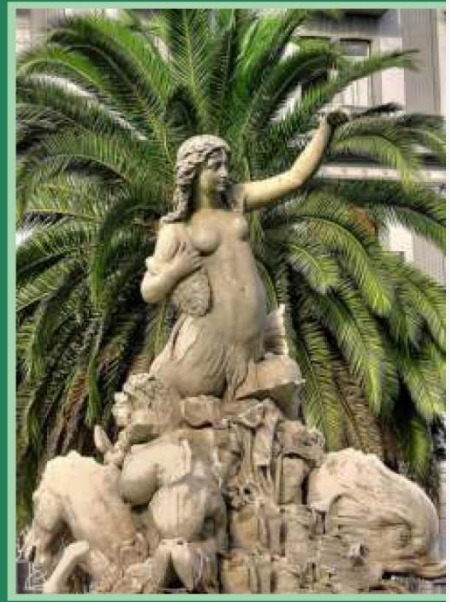
XVI Corso
in Riparazione Tessutale
Formazione e comunicazione
nel Wound Care



19
Aprile
2018

Responsabili scientifici:
Dott. Giuseppe Nebbioso, Dott. Francesco Petrella

Centro Congressi Hotel Gli Dei

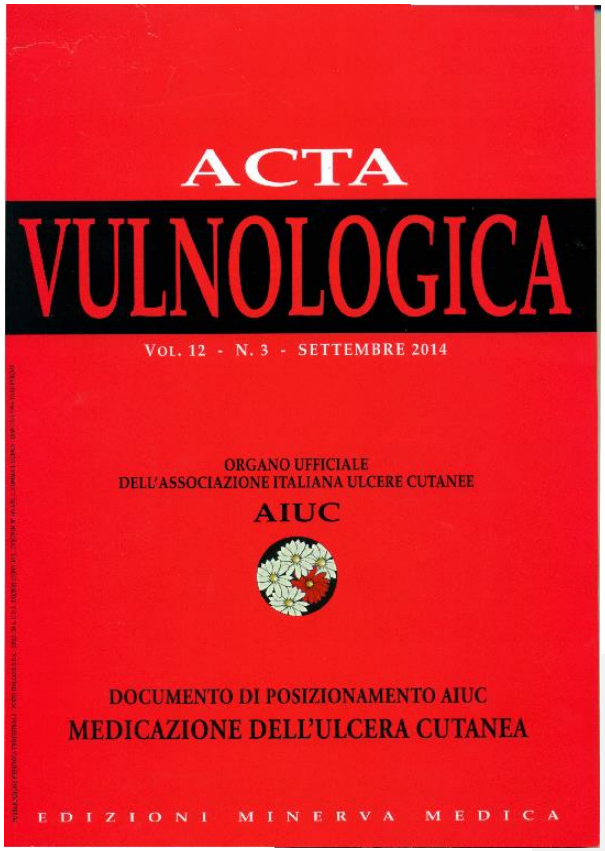
Pozzuoli (NA)

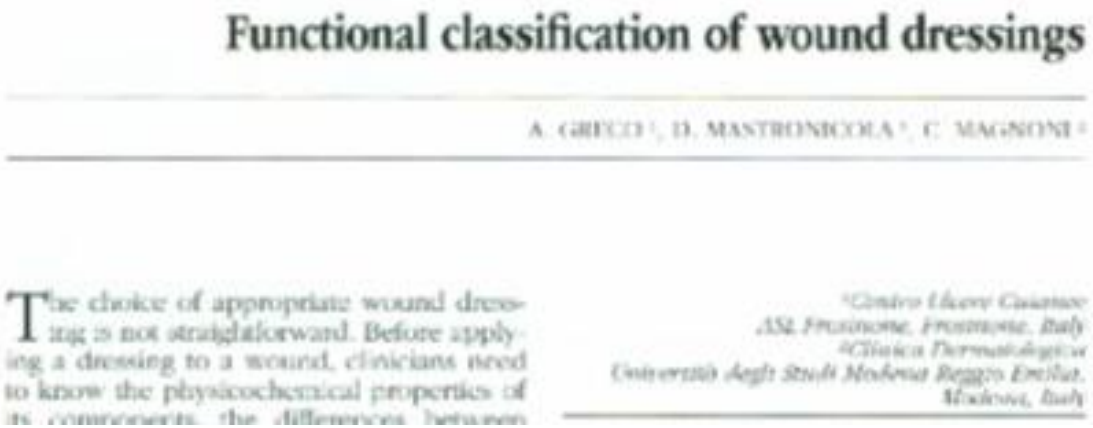
Medicazioni riepitelizzanti eudermiche

Focus su: Medicazioni per bioinduzione

Francesco Petrella
Responsabile Centro Chirurgico Territoriale (CCT)
Centro di II livello per la cura delle Ulcere cutanee
Asl Napoli 3 sud - ds 34- Portici
Presidente Associazione Italiana Ulcere Cutanee (AIUC – onlus

2014



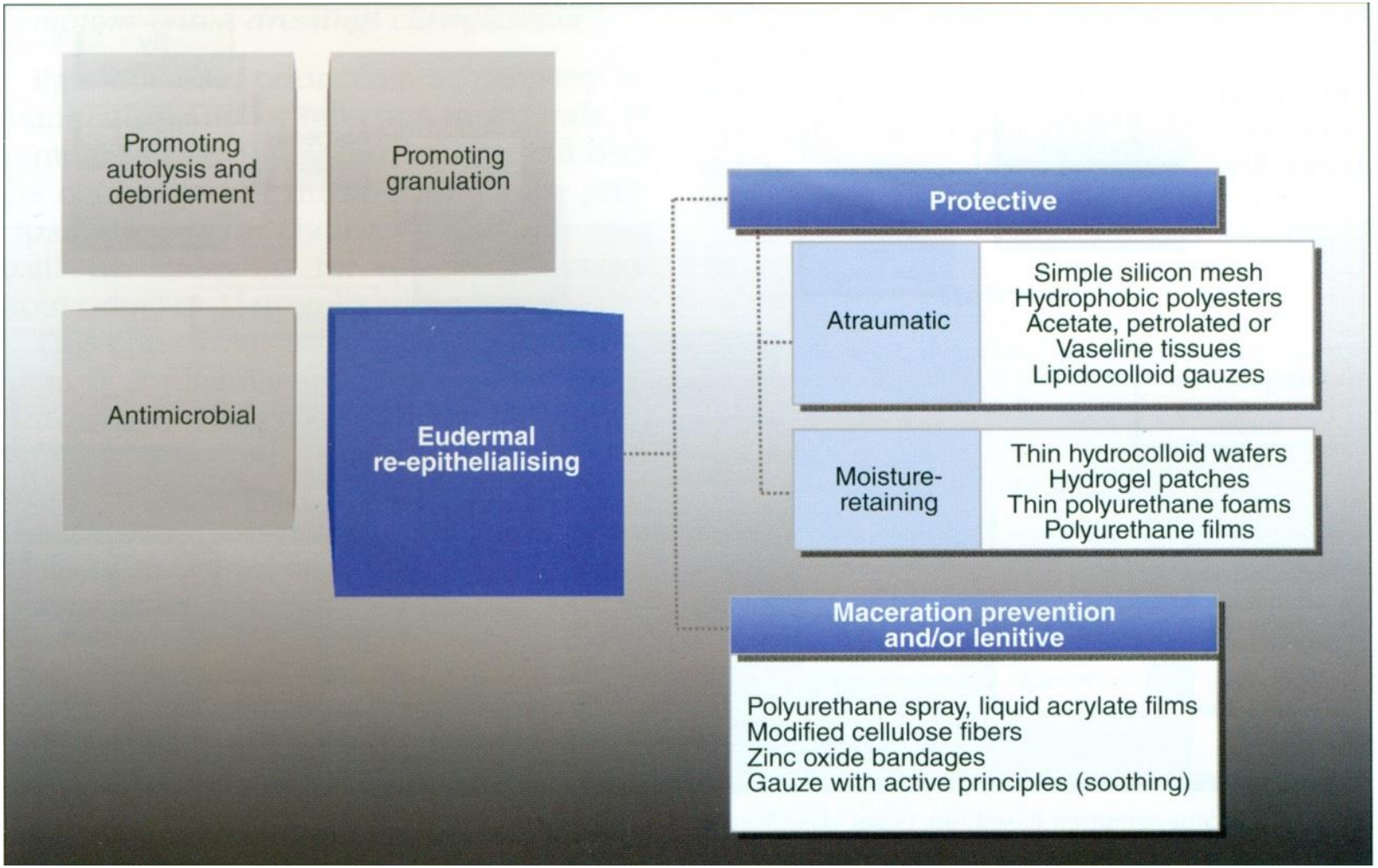

Functional classification of wound dressings

A. GRECO [1], D. MASTRONICOLA [2], C. MAGNONI [1]

The choice of appropriate wound dress-

CLASSIFAZIONE FUNZIONALE DELLE MEDICAZIONI

MEDICAZIONI EUDERMICHE ED RIEPITELIZZANTI



MEDICAZIONI EUDERMICHE ED RIEPITELIZZANTI

MEDICAZIONI EUDERMICHE ED RIEPITELIZZANTI

MEDICAZIONE ATRAUMATIVA

MEDICAZIONI EUDERMICHE ED RIEPITELIZZANTI

MEDICAZIONE
GESTIONE
UMIDITA’

MEDICAZIONI EUDERMICHE ED RIEPITELIZZANTI

MEDICAZIONE PREVENTIVA E LENITIVA

MEDICAZIONI EUDERMICHE ED RIEPITELIZZANTI

MEDICAZIONE PREVENTIVA E LENITIVA

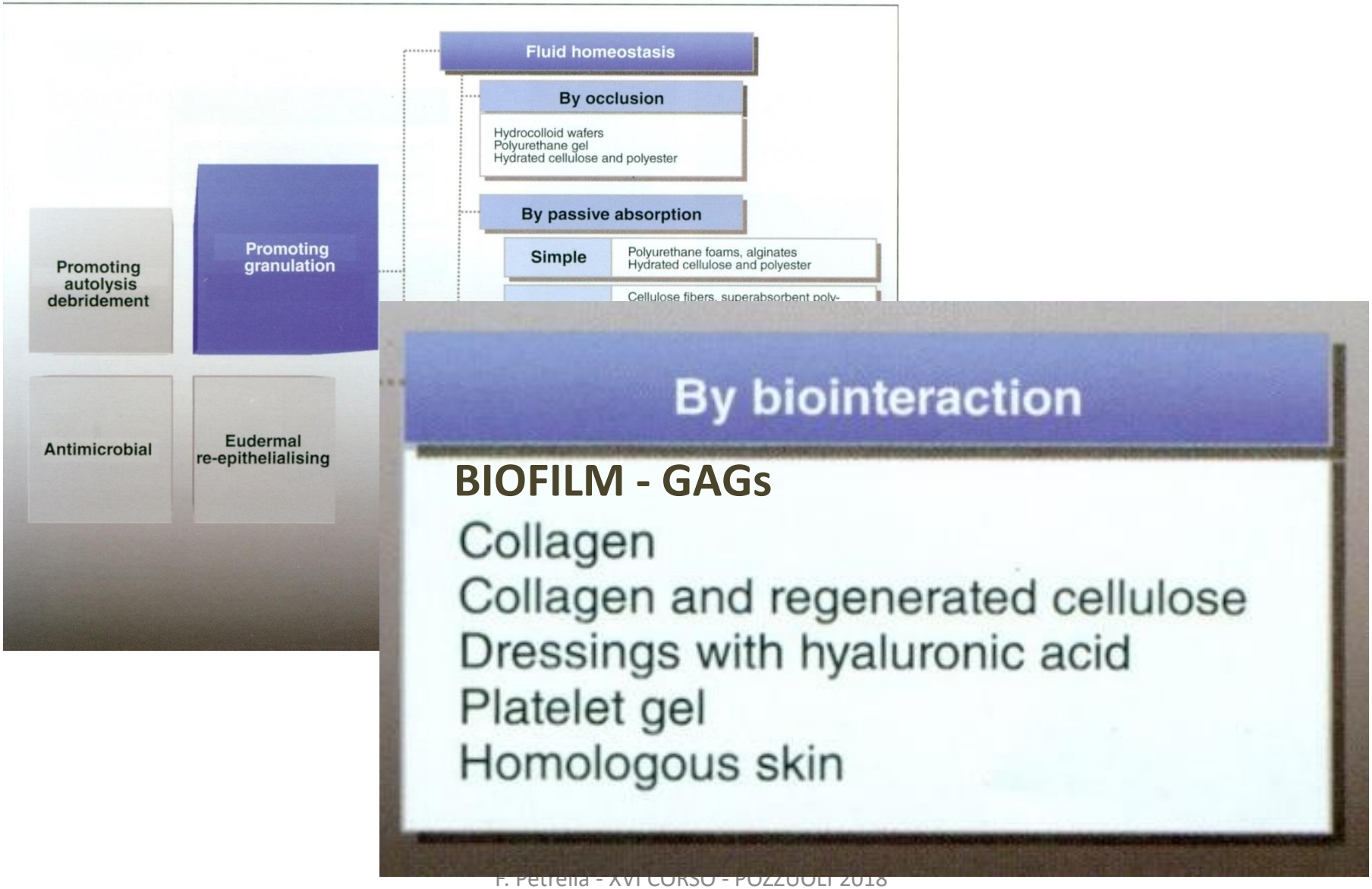
MEDICAZIONI EUDERMICHE ED RIEPITELIZZANTI

MEDICAZIONE PREVENTIVA E LENITIVA

Focus su:

Medicazioni per bioinduzione

MEDICAZIONI PER BIOINDUZIONE



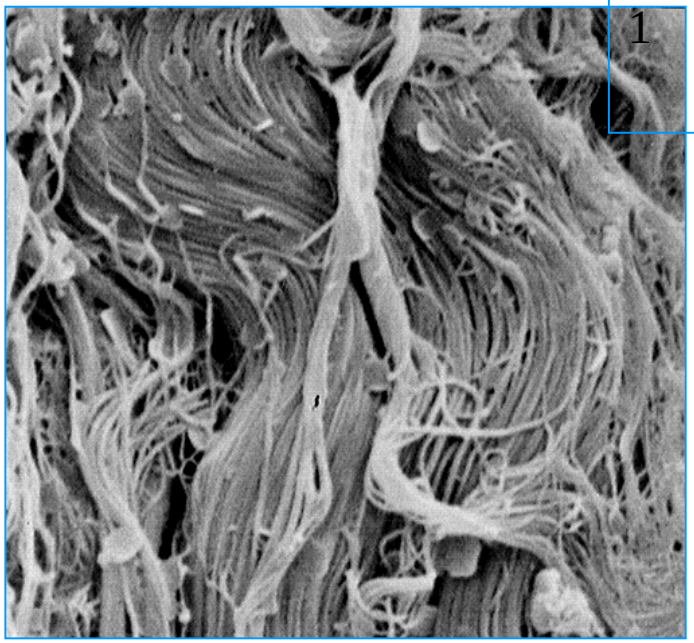
MEDICAZIONI PER BIOINDUZIONE

L'ECM (EXTRACELLULAR MATRIX) è composta da due principali classi di macromolecole:

1. **Glicosamminoglicani (GAG)**
2. **Proteine fibrose**

I primi comprendono:
Ac Ialuronico (HA); Condroitin Solfato (CS); Eparan Solfato (ES); Eparina e Dermatan Solfato (DS).

Le seconde comprendono due gruppi:
Collageni ed Elastina con funzione principalmente strutturale
Fibronectina, Laminine, Entactine o nidogeni e Vitronectina con funzione adesiva



MATRICE EXTRACELLULARE/ ECM

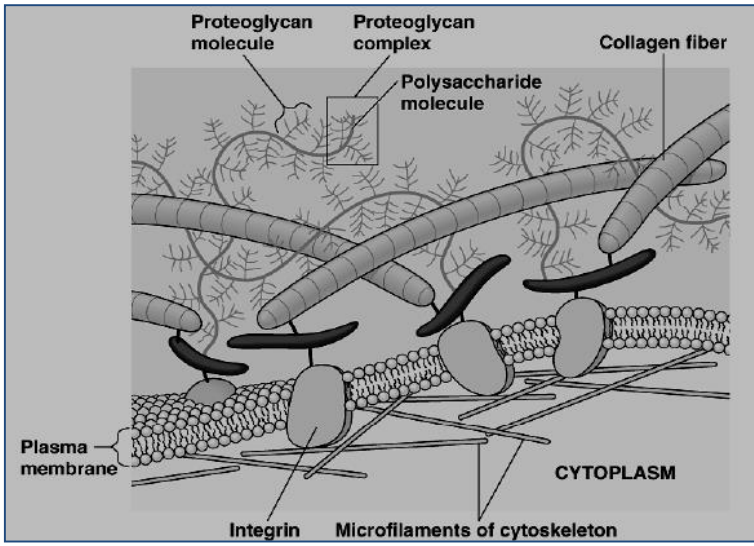
La ECM rappresenta il substrato su cui tutte le cellule dei tessuti possono aderire, migrare, proliferare e differenziare, e che ne influenza inoltre la sopravvivenza, la forma e la funzione. Infatti, le macromolecole dell'ECM sequestrano fattori di crescita, molecole come l'acqua o i minerali, e controllano fenomeni fisiologici, quali la morfogenesi, fisiopatologici, quali la guarigione delle ferite, e patologici, quali l'invasione e la metastatizzazione

MEDICAZIONI PER BIOINDUZIONE

«*Le metalloproteinasi della matrice non sono coinvolte soltanto nella degradazione della matrice, ma giocano anche altri ruoli nella* ***regolazione del comportamento cellulare****, e nella modulazione di molte molecola bioattive sulla superficie cellulare; possono agire in concerto con altri enzimi per influenzare il comportamento delle cellule.*»

Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer. 2002 Mar;2(3):161-74.

SCOMPAGINAMENTO DELLA MATRICE EXTRACELLULARE - ECM



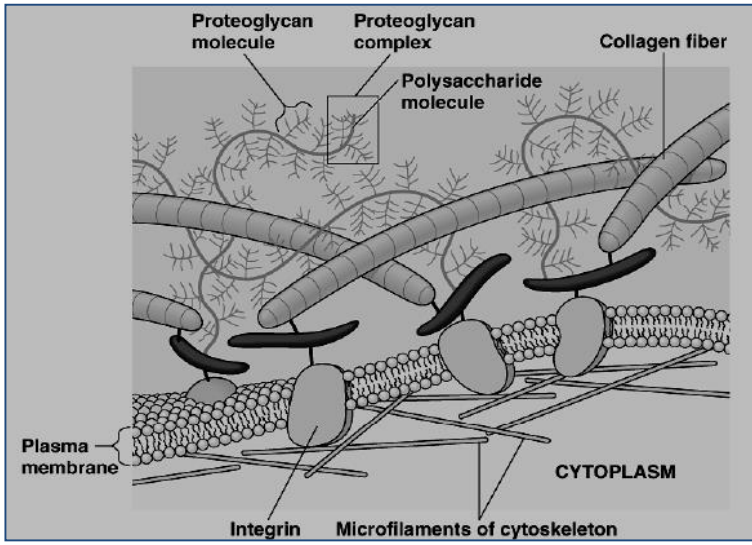
Sottoposta a:

- ➢ Progressiva distruzione dei vari tipi di collagene
- ➢ Proteolisi nei confronti della elastina e proteine fibrillari
- ✓ Reazione dei fibroblasti
- ✓ Incremento dei processi di sintesi,

incompleti e disorganizzati inefficaci

Kessler D, Dethlefsen S, Haase I, Plomann M, Hirche F, Krieg T, Eckes B - Fibroblasts in mechanically stressed collagen lattices assume a "synthetic" phenotype - J Biol Chem 2001 Sep 28;276(39):36575-85

SCOMPAGINAMENTO DELLA MATRICE EXTRACELLULARE - ECM



- Incapacità dei fibroblasti di “percepire” la conformazione della matrice e di interagire con essa
- Difficoltà delle fibre nel disporsi in modo ben orientato
- Deposizione di matrice degenerata, con caratteri morfologici simil-cicatriziali

Fisher GJ, Choi HC, Bata-Csorgo Z, Shao Y, Datta S, Wang ZQ, Kang S, Voorhees JJ - Ultraviolet irradiation increases matrix metalloproteinase-8 protein in human skin *in vivo* - J Invest Dermatol 2001 Aug;117(2):219-26

Le metalloproteasi (MMP) prodotti da cellule del tessuto connettivo e secreti nella matrice extracellulare ad oggi sono state individuate 28

Quattro classi:

1. Gelatinasi (MMP-2, MMP-9)
2. Collagenasi (MMP-1, MMP-8, MMP-13)
3. Stromelisine (MMP-3, MMP-10, MMP-12)
4. Metalloproteasi di membrana (MT1-MMP, MT2-MMP, MT3-MMP, MT4-MMP).

La proteina dopo la sua sintesi viene secreta nella sua forma inattiva (pro-MMP), nella quale l'atomo di zinco, presente nel sito catalitico, non è disponibile alla formazione di legami a causa della presenza di una cisteina.

MEDICAZIONI PER BIOINDUZIONE

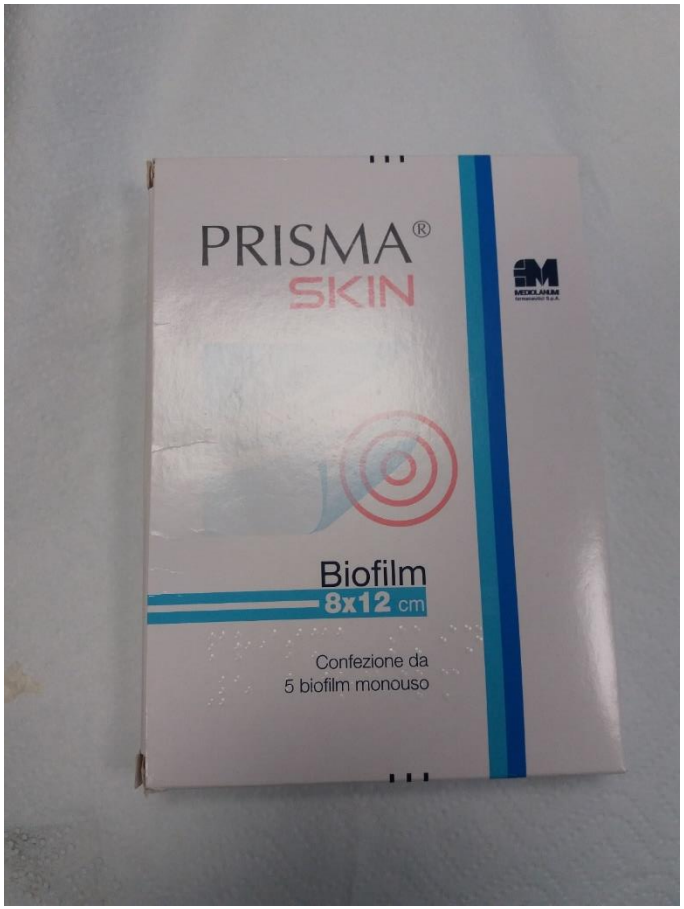


Tabella 4.1: Composizione del DM

MATERIE PRIME	*FUNZIONE*	*Quantità in %*
· Sodium Alginate	idrofilo/filmante	36,59%
· Microcrystalline Cellulose	idrofilo	7,15%
· Vegetable Protein (Pea Protein)	idrofilo	12,62%
· Sorbitol	umettante	7,36%
· Glycerin	umettante	21,03%
· Polysorbate 80	solubilizzante	8,41%
· Polyvinylpyrrolidone	idrofilo	2,1%
· Sodium mesoglycane	idrofilo/filmante	4,63%
· Sodium Hyaluronate	idrofilo/filmante	0,11 %

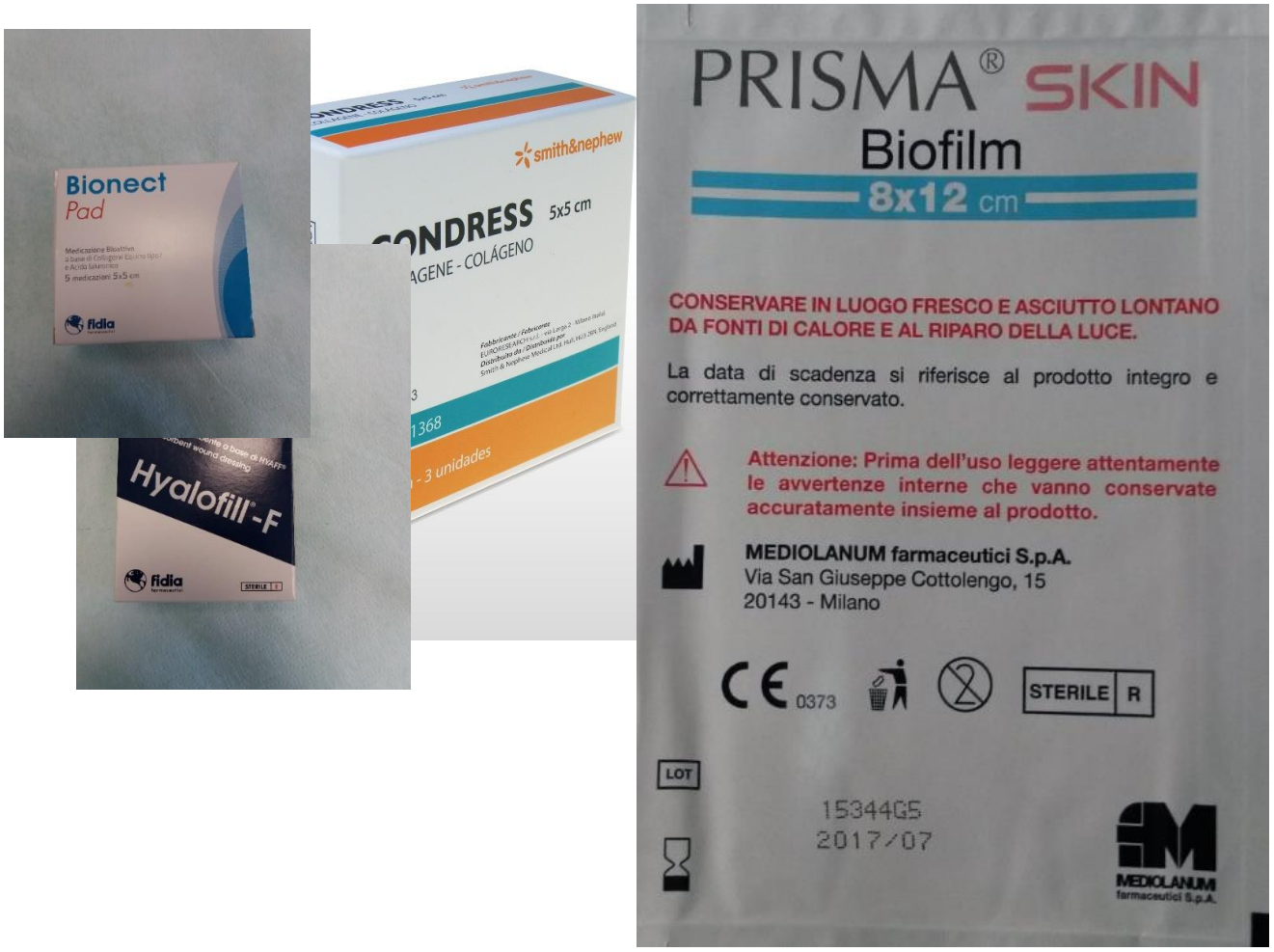
4,63%

MESOGLICANO

Miscela di:

1. Eparansolfato 47.5%,
2. Dermatansolfato 35.5%,
3. Eparina slow moving 8.5%,
4. Condroitinsolfato 8.5%.

MEDICAZIONI PER BIOINDUZIONE



BIOFILM: MADALITA’ DI APPLICAZIONE

RIMOZIONE DELLA PARTE ECCEDENTE SULLA CUTE PERILESIONALE DEL BIOFLIM

MEDICAZIONI PER BIOINDUZIONE

MEDICAZIONI PER BIOINDUZIONE

MEDICAZIONI PER BIOINDUZIONE

MEDICAZIONI PER BIOINDUZIONE

CELL ADHESION & MIGRATION
https://doi.org/10.1080/19336918.2017.1340137

Taylor & Francis
Taylor & Francis Group

RESEARCH PAPER

Check for updates

Effects of Prisma® Skin dermal regeneration device containing glycosaminoglycans on human keratinocytes and fibroblasts

Raffaella Belvedere[a], Valentina Bizzarro[a], Luca Parente[a], Francesco Petrella[b], and Antonello Petrella[a]

[a]Department of Pharmacy, University of Salerno, Fisciano, Salerno, Italy; [b]Primary Care - Wound Care Service, Health Local Agency Naples 3 South, Portici, Napoli, Italy

ABSTRACT

Prisma® Skin is a new pharmaceutical device developed by Mediolanum Farmaceutici S.p.a. It includes alginates, hyaluronic acid and mainly mesoglycan. The latter is a natural glycosaminoglycan preparation containing chondroitin sulfate, dermatan sulfate, heparan sulfate and heparin and it is used in the treatment of vascular disease. Glycosaminoglycans may contribute to the re-epithelialization in the skin wound healing, as components of the extracellular matrix. Here we describe, for the first time, the effects of Prisma® Skin in *in vitro* cultures of adult epidermal keratinocytes and dermal fibroblasts. Once confirmed the lack of cytotoxicity by mesoglycan and Prisma® Skin, we have shown the increase of S and G2 phases of fibroblasts cell cycle distribution. We further report the strong induction of cell migration rate and invasion capability on both cell lines, two key processes of wound repair. In support of these results, we found significant cytoskeletal reorganization, following the treatments with mesoglycan and Prisma® Skin, as confirmed by the formation of F-actin stress fibers. Additionally, together with a significant reduction of E-cadherin, keratinocytes showed an increase of CD44 expression and the translocation of ezrin to the plasma membrane, suggesting the involvement of CD44/ERM (ezrin-radixin-moesin) pathway in the induction of the analyzed processes. Furthermore, as showed by immunofluorescence assay, fibroblasts treated with mesoglycan and Prisma® Skin exhibited the increase of Fibroblast Activated Protein α and a remarkable change in shape and orientation, two common features of reactive stromal fibroblasts. In all experiments Prisma® Skin was slightly more potent than mesoglycan. In conclusion, based on these findings we suggest that Prisma® Skin may be able to accelerate the healing process in venous skin ulcers, principally enhancing re-epithelialization and granulation processes.

ARTICLE HISTORY
Received 22 March 2017
Revised 22 May 2017
Accepted 6 June 2017

KEYWORDS
glycosaminoglycans; mesoglycan; Prisma® Skin; re-epithelialization; skin wound healing

5.169.142.241] at 10:12 10 August 2017

International Journal of *Molecular Sciences*

MDPI

Article

The Pharmaceutical Device Prisma® Skin Promotes in Vitro Angiogenesis through Endothelial to Mesenchymal Transition during Skin Wound Healing

Raffaella Belvedere [1], Valentina Bizzarro [1], Luca Parente [1], Francesco Petrella [2] and Antonello Petrella [1,*]

[1] Department of Pharmacy, University of Salerno, via Giovanni Paolo II 132, 84084 Fisciano (Salerno), Italy; rbelvedere@unisa.it (R.B.); vbizzarro@unisa.it (V.B.); lparente@unisa.it (L.P.)
[2] Primary Care—Wound Care Service, Health Local Agency Naples 3 South, Via Libertà 42, 80055 Portici (Napoli), Italy; dottpetrella@gmail.com
* Correspondence: apetrella@unisa.it; Tel.: +39-089-969762; Fax: +39-089-969602

Received: 29 June 2017; Accepted: 22 July 2017; Published: 25 July 2017

Abstract: Glycosaminoglycans are polysaccharides of the extracellular matrix supporting skin wound closure. Mesoglycan is a mixture of glycosaminoglycans such as chondroitin-, dermatan-, heparan-sulfate and heparin and is the main component of Prisma® Skin, a pharmaceutical device developed by Mediolanum Farmaceutici S.p.a. Here, we show the in vitro effects of this device in the new vessels formation by endothelial cells, since angiogenesis represents a key moment in wound healing. We found a strong increase of migration and invasion rates of these cells treated with mesoglycan and Prisma® Skin which mediate the activation of the pathway triggered by CD44 receptor. Furthermore, endothelial cells form longer capillary-like structures with a great number of branches, in the presence of the same treatments. Thus, the device, thanks to the mesoglycan, leads the cells to the Endothelial-to-Mesenchymal Transition, suggesting the switch to a fibroblast-like phenotype, as shown by immunofluorescence assays. Finally, we found that mesoglycan and Prisma® Skin inhibit inflammatory reactions such as nitric oxide secretion and NF-κB nuclear translocation in endothelial cells and Tumor Necrosis Factor-α production by macrophages. In conclusion, based on our data, we suggest that Prisma® Skin may be able to accelerate angiogenesis in skin wound healing, and regulate inflammation avoiding chronic, thus pathological, responses.

Keywords: mesoglycan; Prisma® Skin; glycosaminoglycans; angiogenesis; skin wound healing

MEDICAZIONI PER BIOINDUZIONE

I Dipartimento di Farmacia dell'Università degli Studi di Salerno

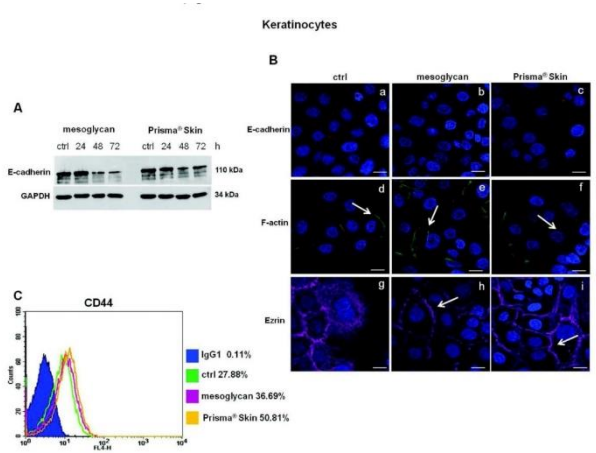


Figure 5. (**A**) Western blots showing E-cadherin and GAPDH expression in HaCaT cells treated or not with 0,3 mg/ml of sodium mesoglycan and Prisma® Skin for 24, 48 and 72h. The blots were exposed to Las4000 (GE Healthcare Life Sciences). (**B**) Immunofluorescence analysis to detect E-cadherin (panels a, b, c), F-actin (panels d, e, f) and ezrin (panels g, h, i) treated with mesoglycan and Prisma® Skin for 48h. Nuclei were stained with DAPI. Magnification 63x1.4 NA. Bar=10 µm. (**C**) Cell surface expression of CD44 was analyzed by flow cytometry. The blue areas in the plots are relative to human IgG1; CD44 signals are showed in green for ctrl HaCaT, in purple for HaCaT in presence of mesoglycan and in yellow for cells with Prisma® Skin, at 24, 48 and 72h.The results are representative ± SEM of almost three analyzed experiments.

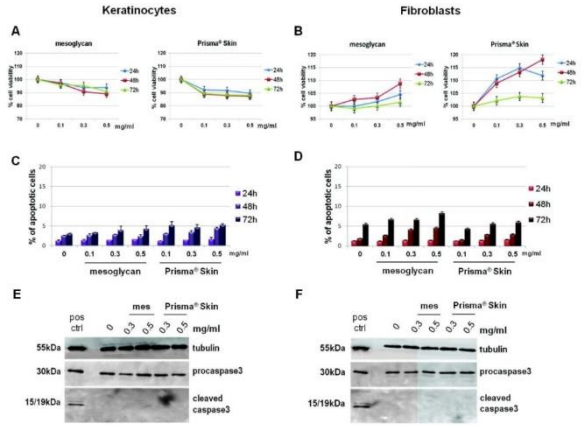
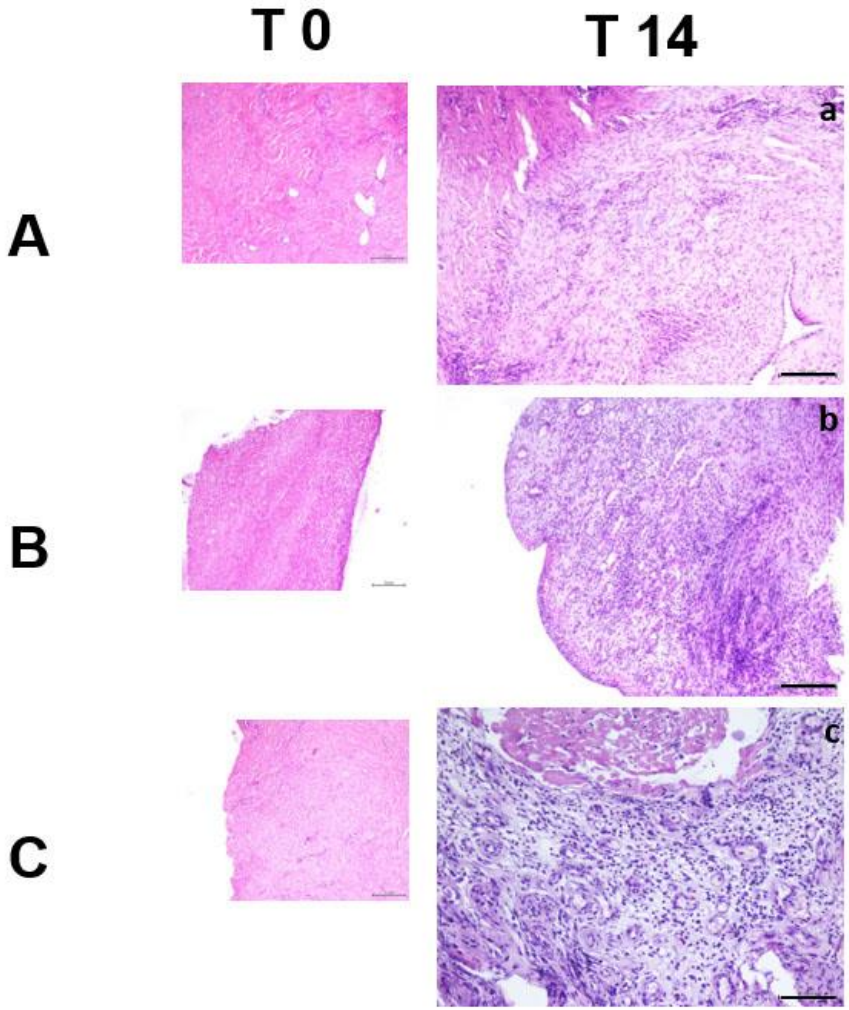


Figure 1. Cell viability was evaluated by MTT colorimetric assay on keratinocytes (**A**) and on fibroblasts (**B**). Absorbance relative to controls was used to determine the percentage of cells treated with varying concentrations of sodium mesoglycan and Prisma® Skin at 24, 48 and 72h. Values are the mean ± S.E.M. from 5 independent experiments performed in triplicates. Analysis of apoptotic cells by cytofluorimetric assay of the effect of 0,1, 0,3 and 0,5 mg/ml of mesoglycan and Prisma® Skin at 24, 48 and 72h on HaCaT (**C**) and on B| (**D**). The data are representative of 5 experiments with similar results. Western blot for pro - and cleaved caspase-3 of keratinocytes (**E**) and fibroblasts (**F**). Both of cell lines were treated or not with mesoglycan and Prisma® Skin for 48h and a positive control of apoptotic cells was used. Protein bands were normalized on tubulin levels. Image is representative of 3 independent experiments.

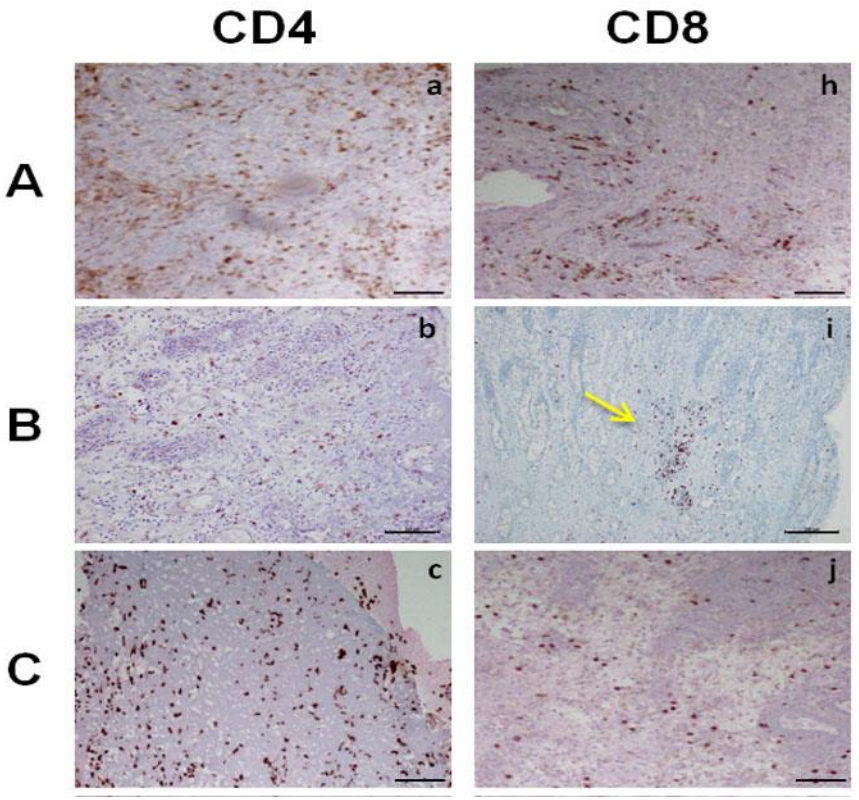
MEDICAZIONI PER BIOINDUZIONE



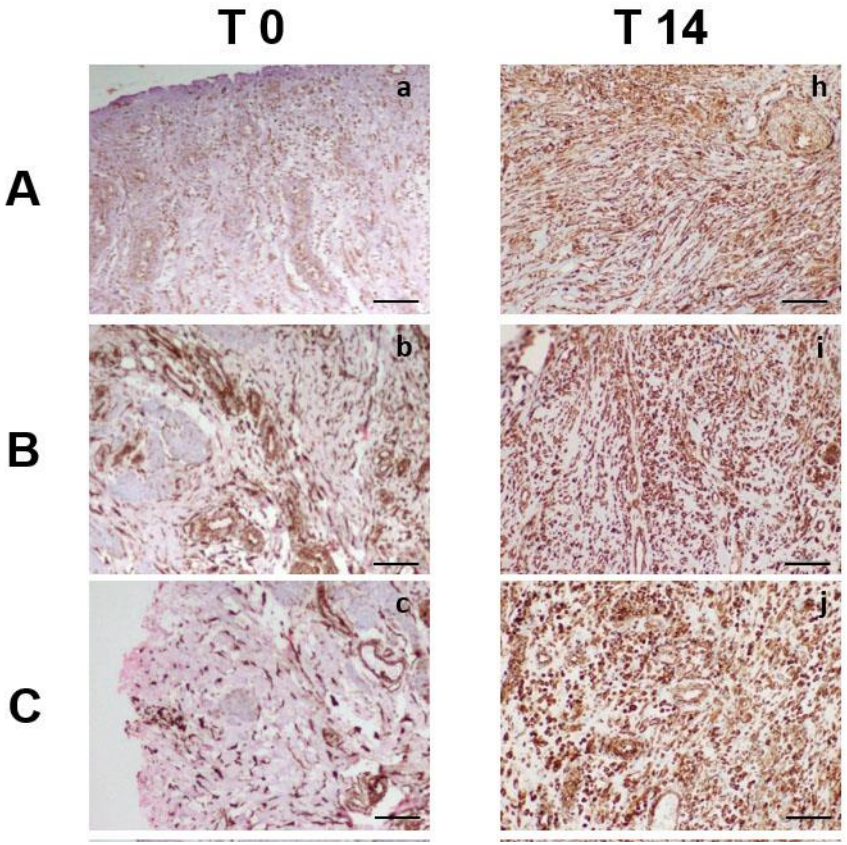
ESAME ISTOLOGICO
COLORAZIONE STANDARD

IMMUNOISTOCHIMICA

RICERCA LINFOCITI



MEDICAZIONI PER BIOINDUZIONE



IMMUNOISTOCHIMICA

RICERCA FIBROBLASTI

RISULTATI

Grazie