SIdEM
Società Italiana
di Emaferesi e
Manipolazione Cellulare

Università degli Studi di Napoli
Federico II

LA MEDICINA RIGENERATIVA: approccio multidisciplinare

Interazione fra fattori di crescita e biopolimeri

Carla M. Caramella
Università di Pavia

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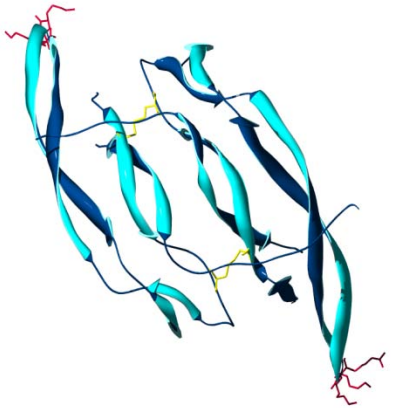
Joint project with the Center for Transplant Immunology of S. Matteo Hospital-Pavia

Treatment of epithelial lesions in transplanted patients

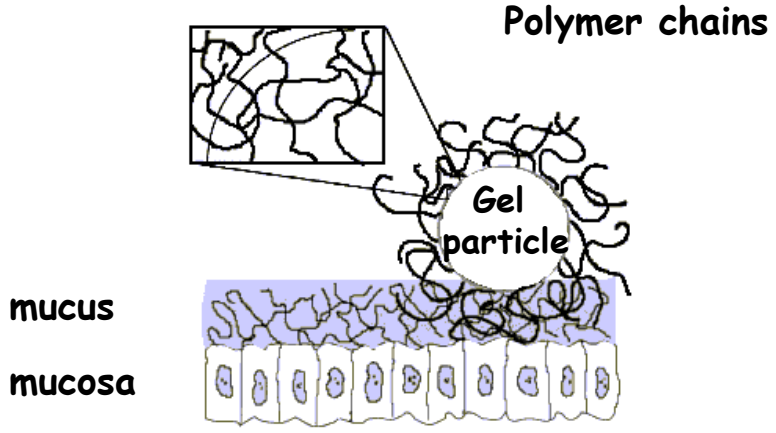
- To combine a palliative approach with a reparative strategy based on the use of ***growth factors*** (in pool)
- To exploit the cicatryzing/repairing properties of ***platelet lysate*** (pool of growth factors in solution)
- To develop suitable formulations for oral mucositis (stability, sterility, accurate dosing, ease of application, convenience for patients)
- To provide ***vehicles/bases*** for easily reconstituting the finished product with either freeze-dried or fresh platelet lysate



Mucoadhesive polymers

- ***Biocompatible polymers*** that interact, by means of weak bonds, in a ***reversible*** way, with the sialic sites of mucin macromolecules or similar receptor sites on the surface of cell membranes thus making and strengthening the so-called ***mucoadhesive joint***.

- ***Carboxyvinylpolymers (Carbopol) PAA***
- ***Chitosan (glutamate) CGS***
- Hyaluronates
- Alginates
- Natural hydrocolloids
- Cellulose derivatives
- ….



PLATELET LYSATE BIOADHESIVE COMPOSITIONS

(PCT/IT2008/000744)

Vehicle 1

carbopol 5% (w/w) in saline
- saccarin 0.2% (w/w)
- flavour 0.2% (w/w)
- NaOH 4 N to pH 7

Vehicle 2

chitosan glutamate 6% (w/w)
- HPMC (K4M) 2% (w/w)
- saccarin 0.2% (w/w)
- flavour 0.2% (w/w)
- Purified water q.s.

Vehicles are autoclaved under nitrogen blanket, mixed with platelet lysate in a 1:1 ratio and stored at 4-8 °C until testing to give:

Formulation 1 (PL PAA)

Formulation 2 (PL CGS)

Monodose preparation, number of doses tailored to the treatment (e.g.15 days), stored at 4-8 C

PLATELET LYSATE BIOADHESIVE COMPOSITIONS
(PCT/IT2008/000744)
In vitro testing for a preliminary screening

- Test of ***cell proliferation (bioassay)***

  The proliferative effect induced by platelet lysate is tested on cell cultures (***fibroblasts*** or ***rabbit epithelial corneal cells).*** The test is used to check maintainance of bioactivity in the formulation and stability

- ***Wound healing test***

  Cells are seeded in ***a micro petri dish*** with a special ***insert;*** *a* ***cell-free gap*** simulating a wound gap (500 µm in width) is created. The ability of the formulation to fill the gap is followed by microscopy

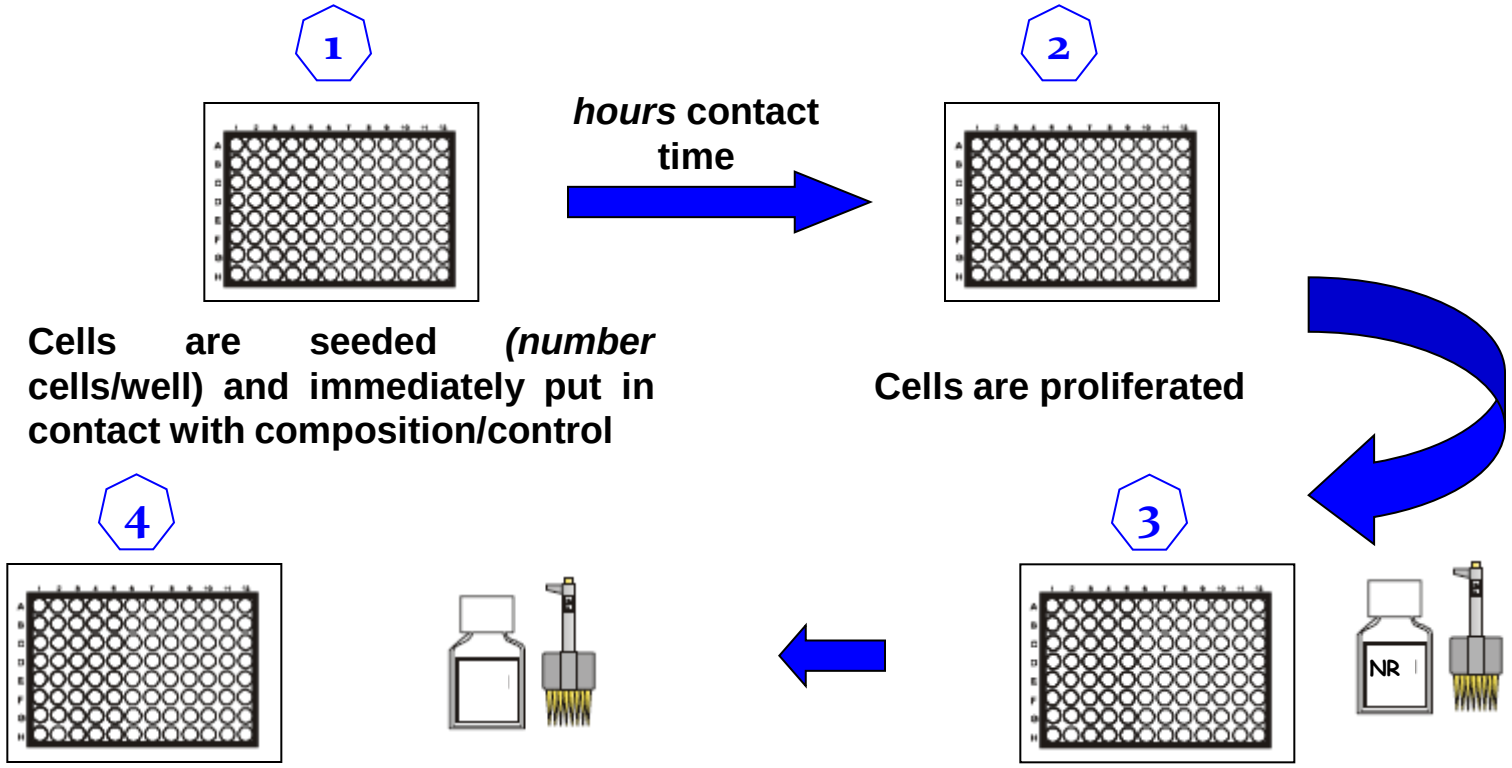
  The test provides an in vitro proof of concept of the repairing effect

- ***Elisa test*** for assay of growth factor content

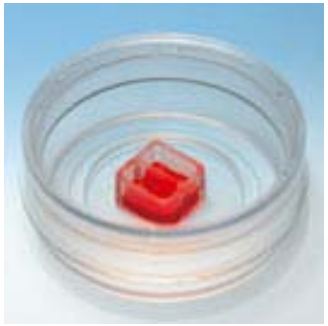
Proliferation test

METHOD: cell viability using Neutral Red (NR) exclusion test or MTT test

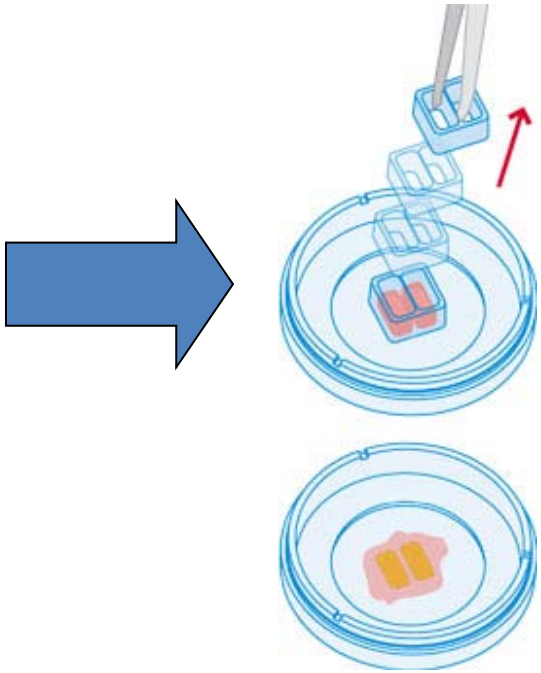
Cell line : Fibroplast or Corneal epithelial cells



Wound healing in vitro test

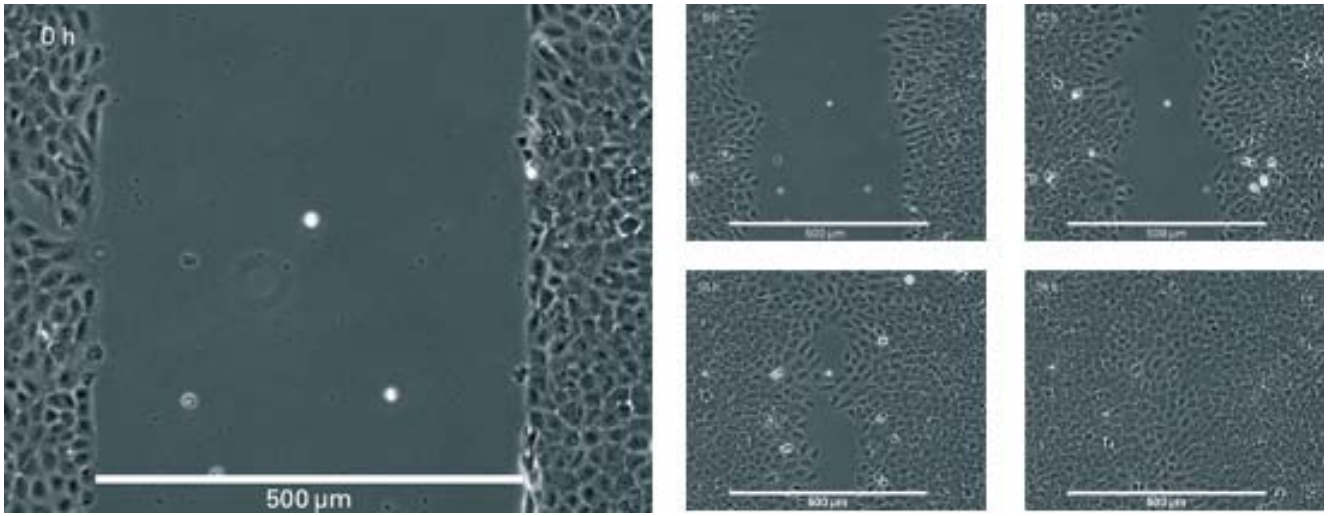


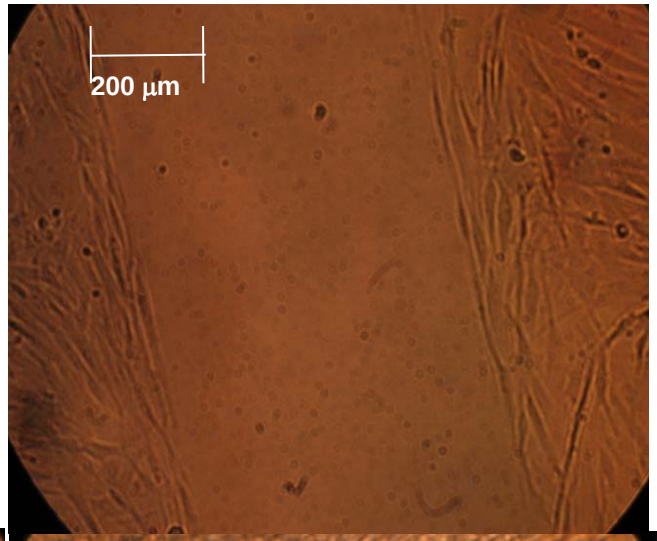
After seeding (10^5 cells/cm^2), the cells grow to form a confluent layer in the growth areas leaving a gap



After confluence, the insert is removed and the composition is put in contact with the cells and incubated.

At fixed time intervals (24, 48, 72, .. hours) the "wound width" is measured. The time required to join the cell substrate is noted





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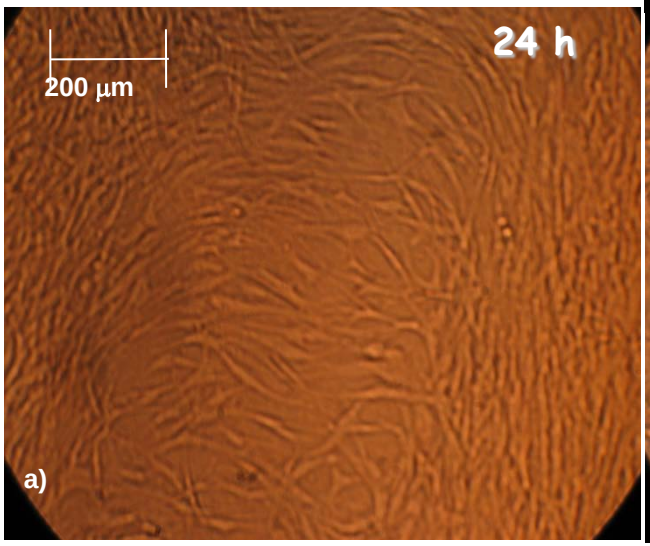
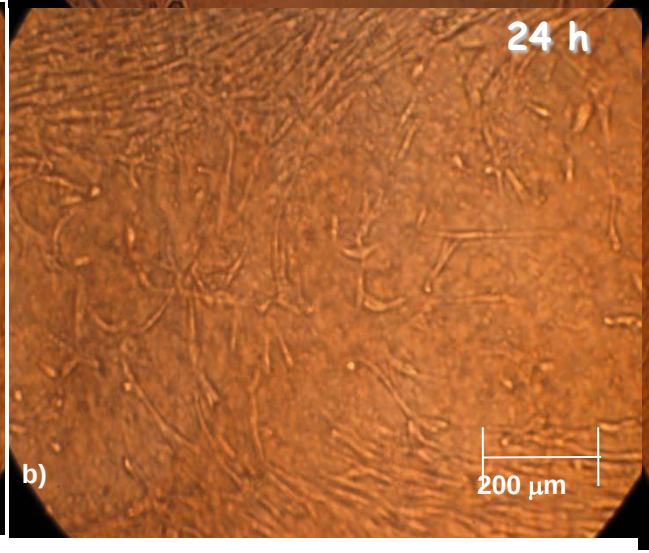
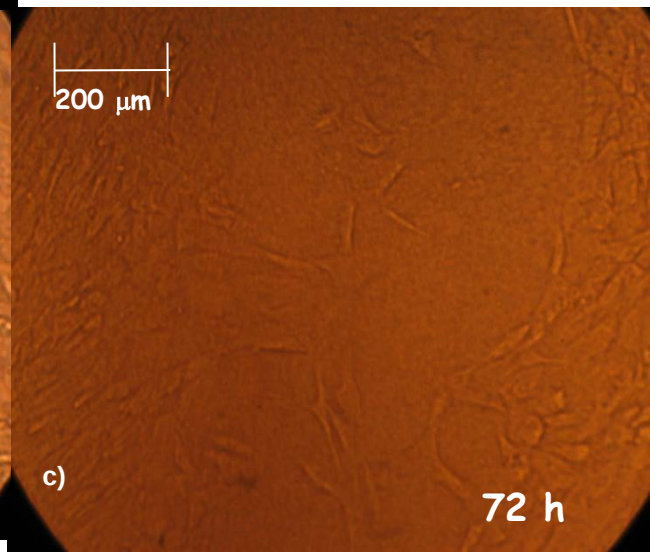






Platelet lysate PL

PL Chitosan Glutammate

PL Carbopol

Wound healing test results on mucoadhesive formulations

PLATELET LYSATE BIOADHESIVE COMPOSITION

(PCT/IT2008/000744)

Preliminary in vivo study - Summary of treatment and results

Pts n.	Age	GvHD	Mucositis (grade)	Plt lysate	Weight (% increase)	Response	Use of analgesics	Oral infection
1	13	Acute (grade III)	IV	allogeneic	0	NR	unchanged	no
2	51	chronic extensive	III	autologous	10	100% R	withdrawal	no
3	34	Chronic extensive	III	allogeneic	7	50% R	reduction	no
4	33	Chronic extensive	III	autologous	2	25 % R	unchanged	no
5	17	Chronic extensive	III	allogeneic	10	100% R	withdrawal	no
6	45	Chronic extensive	II	autologous	3	50% R	reduction	no
7	54	Chronic extensive	III	autologous	6	50% R	reduction	no

NO RESPONSE

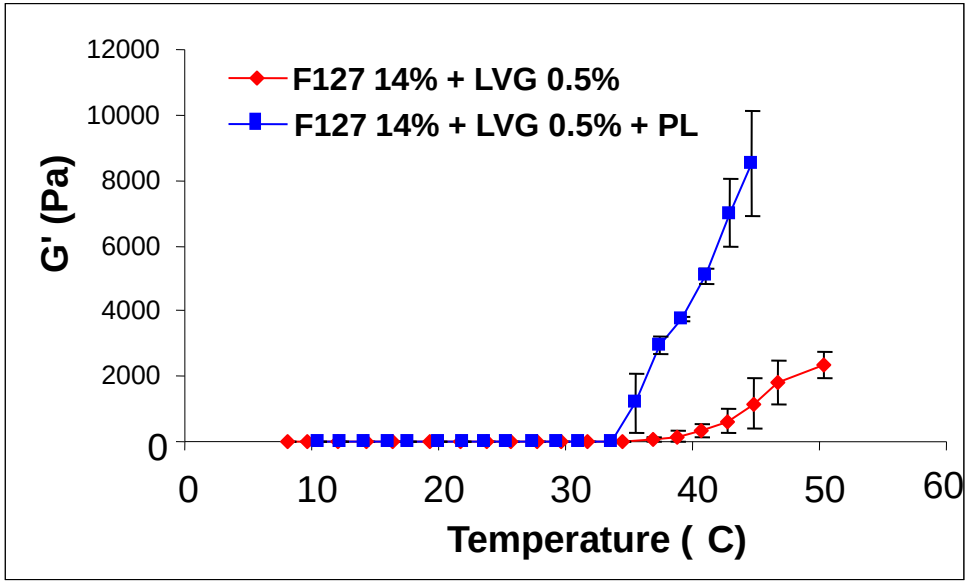
25% RESPONSE

50% RESPONSE

100% RESPONSE

Thermogelling buccal formulation

- based on a mixture of thermosensitive polymers
- liquid at room temperature
- *gelifies at body temperature*
- *It is mucoadhesive*

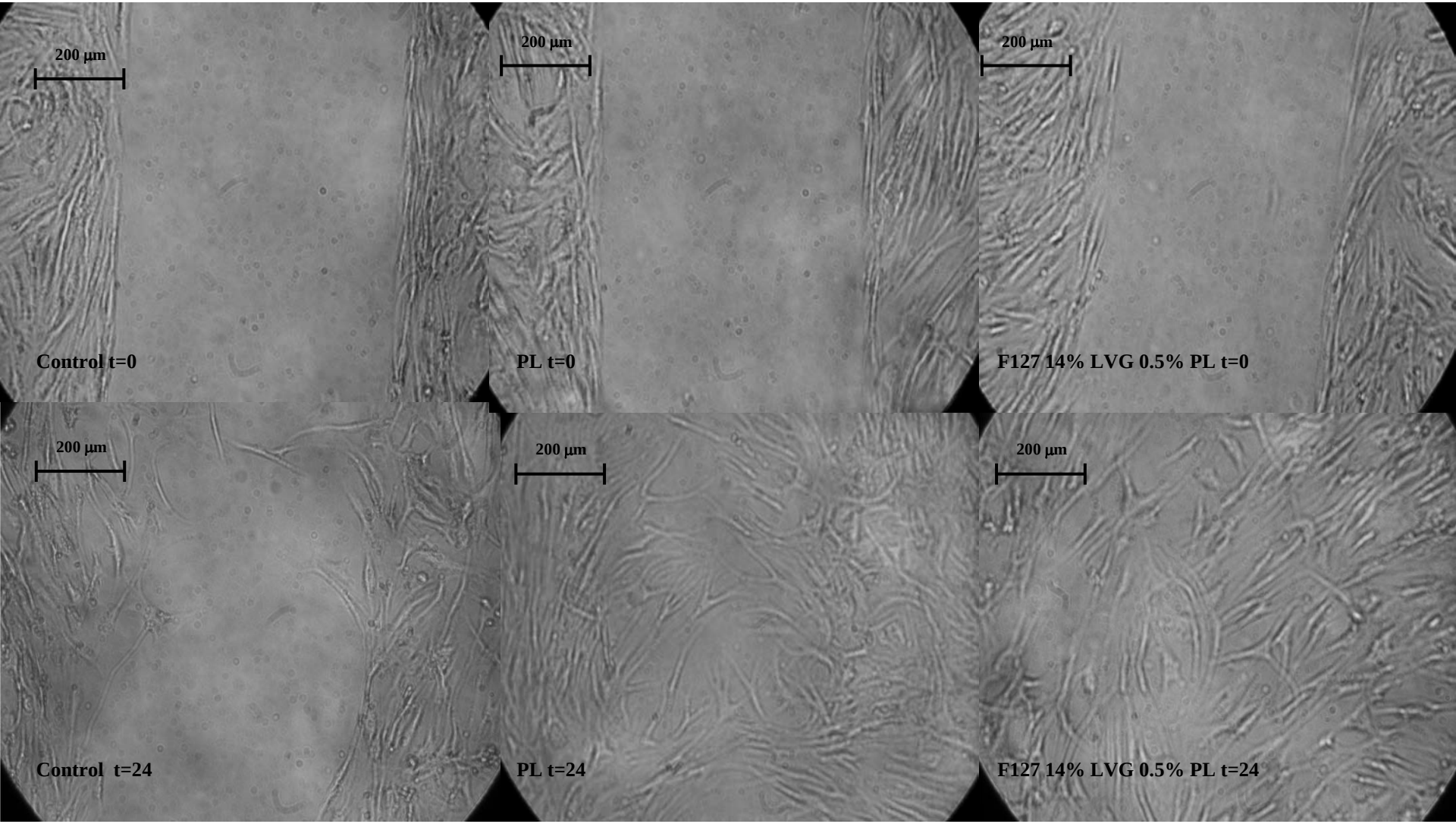


Rheological characterization

♦ **Vehicle**:
-Poloxamer 407 (F127) 14%
-Sodium Alginate (LVG) 0.5%
-Saline solution
■ **+ Platelet Lysate (PL)**

Delivered with a pump spraying device

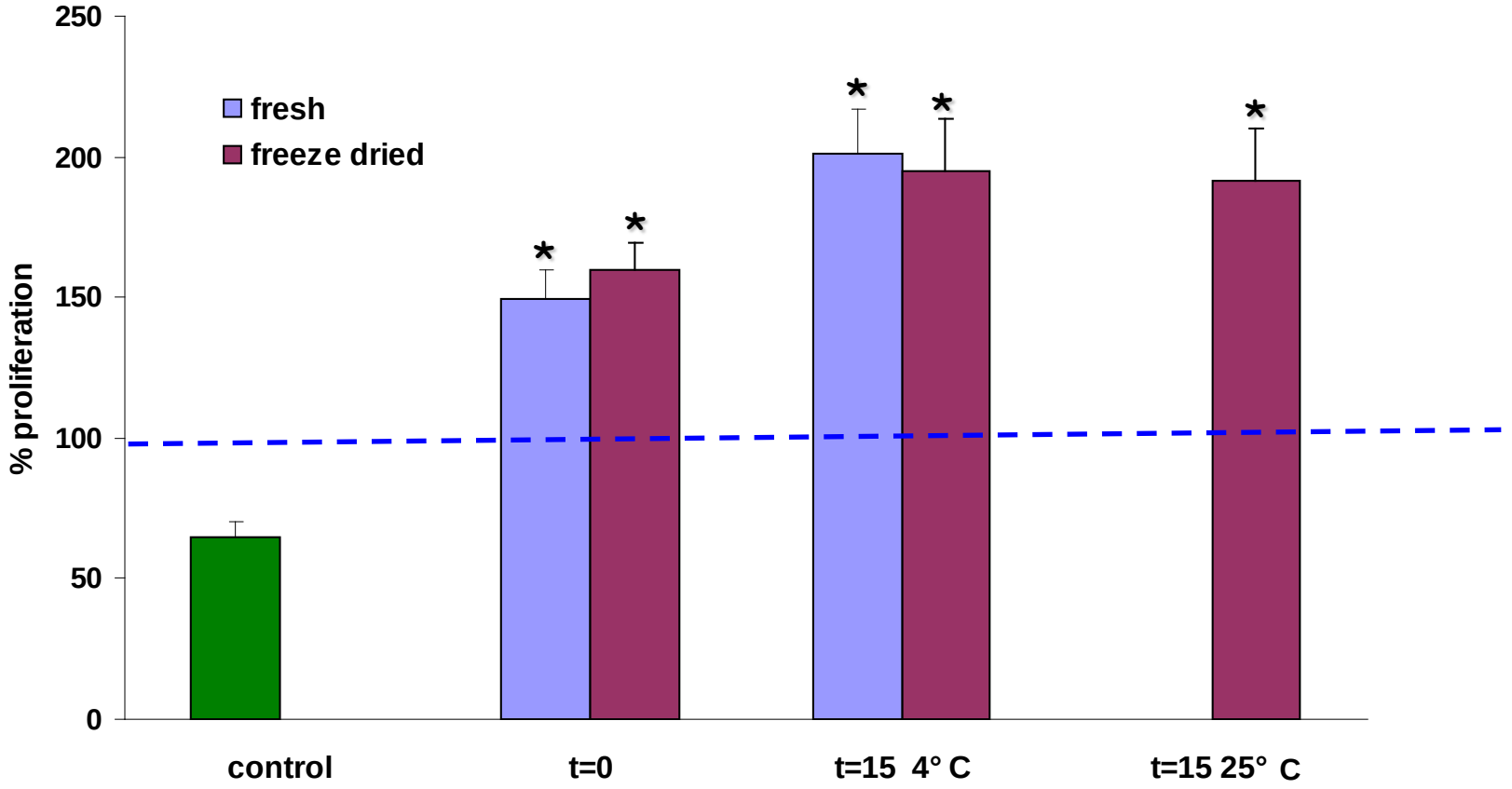
Wound healing test on the thermogelling formulation



Control ***Platelet lisate*** ***Formulation***

Bioactivity of freeze-dried platelet lysate

(freezing at -20 °C for 24 h, then sublimation for 24 h; Heto dryer, Analitica De Mori, I)



% cell proliferation induced by PL (dilution 1/20) in comparison with the *control* as a function of *storage time (days) and temperature* (mean values ± s.e.;n=8)

The dotted line indicates the proliferation of the ***reference*** (assumed as 100%)

* = significant difference from control (Mann Whitney test $p<0.05$)

New Project with the Vascular Surgery Unit : ***treatment of chronic wounds***

Development of a formulation suitable for the release of platelet lysate

to assure stability (maintainance of the activity of the growth factors) during the preparation, storage and use

to feature suitable hydration properties for exudating lesions

To assure a suitable therapeutic efficacy of platelet lysate by prolonging and making more intimate the contact with the injured tissue

to assure the *microbiological quality* of the product

to improve compliance by facilitating application and painless removal

to exploit the synergistic effect with biopolymers endowed with tissue repair properties

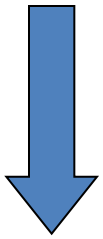
"Sponge-like" dressings based on chitosan and hyaluronic acid loaded with platelet lysate

Wound dressings based on chitosan (CGS) and sodium hyaluronate (SH)

chitosan glutammate (CGS) 3% (w/w)- glycine 2% (w/w)

Sodium hyaluronate (SH) 3% (w/w) - glycine 2% (w/w)

Ultraturrax 24000 g.min-1



NaOH 2M to adjust pH (6.3 for CHS, 7.3 for SH)

1: 1 dilution with:

saline solution
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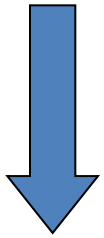
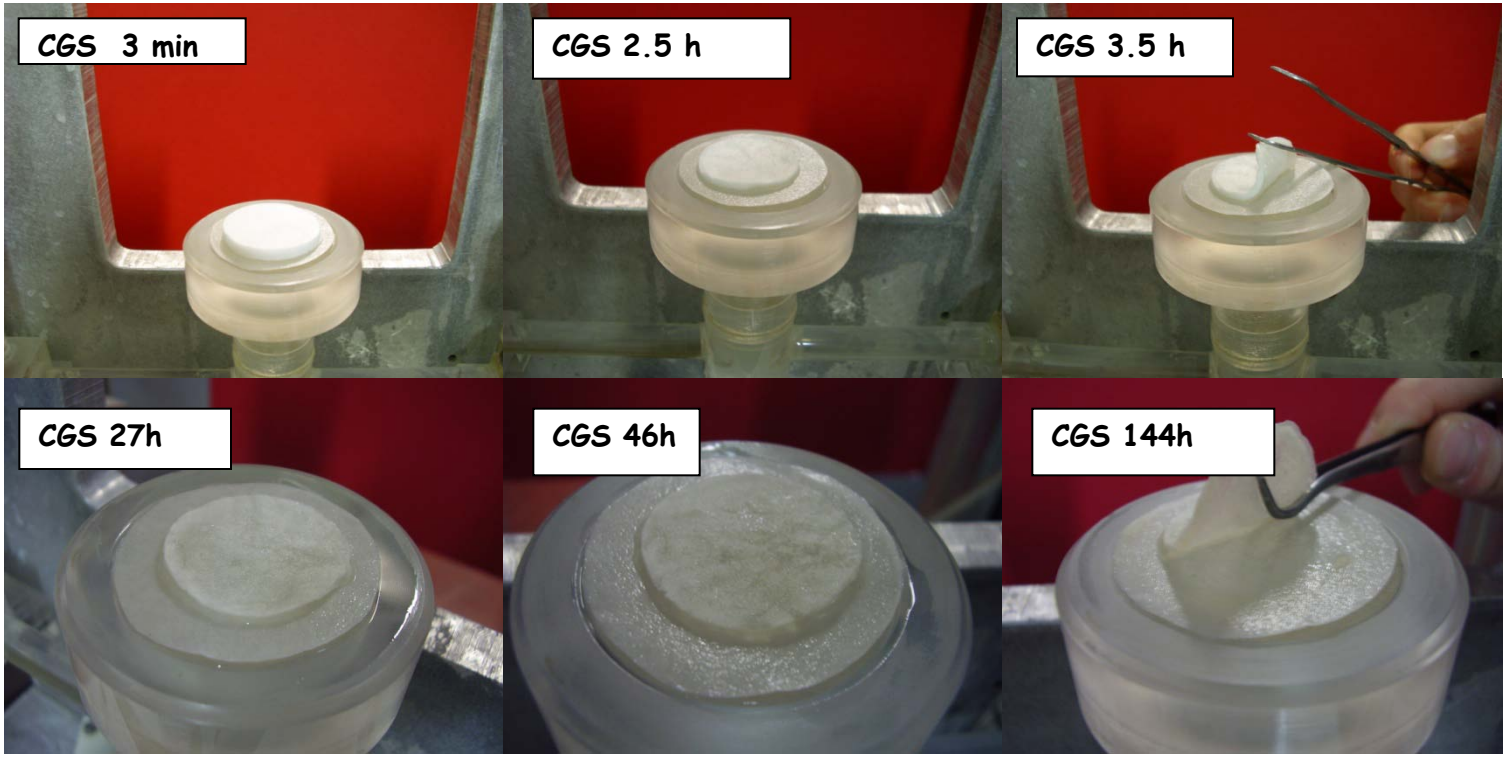


Mixture
saline solution/platelet lysate
LOADED

4g in plastic cylindrical container (∅ 40mm)
frozen – 40 C
Freeze dried for 24h

Hydration properties of sponge-like dressings based on Chitosan glutamate salt

CGS 1.5% (w/w)/Gly1% (w/w)/10% water



The dressings keep absorbing physiological solution up to six days without loosing integrity (suitable for exudating wounds)

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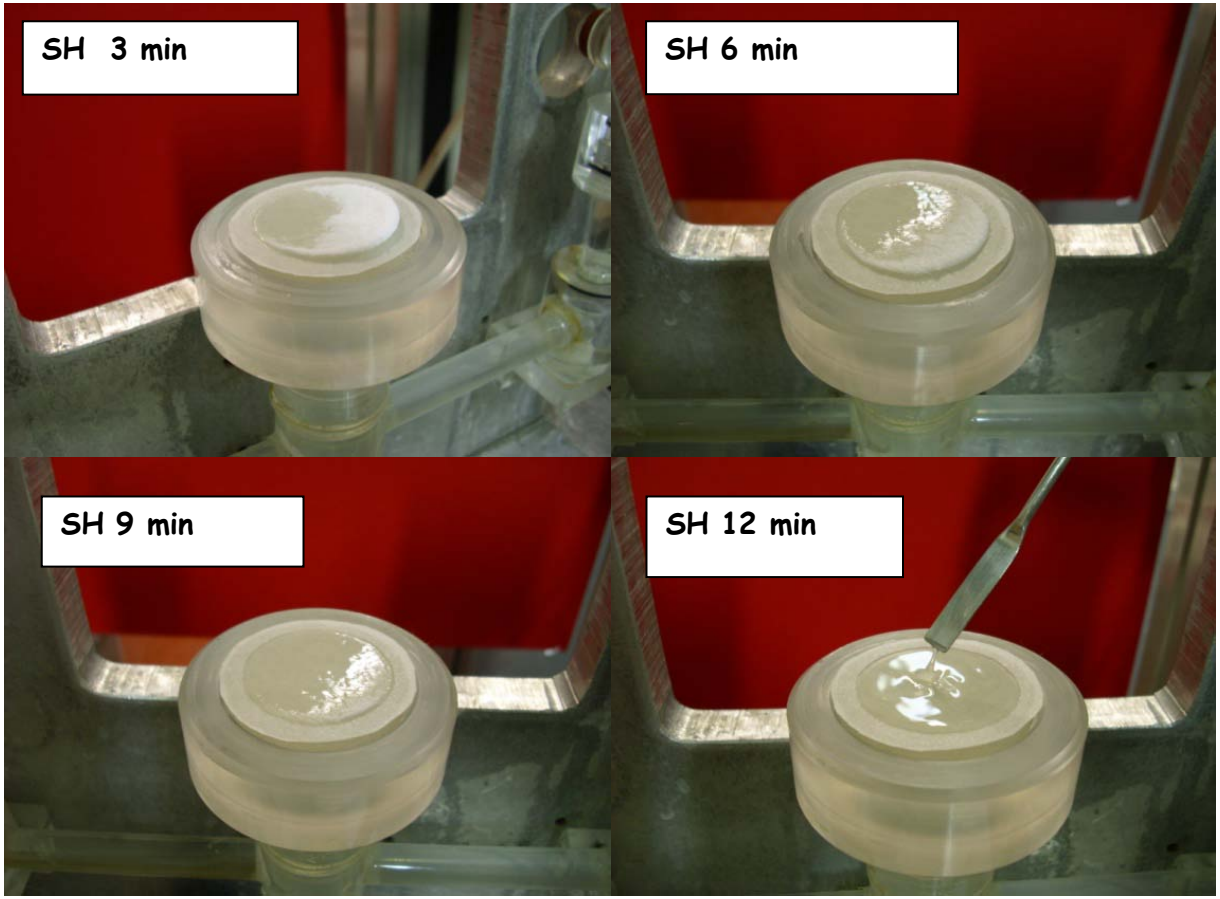
Hydration properties of sponge-like dressings based on sodium hyaluronate

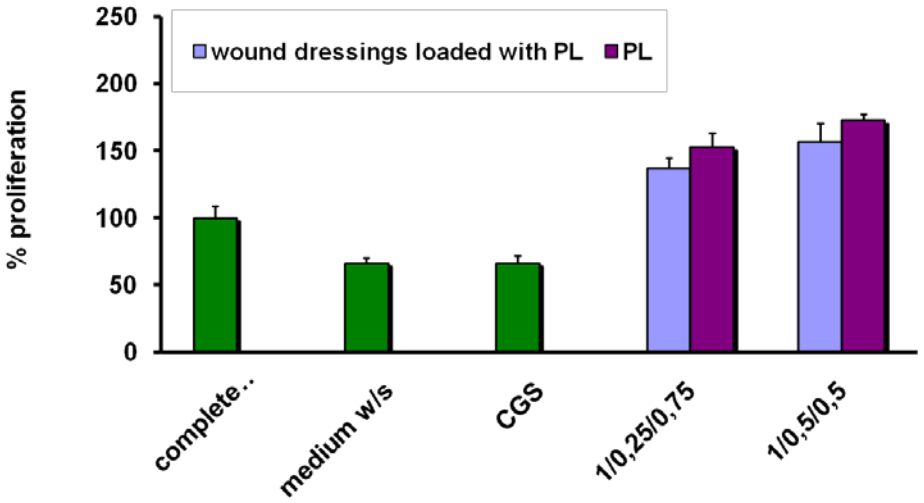
SH 1.5% (w/w)/Gly1% (w/w)/10% water



The dressings absorb physiological solution rapidly becaming hydrated (suitable for dry wounds)

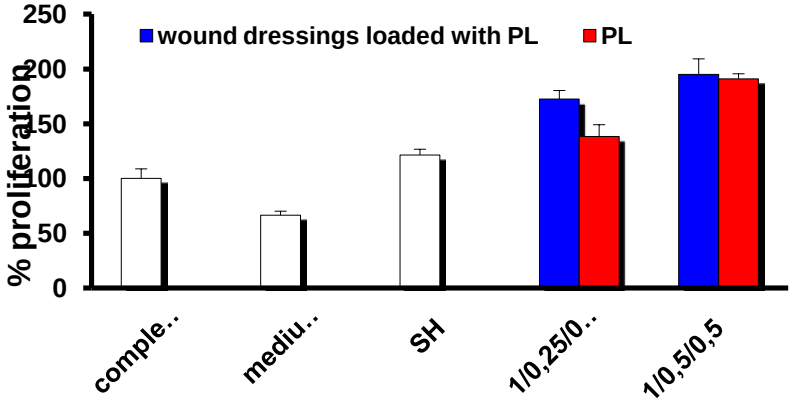
dressings loaded with PL

Cell proliferation test on human fibroblasts



Chitosan Glutamate /Glycine dressings

Sodium hyaluronate/Glycine dressings



Sponge-like dressings based on biopolymers for chronic ulcers

- Prepared by ***freeze-drying***
- Suitable ***mechanical properties*** for storage and handling
- ***Hydration properties*** tailored to the required exudate absorption capacity
- Platelet lysate can be either loaded during freeze-drying or imbibed as a solution just before the application
- ***Synergistic effect*** between PL and biopolymers

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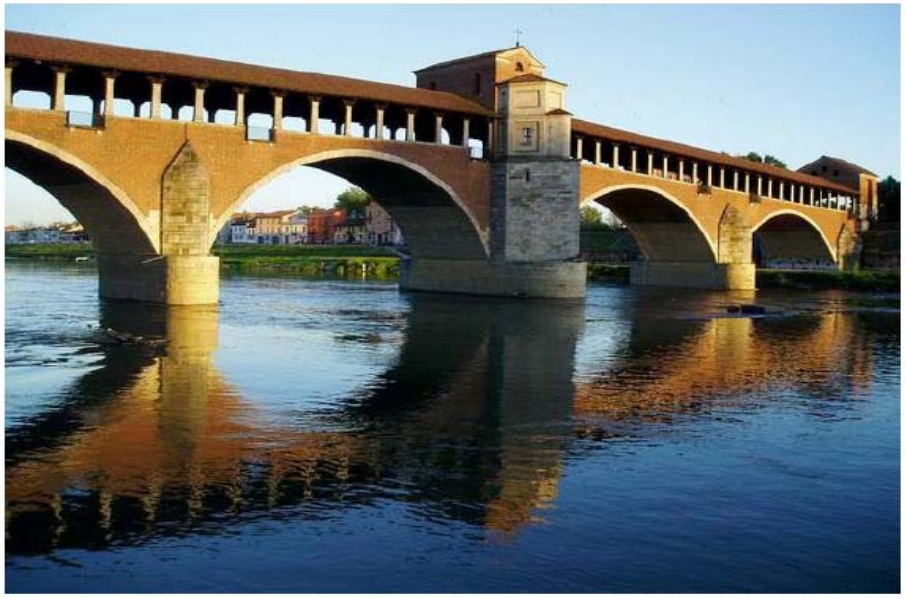

Center for Transplant Immunology, S.Matteo Hospital, Pavia

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