# AOAPRM Conference
# Kansas City, MO May 8-10

# *"Update On Biocellular Regenerative Medicine 2014"*

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# Goals in Biocellular Regenerative Medicine

- ✓ Return To Full Function
- ✓ Eliminate Or Markedly Decrease Pain
- ✓ Resist Recurrence Of Injury
- ✓ Reverse, Stabilize, or Resist Degeneration
- ✓ Utilize Autologous Tissues For Repair
- ✓ Accelerate Healing Processes
- ✓ Enhance Results Of *or* Reduce Surgical Requirements (Shorten PT Need)
- ✓ Restore Tissues With Minimal Scar Formation

# "Ideal" Cell-Based Therapy

- ✓ *Autologous* Source Via Closed System
- ✓ Harvest Desired Cells/Matrix On Site
- ✓ Transplant In *Same Surgical Session*
- ✓ Avoid Need For Manipulation (Chemical)
- ✓ Provides Stromal Cells + Matrix
- ✓ Easily Allows HD-Ultrasound Placement
- ✓ Density Visible At Time Of Injection
- ✓ Does Not Require Artificial Scaffolding
- ✓ Optional Ability To Culture & Expansion Needs Prior To Placement or IV Uses

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# Evolution of Biocellular Therapy

- Study Mechanisms of *Homeostasis* Revealing
- Examine Processes of *Remodeling & Repair*
- Locating Highest Numbers of Undesignated Cells In Body *(Microvascular Locations)*
- Safe & Effective Means Of Access To High *Numbers Stem/Stromal Cell* Populations

# Evolution of Biocellular Therapy

- Critical Importance of *Matrix To Repair*
- Understanding Value Of *Biologics* In Sites
- Finding That *Site Specific Changes* Depend On *Microenvironment* & *Paracrine Functions*
- Streamline Delivery of Concentrates To Sites

# Homeostasis, Remodeling & Self Repair Components

- Involves *Cellular Elements* (Heterogeneous)
- Involves *Biologic Elements*
  - Growth Factors
  - Signal Proteins (Cytokines, Chemokines, Etc)
- Exhibits Microenvironmental Controls
  - *Paracrine Secretion ("Bioactive" Chemical Influences)*
  - *Cell-to-Cell* & *Cell-to-Matrix* Communication

# Choosing Ideal *Cell Source* Biocellular Medicine (AD-tSVF)

- Ease Of Accessibility- Simple Harvest
- Quantity Of Cells Available
- Minimum Morbidity Of Donor Site
- Safety After Implantation
- Degree Of Proliferative Capacity
- Immunopriviledged Cells Preferred
- Secrete Immunomodulatory Factors

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# TERMS: tSVF & cSVF

## Tissue Stromal Vascular Fraction *(tSVF)*

- Includes ALL Cellular Components Of Tissue
- Includes ALL Biologic Components
- Includes Native Bioactive Matrix (Secretive)
- Requires NO Manipulation

## Cellular Stromal Vascular Fraction *(cSVF)*

- *Requires Digestion, Incubation, Isolation*
- *NOT Compliant With Current FDA Regs*
- *Common Use Reported In Research Settings*

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# Why AD-tSVF As Primary Cellular Source?

- Living, Native Bioscaffolding Accompanies Cells
- *tSVF* Offers Very Heterogeneous Cell Population
- Higher Mesenchymal Stem Cell Counts (>2000X)
- Readily Available at Minimal Cost, Invasion, Risks
- Strongly Overlapping Cell Differentiation Abilities
- Actually Placing "Intact Microenvironment" In Graft Of Adipose Tissue Complex (Strings Of ECM/Cells)

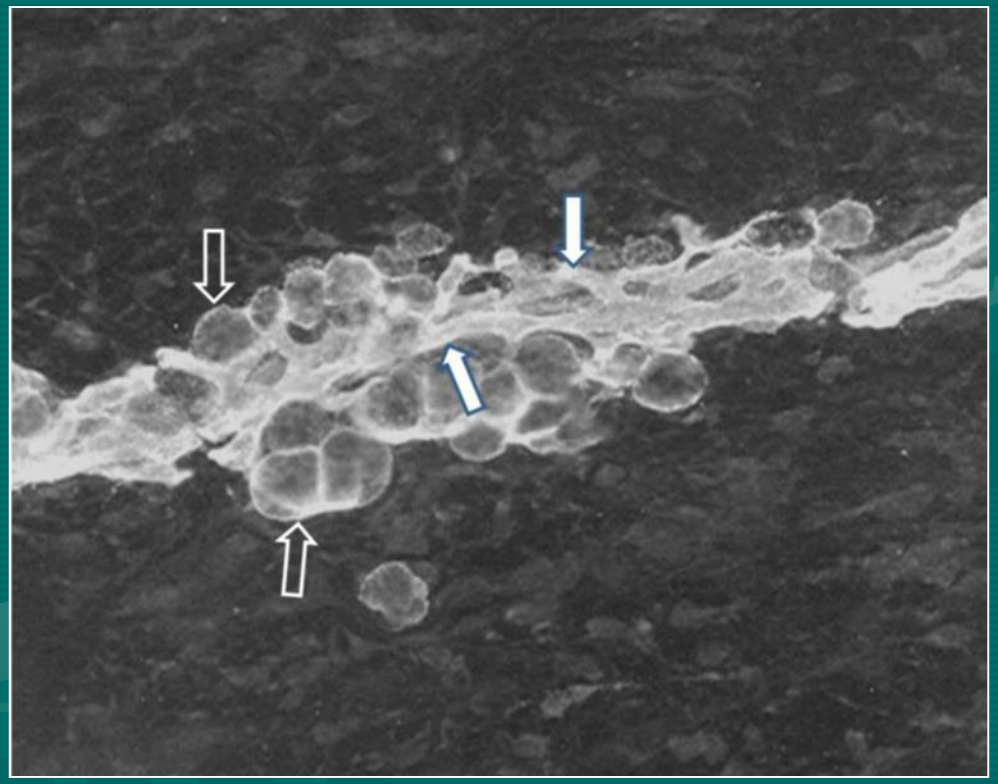
# "Stromal Vascular Fraction"
# *AD-tSVF - Very Heterogeneous*

- Mesenchymal Stem Cells (A Key Cell Group)
- Pericytes/Endothelial Cells & Adventitial Cells
- Pre-Adipocytes (Adipose Progenitors)
- Fibroblasts
- Macrophages
- Vascular Smooth Muscle Cells
- Miscellaneous Native Blood Derived Cells
- Extensive "Bioactive Secreting" ECM

# Import of Microvasculature

- Key Locations of Undifferentiated Cells in Adults *(All Tissues Have Some)*
- Provides Repository of "On Call" Cells Available in Nearly ALL Tissues
- Every Tissue With Vessels Contain Stem-Stromal Cells
- Adipose Is Largest Microvascular Organ In The Body

# Microvascular Relationship Adipose tSVF



Black Arrows = Adipocytes
White Arrows = Microcapillaries

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# Importance Of Stroma In Biocellular Therapy?

- Provides Needed Attachment Sites
  - *Required* for Cell Activation and Proliferation
  - Undifferentiated Cells Must Attach To Activate, Proliferate & Differentiate
- Native Scaffolding Of Adipose *Bioactive*
  - Participate In Paracrine Secretory Activities
  - Permits Early Attachment & Activation of Cells
- Highly Heterogeneous Population Considered Important To Provide "Site Specific" Needs

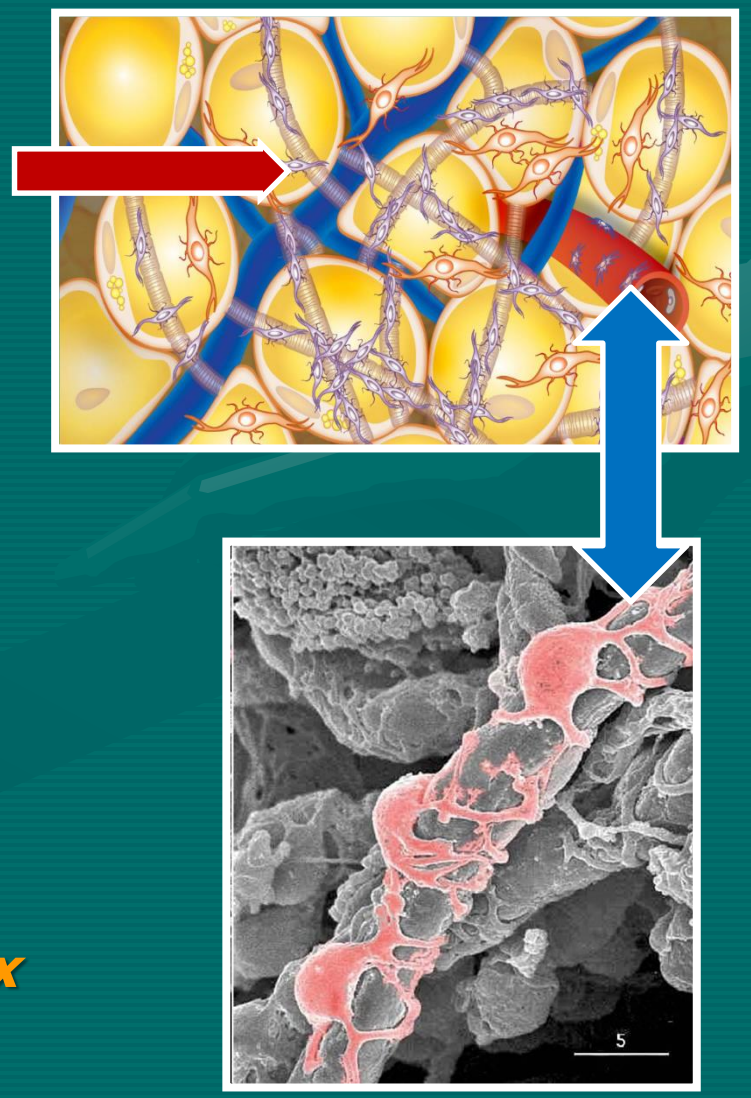
# Components Of AD-tSVF

***KEY Multipotent Cells Found In AD-tSVF***

- ❑ **Mesenchymal Stem/Stromal Cells**
- ❑ **Pericyte-Endothelial Cells****
- ❑ **Adipocyte Progenitor Cells**
- ❑ **Adipocytes (Temporary But Important)**

***Tissue Resident Cell Populations***

***+ Bioactive NATIVE Structural Matrix***

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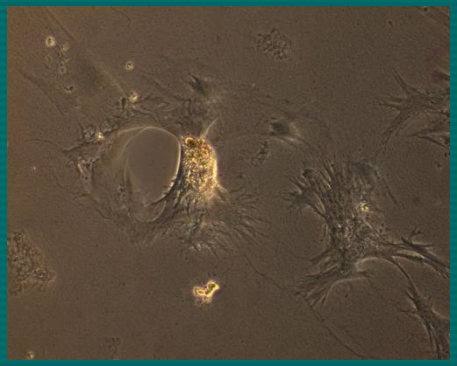
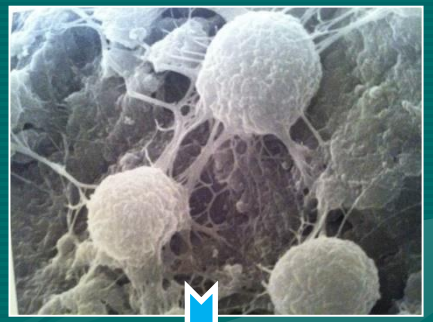
# AD-Mesenchymal Stem Cells

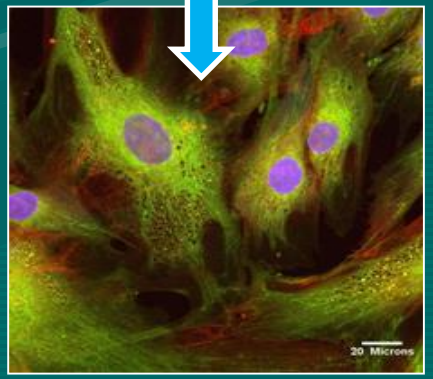


AD- MSC in 3D Culture
Treated With HD PRP Concentrate

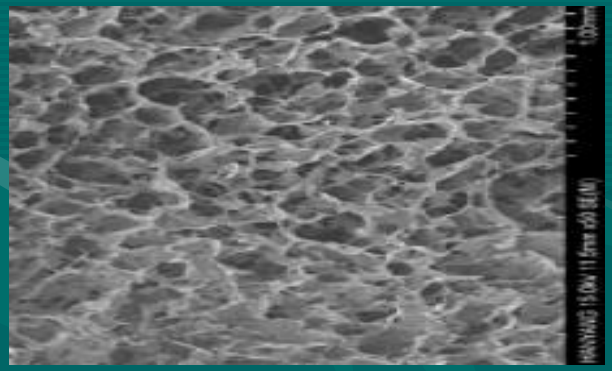
MSCs Cell-To-Matrix
& Cell-To-Cell



Cell-To-Cell Contacts




Calcein AM Dye – DAPI Nuclear Stain



Native Adipose Matrix
(Bioactive – Secretive)

Alexander, R., Mandle, R. 2012

# AD-MSC Differentiation

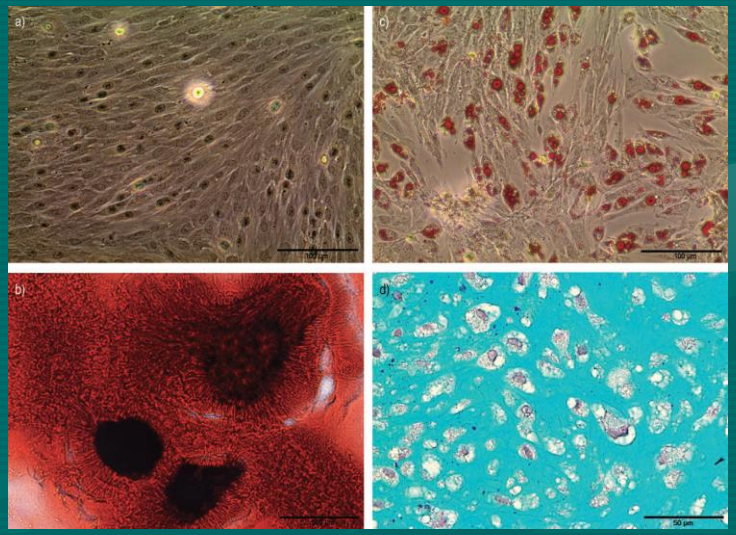
## *IMPORTANT:*

Mesenchymal Stem-Stromal Cells Capabilities Overlap >95+% Regardless Of Tissue Origin

Adipose & Bone Marrow MSCs Are Virtually *Interchangeable* In Capabilities *In Vitro*

Adipose Provides >2000 ***TIMES*** The Actual MSC Numbers Compared To Bone Marrow (per cc)

Adipose Does *NOT* Require Isolation, Culture-Expansion To Achieve Therapeutic Numbers




**Osteogenic, Adipogenic & Chondrogenic differentiation in AD-MSC:** a) Control MSCs basal medium (10X); b) Alizarin red staining of cells cultured for 7 days in osteogenic differentiation medium (magnification 4X). c) Oil red O staining of cells cultured for 15 days in adipogenic medium (magnification (10X). d) Haematoxylin Mayer's and Alcian Blue stainings of cells cultured for 21 days in chondrogenic medium (40X).

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# Why Platelet Concentrates?

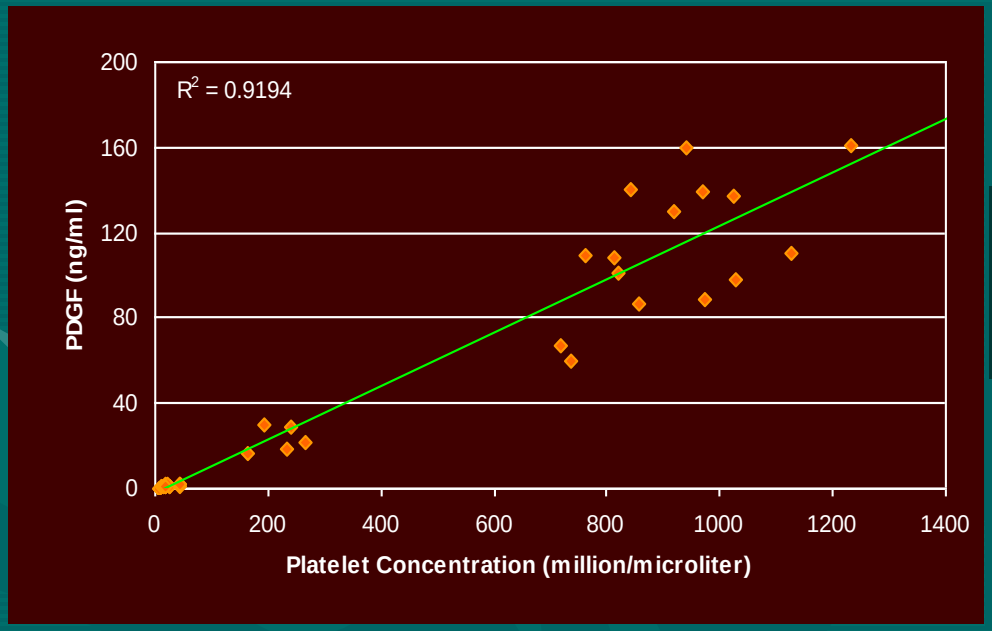
"Not All Platelet Concentrates Are Created Equal"

# HD Platelet Concentrates

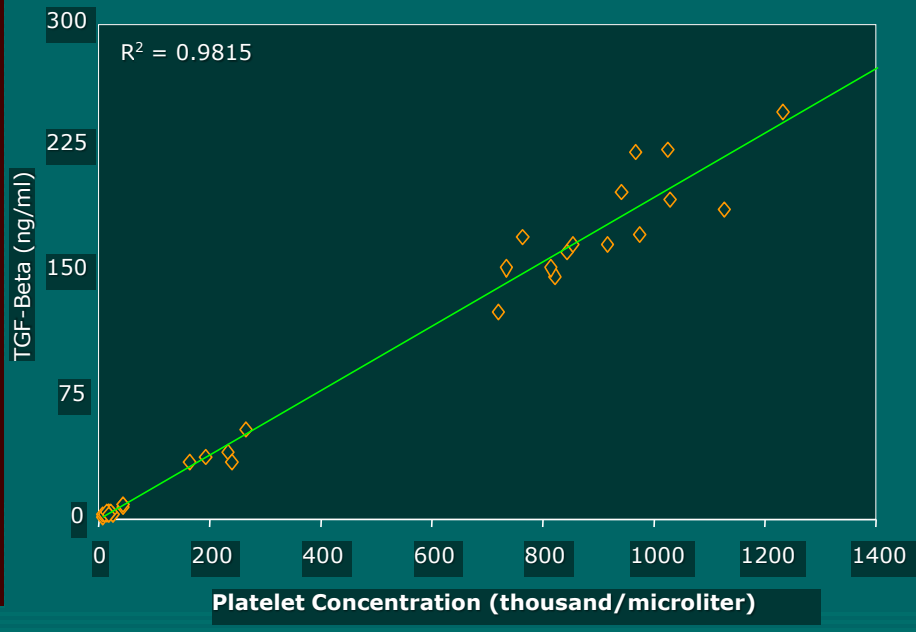
- Offers Important Biological Contributions of Growth Factors & Signal Proteins
- Directly Impact *Proliferation* & *Migration* of Stem/Stromal Cells
- Platelets Contribute Vital Healing Chemicals To Healing Sites ("Quarterbacks" Cascade)
- Contributes To Tissue "Autoamplification" System Within Local Sites

# PLATELET CONCENTRATES GF LEVELS INCREASE *LINEARLY* with HD-PRP

Platelet-Derived Growth Factor (PDGF)




Transforming Growth Factor-ß (TGF-ß)




*S. KEVY, et al, HAVARD CENTER FOR BLOOD RESEARCH; BIOMATERIALS, APR 2001*

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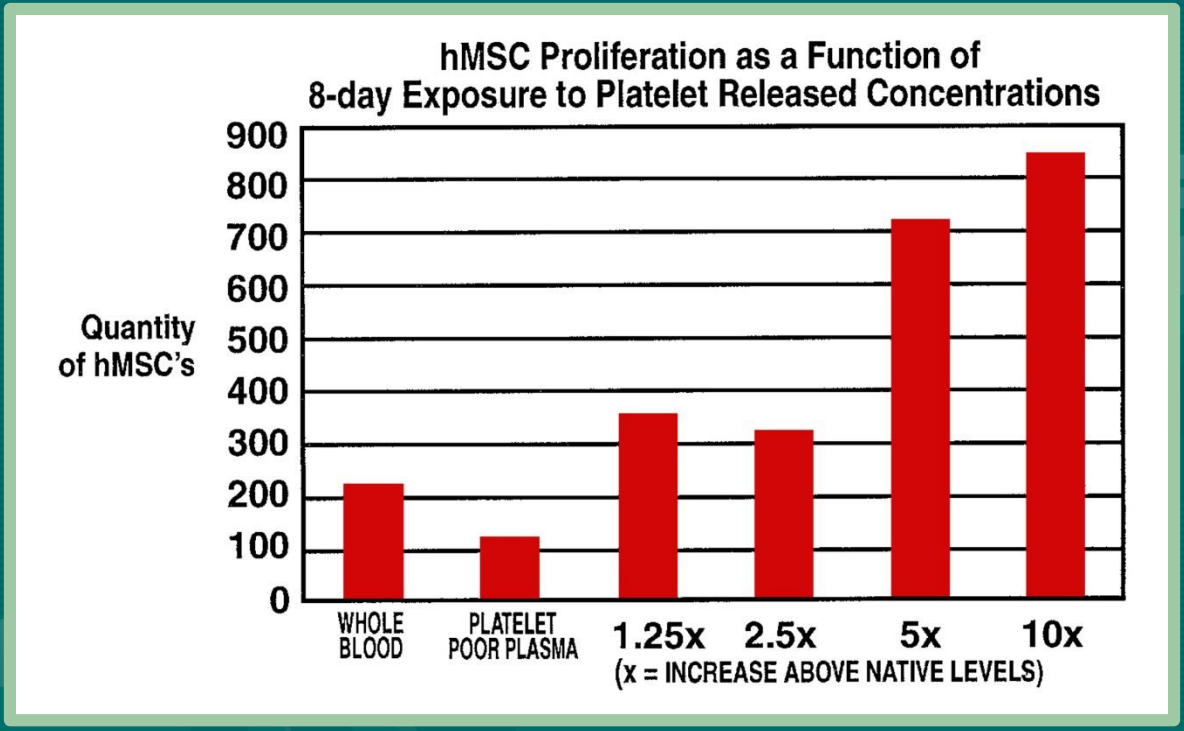
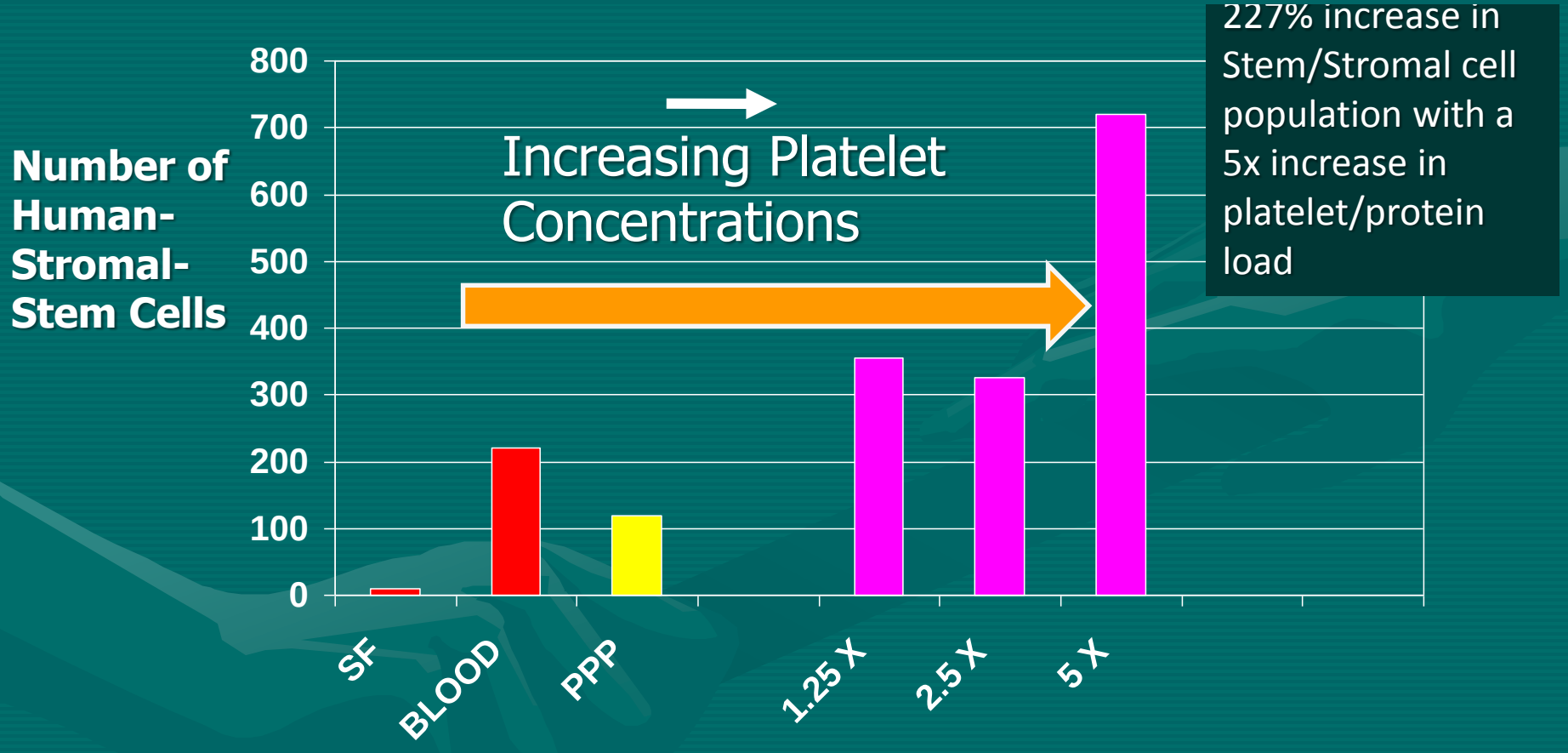
# AD-MSCs + PRP Exposure

## *"Proliferation Effects"*




Higher Concentrations HD PRP Achieved = *MUCH*
Higher MSC Proliferation Rates In Tissues

# *Migration* of Repair Cells (Stromal-stem cells) To Injury Site *INCREASES* Directly With *HIGHER* Platelet Concentrations




Haynesworth, SE, Bruder, SP, et al; "Mitogenic Stimulation of Human Mesenchymal Stem Cells by PRP Suggests a Mechanism for Enhancement of Bone Repair", Presented at 48th Orthopaedic Research Society Meeting, Dallas, TX, 2002

# Why Combine Cells With HD PRP?

By Definition: This IS The Components of "Biocellular Regenerative Therapies"

# "Workers & Bricks" Analogy

*How To Decide On Use Of Biologics ONLY (PRP or BMA) vs Use of Biologics + Cellular Elements?*

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# WHY HD-PRP + AD-tSVF?

- Immediate Availability And Much Higher Growth Factor Load Added To Target Tissues
- More Signal Proteins And Cytokines/Chemokines
- *Both* Stimulate *Angiogenesis* - (Key Element !)
- Stimulates AD-MSCs & All Other tSVF Elements
- Enhances The Microenvironment (Niche) To Encourage Stem Cell Proliferation, Migration, & Site Specific Differentiation
- Actively Participate in Signaling Processes, Cellular Recruitment, And Migration Of Other Needed Cells To Target Site

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# Important Concepts!

*Chronic inflammation & re-injury "uses up" the local repair cells (Depletion of Regenerative Capable Cells).*

*This is why/when the need to add repair cells (adipose-derived stromal/stem cells) PLUS biologics (either HD PRP and/or BMA) to the target site*

*Niche (microenvironment) becomes very important & site specific for wound healing or regeneration.*

# Why Centrifugation?

# Centrifugation Of Autologous Fat Grafts

- Creates A "Density Gradient" Of SVF
- MARKEDLY Improved Layer Separation Of :
  - Infranatant Fluids
  - Red Blood Cells & Cellular Debris
  - Lipids, Proteases, & Lipases (Separator Disk)
- Optimal Centrifugation *1000 g* For 3-4 Minutes
- Favors Transplantation Of Maximum Stroma
- Decreases Fluid Load To Site & Reduces Exposure To Local Anesthetics

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# Pre-Centrifugation

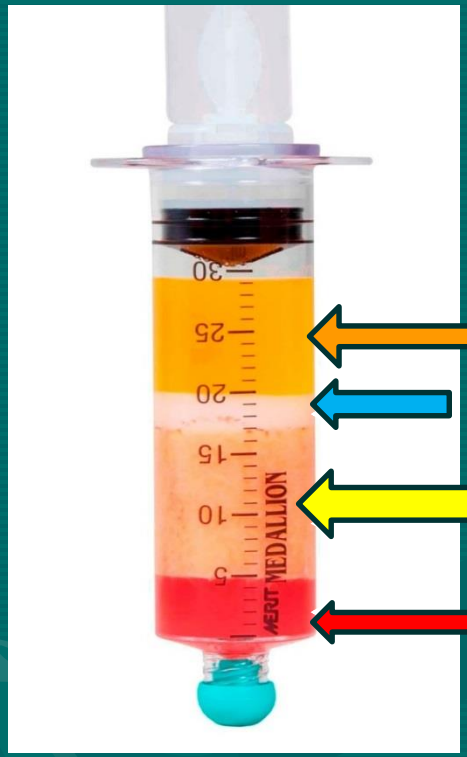
Adiprep Kit System

(FDA 510k Approved by Terumo-Harvest, Plymouth, MA)

# *Not* All Autologous Fat Grafts Are *Equal*

**Gravity Decantation 30 Min**

**Centrifuged Graft 1000 g 4 Minutes**




Oil and Lipid Fraction

Lipid Barrier Disk

Compressed Adipose Tissue (tSVF)

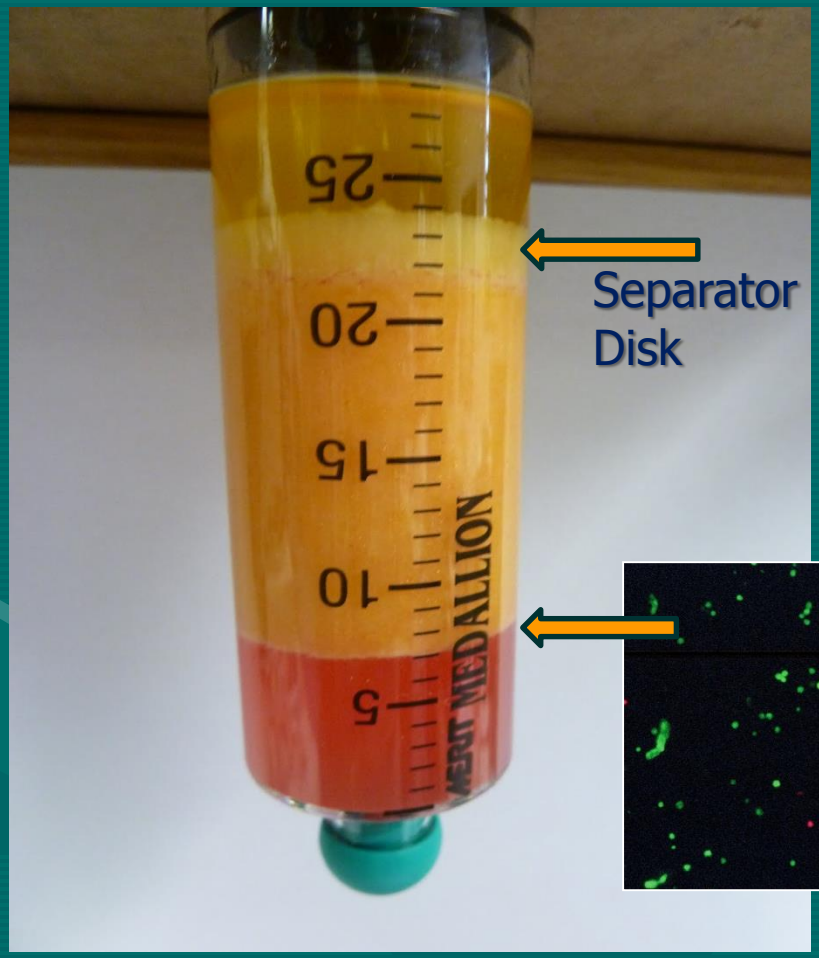
Excess Fluid – Few Cells & Extensive Debris

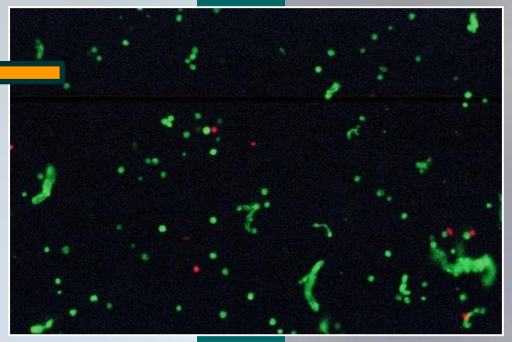
Decant ONLY

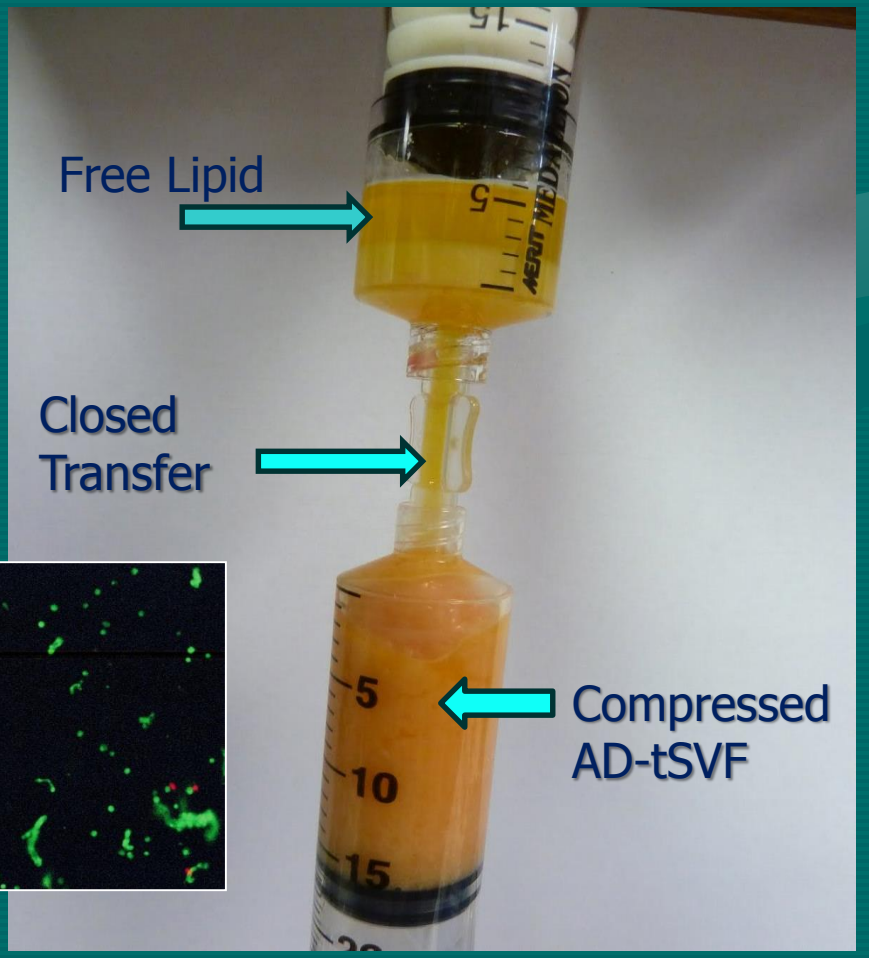
*Important Advantages of Centrifugation:*

1. Compressed Graft (Less Volume Required);
2. Effectively Eliminates Free Lipid Layer;
3. Easy Discard of Infranatant Fluid and Debris;
4. Reduces Residual Lidocaine In Graft;
5. NOT Damaging To Cell Viability (Optimal g-Force of 800-1200 g)

# AFG Separation-Density Gradient






Centrifugation 1000g 4 Minutes

"Anaerobic" Transfer Loading

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# Create Biocellular Mixture

Normal Ratios Vary
From 50:50
To
30% HD PRP to 70%
tSVF By Volume.

*If Suspicious Of Cell Depletion…..*
*Use More tSVF Cells !*

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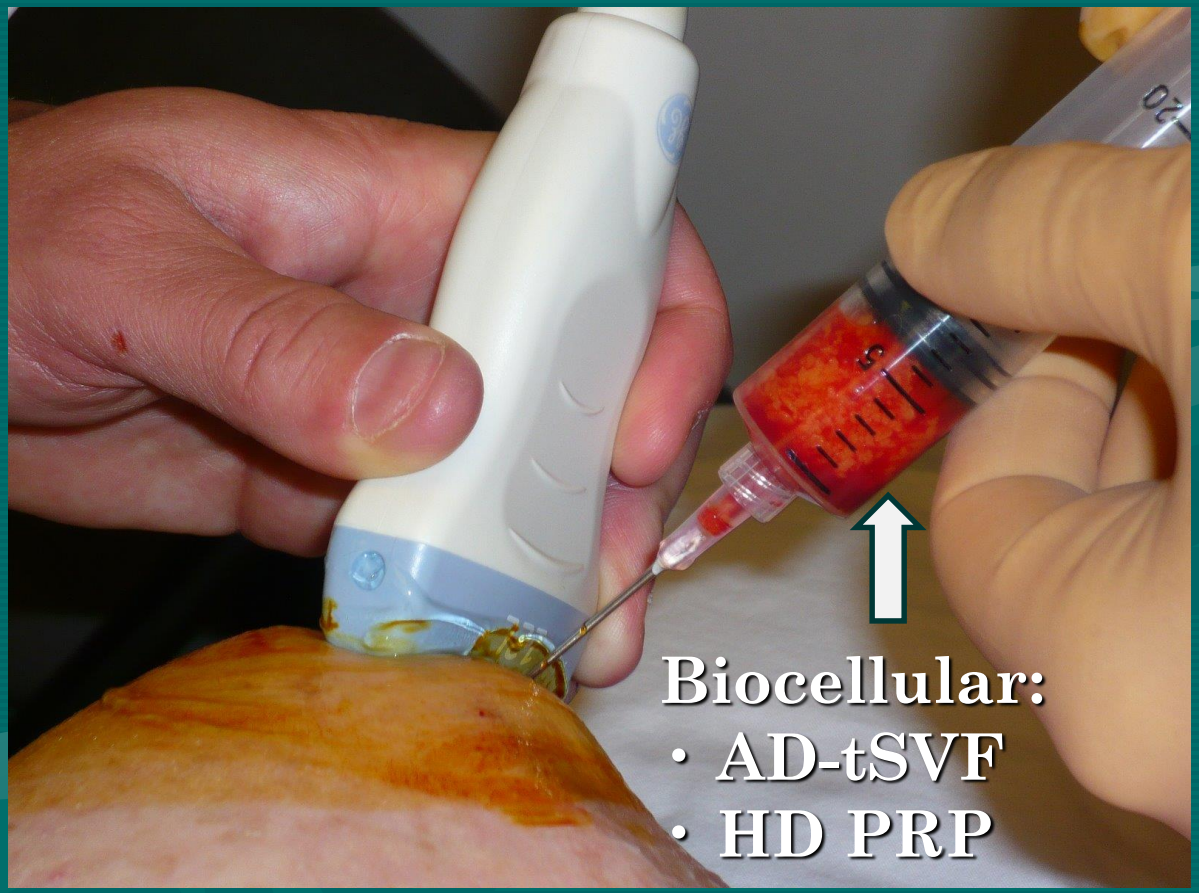
# U/S Guided Injection Therapy




Accurate Placement *Critical* To Outcome Improvement

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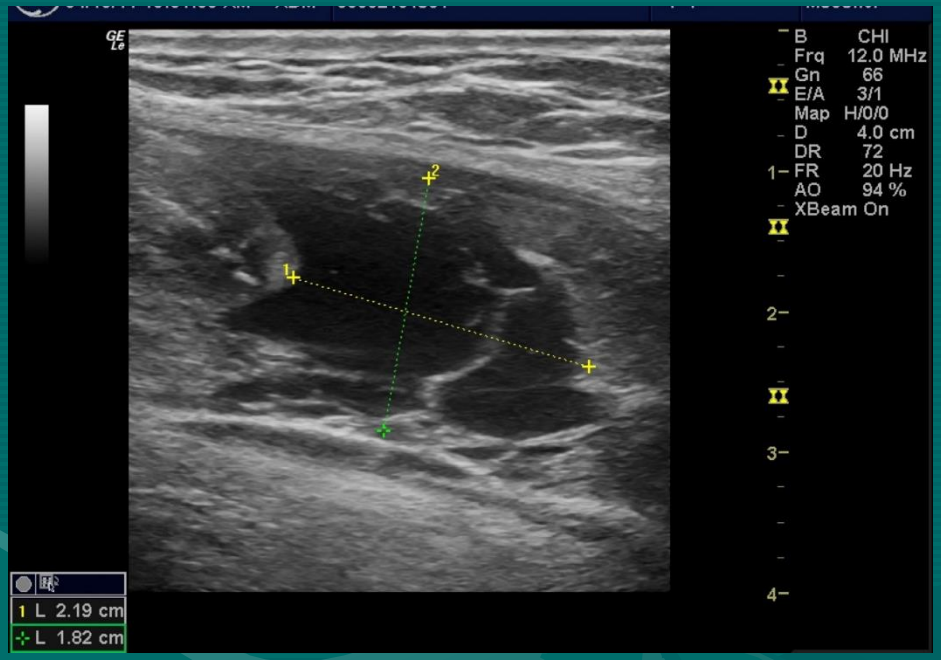
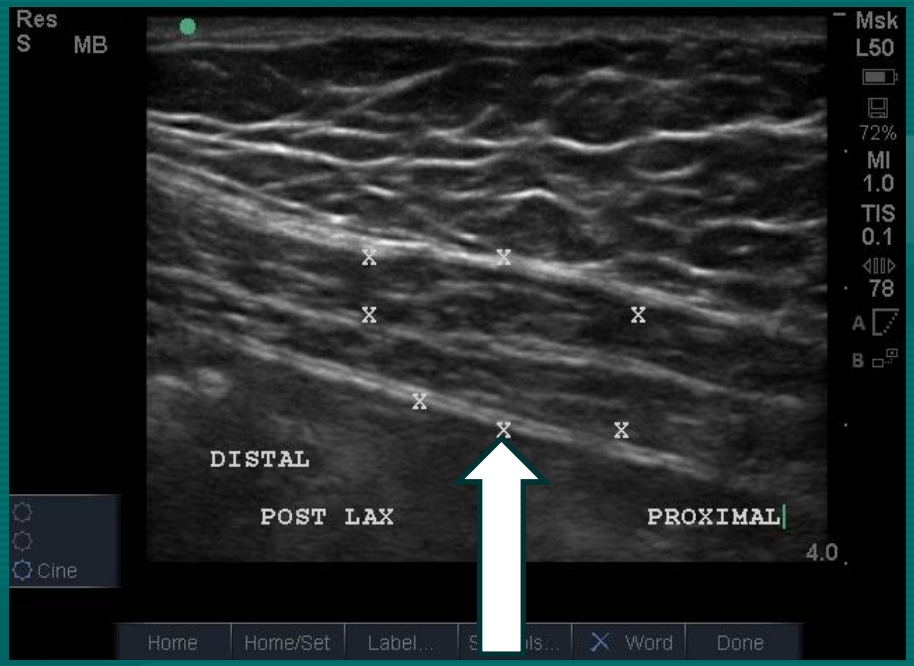
# Use of AFG + HD PRP In Muscle




48 Hours Post Blunt Trauma Injury (Rectus)




UltraSound Image 5 Weeks (Outline of Defect Marked)

*Note: Minimal Scar Evidence Residual*

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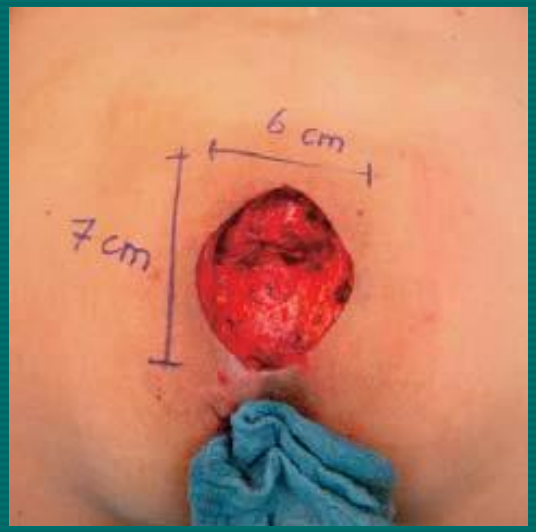
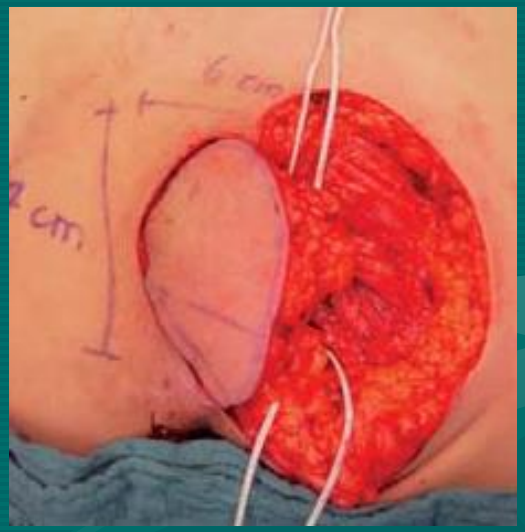
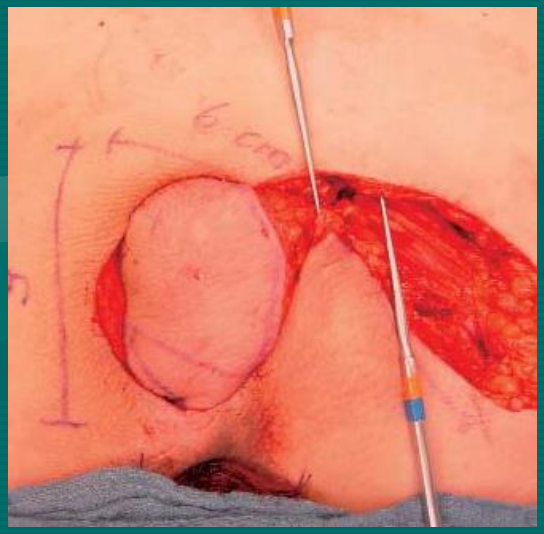
# Uses In Wound Healing

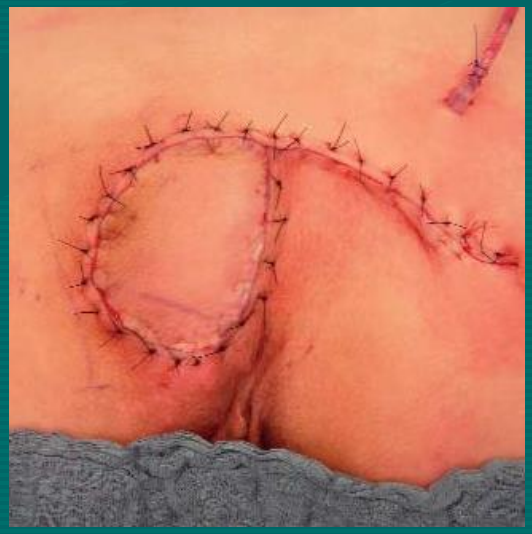
- Biocellular Elements Strongly Encourage Wound Vascularization (*Angiogenesis*)
- Supports Cell & Tissue Repair
  - Directly Via Growth Factors & Cells
  - Indirectly Via Stimulating Site Tissues
- NOT Substitute For Debridement
- Often Helps In Bacterial Control

# Sacral Ulcer Flap With PRP+

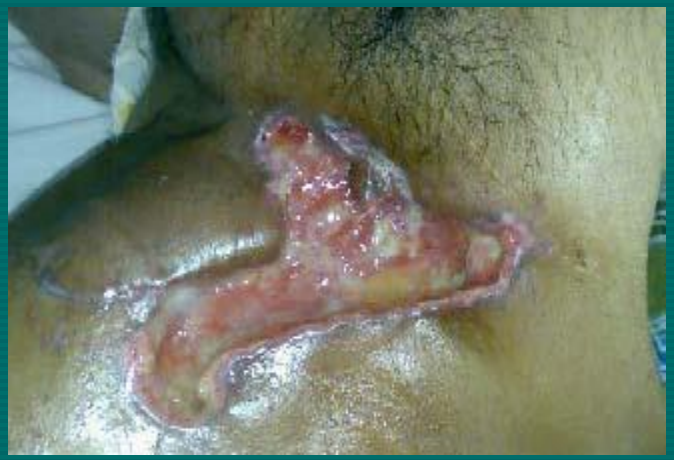




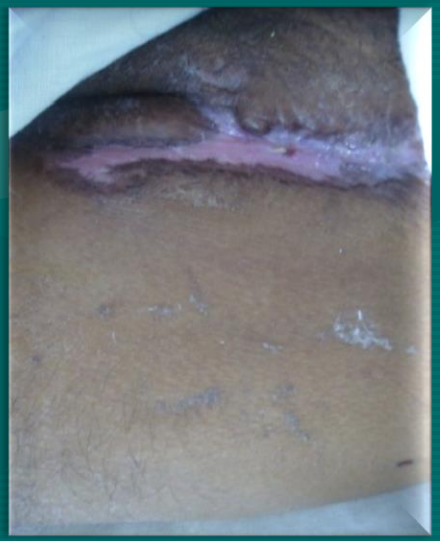


# Chronic Wound Healing



De-vascularized Flap, "Wet"

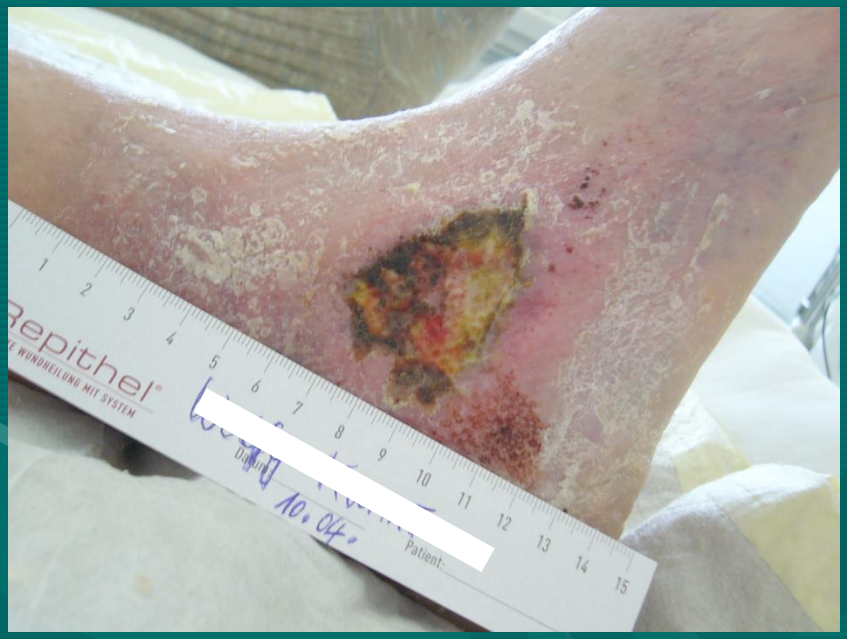
Debrided, AD-tSVF + HD PRP, 2 Weeks, Margins Active; Granulating



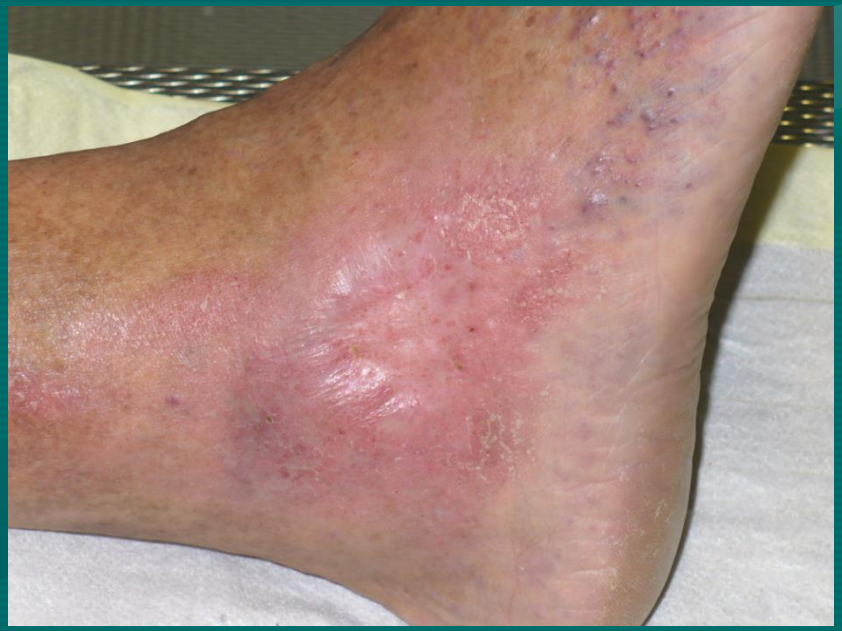
Closed, 6 Weeks; Early Wound Contracture

*Note: Valuable Also For <u>EARLY</u> Pain Relief In Majority of Patients!*

# AD-tSVF + HD PRP



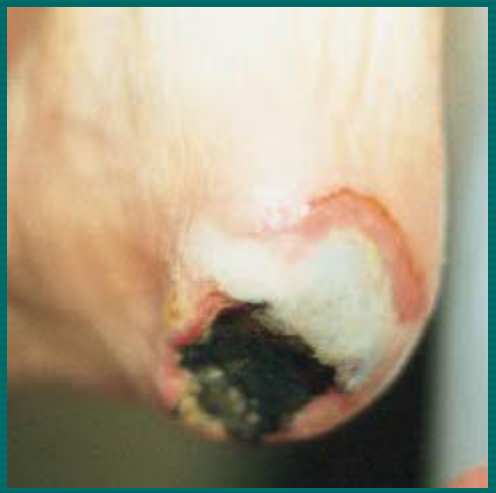

Pre-Operative Stasis Ulcer, Two Year Duration



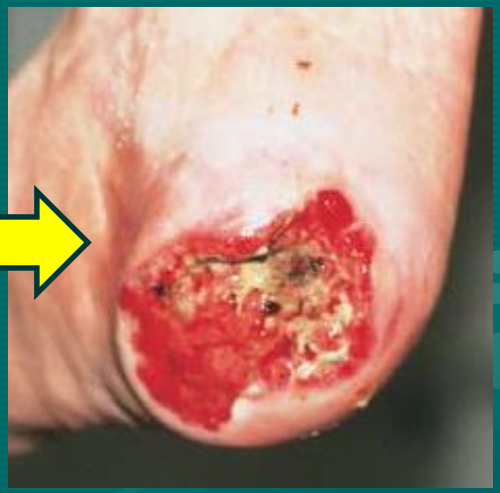
Ten Weeks Post-debridement, AD-tSVF + HD PRP, 2 Applications; NO Grafting

# Heel Pressure Sore

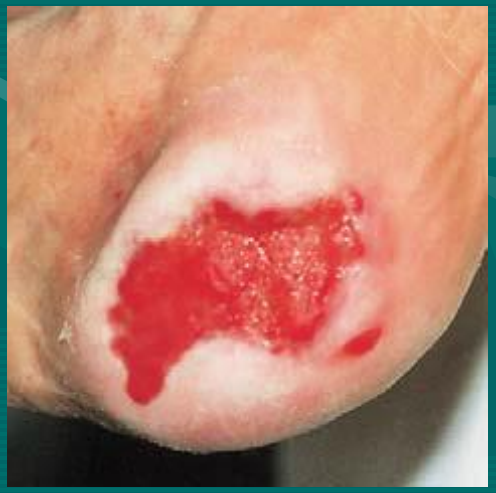
## 84 Year Old Female; CVD, HTN,



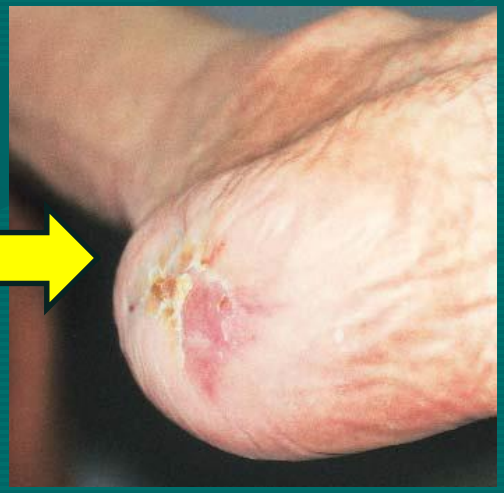
Evaluation
Grade IV &
Debridement



Biweekly
Debridement;
PRP+ & Gel
Apps



Four Weekly
Debridements;
Two AD-SVF +
PRP Injection
Of Margins &
Deep (14 Day
Intervals



Tissue Closure,
NO GRAFT

# *Things Have Changed !*

- *Began With Prolotherapy To Stimulate Healing*

- *Advanced To "PRP Prolotherapy"*
  - Potency Proportional To PRP Density & Configuration
  - Often Requires a "Series" of Injections To Reach Goal
  - Useful In Acute or Subacute Conditions

- *"Biocellular Guided Therapy"*
  - Now Shown To Be The Most Potent Regimen
  - Offers Reduction "Toxic" Inflammation
  - Provides Cells For Site Specific Needs

# Hypotheses Of tSVF Effects

- Originally: Thought MOST Effects Were Due To Stem Cell Survival & Differentiation ***ONLY***

- Now Believed: ***Most*** Important Effects Are Due To Autocrine & Paracrine Secretory Effects On Microenvironment & Vice Versa

- ***Both* Effects Are:** Complimented With Addition Of Elements Of HD PRP+/BMAC (Cytokines, Growth Factors, Etc.) Additives

# Current Trends In Orthopaedic Regenerative Medicine-Surgery

- Expanding Adipose Tissue + Biological Applications To Regenerative Needs *("Workers + Bricks")*
- Cell-Assisted Concentrates – More Cells To Graft
- Increased Uses In Musculoskeletal, Chronic Wound, Inflammatory Sites, & Soft Tissue Flap Surgeries
- Many Case Series & Clinical Studies Confirming *Safety and Efficacy* of Uses In Variety of Regenerative & Reconstructive MSK Applications *(Leaving Translational Phase – Entering Clinical Trial Phase (IRB)*

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# Look To The Future

*Thank You For Your Attention ☺ !*

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# Thank You!

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