

# *In vitro Transcript Expression by Gingival Fibroblasts on Barrier Membranes*

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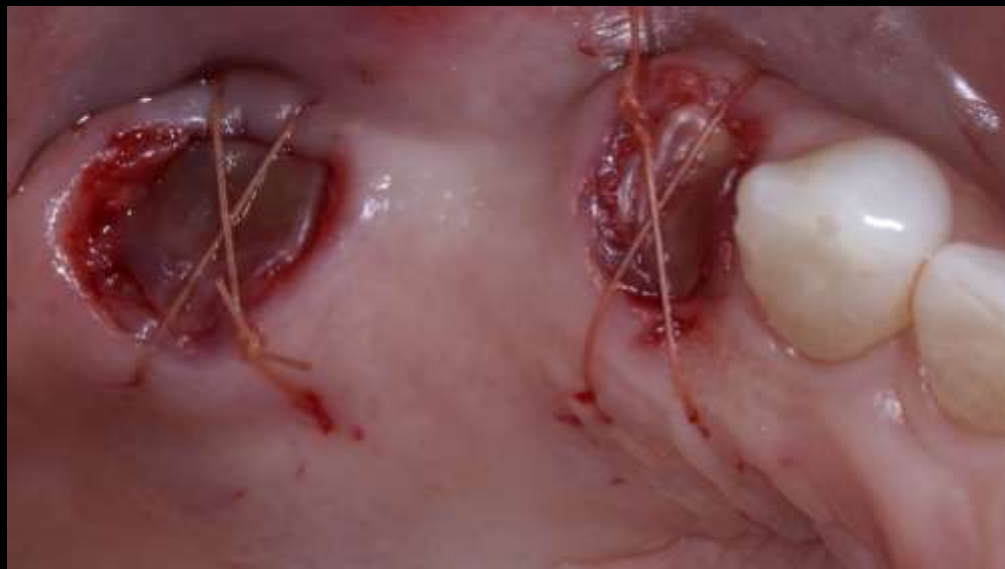
Department of Periodontics



# Barrier Membranes in Periodontics

Why do we need them?

When do we choose one over another?





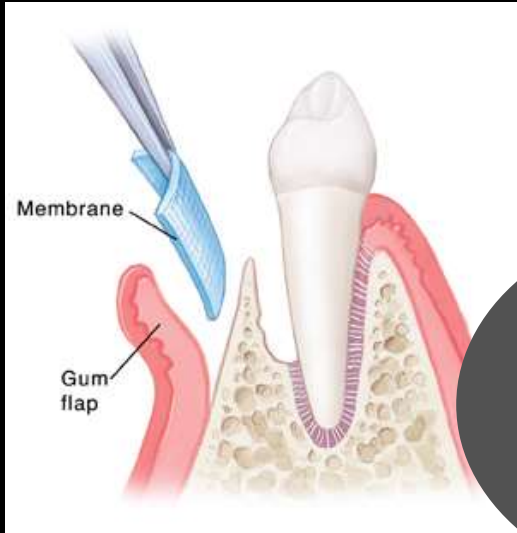
Epithelial Exclusion

Tissue Compatibility



Promote Healing

Ideal Membrane Properties



Maintain Space

Good Handling



## *Key Question:*

Do different bioabsorbable membranes promote the same tissue response from gingival fibroblasts?

# Collagen Membranes

## Cross-Linked Collagen

- Bovine Type 1 collagen cross-linked with glutaraldehyde
- Delays the breakdown of the membrane
- Increases stiffness

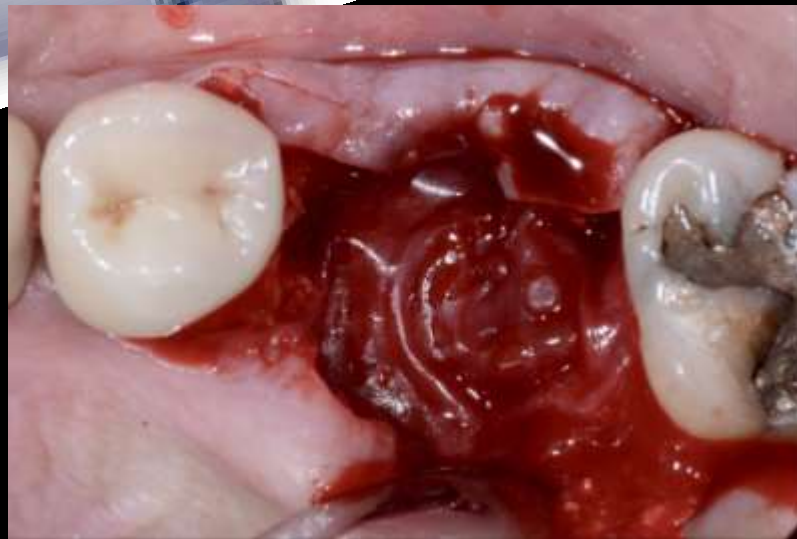


## Non-Cross-Linked Collagen

- Porcine Type 1 and 3 collagen
- Rapid resorption of membrane if exposed
- More pliable



# Amnion Chorion Membranes



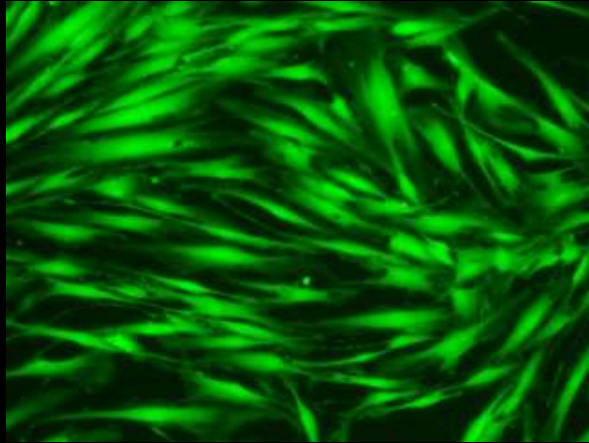
## Properties

- Dehydrated human amnion-chorion membrane
- Laminin-rich scaffold
- Contains growth factors:
  - PDGF-AA, PDGF-BB, TGF $\alpha$ , TGF $\beta$ 1, bFGF, and interleukins (IL) -4,-6,-8, and -10
- *In vitro* studies have demonstrated increased fibroblast migration with these growth factors but not on intact membranes
- Clinical studies claim rapid wound closure when left exposed

Objective: To compare transcript expression of gingival fibroblasts when exposed to three different intact bioabsorbable barrier membranes

Hypothesis: There will be differences in transcript expression of gingival fibroblasts exposed to collagen or amnion chorion membranes.

# Study design:



Vs.



Vs.



Vs.



Control

Amnion Chorion Membrane  
(AC)

Cross-Linked Bovine  
Collagen Membrane  
(BM)

Non-Cross-Linked Porcine  
Collagen Membrane  
(BG)



## Measuring transcript expression:

- Gingival fibroblasts incubated for 72 hours
  - +/- Barrier Membranes
- Cells harvested and RNA extracted
- cDNA made
- PCR amplification
- Transcripts expression analysis and quantification (Image J)
- Statistically analyzed with Anova and paired t-test ( $p < 0.05$ ) for individual samples



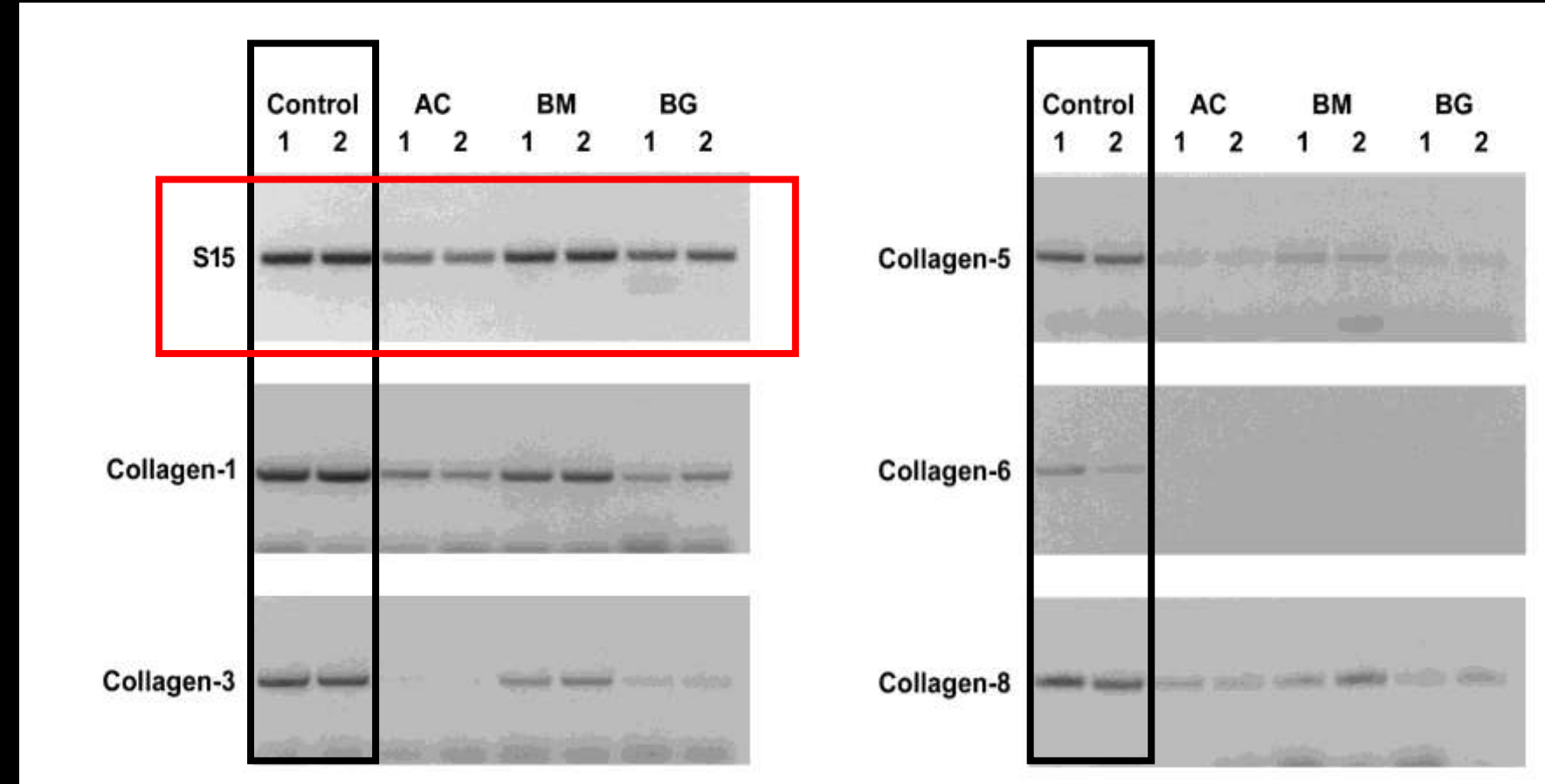
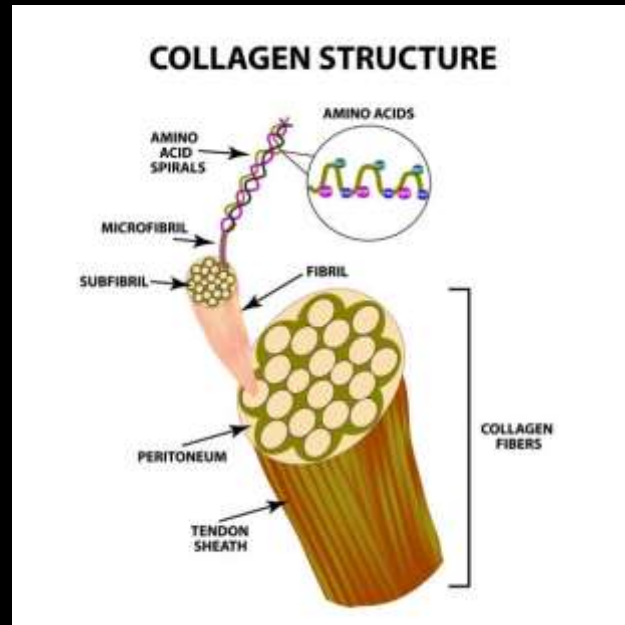
# Transcripts examined:



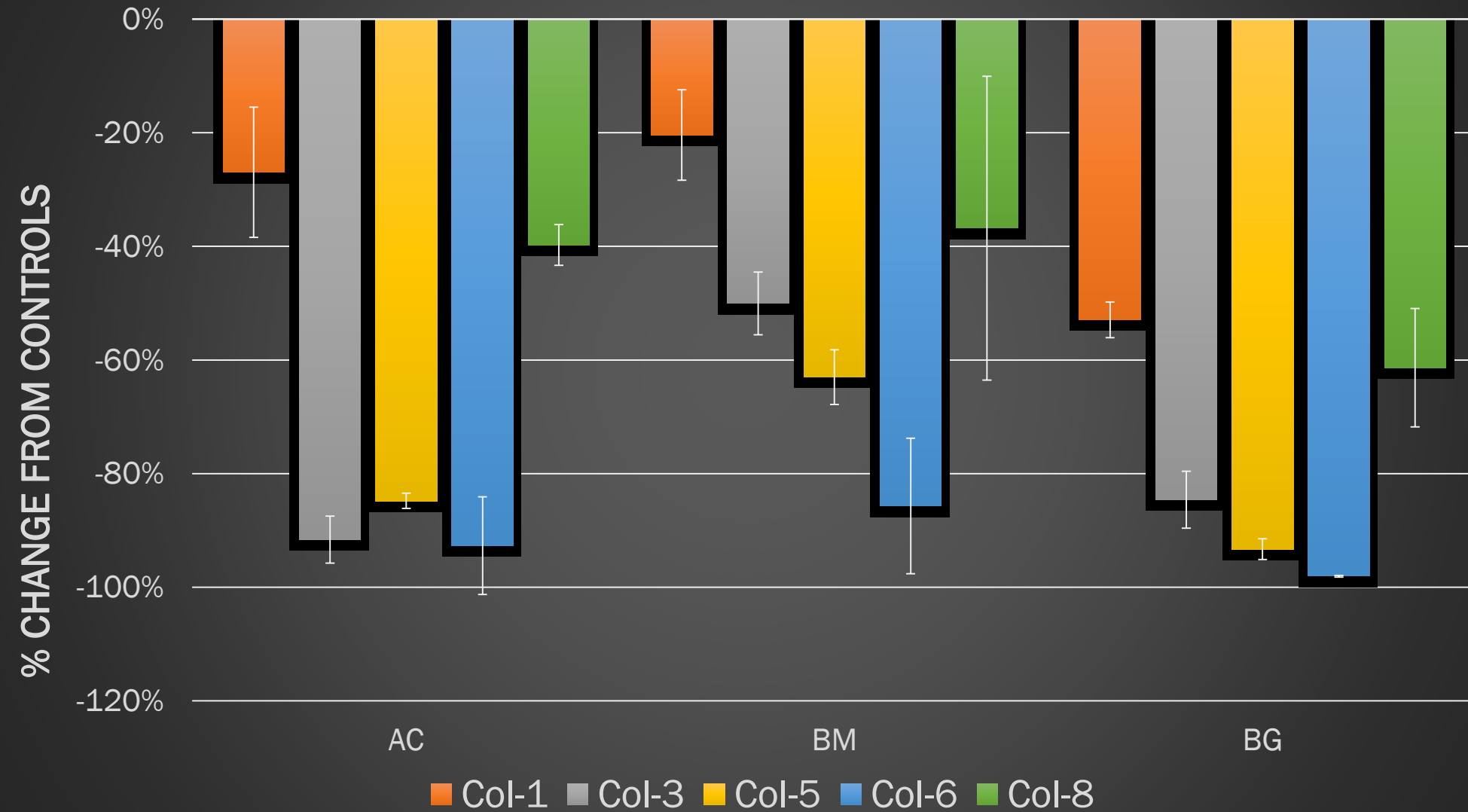
Gene group:	Functions:
S15	Ribosomal gene, responsible for protein production (control)
Collagen I, III, V, VI, VIII	Variants of collagen transcripts for collagen synthesis
TGFβ1	Transforming growth factor; controls proliferation, differentiation and other functions in many cell types
BMP2/BMP4	Bone morphogenic protein; induce formation of bone and cartilage
GDF5	Growth differentiation factor; regulators of cell growth and differentiation
FGF2/FGF5	Fibroblast growth factor; have regulatory, morphological, and endocrine effects
CTGF	Connective tissue growth factor; plays a role in cell adhesion, migration, proliferation, angiogenesis, skeletal development, and tissue wound repair
IL-6, IL-8, TNF-α	Pro-inflammatory and anti-inflammatory cytokines with roles for multiple cell types, IL-8 is linked to angiogenesis

# Results:

- Expression of Collagen Transcripts



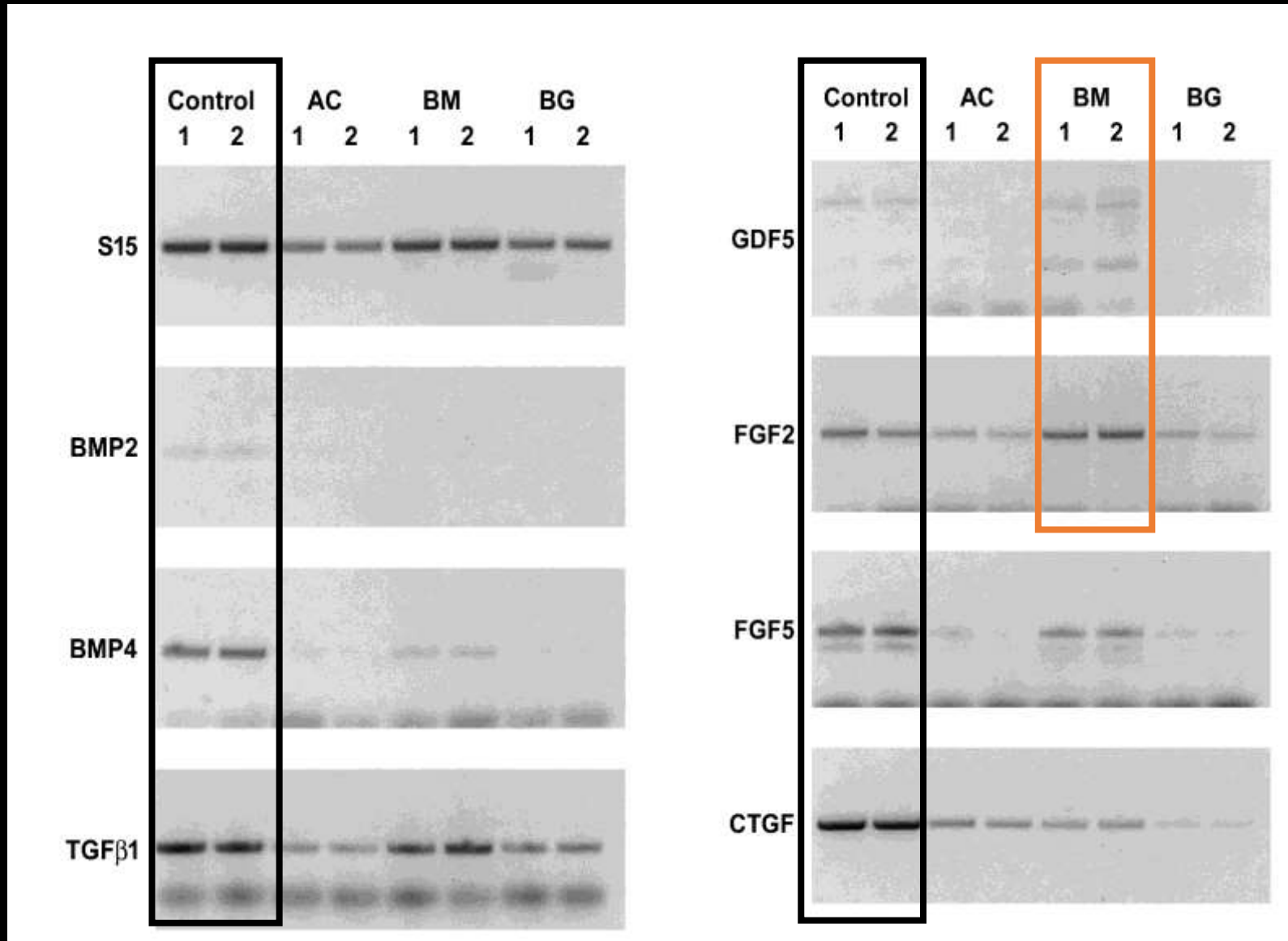
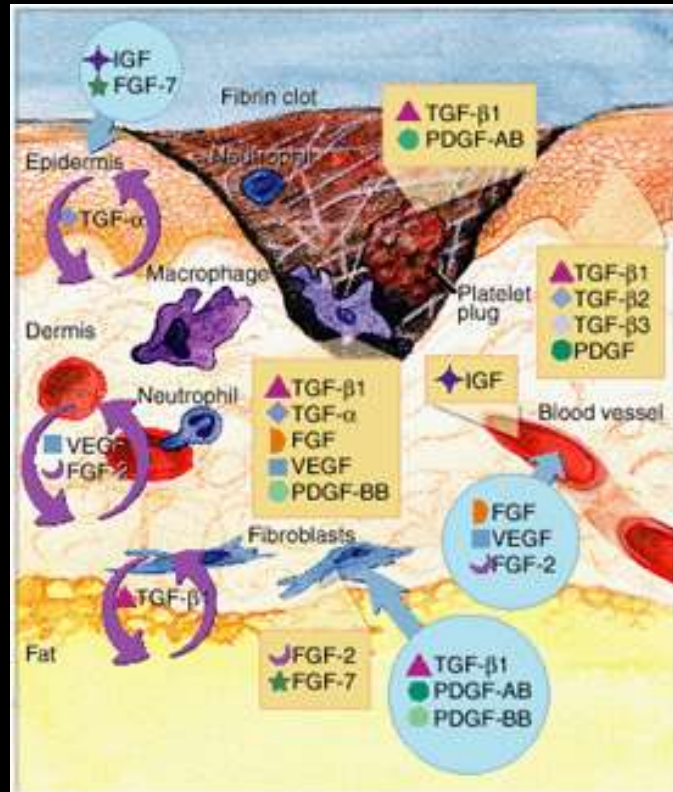
## Expression of Collagen Transcripts



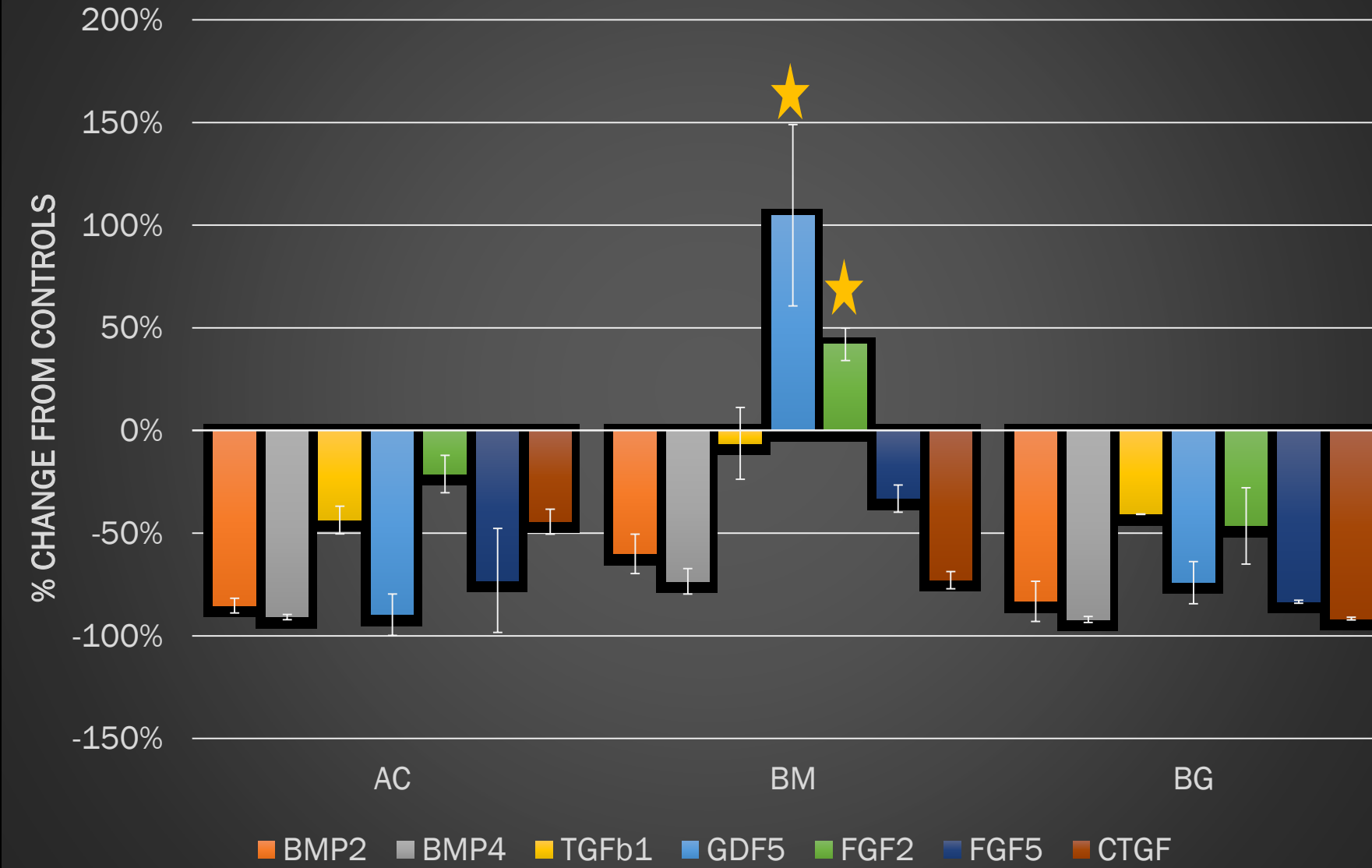
★ All GF grown on membranes had a significant reduction of all collagen transcripts.

# Results:

- Expression of Growth Factor Transcripts



## Expression of Growth Factor Transcripts



★ GF grown on BM membranes had a significant increase of GDF5 and FGF2

# Results:

- Expression of Pro-inflammatory cytokines

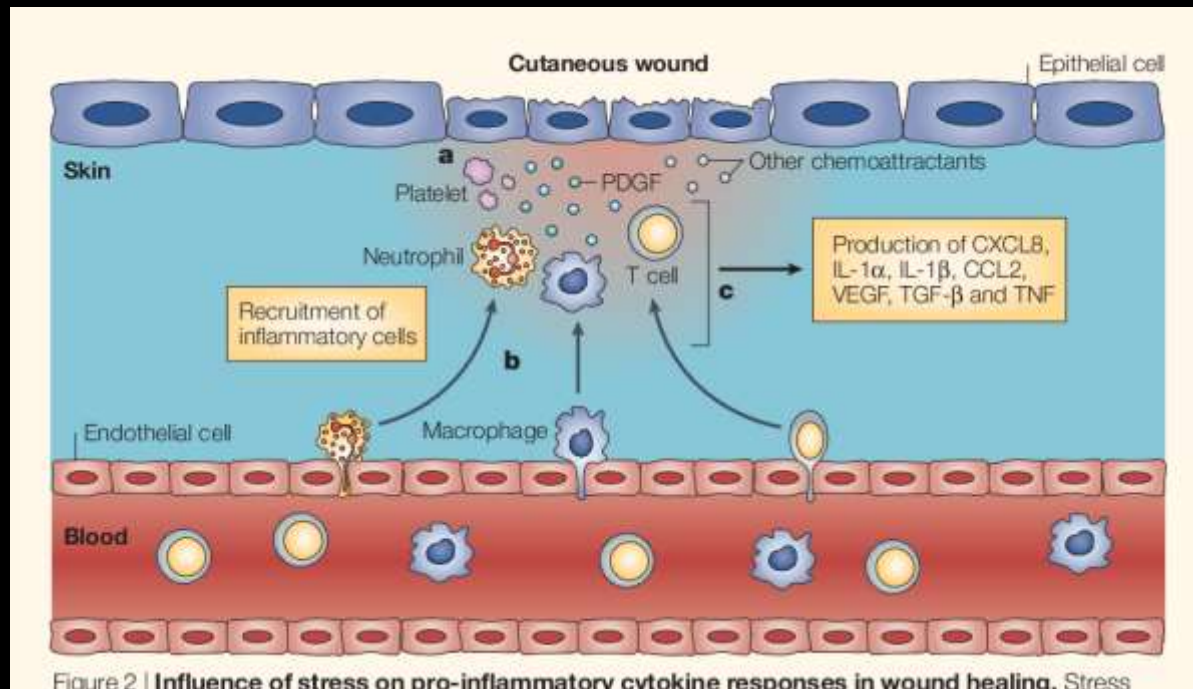
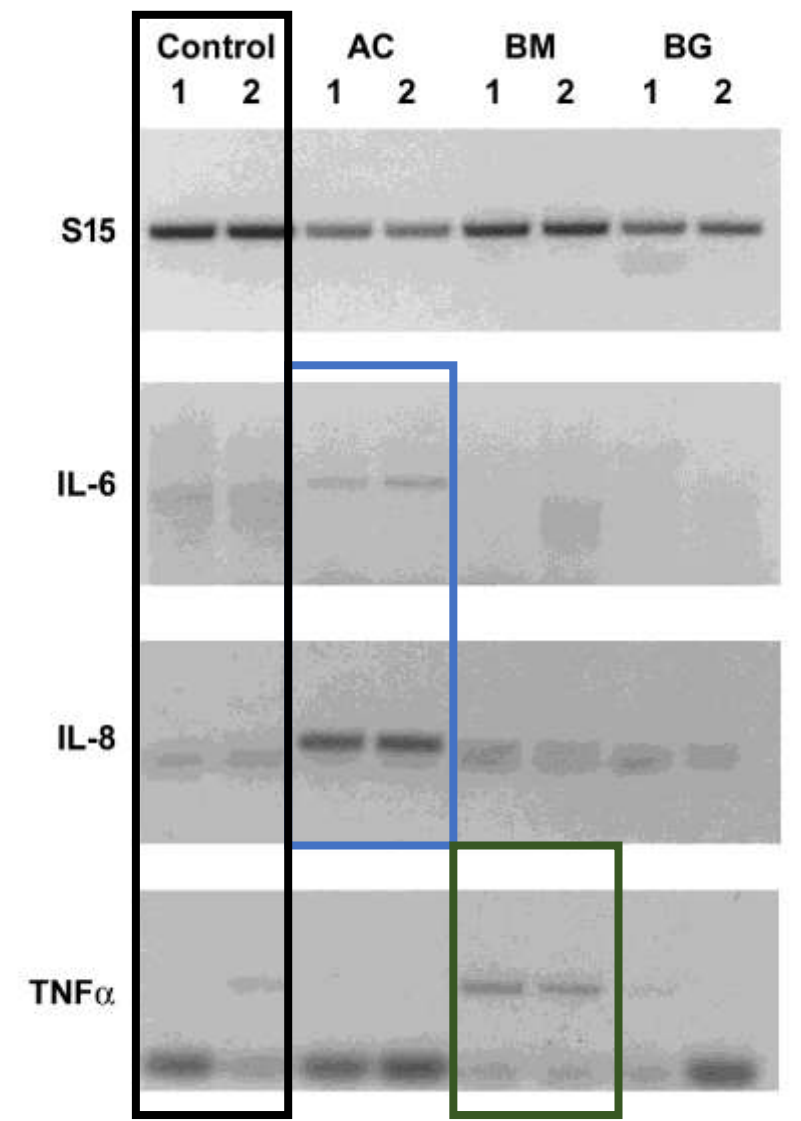
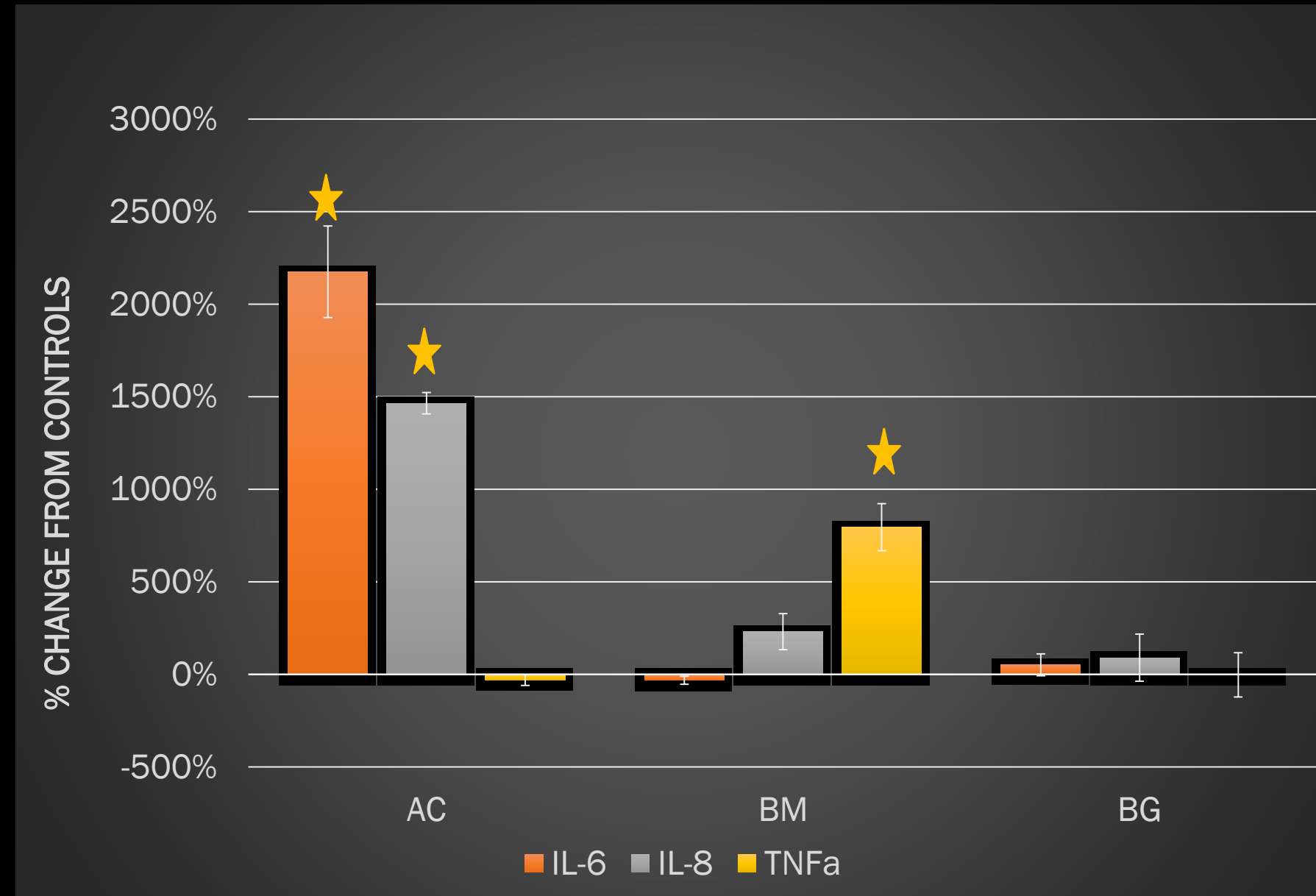


Figure 2 | Influence of stress on pro-inflammatory cytokine responses in wound healing. Stress



## Results:

- Expression of Pro-inflammatory cytokines



★ GF grown on AC membranes had a significant increase of IL-6 and IL-8

★ GF grown on BM had a significant increase of TNF-a



## *Key Findings:*

Gingival fibroblasts shown to attach and survive on intact barrier membranes.

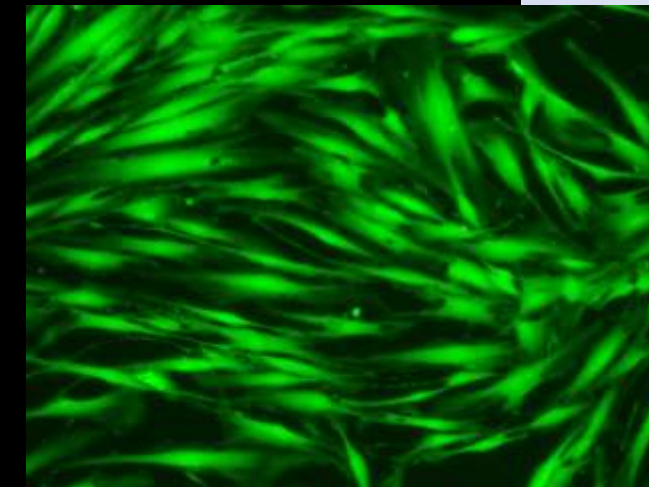
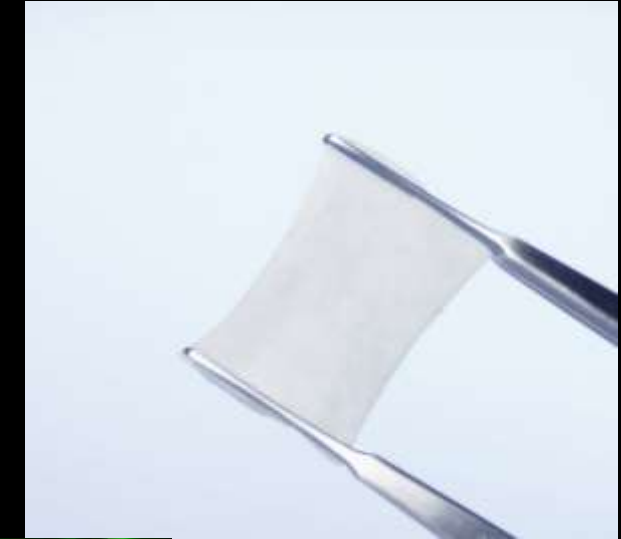
The three barrier membranes differentially promoted expression of cytokines and growth factors important to wound healing.

## *Conclusions:*

- Supports data from other studies that gingival fibroblasts attach to amnion-chorion membranes.
- Generally collagen transcript production is reduced for fibroblasts attached to membranes.
- Significantly increased cytokine activity with amnion-chorion and cross-linked bovine collagen but not with the porcine collagen.

## *Future Research*

- Evaluate additional transcripts related to wound healing
- Quantify cell attachment to intact membranes
- Determine fibroblast migration across intact membranes



*Thank you!*

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