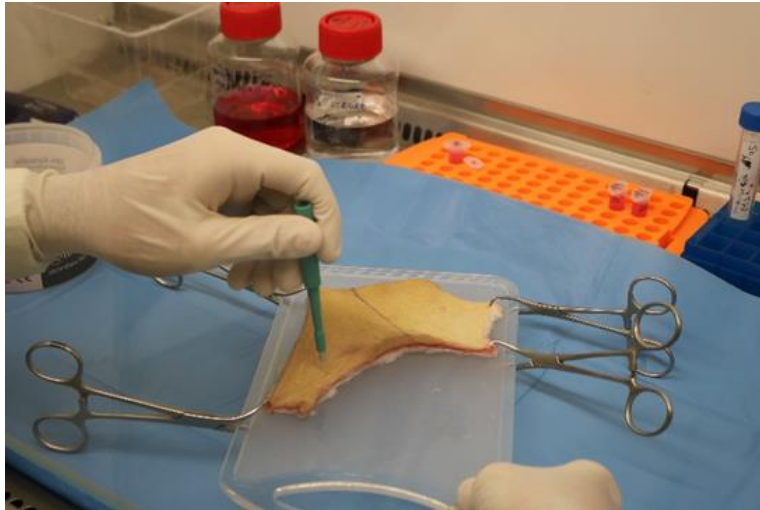
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Modello umano *ex-vivo*



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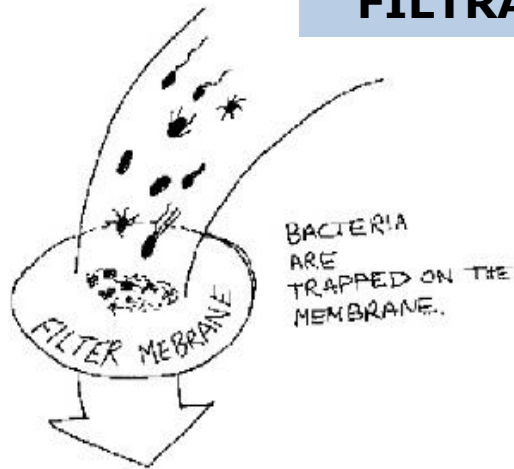
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Acqua
termale

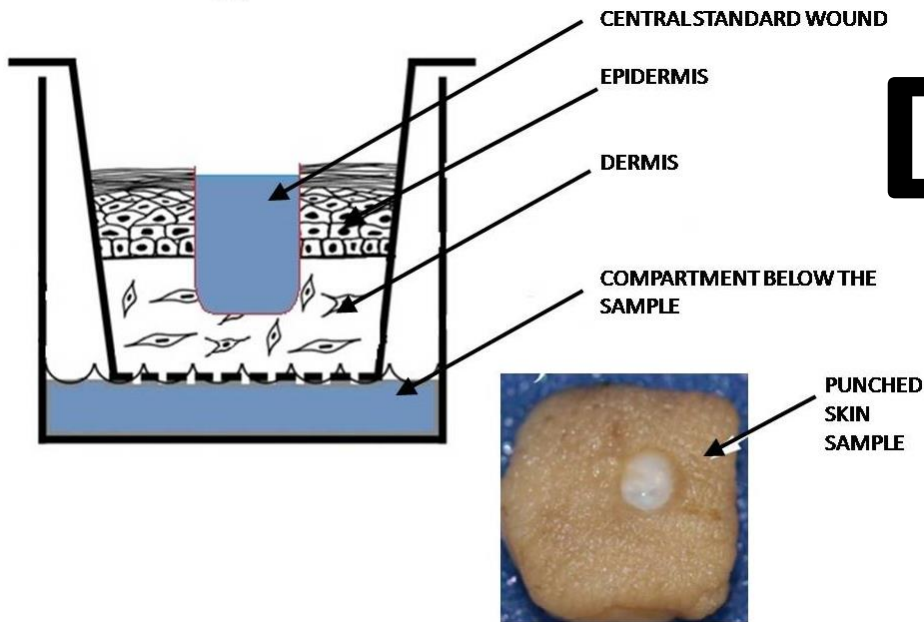
FILTRATA



DMEM

VS

DMEM +



24h

48h

72h



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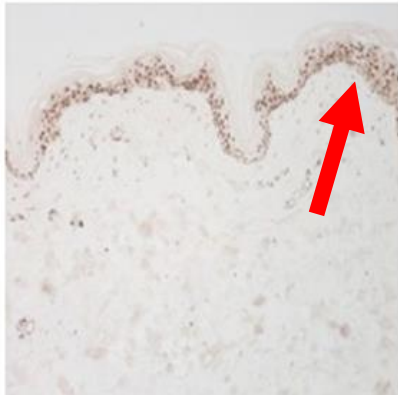
Risultati

DMEM+  **vs** **DMEM**

- Riduzione infiammazione
- Reclutamento fibroblasti
- Stimolazione proliferazione cellulare
- Incremento derma papillare
- Rigenerazione fibre collagene

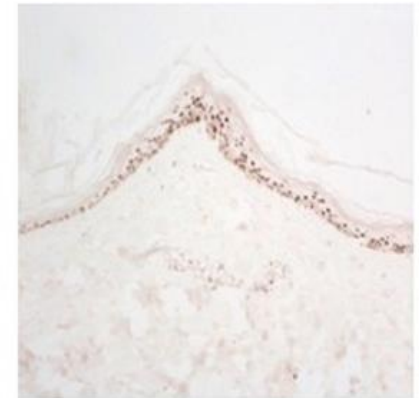
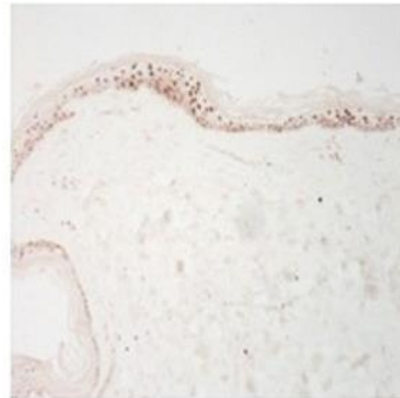
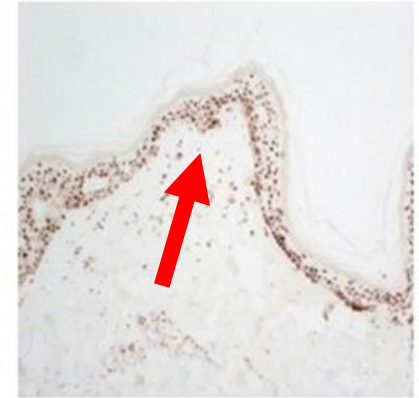
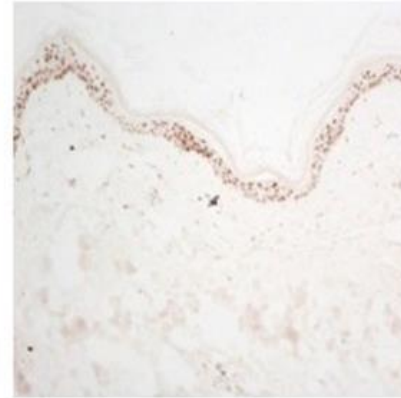
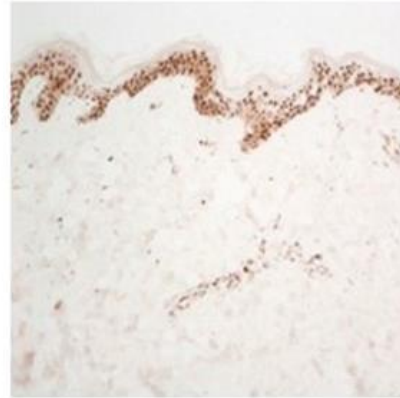
Proliferazione cellulare

Acqua termale



Cute non trattata

Controllo



T₁ (24 h)

T₂ (48 h)

T₃ (72 h)



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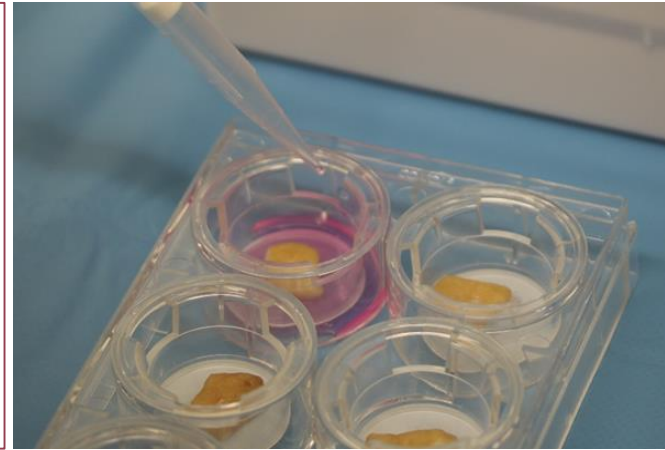
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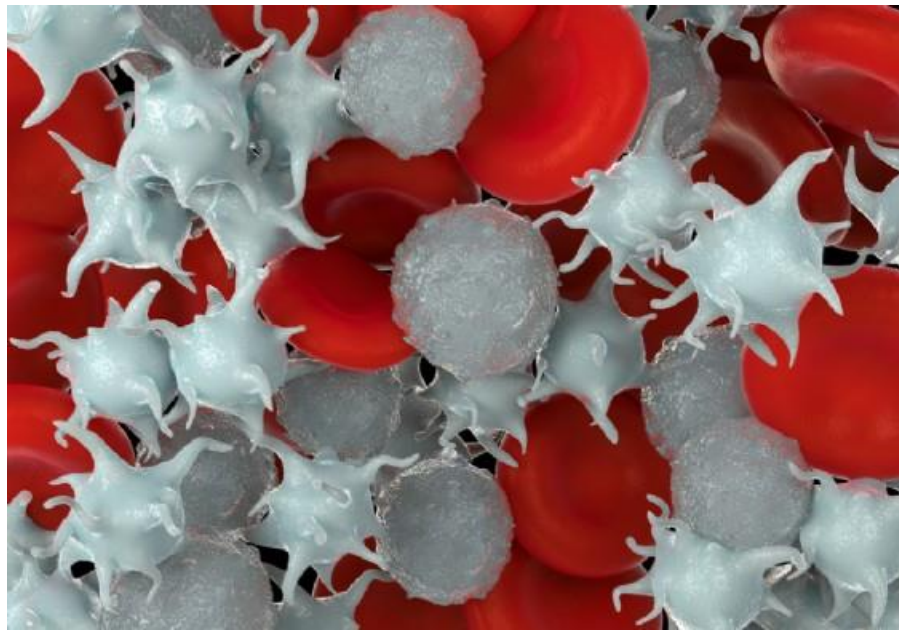
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Limite modello sperimentale organotipico *ex-vivo*



- Breve vitalità (3-4 giorni)
- Limitato tempo osservazione
- Modello insufficiente a riprodurre fedelmente fisiologia tessuto/organo

L'evoluzione del modello sperimentale

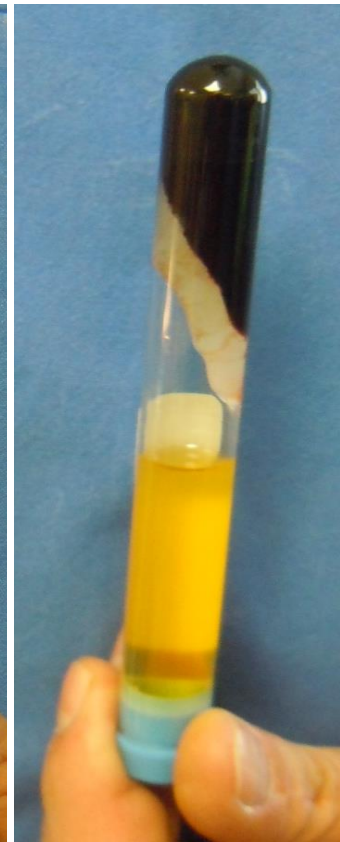


- Rimodellamento cicatrici
- Sintesi matrice extracellulare
- Neoangiogenesi
- Riepitelizzazione
- Differenziazione cellule staminali

Prodotti cellulari

Fattori di crescita

PDGF TGF β PF4 IL-1 PDAF VEGF EGF PDEGF ECGF IGF TSP1 Osteocalcina Osteonectina Fibronectina Vitronectina

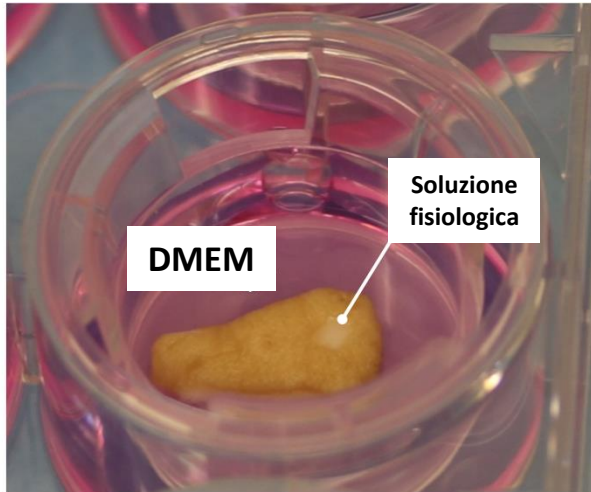


PRP

Alsousou J, Ali A, Willet K, Harrison P. The role of platelet rich plasma in tissue regeneration. *Platelets*. 2013;24(3):173-182.

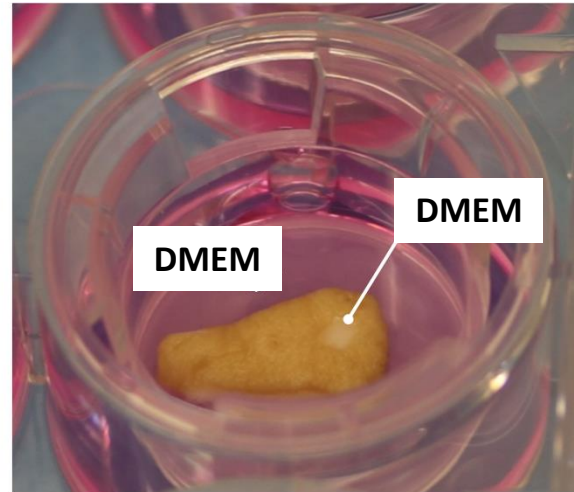
Saggio variante colturale con PRP

C



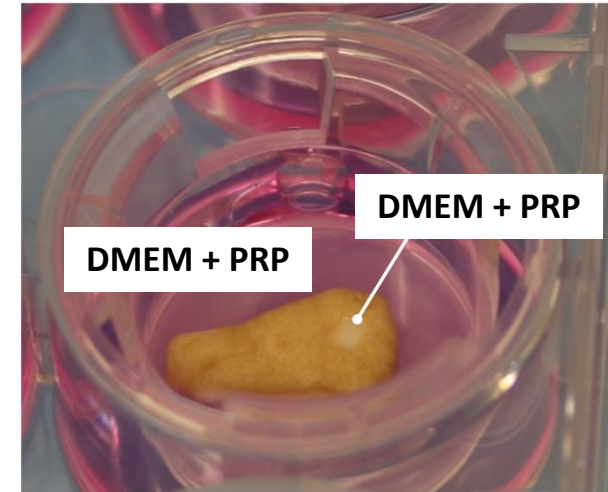
Controllo

D



DMEM

DP



DMEM + PRP



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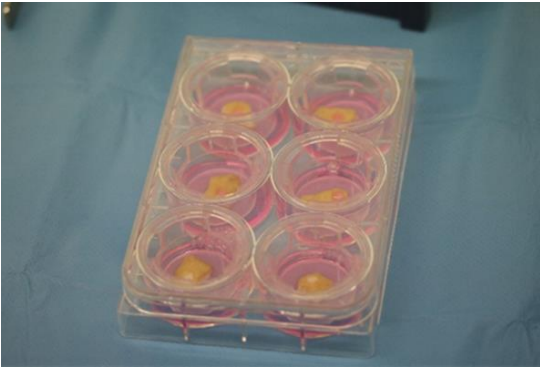
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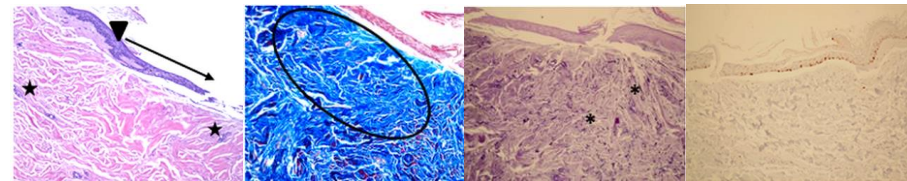
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Tempi di osservazione

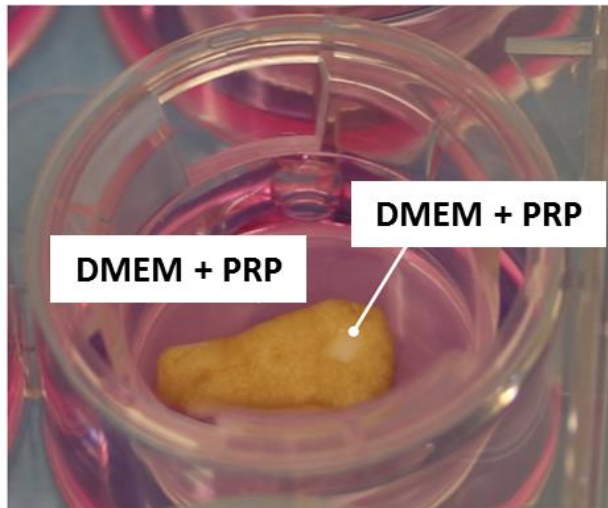


Modalità di osservazione

- Ematossilina/Eosina: cellularità
- Masson: fibre collagene
- Weigert: fibre elastiche
- Ki-67: proliferazione cellulare



Risultati



VS

**ALTRE
CONDIZIONI
COLTURALI**

- Piena vitalità campioni oltre **10 giorni**
- Modulazione favorevole proliferazione cellule epiteliali e fibroblasti
- Modulazione favorevole destrutturazione-riorganizzazione-rigenerazione fibre collagene ed elastiche
- Visualizzazione step-by-step processo rigenerazione cute

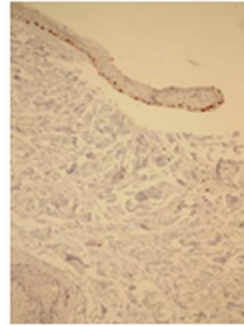
T₁



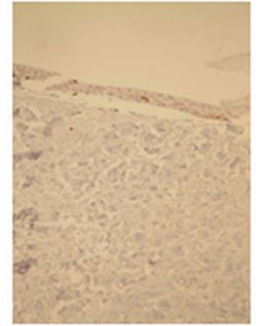
T₃



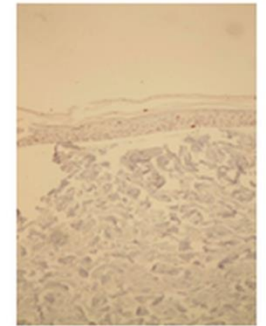
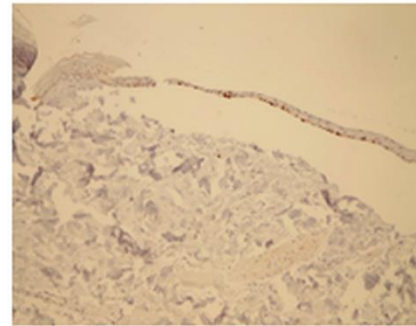
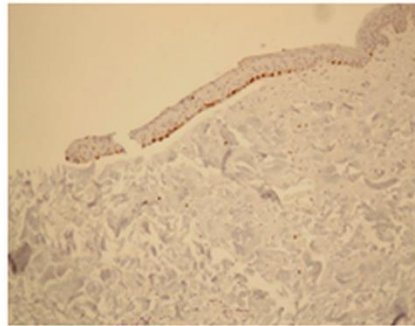
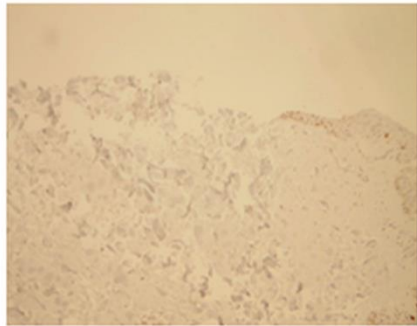
T₅



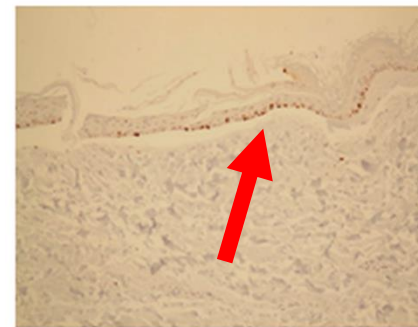
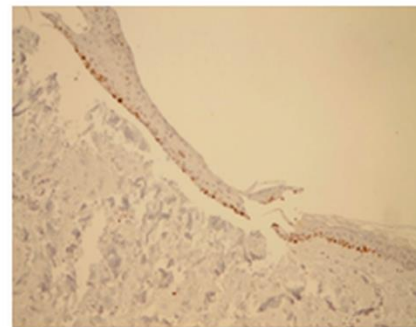
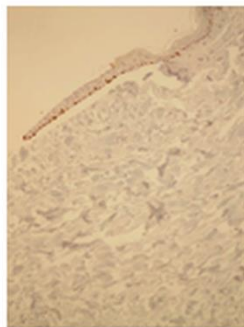
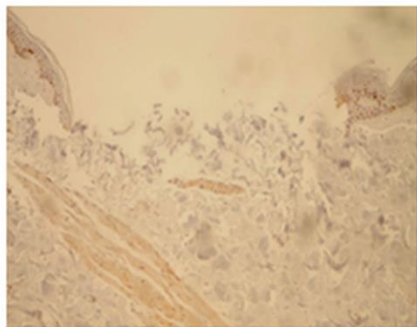
T₁₀



C



D



DP

Ki-67: la proliferazione cellulare



Platelet Rich Plasma Enhancement of Skin Regeneration in an *ex-vivo* Human Experimental Model

Giovanni Nicoletti^{1,2,3*}, Marco Saler¹, Laura Villani⁴, Agnese Rumolo¹,
Marco Mario Tresoldi^{1,3} and Angela Faga^{2,3,5}

¹ Plastic and Reconstructive Surgery, Department of Clinical Surgical, Diagnostic and Pediatric Sciences, University of Pavia, Pavia, Italy, ² Advanced Technologies for Regenerative Medicine and Inductive Surgery Research Center, University of Pavia, Pavia, Italy, ³ Plastic and Reconstructive Surgery Unit, Department of Surgery, Istituti Clinici Scientifici Maugeri, Pavia, Italy, ⁴ Pathological Anatomy and Histology Unit, Istituti Clinici Scientifici Maugeri, Pavia, Italy, ⁵ Department of Molecular Medicine, University of Pavia, Pavia, Italy

Conclusioni

- Sviluppo modello colturale cute umana *ex-vivo* a vitalità estesa
- Valutazione approfondita fisiologia rigenerazione cute umana
- Modello base studio proprietà rigenerative principi attivi farmacologici



Refinement

Grazie



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