

# Hands-On Cadaver Seminar

February 21-23, 2024  
Celebration, Florida

## The Role of Proteins and Scaffolds in Regenerative Healing

John Starinski, DPM



# Conflict of Interest Disclosure

*John Starinski, DPM has a financial relationship with the following companies and/or products. BioStem Technologies, Progenacare Global. These relationships may or may not apply to this lecture.*

The content of this presentation reflect the opinions of the speaker alone and any products or services mentioned are not endorsed by the AMIFAS.

## Learning Objectives

- 1.) Identify the key proteins (growth factors and cytokines as well as scaffold proteins involved in regenerative healing.
- 2.) How these essential proteins are preserved.
- 3.) How do we know these proteins are preserved? Assay technique

## The Players:

- PDGF-BB
- Fibronectin
- IL-1ra

## PDGF-BB

### **Platelet Derived Growth Factor BB**

- PDGF-BB increased the depth and area of new granulation tissue, the influx of fibroblasts, and the deposition of new matrix into wounds.

[Principles of Tissue Engineering \(Second Edition\), 2000](#)

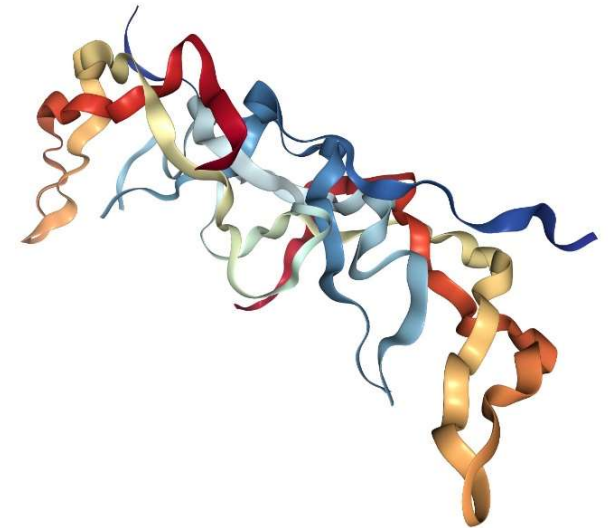
Why is PDGF-BB so effective at this task?

## PDGF-BB

The crystal structure of the homodimeric BB isoform of human recombinant platelet-derived growth factor (PDGF-BB) has been determined by X-ray analysis to 3.0 Å resolution. The polypeptide chain is folded into **two highly twisted antiparallel pairs of beta-strands** and contains an unusual knotted arrangement of three intramolecular disulfide bonds.

**Author:** C. Oefner, A. D'Arcy, F.K. Winkler, B. Eggimann, M. Hosang

**Publish Year:** 1992

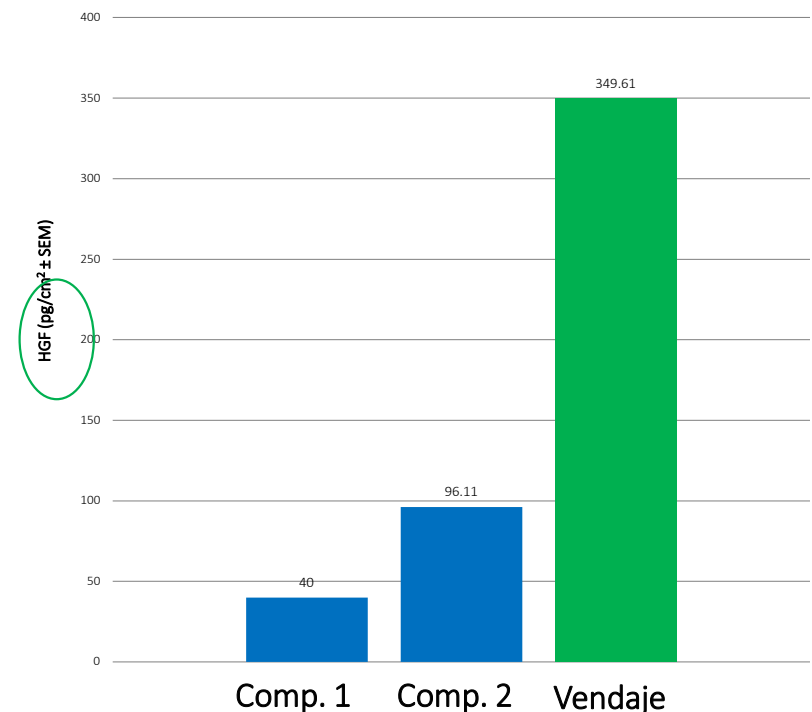


Why is the structure information important?

## PDGF-BB

(Platelet-derived growth factor subunit B homodimer)

- Required element for division of fibroblasts.<sup>1</sup>
- Exogenous PDGF significantly reduces inflammatory cells → accelerates ECM and collagen formation → reduces the time for healing.<sup>2</sup>
- Powerful promoter of cell proliferation.
- Increases the expression of stem cell markers.<sup>3</sup>
- Significant role in blood vessel formation and proliferation and directed migration of mesenchymal stem cells.<sup>2</sup>
- In combination with other growth factors, stimulates soft and hard tissue healing.<sup>4-7</sup>

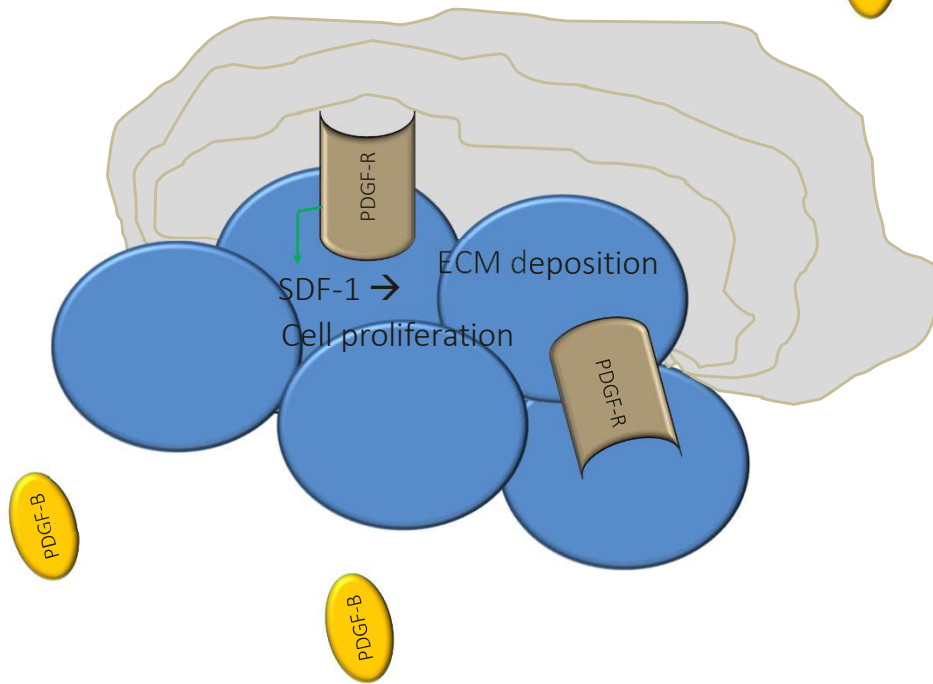


<sup>1</sup>[Alvarez et.al. 2006], <sup>2</sup>[Pierce et. al. 1991], <sup>3</sup>[Mihaylova 2018], <sup>4-7</sup>Lynch et al. 1987, 1989, 1991, 1995.

# PDGF-BB



*Platelet-derived growth factor subunit B homodimer*





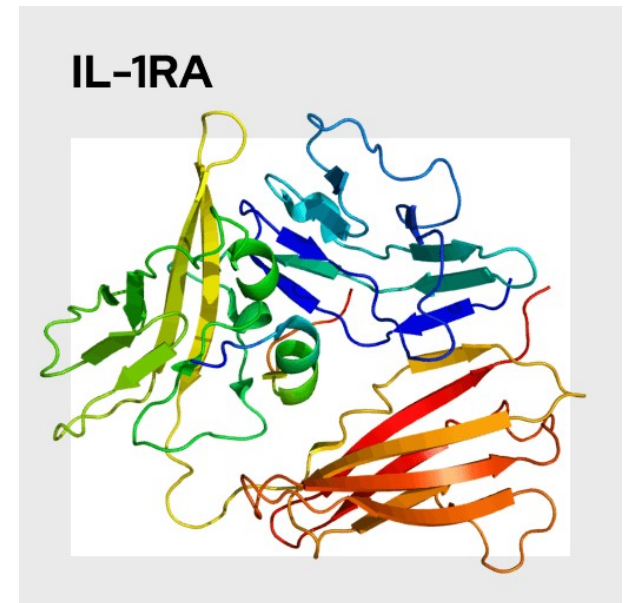
## IL-1ra

- IL-1RA is a member of the interleukin 1 cytokine family. IL1Ra is secreted by various types of cells including immune cells, epithelial cells, and adipocytes, and is a natural inhibitor of the pro-inflammatory effect of IL1 $\beta$ .

Why is this important in regenerative healing?

## IL-1ra

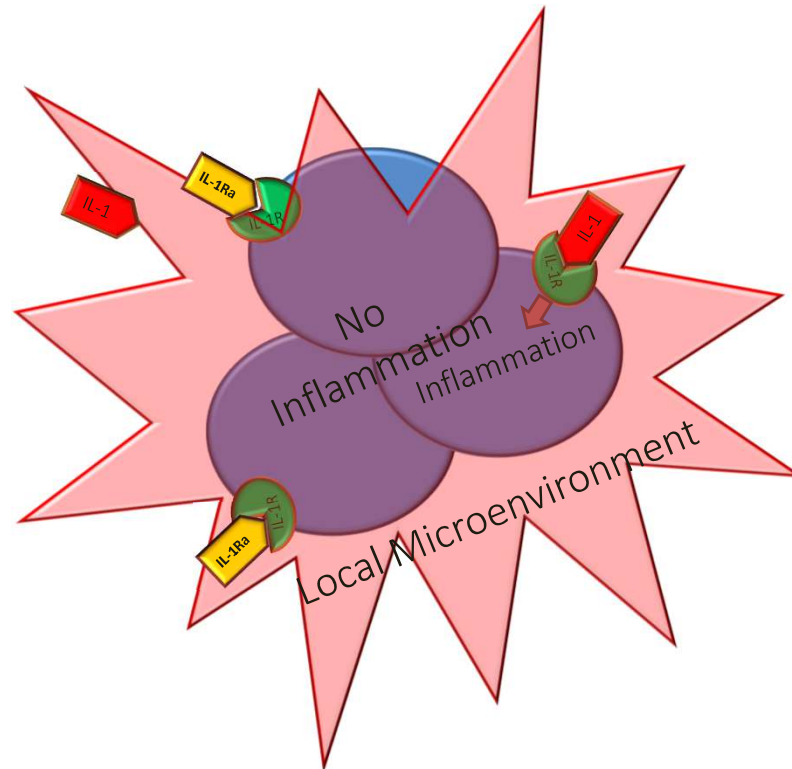
IL-1ra has the same fold as IL-1 alpha and IL-1 beta. The fold consists of **twelve beta-strands** which form a six-stranded beta-barrel, closed on one side by three beta-hairpin loops. Cys69 and Cys116 are linked via a disulfide bond and Pro53 has been built in the cis-conformation.



Why is the structure information important?

# IL-1ra

*Interleukin-1 receptor antagonist*



## Fibronectin

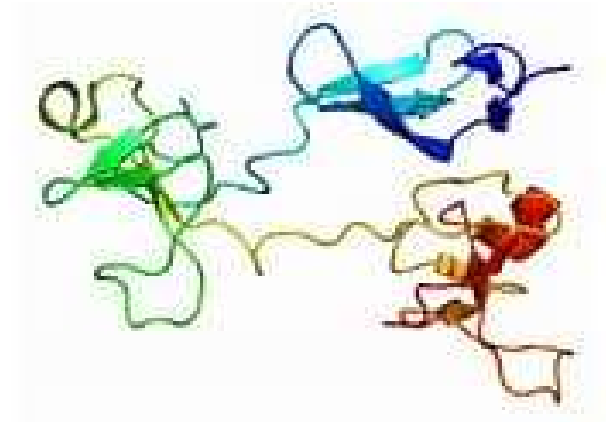
- Fibronectin (FN) is a multifunctional adhesive glycoprotein that plays an important role in tissue repair, in regulating cell attachment and motility, and in embryogenesis.

[Comprehensive Biomaterials II, 2017](#)

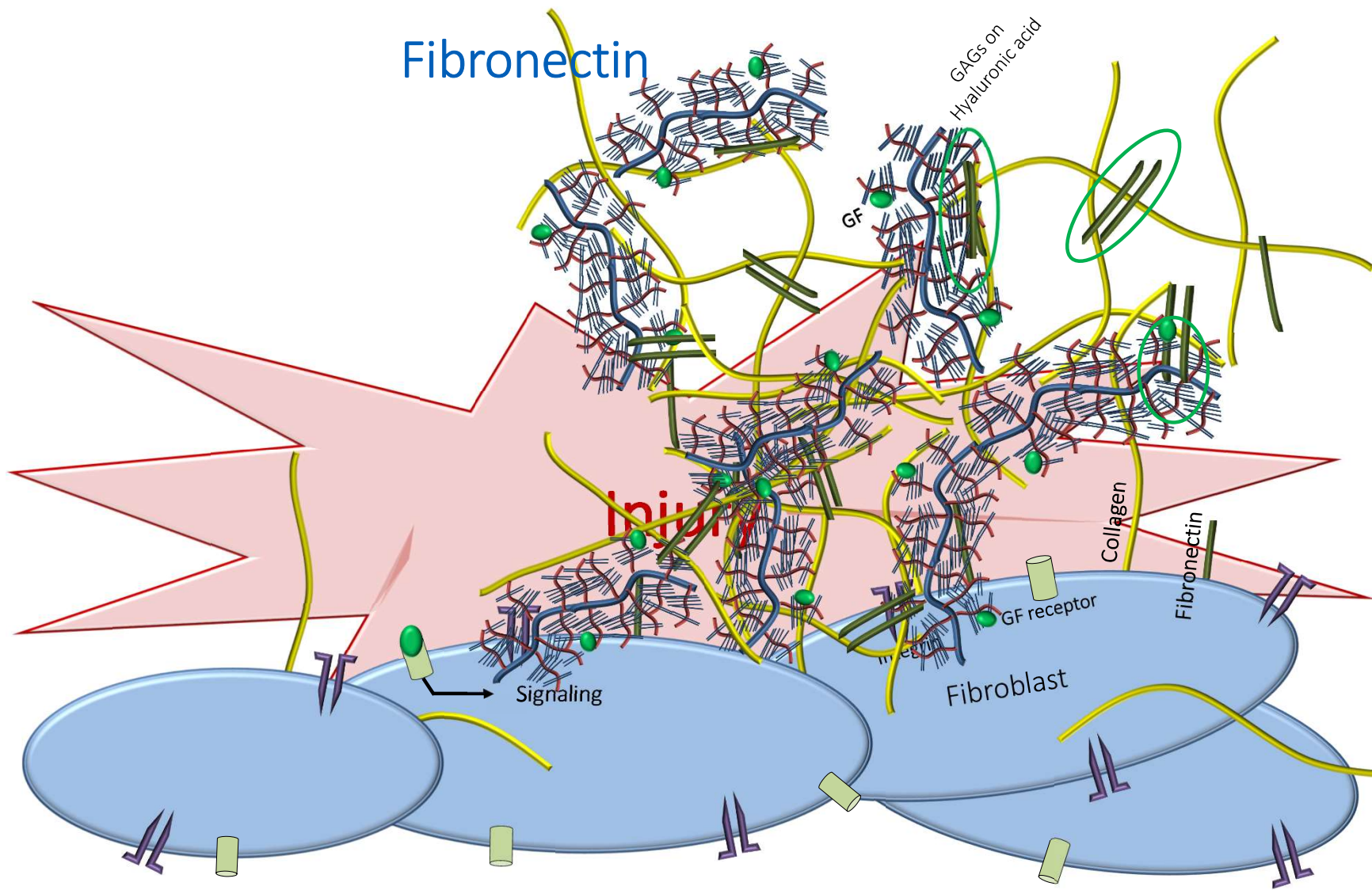
How does it work in cell attachment?

## Fibronectin

Fibronectin exists as a protein dimer, consisting of two nearly identical polypeptide chains linked by a pair of C-terminal disulfide bonds.



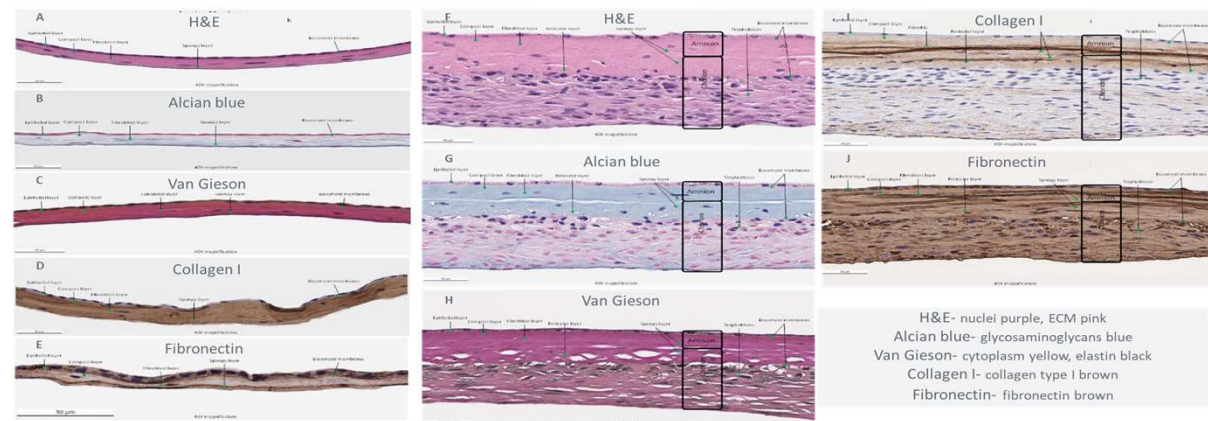
Why is the structure information important?



# A Word About Structural Proteins:

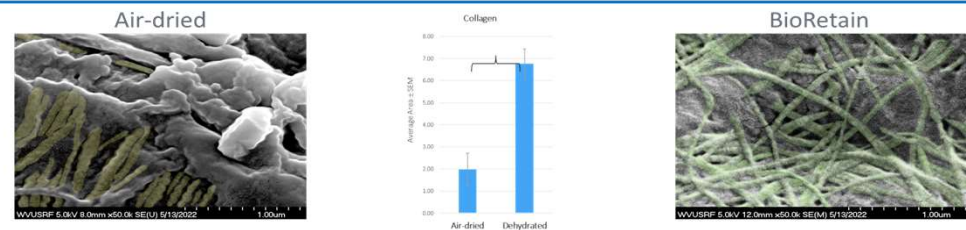
- Collagen in regeneration must be in a native alignment and maintain the interfibrillar bonds.
- Maintain it's Fibronectin relationships
- Provide a scaffolding effect

**Histology of BioRetain-processed stroma.** **A-E** Amnion membrane, **F-J** Amnion/Chorion membrane **A,F)** Hematoxylin and eosin stain for structure. **B,G)** Alcian blue staining for glycosaminoglycans. **C,H)** Van Gieson staining for elastin and collagen. **D,I)** Staining for collagen I in brown. **E,J)** Staining for fibronectin in brown. Performed by HistoWiz, Inc.



## Collagen Structure of BioRetain-processed and air-dried stroma.

Each amnion/chorion was cut into 2 pieces. SEM Images from WVU Electron Microscopy Facilities, Marcela Redigolo, PhD. Collagen was colored based in striation. Image J analysis quantified the collagen. N=3



## Processing:

### 1.) Disinfection

### 2.) Reduction

### 3.) Cleansing

### 4.) Dehydration

### 5.) Placement

### 6.) Sterilization



#### Minimally damaging **Disinfection**

The membrane is submerged in an effective bactericidal, tuberculocidal, fungicidal and virucidal solution to eliminate pathogens



#### Manual hematopoietic **Reduction**

Blood and blood-producing components are removed by hand without scraping or scrubbing to preserve membrane structure



#### Cold isotonic **Cleansing**

A series of washes in clean, low-temperature, pH-balanced solution with gentle stirring remove remaining debris while minimizing tissue and growth factor breakdown



#### Gentle and gradual **Dehydration**

Slow drying of the membrane using physiological temperature preserves tissue structure and natural growth factors, preventing damage from freezing or high temperatures



#### Precise cutting die **Placement**

Precise, manual placement of cutting dies helps ensure optimal tissue quality for each individual allograft



#### Low-dose E-beam terminal **Sterilization**

Crafts are individually packed and terminally sterilized to an assurance level of  $10^{-6}$  via electron beam\*, avoiding harmful byproducts or excessive irradiation associated with other methods



## Disinfection

- The use of a solution using pathogen specific cidals instead of harsh chemicals



Minimally damaging  
**Disinfection**

The membrane is submerged in an effective bactericidal, tuberculocidal, fungicidal and virucidal solution to eliminate pathogens

## Reduction

- Hand removal of blood components as opposed to scraping or scrubbing



### Manual hematopoietic **Reduction**

Blood and blood-producing components are removed by hand without scraping or scrubbing to preserve membrane structure

## Cleansing

- A series of washes in low temperature pH balanced solution with gentle stirring to remove debris

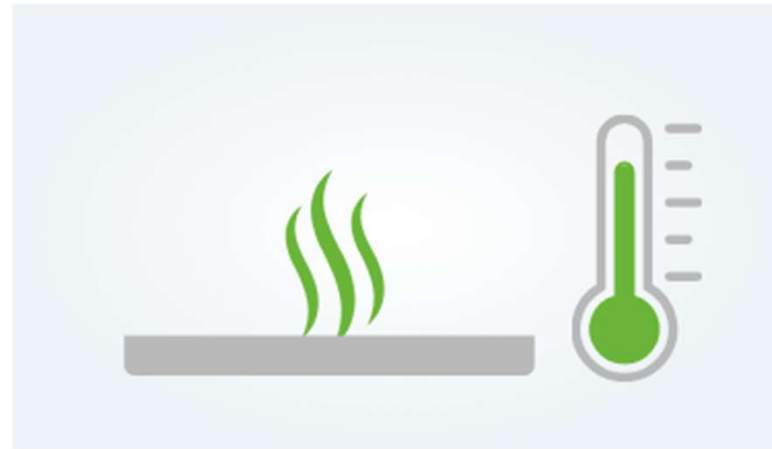


### Cold isotonic **Cleansing**

A series of washes in clean, low-temperature, pH-balanced solution with gentle stirring remove remaining debris while minimizing tissue and growth factor breakdown

## Dehydration

- Slow drying at physiological temperature



### Gentle and gradual **Dehydration**

Slow drying of the membrane using physiological temperature preserves tissue structure and natural growth factors, preventing damage from freezing or high temperatures

## Placement

- Manual cutting die placement



### Precise cutting die **Placement**

Precise, manual placement of cutting dies helps ensure optimal tissue quality for each individual allograft

## Sterilization

- Grafts can be sterilized to an acceptable assurance level with low dose E-Beam



### Low-dose E-beam terminal **Sterilization**

Grafts are individually packed and terminally sterilized to an assurance level of  $10^{-6}$  via electron beam\*, avoiding harmful biproducts or excessive irradiation associated with other methods

## Appropriate Assay Techniques

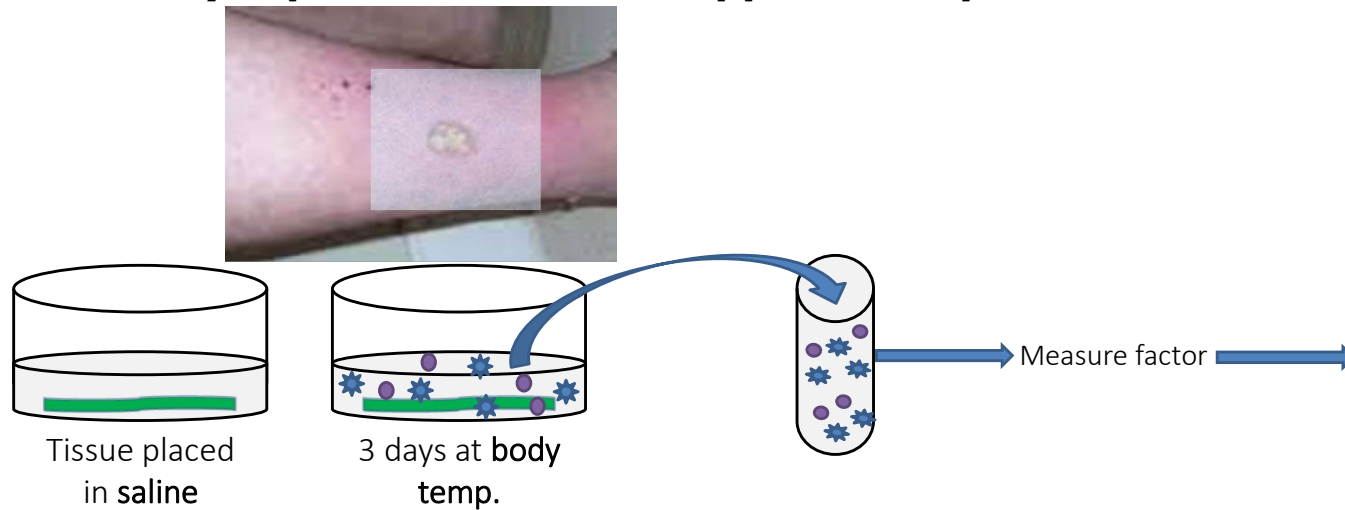
- Important not only to process but to Assay in physiologically consistent ways

## COLLECTION of DATA REPRESENTS *in vivo* AVAILABILITY

**How most tissue is processed to get data?**



**Does this really represent what will happen in the patient?**





## MEASUREMENT REPORTED *for CLARITY*



I have a 4 x 4 cm allograft:

? How much "X Factor" is in this?

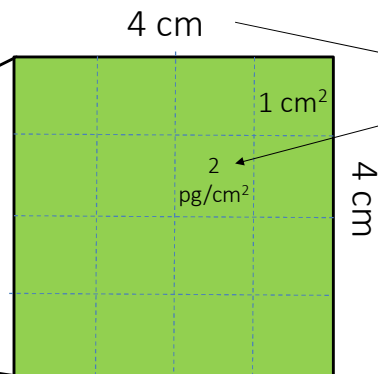
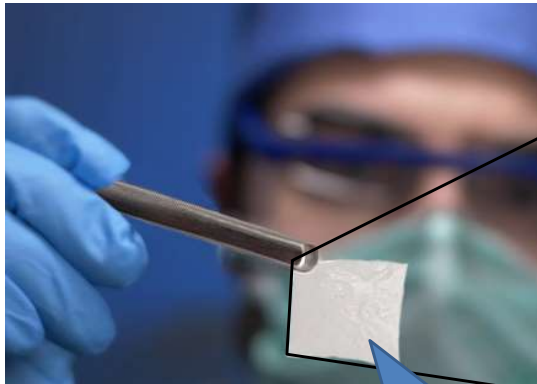
I can't weigh it in the surgical room (e.g., mg)....

There is no way to determine its volume (e.g., mL)....

Relate to saline (x 500  $\mu$ L)  
Relate to tissue ( $\div$  4  $\text{cm}^2$ )



**Factor transferred from  
1  $\text{cm}^2$  of intact membrane**



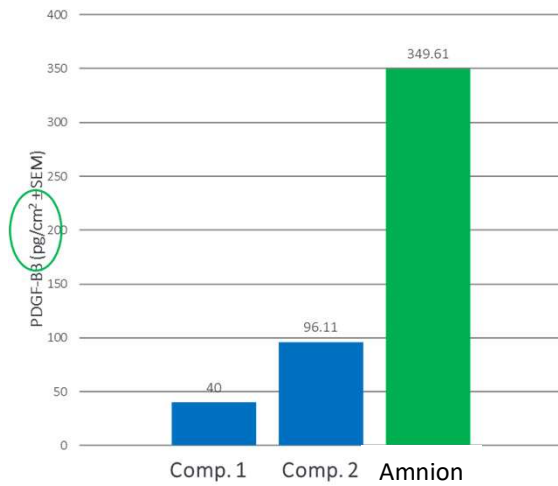
For example:  
2  $\text{pg}/\text{cm}^2$  X Factor

16 square cm

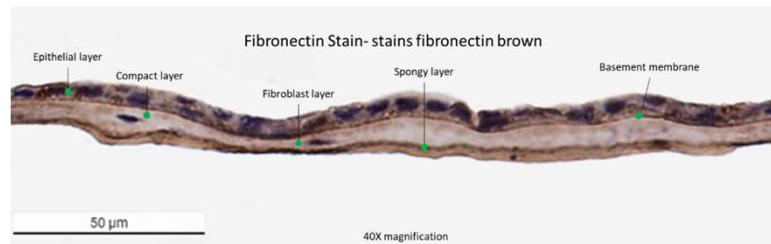


32 pg of "X Factor"  
in this allograft

## PDGF-BB

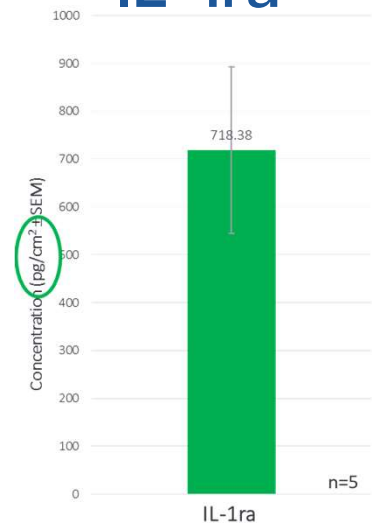


## Fibronectin

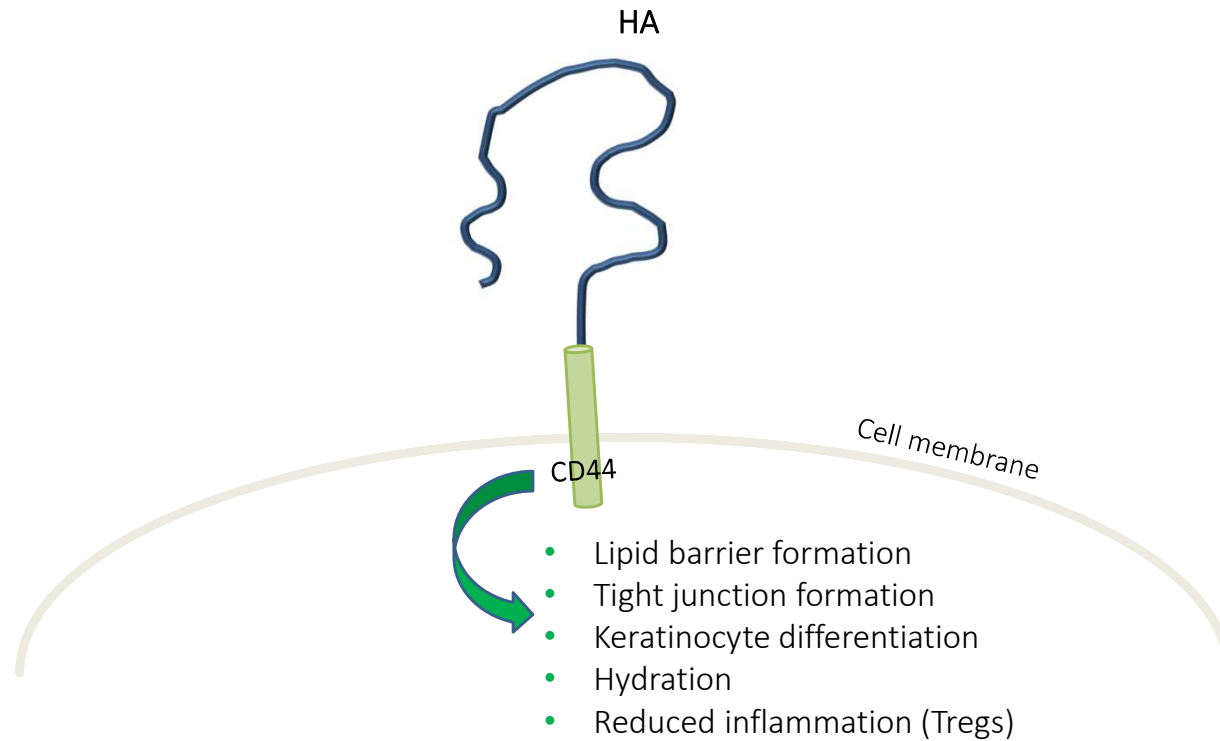


Fibronectin stains brown

## IL-1ra

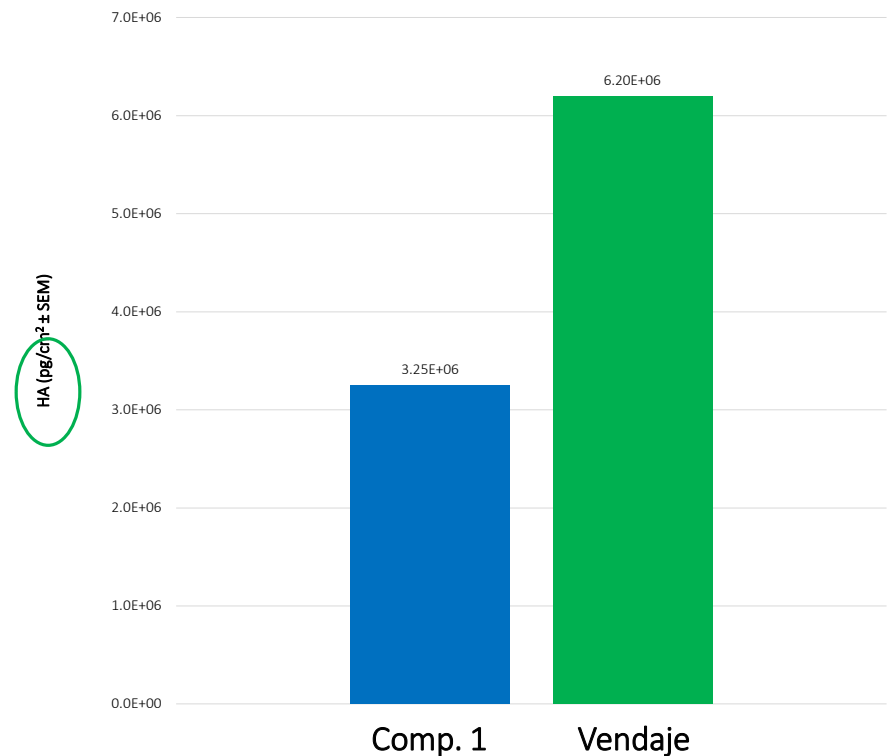


# Hyaluronic Acid- Skin



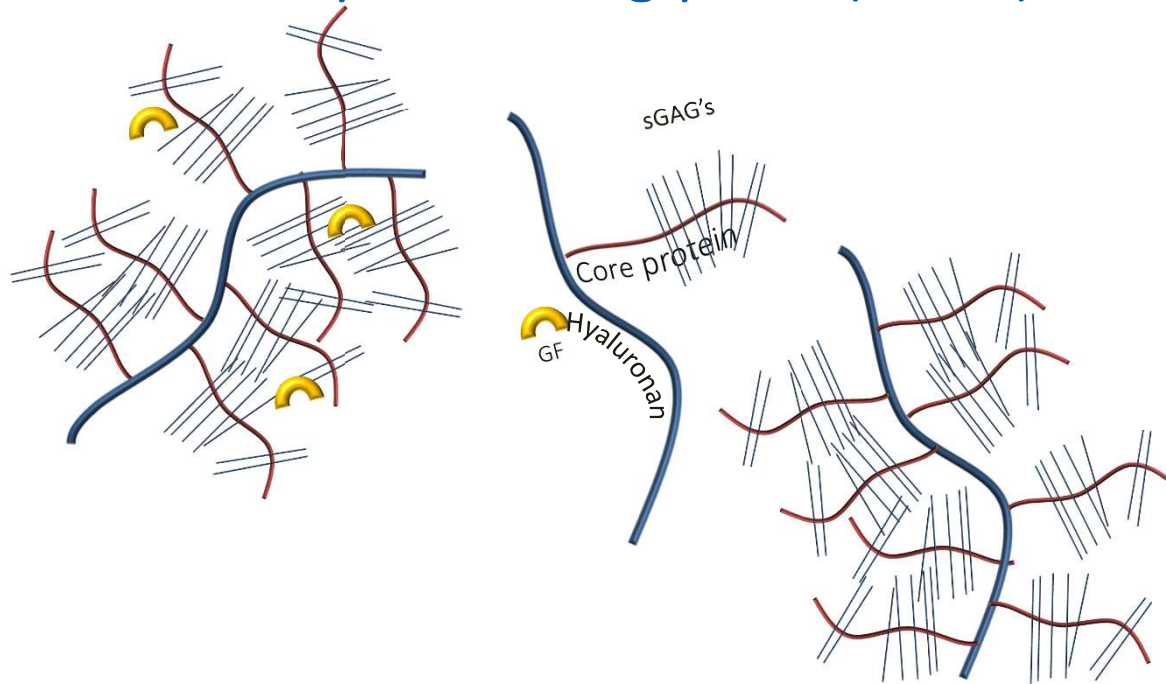
## Hyaluronic Acid (HA)

- One of the chief components of the extracellular matrix<sup>1</sup>.
- Has a key role in tissue regeneration<sup>2</sup>.
- Controls inflammation<sup>3,4</sup>.
- Angiogenesis- stimulates endothelial cells to proliferate<sup>5</sup>.
- Conducive environment for the migration of cells into the temporary wound matrix<sup>6</sup>.

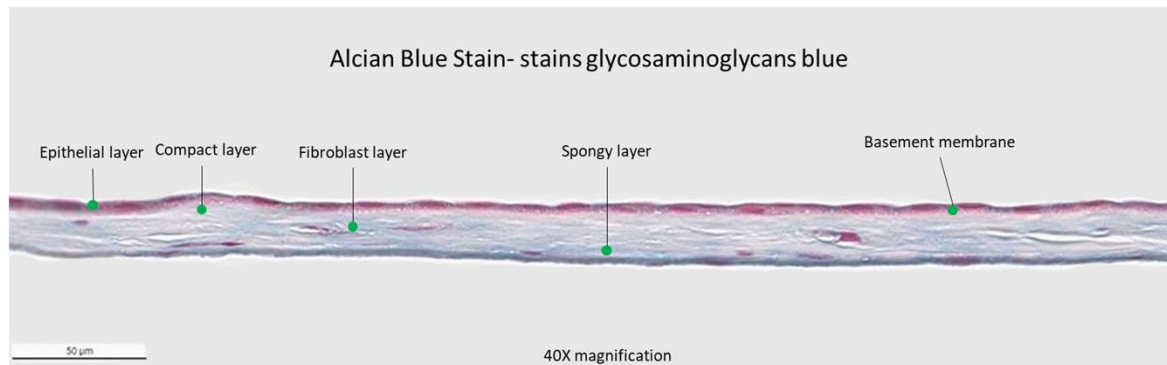


<sup>1</sup>[Toole 2000], <sup>2</sup>[Shaharudin et al, 2016], <sup>3</sup>[Bollyky 2009], <sup>4</sup>[Bollyky 2007], <sup>5</sup>[Genasetti 2008], <sup>6</sup>[Litwiniuk 2016].

# Glycosaminoglycans (GAGs)



## Glycosaminoglycans (GAGs)



### Important in the processes of wound healing:

- Platform for cells and molecules that regulate signaling.
- Interacts with other ECM components to organize the matrix.
- Form a matrix suitable for remodeling.
- Upregulates anti-inflammatory cytokines.
- Promotes endothelial cell proliferation and migration → increasing the secretion of angiogenesis-related cytokines.
- Provides support and adhesiveness.

# Hands-On Cadaver Seminar

February 21-23, 2024  
Celebration, Florida

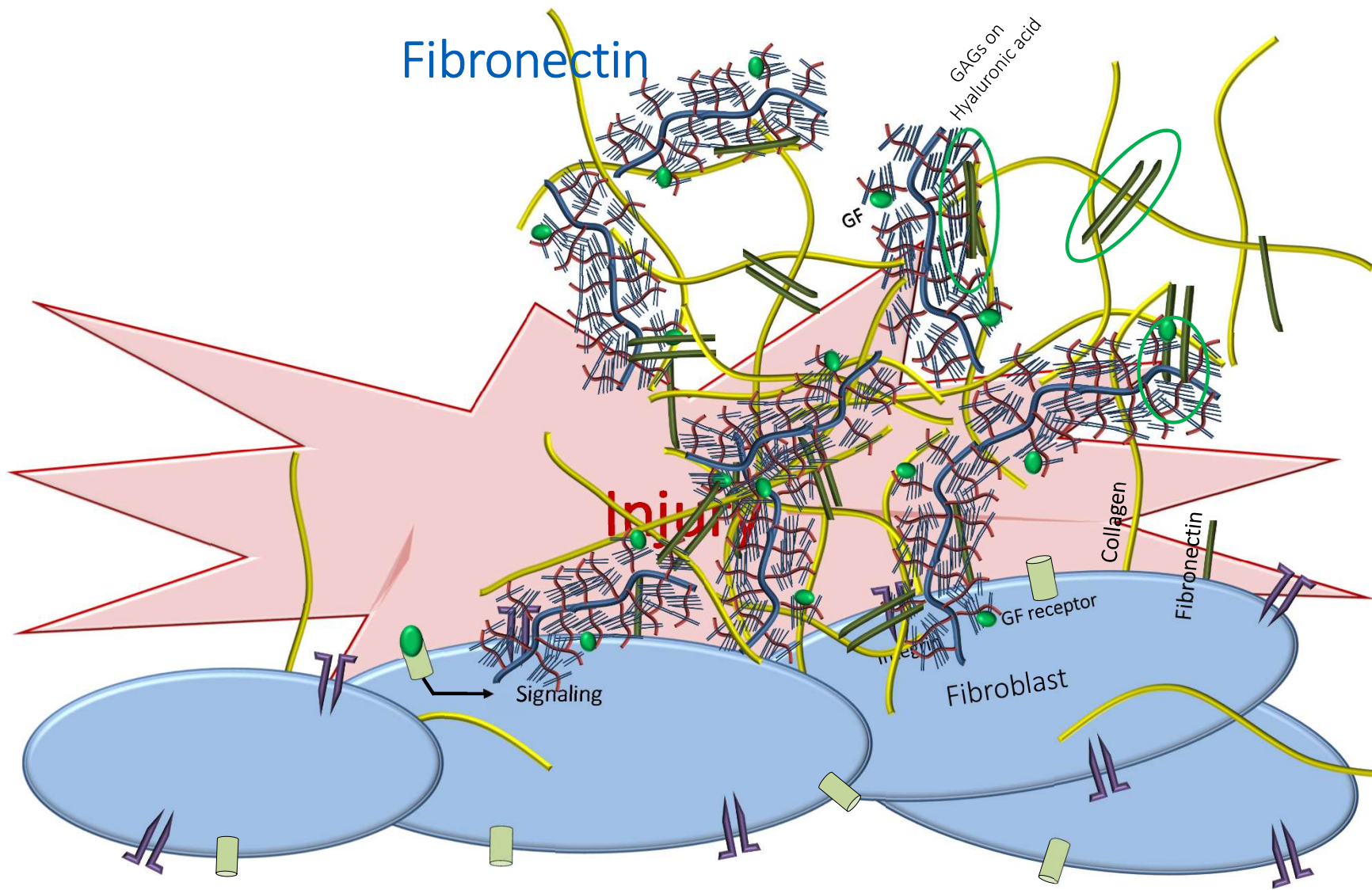
## THANK YOU

### CONTACT ME:

[johns@iuhs.edu](mailto:johns@iuhs.edu) or  
[jstarinski@biostemtech.com](mailto:jstarinski@biostemtech.com)

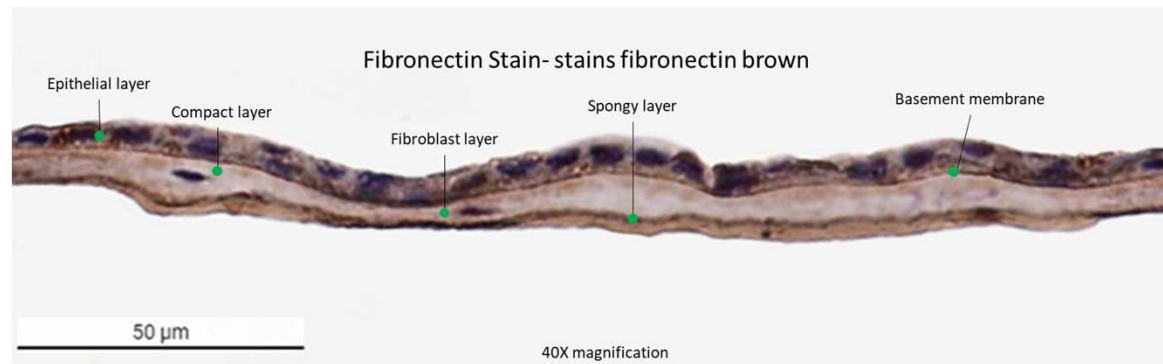
Cell: 570.236.5300







# Fibronectin



- Located in the ECM of embryonic and adult tissues → binds to integrins<sup>1</sup>.
  - Also binds to collagen, fibrin, and sGAG.
- Major role in cell adhesion, growth, migration, and differentiation.
- **Important for the processes of wound healing.**<sup>1,2,3</sup>
  - Formation of proper base for migration and growth of cells during development and organization of granulation tissue, as well as remodeling and resynthesis of the connective tissue matrix<sup>4</sup>.

<sup>1</sup>[[Pankov 2002](#)], <sup>2</sup>[[Grinnel 1984](#)], <sup>3</sup>[[Valenick 2005](#)], <sup>4</sup>[[Grinnel 1981](#)].

# IL-1ra

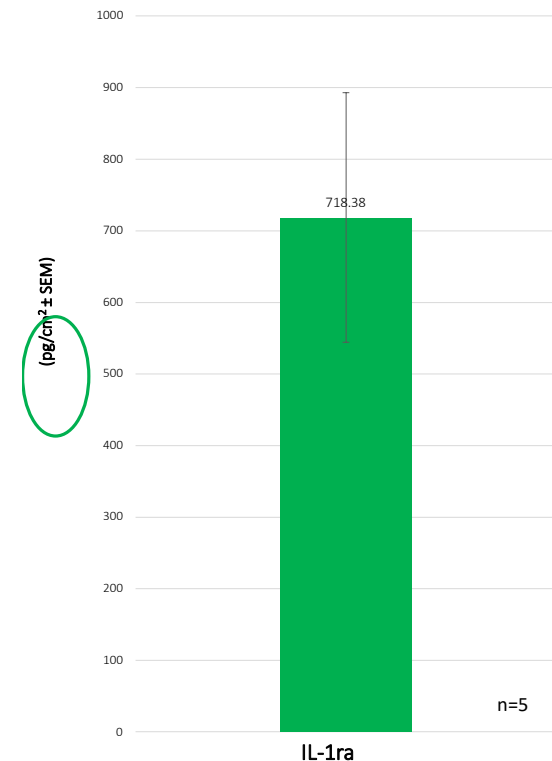
*Interleukin-1 receptor antagonist*



## IL-1ra

(Interleukin-1 receptor antagonist)

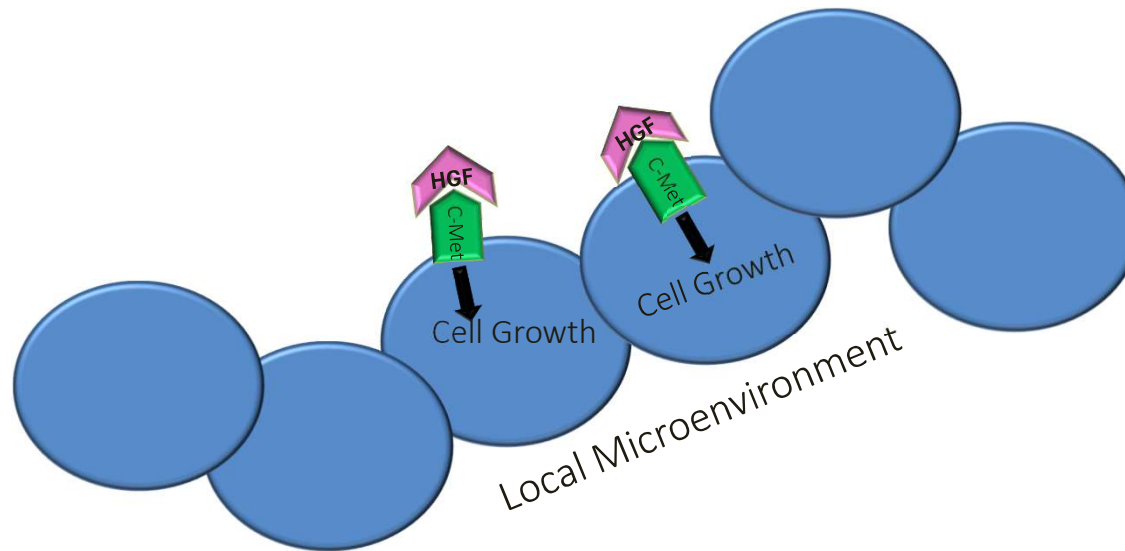
- Binds non-productively to the pro-inflammatory IL-1 receptor → preventing IL-1 from sending a signal.
- Hence, IL-1ra contributes to the inhibition of the immune and inflammatory responses.
- Has been targeted as a drug for the treatment of severely active rheumatoid arthritis<sup>1</sup>.
- Modulates a variety of IL-1 related inflammatory responses.



<sup>1</sup>[[Barron, et.al 2011](#)].

# HGF

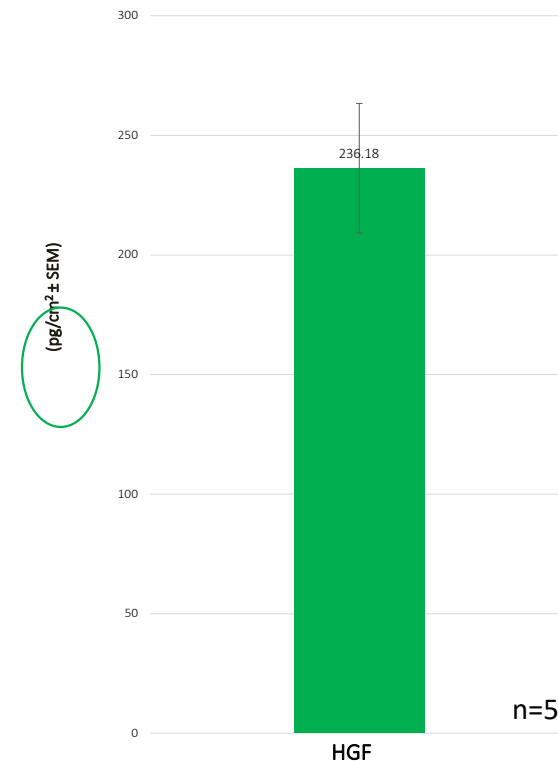
*Hepatocyte growth factor (Scatter factor)*



# HGF

(Hepatocyte growth factor)

- A cellular growth, motility and morphogenic factor secreted by mesenchymal cells
- Acts as a multi-functional cytokine on mainly epithelial and endothelial cells.
- Plays a positive role in angiogenesis, tissue/organ regeneration and wound healing.<sup>1,2</sup>
- Increased expression of HGF has been associated with the **enhanced and scarless wound healing**.<sup>3</sup>

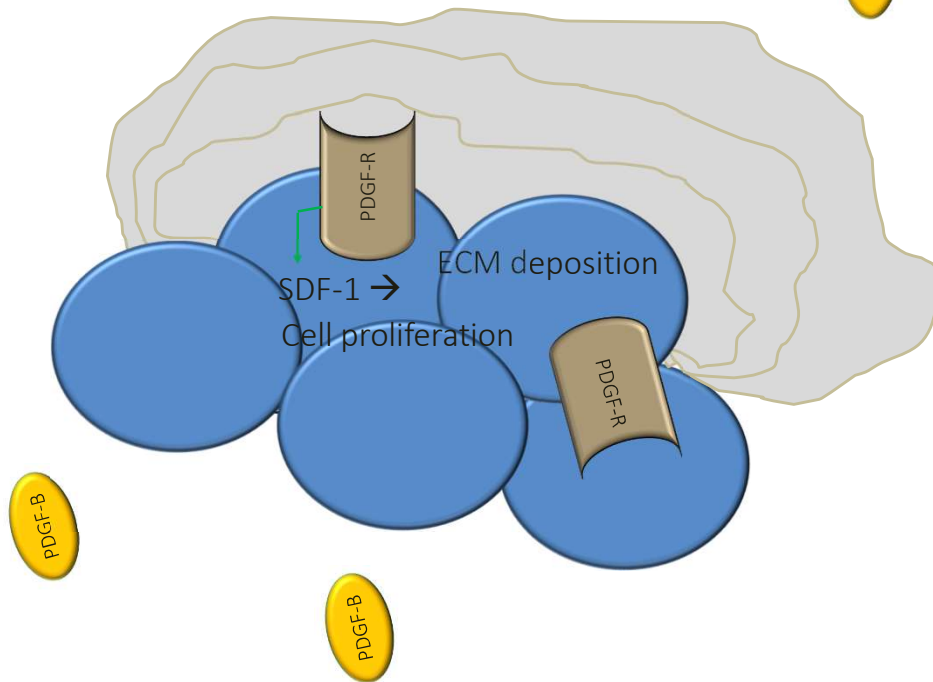


<sup>1</sup>[GeneCards], <sup>2</sup>[Gallagher and Lyon. 2000], <sup>3</sup>[Dally 2017].

# PDGF-BB



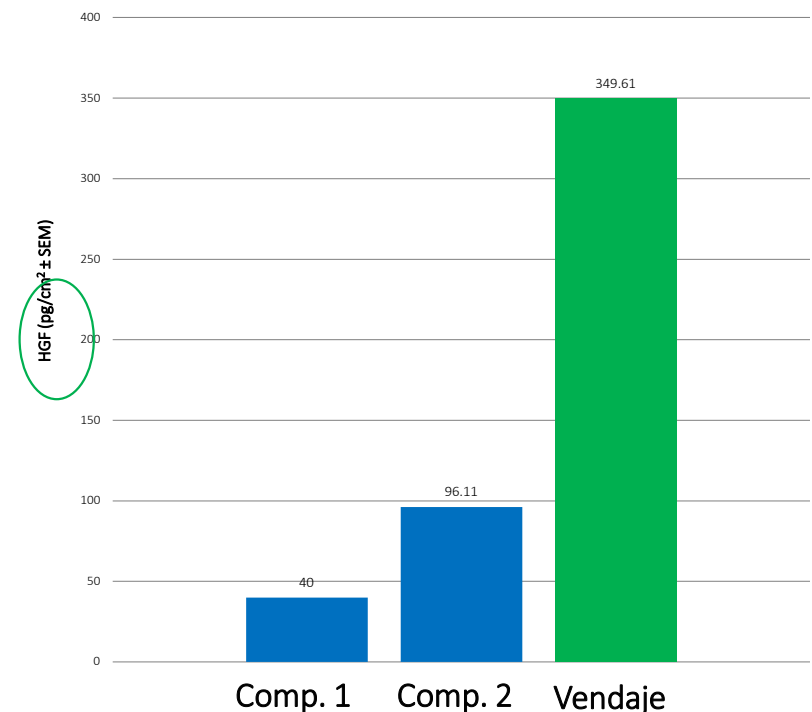
*Platelet-derived growth factor subunit B homodimer*



## PDGF-BB

(Platelet-derived growth factor subunit B homodimer)

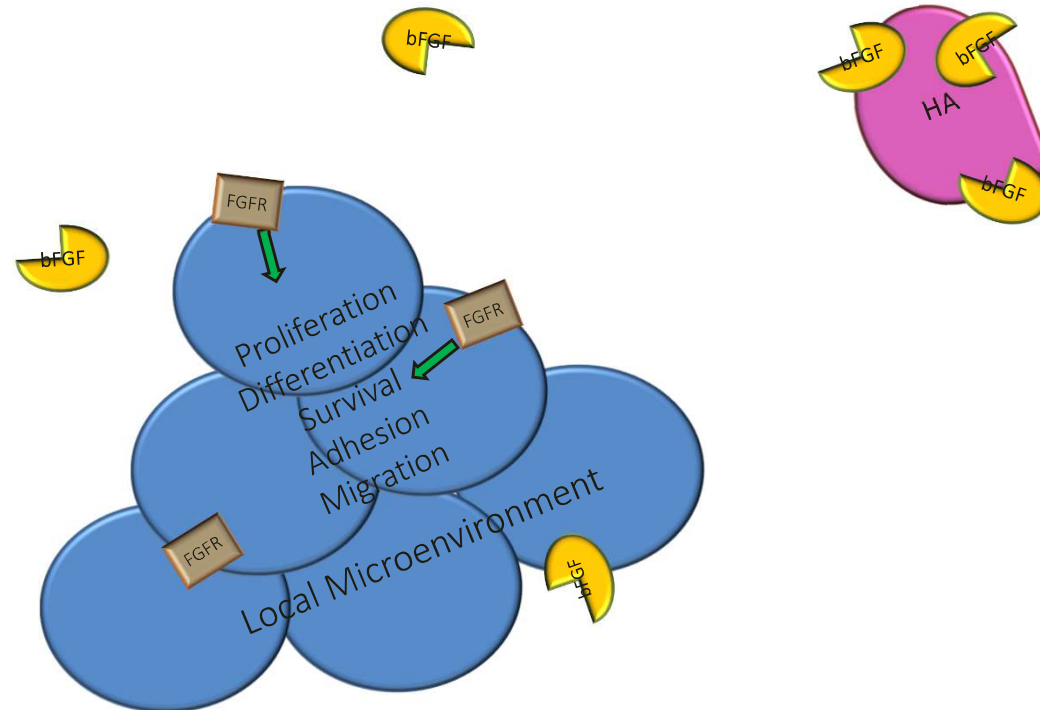
- Required element for division of fibroblasts.<sup>1</sup>
- Exogenous PDGF significantly reduces inflammatory cells → accelerates ECM and collagen formation → reduces the time for healing.<sup>2</sup>
- Powerful promoter of cell proliferation.
- Increases the expression of stem cell markers.<sup>3</sup>
- Significant role in blood vessel formation and proliferation and directed migration of mesenchymal stem cells.<sup>2</sup>
- In combination with other growth factors, stimulates soft and hard tissue healing.<sup>4-7</sup>



<sup>1</sup>[Alvarez et.al. 2006], <sup>2</sup>[Pierce et. al. 1991], <sup>3</sup>[Mihaylova 2018], <sup>4-7</sup>Lynch et al. 1987, 1989, 1991, 1995.

# bFGF

*Basic fibroblast growth factor, FGF2, FGF-β*

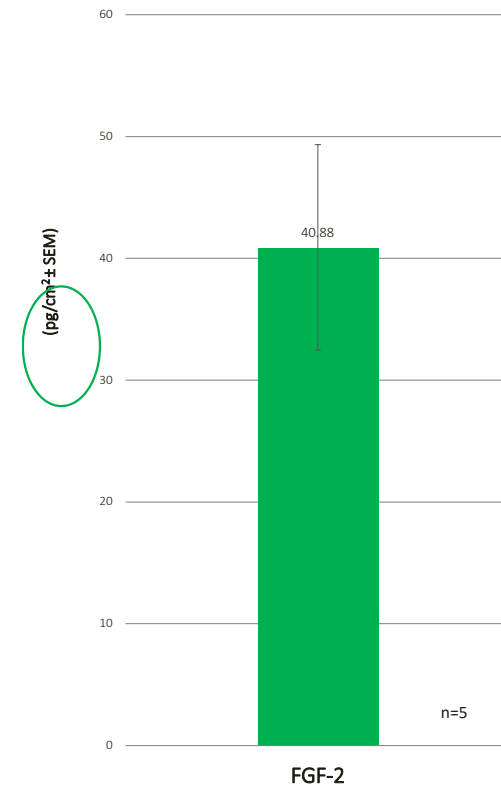




## bFGF

(Basic fibroblast growth factor, FGF2, FGF- $\beta$ )

- Involved in embryonic development, cell growth, morphogenesis and **tissue repair**. Most significant biological function is to promote proliferation.
- Growth factor and signaling protein during **wound healing** of normal tissues, mediating the formation of new blood vessels.



## Migration Assay

- ✓ Migration assay shows that the membrane elutes factors that attract fibroblasts (the key factor in wound healing).
- ❖ Assay was done utilizing DMEM with no added factors (e.g. FBS), hence membrane provided all the factors necessary for healthy cells and migration.

