Type 1 Collagen and Wound Healing

Jane Fore MD, FAPWCA, FACCWS
Highlights of Discussion

- Overview of Type 1 Collagen
- Overview of wounds
  - Chronic and acute
- Where does collagen fit in to healing?
  - Provisional wound matrix- ECM and cellular interaction
  - How collagen effects the wound
- Types of collagen for use
  - Differences in collagen types
  - Economics
  - Biocompatibility
- Clinical uses and applications with collagen
Collagen is....

- Is active biologically on the wound tissue. It is not just a covering.

- Is naturally present in wounds, but chronic wounds and deeper wounds may lack intact collagen structure.

- Very favorable for all of the phases of wound healing.
## Collagen Subtypes

| Fibrillar Collages With Uninterrupted Triple Helices | **Types I, II, III, V, XI** | Collagen Monomers, Basic Unit Is The 300 nm Helical Rod, Aggregate Head-To-Tail And Longitudinally |
| Collagens With Interrupted Triple Helices | Type IV | Most Abundant Collagen In The Dermal-Epidermal Junction |
| Fibrillar Collagens With Interrupted Helices | Types VI, VII | Ubiquitous Component Of Extracellular Matrices |
| Nonfibrillar Collagens | Types VIII- XVIII | Include Fibril-Associated Collagens (FACIT) And Short-Chain Collagens |
Type 1 Collagen Structure

- Three strand chain- 2 α-1 chains and 1 α-2 chain as a helix basic unit
- The Tropocollagen Winds Together Forming A Microfibril, a monomer
- The Collagen Monomer Is About 300,000 Daltons
- Then Microfibrils Join To Produce Collagen Fibrils, 5-stranded rope
- Repeating Sequence Of The Amino Acids Gly-X-Y That Induces the Protein To Form The Helical Pattern
- The Fibrils Of Collagen Become Cross-linked Over Time Producing A Collagen That Is Increasingly Resistant To Breakdown Through Usual Cellular Mechanisms
Science

• Type 1 Collagen is the most abundant collagen, over 90% of collagen is type 1 and 30% of total body protein is collagen.

• Collagen is synthesized by fibroblasts.

• Native collagen has many cross-links of fibrils that increase resistance to breakdown – inter and intra molecular connections.
## Characteristics of Collagen

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<thead>
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<tr>
<td>• Extremely low hypersensitivity</td>
<td>• Compatible with other products applied to the wound</td>
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<td>• Soothing to the wound</td>
<td>• Needs no preservatives</td>
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<td>• Collagen does not support bacterial growth</td>
<td>• Classified as a device not drug</td>
</tr>
<tr>
<td>• Biocompatible</td>
<td>• Compatible for any wound type</td>
</tr>
<tr>
<td>• Safe for all ages</td>
<td>• Promotes autolytic debridement by several mechanisms</td>
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<tr>
<td>• No need to remove</td>
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Collagen and wound repair

- Overall appearance of granulation and degree of cellular migration into the wounds with a collagen matrix is better.
- Collagen products are a poor culture medium and do not support or enhance bacterial growth.
- There is less wound contraction with the use of collagen matrix with wound healing.
- Diabetic wounds, esp type 1, have lower amounts of type 1 collagen (partially on basis of decrease in fibroblast proliferation)


Differences in clot formation, inflammation, capillary migration, granulation tissue, ECM, Keratinocyte migration, scar formation, bacterial infection, biofilm.

Chronic vs Acute Wounds

Initiation of wound repair

- Biofilm
- Granulated tissue
- Proteases
- Neutrophil
- Free radicals
- Bacterial pathogens

Repair of acute wound

Failure to heal: chronic wound

JID, 127, 2007
Chronic Wound Model

Eming, Krieg, Davidson, JID, Vol 127, 2007
Dynamic Reciprocity

Cells and ECM interact in an ongoing, mutually influential series of bidirectional, interdependent events that have been referred to as dynamic reciprocity. ¹ ECM, extracellular matrix.

ECM = Structural proteins (Collagens, esp type 1) and elastin
Multidomain adhesive glycoproteins (fibronectin, vitronectin and laminin)
Glycosaminoglycans (hyaluronan and proteoglycans)
Matricellular proteins (secreted protein acidic and rich in cysteine like tenascin C and X and osteopontin)

Schultz G, Wysocki WRR 2009, vol 17
**ECM**

- Binds to and releases GFs
  - Sequesters and stores and protects GFs
  - Prolongs and localizes GF activity
  - Interacts with GF on cell receptor
  - Provides cell adhesion sites

- Chronic wounds- disrupted ECM↔GF interplay
  - Loss of tissue- debridement, lack of dermis and epidermis, bacterial overgrowth
  - Loss of ECM- debridement, ↑ protease, ↓GF, bacteria
  - Abnormal ECM- i.e. glycation of protein i.e. collagen
  - Disrupted interaction leads to stalled healing
ECM↔Cellular↔GF interaction

Schultz G, Wysocki A, WRR vol 17, 2009
Integrins

- Cell attachment to ECM by integrins essential for cell survival
- Cells interact with the ECM by integrins – like a handshake
- Extracellular portion binds to ECM
- Intracellular domain associates with cytoskeleton
- Regulates cell shape and architecture, migration, ECM remodeling, growth
- Affects intracellular signaling via MAP kinases and other pathways

Fibrillar collagen binding to integrin and DDR2 sites on a fibroblast.

Schultz G, Wysocki A, WRR vol 17, 2009
Integrins And The Extracellular Matrix

- Cell Adhesion Is Intimately Associated With The Process Of Cell Motility
- Integrins Are A Cell-Surface Associated Protein That Recognizes Specific Components/Ligands Of The Extracellular Matrix
- Integrin Interaction With Their Specific Target Molecules Induces Outside-In Signals, Causing Many Intracellular Changes
Type I Collagen And Integrins

- Binding To Denatured Collagen Is Mediated by $\alpha_5-\beta_3$ and $\alpha_5-\beta_1$ Integrins and non-denatured collagen by $\alpha_1-\beta_2$ and $\alpha_1-\beta_1$ integrins

- Endothelial Cells Express Both $\alpha_5-\beta_3$ and $\alpha_5-\beta_1$ integrins

- Integrin interaction with collagen influences the cells that attach to it - macrophages, fibroblasts, endothelial cells
From Falanga at Wound Biotech
Collagen Fragments Stimulate Monocyte Migration

- Collagen I and Collagen I Fragments Are Chemotactic For Macrophages, Not PMNs

- The Chemotaxis Is Concentration Dependent - the more collagen, the stronger the stimulus

- Recruitment Of Monocytes Play A Role In Initiation Of The Wound Healing Process

- Monocytes Aid Autolytic Debridement, Release GFs

Dynamic Reciprocity of Type 1 Collagen and Monocytes

- Chemo attractants for monocytes that then bind to the ECM proteins - intact and especially fragments
- Provisional wound matrix stimulates regeneration
- Monocyte binding stimulates phagocytosis, essential to becoming a macrophage
- Adherence of monocytes stimulates GF expression

Monocyte migration
Monocytes bind to ECM
Monocyte interaction with ECM
GF expression builds ECM
Metalloproteases And Type I Collagen

- Degradation Of The Intact Collagen Molecule Is The Activity Of Collagenase (MMP 1,2,9)

- Initial cleavage of the large intact collagen is a RATE-LIMITING STEP taking up to 14 days to breakdown

- Following The Initial Cleavage, Denaturation Occurs And The Resultant Gelatin Chains Are Susceptible To Additional Proteases or Endopeptidases

- The Cleavage Of The Collagen Proceeds More Slowly As The Size Of The Fibrillar Collagen Increases
Effects Of Collagen Breakdown

- Collagen Fragments Stimulate The Infiltration Of Macrophages And Fibroblasts Into The Wound Bed

- Collagen And The Resultant Peptide Fragments Modulate The Wound Healing Activities

- Collagen and Collagen Peptides Are Recognized By Cells Through Integrins And Modulate Intracellular Activities

- Amino Acids from Collagen Breakdown Are Recycled, used for Production of New Proteins Necessary for healing
ROLE OF FIBROBLASTS IN WOUND HEALING

**Migration/Proliferation**
- From surrounding tissues influenced by growth factors/cytokines
  - PDGF
  - EGF
  - FGF-7
  - CTGF
  - Activin

**ECM production**
- Linkage between actin bundles and ECM
- Serves as scaffold
- Tensile strength

**Growth factor/cytokine production**
- PDGF
- EGF
- FGF-7
- CTGF
- Activin

**Angiogenesis**
- Growth Factors/cytokines
  - FGF-7
  - EGF
  - Activin

**Protease release**
- ECM remodeling
- Dissolves
  - Nonviable tissue
  - Fibrin barrier

ECM = extracellular matrix.
Fibroblasts Interact with Collagen

2D vs 3D collagen matrix w/ and w/o PDGF BB
Dendritic network associated with microtubules cores in 3 D, PDGF promoted formation of the network and supported metabolic coupling.

Collagen I Fragments And Fibroblasts

- Recruitment of fibroblasts is enhanced by collagen and collagen fragments.

- Repair Of The Dermal Component Is Necessary For Progression Of The Epidermal Regeneration.

- Fibroblast Collagenase I Works To Degrade Collagen Fragments As Well.

Wound Care Innovations
Medical Director Dr. Jane Pfliger MD, CWS
Other Activities Of Collagen I

- Collagen Matrix Fibers Bind Fibronectin Enhancing Fibroblast Infiltration
- The Fragmentation Of Collagen Exposes More Active Sites For The Binding Of Fibronectin
- Platelet Derived Growth Factor-BB Has A Synergistic Role With Collagen On Fibroblast Migration
- Keratinocytes migrate over type 1 collagen

References:
<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>COLLAGEN SOURCE</th>
<th>TYPE</th>
<th>FORM</th>
<th>MANUFACTURER/Distributor</th>
</tr>
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<tbody>
<tr>
<td>BioPad</td>
<td>Equine (horse)</td>
<td>I Native</td>
<td>Matrix 100%</td>
<td>Silverlon</td>
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<tr>
<td>BioStep</td>
<td>Porcine (pig)</td>
<td>I Denatured</td>
<td>Matrix 55%</td>
<td>Smith &amp; Nephew</td>
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<td>Fibracol Plus</td>
<td>Bovine (cow) /Alginate</td>
<td>I Native</td>
<td>Pad &amp; Rope 95%</td>
<td>Systagenix</td>
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<td>Cellerate</td>
<td>Bovine- young calves</td>
<td>I Hydrolyzed</td>
<td>Gel 65%</td>
<td>Wound Care Innovations</td>
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<td>Stimulen</td>
<td>Bovine</td>
<td>I Modified</td>
<td>Powder 100% &amp; Gel</td>
<td>Southwest Technologies</td>
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Which Collagen?

- **COST vs. EFFECTIVENESS**
- Flexibility and ease of use-
  - gel and powder no waste
- **Bioactivity**
  - Size of the molecule and type of collagen- hydrolyzed, denatured, intact
  - Especially **collagen fragments** improve fibroblast viability, more rapidly utilized, more available, many enzymes interact, improved cellular response to stressors and rate of cellular aging
  - **Intact** slower to breakdown, stimulates keratinocyte migration through integrins and induction of collagenase-1 or MMP-1
  - Active role in matrix replacement and matrix enhancement to supplement defects and enhance repair

Volloch V, Kaplan D Matrix Biology, 2002
Why Collagen?

- Time-release for GF and Drugs
- Matrix replacement
- Cell recruitment
- Activate healing
- Modulate protease activity ↑TIMP, Bind MMP, Sacrificial Substrate, direct to cells in wound bed
- Local nutrition for cell growth
Be creative with collagen

- Break apart and stuff in holes
- Combine with topical antimicrobials, antibiotics
- Use to fill dead space
- Use under any secondary dressing
- Combine with growth factors and hyaluronic acid, fibronectin etc
- Use under compression wraps
- Combine with other topical products ie Vit A
Applications

- Ulcers with undermining
- Tunnels
- Combining with antibiotics and other biological dressings
- Skin Tears and Flaps
- Burns
Application Options

- **Gel** – less exudates, thinner application of collagen, place in curved-tipped syringe or catheter application

- **Powder** – “puffed” into the wound, use of a tongue blade, thicker application of collagen, applied as often as secondary dressing dictates or as desired

- **Paste** – vary thickness depending on need, easy to apply, liquid with greater percentage of collagen

- **Sheets**
Creative Collagen Applications

- Combine with topical antimicrobials – tobramycin, vancomycin, polymyxin, doxycycline, gentamicin
- Combine with Collagenase Santyl, Regranex, living skin equivalents
- Use under VAC, Altrazeal
Combining with antibiotics

- Delivery of antibiotics is difficult with decrease in blood supply and disruption of tissue
- Allows high concentration of antibiotic in areas where there may be a cavity
- Chemical property of collagen sustains activity of antibiotic release
- Dual activity of improvement of wound healing and antibiotic delivery in a biocompatible form
Combine with antibiotics

Vancomycin

- Use the powder and combine with powder, gel or paste of Cellerate Rx
- If pharmacist needs to formulate – 50 mg/cc of Cellerate Rx gel
- Order for nurses to apply powder to wound bed and then add the Cellerate Rx if questioned about nurses compounding
- Inject into tunnels
Combine with antibiotics

Tobramycin, Gentamicin, Polymyxin

- Done in a similar fashion as the vancomycin
- Tobramycin mixes very quickly and needs very little gel if making a paste
- Polymyxin topically combined with cellerate to the wound bed of burns is a great dressing
Combine with antibiotics

Doxycycline

- Powder, not crushed tablets, pharmacist will make up the powder capsules, open and add small amount to Cellerate Rx

- Do not combine with silver due to chelation

- Anti-inflammatory and antimicrobial

- 5-10% gels in literature, make a lightly colored gel or powder combination with doxycycline
Emerging Use of Topical “Biologics” in Limb Salvage Role of “Activated Collagen” in Multimodality Treatment

- Gary M. Rothenberg, DPM
- Attending Podiatrist
- VAMC Miami Florida
- Gary.rothenberg@med.va.gov
- (305) 324 4455 x4920
The Role of Activated Collagen on the Healing of Diabetic Foot Wounds

Purpose: To evaluate activated collagen in the practice of medicine in general and in particular, the healing of diabetic foot wounds. It is our hypothesis that patients treated with activated collagen gel will heal more rapidly than those receiving standard of care therapy alone.

- Physician driven study
- Open label, randomized
- Control group – hydrogel
- Treatment group – CellerateRx gel
- Routine clinic visits maintaining all tenants of “good wound care” practices – debridements and measurements
- Standardize secondary dressings, dressing change frequency and offloading
- 16 weeks or closure
Activated Collagen
CellerateRx

- “Activated” collagen = “Hydrolyzed”
- Collagen fragments 1/100\textsuperscript{th} native collagen – 1000 daltons, maintains basic collagen structure
- >65\% hydrolyzed Type I collagen derived from bovine source in gel form and 96\% in powder

Li F, et al “Low molecular weight peptides derived from extracellular matrix as chemoattractants for primary endothelial cells” *Endothelium*, Vol 11(3-4) 2004
Case - JB

61 yo male
PMH: DM, HTN, Depression, Dyslipidemia, other social stressors followed x 1 month in OPC
Trauma to **RIGHT** foot great toe
Soaked and noticed a “blood blister” to **LEFT** heel
Self treated x 3 days then PCP due to worsening wound, pain, and constitutional symptoms

PCP soak in vinegar and water, labwork, Rx Ciprofloxacin and Azithromycin, referral to DPM
WBC 13.5
HbA1C 6.1%
Alb 3.3 g/dl
Podiatrist started cadexomer iodine x 3 weeks and referred to our wound clinic
Case - JB

Limping
0.3cm ulcer to plantar central hallux IPJ right great toe
LEFT plantar heel 5.5cm x 3.5cm x 1.0cm probing to plantar fascia and covered bone
40% red granular and 60% fibrous, fat, and fascia
+ pedal pulses
Absent protective threshold
Case - JB

- Rx radiographs
- Rx Reverse IPOS shoe
- Debridement
- Rx wound care supplies – CellerateRx
- Rx Provant bid
- Called Social Worker
- 40 minutes teaching and explaining the what, why, and how
- Provide written handouts

9/2/08

1.7cm x 1.0cm
Case Study

Gary M. Rothenberg, DPM
Attending Podiatrist
VAMC Miami Florida
Gary.rothenberg@med.va.gov
(305) 324 4455 x4920
HM

- 60 year old male referred to Miami VA with left foot / ankle wound
- 6 month history of dorsal left foot / ankle wound after blister formation. Multiple previous treatments including antibiotics, surgery, debridements and local care
- Past Med History: Vasculitis, Acute Myeloid Leukemia, Anemia, Thrombocytopenia
- No Known Drug Allergies
Visit Date 5/6/08

- Extensive full thickness wound dorsal left ankle 18cm wide x 17cm tall
- 90% red, granular base with extensor tendons exposed
- No clinical sign of infection
Over the next several months, several applications of Skin substitutes were used (Apligraf application x 3 over 3 months) and the wound became smaller.

5/6/08-9/23/08, **14 wk, 50% closure**
HM

- 9/23/08 as wound stalled at 14cm tall x 11cm wide –
- Cellerate Rx prescribed to stalled wound and at last visit 10/28/08 wound was 8cm tall x 8 cm wide
- 41% closure, 4 wks
Unfortunately, the patient passed away from his leukemia 12/9/08.
Additional therapies included:
- Oral antibiotics
- Topical wound care therapy including silver dressings prior to grafting
- Provant Radiofrequency therapy
- Compression
- Limited weight bearing
ACTIVATED COLLAGEN ACCELERATES WOUND REPAIR AND MODULATES CYTOKINE PRODUCTION IN WHOLE BLOOD AND PBMC CULTURES.

Gregory B. Pott¹², K. Scott Beard¹², Matthew Regulski³, and Leland Shapiro¹².

¹Department of Medicine, Denver Veterans Affairs Medical Center, Denver, CO, USA, ²Department of Medicine, University of Colorado Denver, Aurora, CO, ³Wound Care Center of Ocean County, NJ.

- 16 DM with LE wounds
  - 14 wk
    - Control- Mean reduction 59% and median 73.5%
    - Treatment- 100% healed
- Exposed whole blood with, w/o cellerate to S. epidermidis and PBMC to LPS
  - Concentration dependent reduction in TNF-α, ↑IL-8, IL-6, IL-1β in whole blood tests
  - Significant reduction in LPS stimulated TNF-α and ↑IL-8, IL-6, IL-1β from PBMC

Presented as abstract in Lisbon, Portugal at international cytokine meeting, October, 2009
Activated Collagen Skin Tear Study

Dr. Jane Fore MD
Cherie Rash RN
Tri-State Wound Care and Hyperbaric Center, Clarkston, Washington
Patient Description

- Patient 93 years of age

- Co-morbidities - Congestive heart failure, mild dementia, Type 2 DM, insulin requiring, CAD post bypass and recent angioplasty

- Medications - duragesic patch, lantus insulin, atenolol, multivitamin, PM oxygen, lasix, spironolactone, zocor, zaroxolyn, plavix
Day 7 100% epithelialized
Skin Flaps
Great salvage for skin flaps

1. Clean flap and wound bed with saline
2. Cavilon skin prep to periwound
3. Activated Collagen gel or powder to flap and wound bed
4. Flatten out the flap and especially edges to approximate to the skin edge
5. Secure with steri-strips
6. Cover with Mepilex foam or Telfa and cover with a film
7. Secure with comperm if needed also, helps edema and bruising and leaving dressing alone
8. Replace every seven days
9. Usually healed in seven days.
Looking on the bright side

We’re going to pass a health care plan;
written by a committee whose head says he doesn’t understand it,
passed by a Congress that hasn’t read it but exempts themselves from it,
signed by a president that also hasn’t read it, and who smokes,
with funding administered by a treasury chief who didn’t pay his taxes,
overseen by a surgeon general who is obese,
and financed by a country that’s nearly broke.

What possibly could go wrong?

Dr. Jane Fore
Tri-State Wound Care and
Hyperbaric Center,
Clarkston, Wa
docjanep@aol.com