INSTITUTE OF REGENERATIVE AND MOLECULAR ORTHOPEDICS

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www.stemcellorthopedic.com and STEM CELL CENTERS OF AMERICA

STEM CELL AND PLATELET RICH PLASMA INJECTIONS



Cells, not doctors heal patients

No physician in the history of humanity has ever healed a patient. Only the cells of the patient can heal the patient. Only cells know how to close wounds, understand what to do with insulin and how to destroy pathogens. The best a physician can do, is to move obstacles out of the way of cells (e.g. by surgery), supply materials and weapons to the cells (e.g. drugs and building blocks of life) and leave the fight against disease to the cells. Harnessing the power of the cells is the fundamental basis of Regenerative Medicine

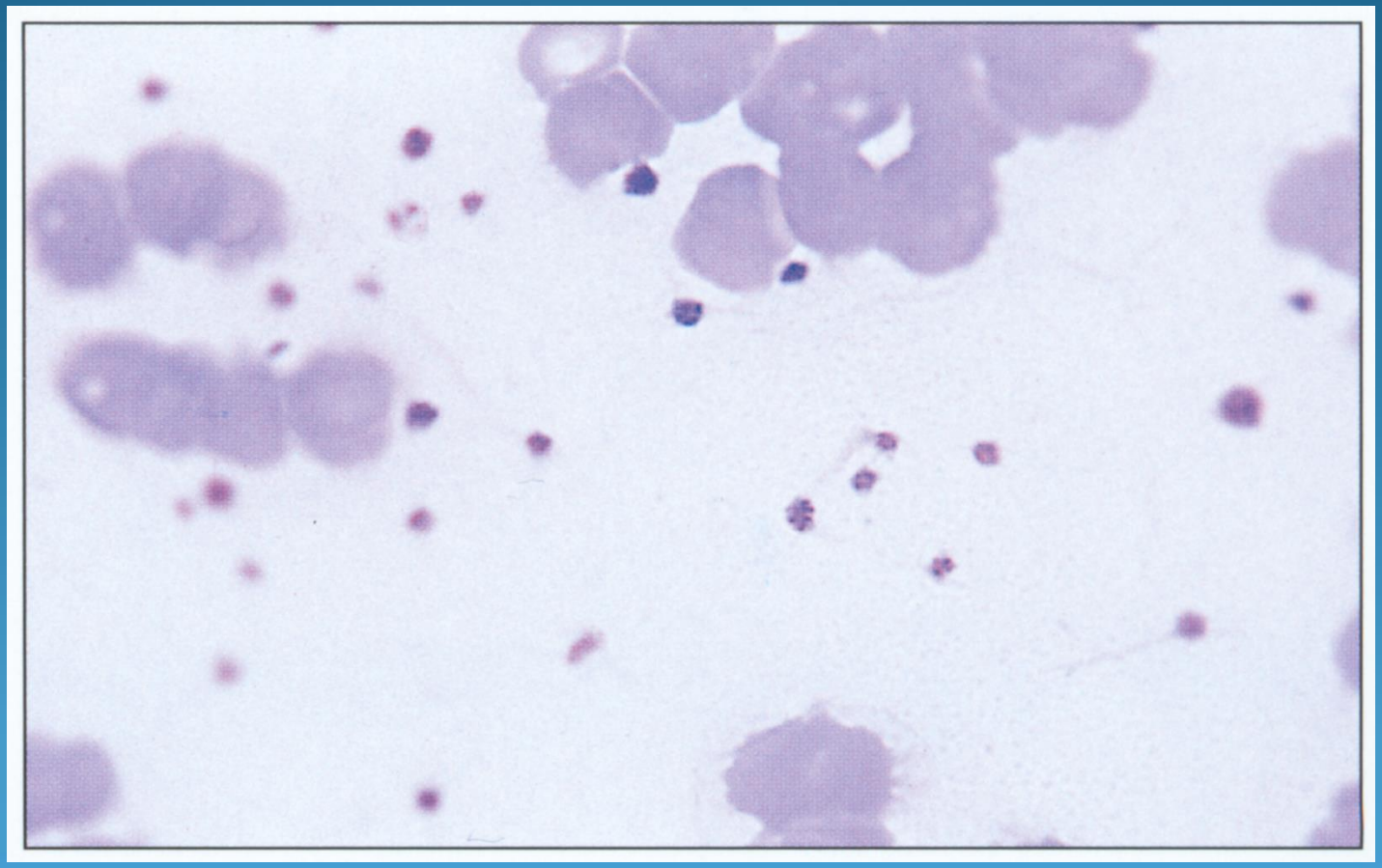
PLATELET RICH PLASMA HIGHLIGHTS

PLATELET RICH PLASMA

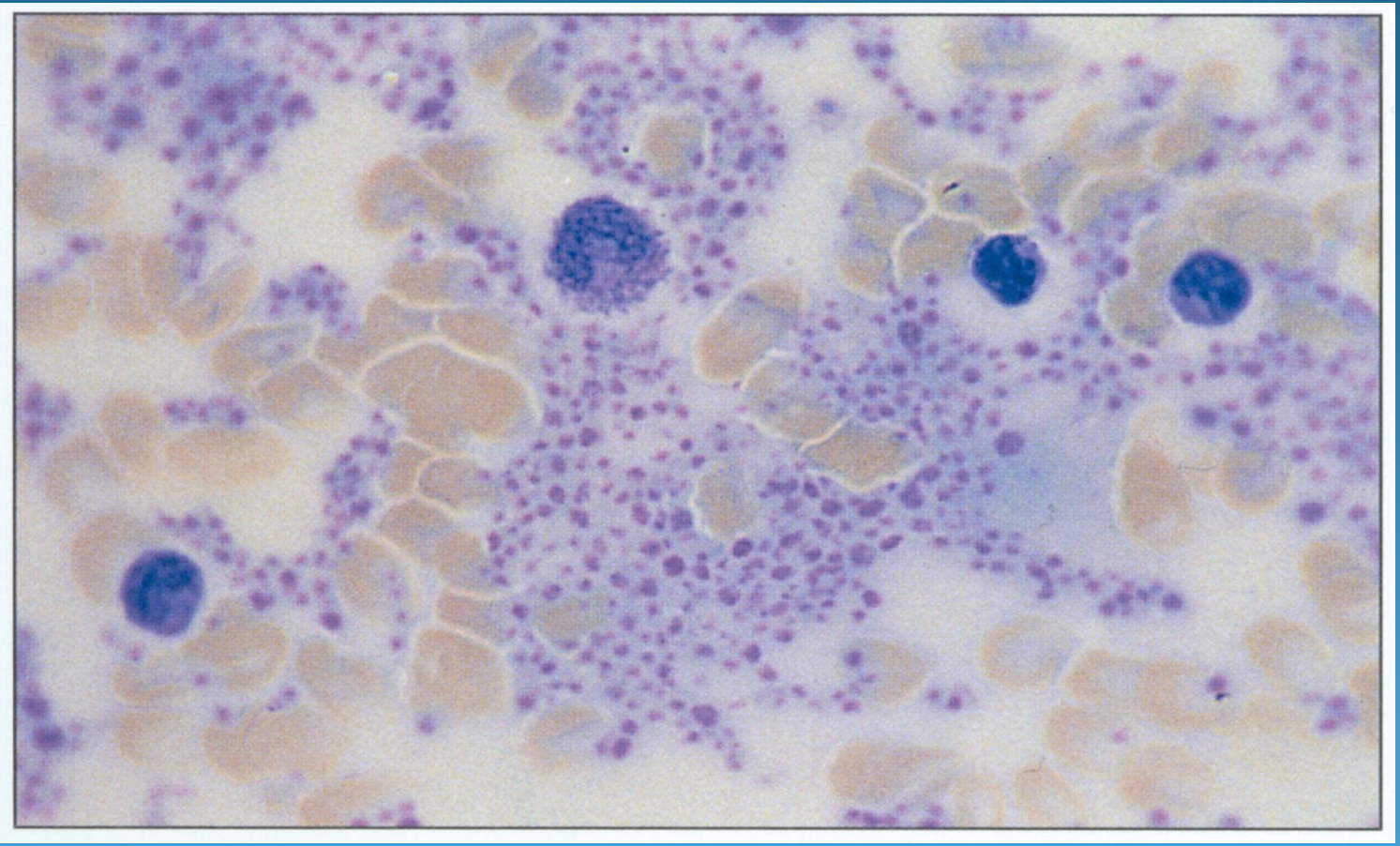
THE MORE WE KNOW THE MORE WE DON'T KNOW!!!

BUT WHAT DO WE THINK WE KNOW?

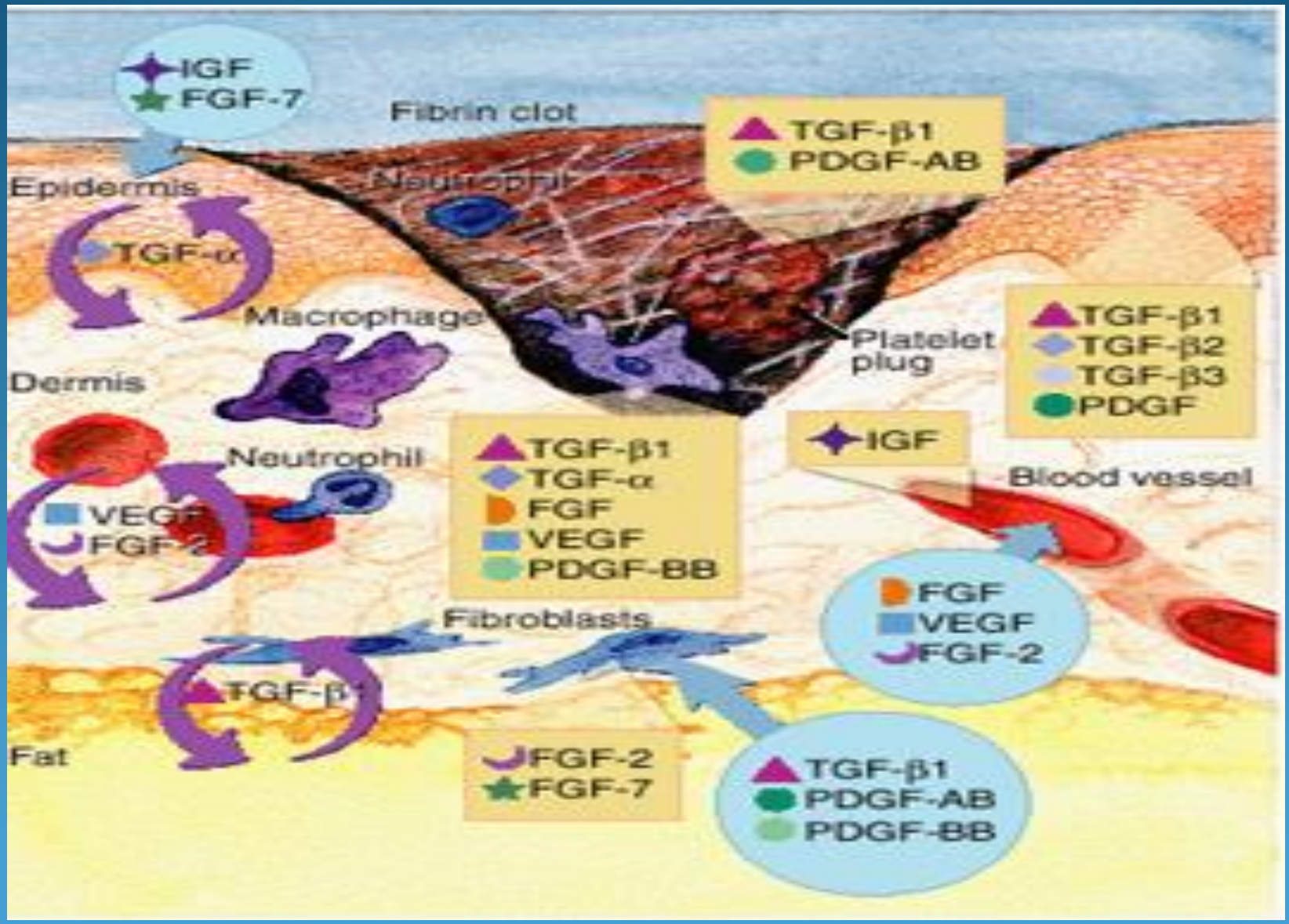
A normal platelet count…



Autologous platelet concentrate…



Power of Platelets



PRP CONTENTS

1. **Platelets**
2. **Neutrophil** (PMN) - 40-75% of circulating leukocytes
3. **Monocyte macrophage** - 2-10% of circulating leukocytes. Highly motile and migrate to soft tissues
4. **Fibroblast -** produce collagen, reticular fibers, glycosaminoglycans, glycoprotein
5. **Endothelial Cell** - permeability barrier, regulate blood flow and vascular reactivity, vasodilators, vasoconstrictors, regulate inflammation and immunity
6. **Keratinocyte** - Stratified, squamous epithelial cells Primary function is to act as a barrier
7. Very small embryonic like stem cells **(VSEL)**

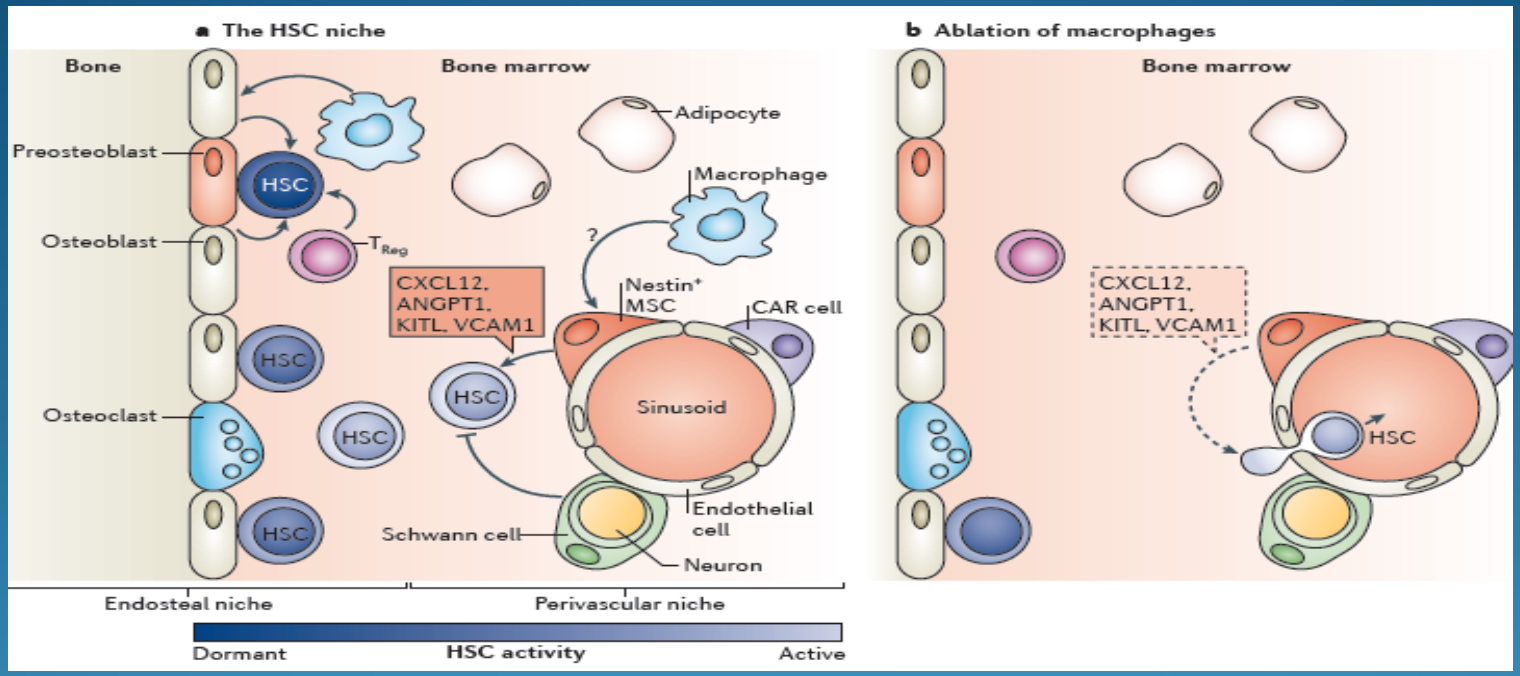
Efficacy of the Platelet Product

1) Depends upon the concentration and composition of the releasate components at the site of application
2) **Optimal concentration of a Platelet-Rich Plasma (PRP) for angiogenesis is 1.5 – 3.0 million platelets/µL**
3) Inhibition was demonstrated at platelet concentrations of 5 million/µL or greater
4) No point of care PRP system can attain a level that will result in inhibition
5) Systems that produce platelet concentrations $<500X10^3/\mu L$ support proliferation no better than platelet-poor plasma
6) **Very Small Embryonic Stem Cells (VSEL)**

Efficacy of the Platelet Product (cont.)

1. Clinically effective PRP' s contain both stem cells and their homing agent SDF-1$^{\alpha}$ dependent upon WBCs
2. Homing is a multistep process signaled by stromal derived factor 1 alpha (SDF-1α), stem cell factor (SCF) also called CXCL**-12**
3. RBCs may not play an important role in the PRP product. There presence probably has no effect on the joint.
4. Inflammation is probably not RBC mediated examples include Micro fracture technique and acute traumatic joint effusion which causes little inflammation

Why WBCs are important



Hsu & Fuchs. (2012). A family business: stem cell progeny join the niche to regulate homeostasis. *Nature Reviews Molecular Cell Biology*; 13:103-114

1. **Depleting macrophages down regulates genes for secreting factors in nestin⁺ MSCs (CXCL12, ANGPT1, KITL, VCAM1) nestin MSCs lead to HSCs homing**

2. **Macrophages affect HSC retention through regulating critical retention factors in nestin⁺ MSCs**

VASCULOGENESIS VS. ANGIOGENESIS

1. ANGIOGENESIS DENOTES THE FORMATION OF NEW BLOOD VESSELS FROM PRE-EXISTING ONES
2. VASCULOGENESIS IS A TERM USED TO DESCRIBE THE FORMATION OF NEW BLOOD VESSELS WHEN THER WERE NO PRE-EXISTING ONES. THIS OCCURS WHEN ENDOTHELIAL PRECURSOR CELLS MIGRATE TO AN AREA AND DIFFERENTIATE.

CYTOKINES

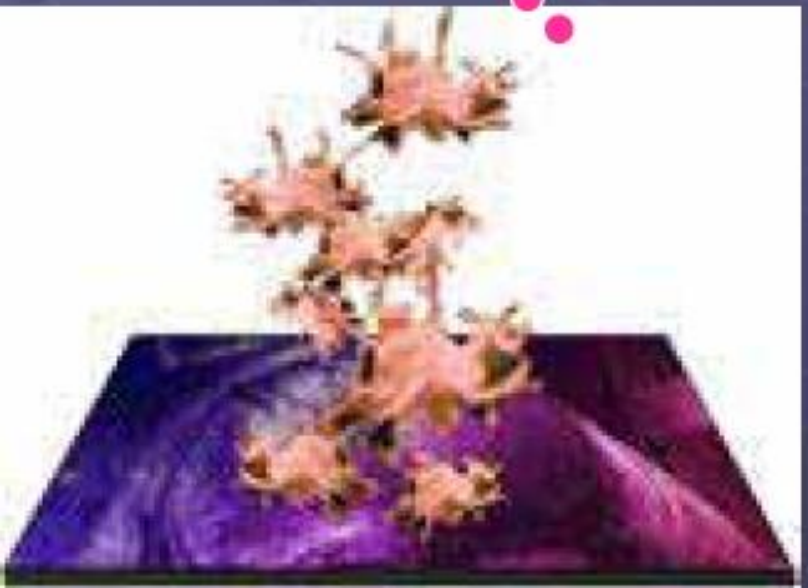
THE METHOD OF INTERCELLULAR COMMUNICATION

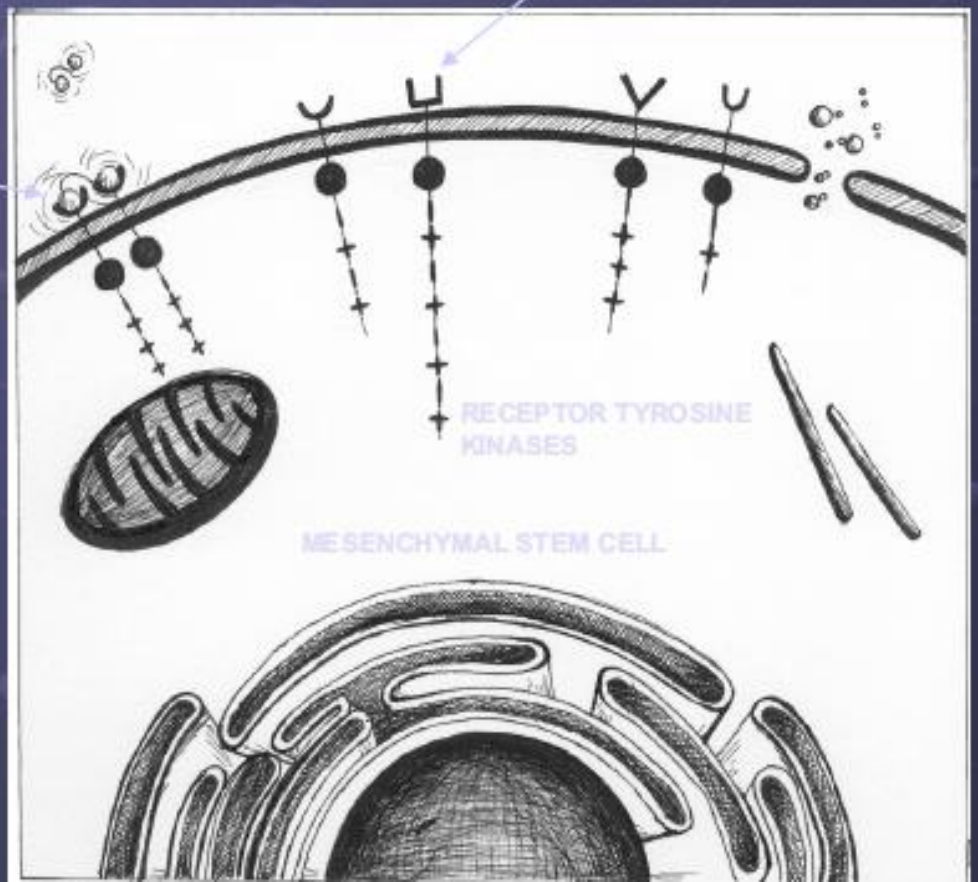


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Growth Factor release from an Activated Platelet

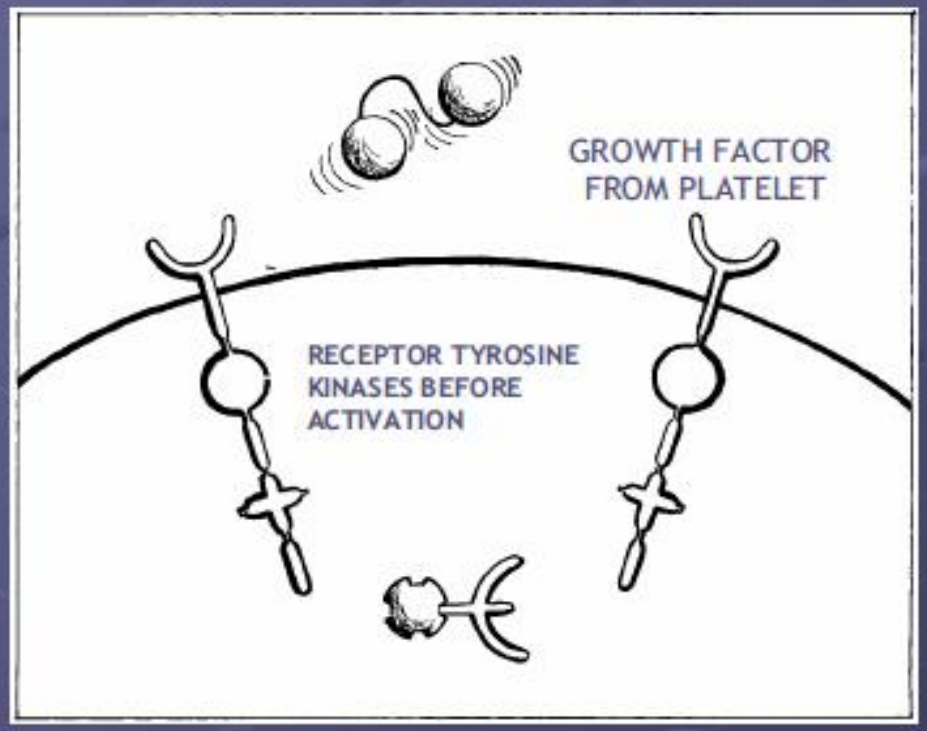
GROWTH FACTORS RELEASED FROM ACTIVATED PLATELETS BIND TO CELL MEMBRANE TO ACTIVATE GENES CONTROLING MITOSIS



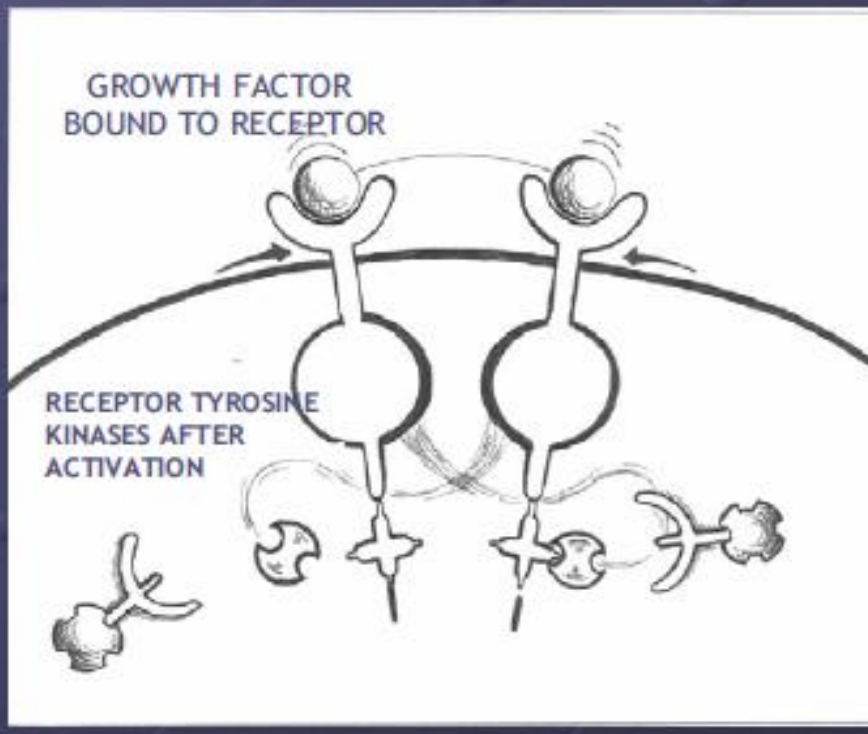


Mechanism of Action for Growth Factor Signaling

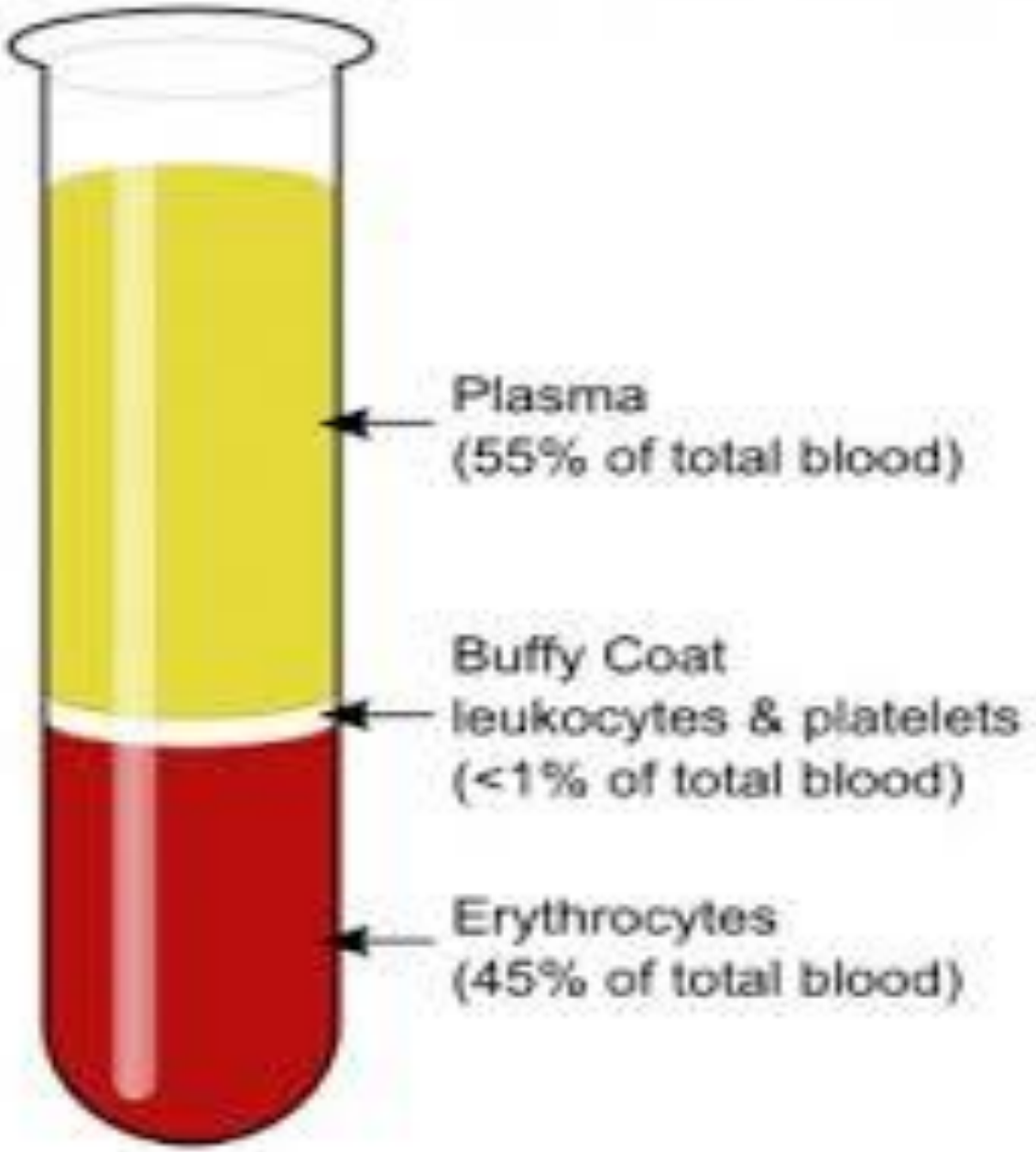
1. BEFORE SIGNALING



2. GROWTH FACTOR ACTIVATES MEMBRANE RECEPTOR



SCIENTIFIC AMERICA JUNE 2000, VOL.282, NO 6


Plasma
(55% of total blood)
Buffy Coat
leukocytes & platelets
(<1% of total blood)
Erythrocytes
(45% of total blood)

COMMON CONDITIONS TREATED WITH PRP

1) Disorders of the shoulder including bursitis and rotator cuff tears
2) Tendonitis of a variety of tendons including tennis elbow, Achilles tendonitis, and heel spur syndrome
3) Muscle tears, sprains, trigger points
4) Meniscus tears of the knee
5) Mild to moderate degenerative arthritis of various joints
6) Disorders of the spine especially facet joints

SPORTS MEDICINE CLASSIFICATION OF PLATELET RICH PLASMA

	White Blood Cells	Activation?	Platelet Concentration		
Type 1	Increased	No Activation	A, 5x or more	or	B, less than 5x
Type 2	Increased	Activated	A, 5x or more	or	B, less than 5x
Type 3	Minimal or No WBC's	No Activation	A, 5x or more	or	B, less than 5x
Type 4	Minimal or No WBC's	Activated	A, 5x or more	or	B, less than 5x

1. **Subtype A contains an increased platelet concentration at or above five times baseline.**
2. **Subtype B contains an increased platelet concentration less than five times baseline.**

activated by an exogenous activator such as thrombin or calcium

"Mishra, et al. "Sports Medicine Applications of Platelet Rich Plasma", *Current Pharmaceutical Biotechnology*, 2012, 13, 1185-1195.

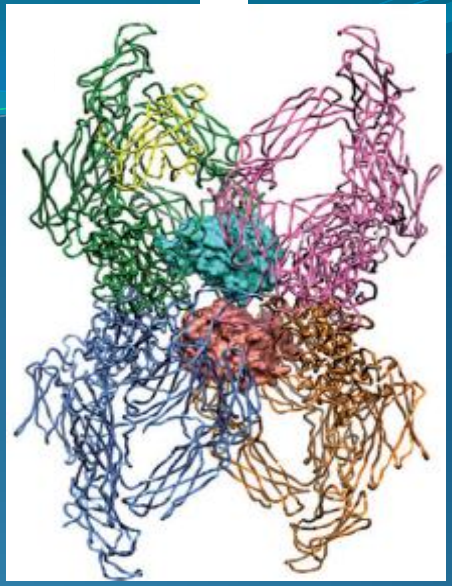
WHAT IS GOING TO BE THE NEXT PRP?

Autologous Protease Inhibitor Concentrate

APIC

The Secret Behind APIC:

Our Body's Own Defense
α-2-Macroglobulin (A2M)



- Broad Spectrum Multi-Purpose Protease Inhibitor
- High Concentration in Blood (*up to 6mg/ml*)
- Inhibits MMPs & ADAMTS Degradation Proteases
- Binds / Regulates Cytokines and Growth Factors

STEM CELLS

Why Adult Stem Cells?

"Basically, our bodies are constantly undergoing stem cell therapy," said Spradling.
"We would live one or two days without (adult) stem cells. It's essential to have these cells doing their thing."

Allan C. Spradling, Ph.D.

Howard Hughes Med. Inst.
HHI Newsletter Feb.16, 2007

Adult Stem Cells

Adult stem cells are found in many tissues

- There are undifferentiated cells and differentiated cells in tissue samples

The primary role of stem cells is to maintain and repair the tissue in which they are found

Adult stem cells are multipotent, not pluripotent

- **Pluripotent: can differentiate into any cell type**
- **Multipotent: can differentiate into a subset of cell types**

Adult stem cells may exhibit plasticity

WHAT TYPES OF STEM CELLS EXIST?

1) Embryonic stem cells
2) Adult mesenchymal stem cells
3) Hematopoietic stem cells
4) IPS cells induced pluripotential stem cells
5) Various other more specific type of stem cells
6) Very Small Embryonic Like Stem cells (VSEL)

EMBRYONIC STEM CELLS

1) By far the most controversial stem cells. U.S. government has lifted some bans but FDA has still significantly restricts use in people.
2) These cells seem to present the most potential for correcting and curing certain conditions due to their plasticity or ability to morph into many cell types.
3) There are ethical issues.

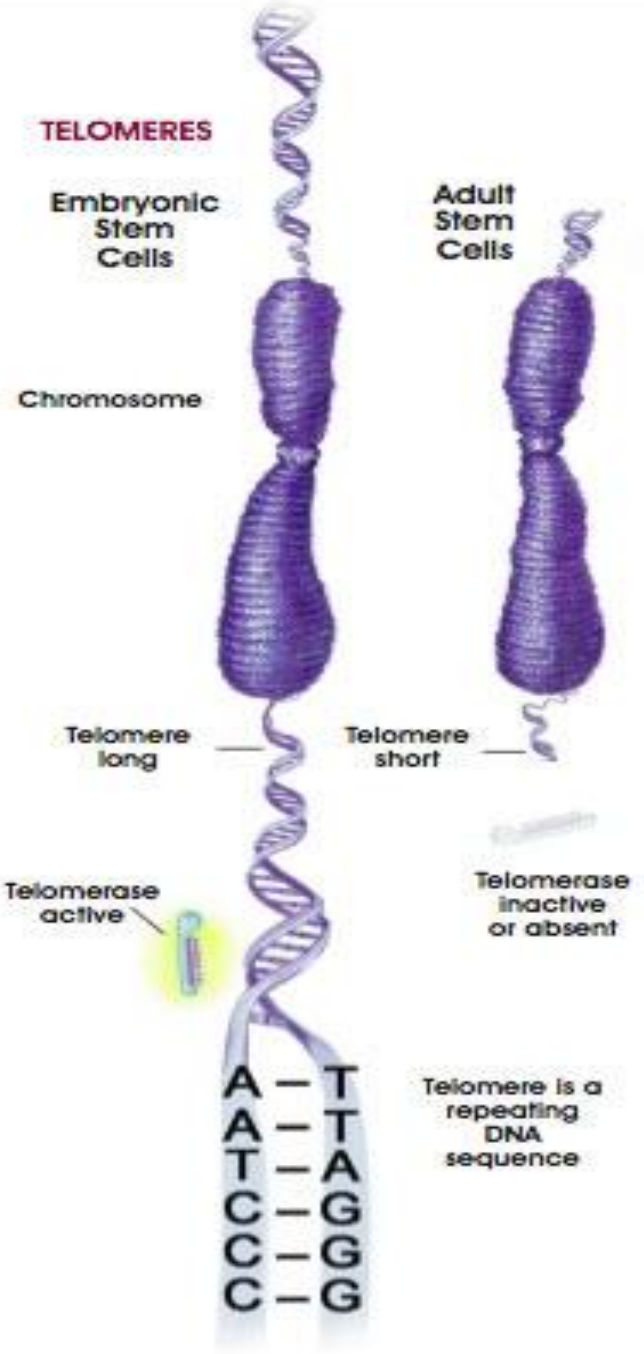
POTENTIAL PROBLEMS WITH EMBRYONIC STEM CELLS

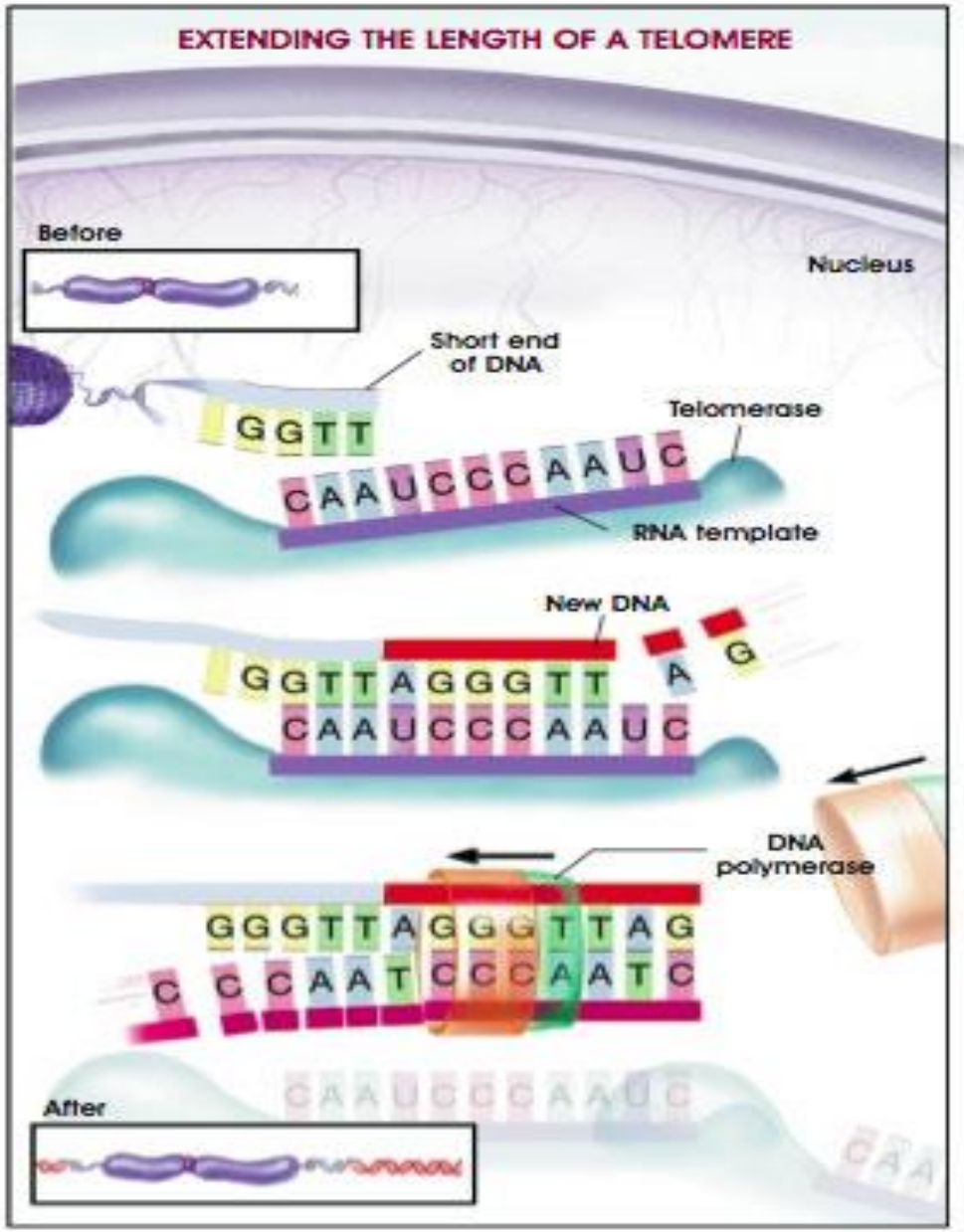
1) Patient will inherit any potential diseases that the embryo may have.
2) There is a significant potential that the cells can grow unchecked and essentially act as a tumor.
3) There are certain immunogenic factors. Will the body attack the stem cells as being foreign invaders? The patient may be required to take drugs to ward off cell rejection.

IPS CELLS



1) THESE CELLS ARE PRODUCED FROM ADULT CELLS WHICH ARE MANIPULATED INTO BECOMING STEM CELLS BY ENZYMATIC OR VIRAL MEANS
2) THE PROBLEM WITH IPS CELLS IS THAT THEIR TELOMERES (DNA ENDS) ARE OLD AND SHORTENED
3) THINK OF DOLLY THE CLONED SHEEP. DOLLY DIED AT A YOUNG AGE OF OLD AGE DUE TO THE FACT OF TELOMERE AGING.
4) THERE IS POSSIBILITY OF ACTIVATING ONCOGENES WHICH PRODUCE CANCER
5) NOBLE PRIZE IN MEDICINE 2012

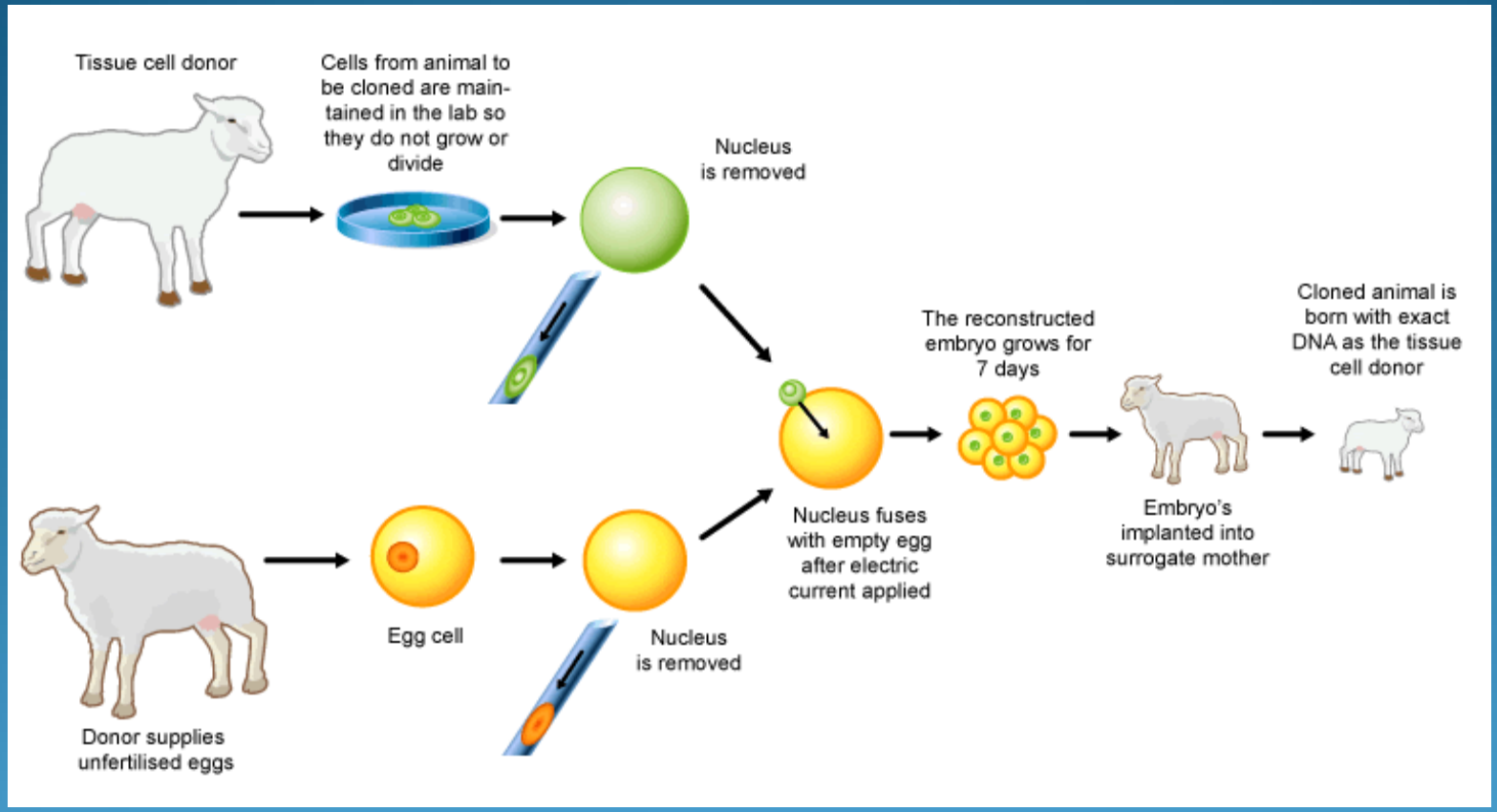

TELOMERES
Embryonic Stem Cells
Adult Stem Cells
Chromosome
Telomere long
Telomere short
Telomerase active
Telomerase inactive or absent
A – T
A – T
T – A
C – G
C – G
C – G
Telomere is a repeating DNA sequence


EXTENDING THE LENGTH OF A TELOMERE
Before
Nucleus
Short end of DNA
G G T T
Telomerase
C A A U C C C A A U C
RNA template
New DNA
G G T T A G G G T T
A
G
C A A U C C C A A U C
DNA polymerase
G G G T T A G G G T T A G
C C C A A T C C C A A T C
After

“NEW SCIENTIST” JULY 2015

1. Mutation alert halted IPS stem cell trial to cure blindness
2. Six mutations were observed in the IPS induced cells
3. One mutation involved an activation of an Oncogene associated with a cancer risk.
4. It is believed that the mutations were related to the IPS cell technology.

Somatic Nuclear Transfer Cells



Ethically =Cloning

CAN NUCLEAR TRANSFER REVERSE AGING ??? YES BUT

1. It appears that the nuclear transfer will add on telomere length to the existing adult DNA (research has been performed by Oregon Health & Science University)
2. The imperfect embryos prevented the acquisition of human ESC. The ESC obtain were found to be capable of producing teratomas, expressed pluripotent transcription factors, and expressed a normal 46XX karyotype, indicating these SCNT were in fact ESC-like. This was the first instance of successfully using SCNT to reprogram human somatic cells.

VERY SMALL EMBRYONIC LIKE STEM CELLS

1. ALSO CALLED BLASTOMERES,STEMBIOS CELLS, OR V Cells
2. FOR TISSUE REGENERATION AND ANTI-AGING APPLICATIONS
3. THESE CELLS ARE PLURIPOTENT
4. THEY MAY ELIMINATE THE NEED TO MANIPULATE OR CULTURE CELLS

Human VERY SMALL EMBRYONIC Like Stem Cells Primordial Adult Stem Cells

1. VSEL- a unique type of adult-derived stem cell.
   - Nearly Totipotent
   - Self-renewal
   - Clonal populations

2. Unique Physical Properties.
   - Display distinct cell surface marker
   - Very small cells (≤3 μm)

3. *In vitro* cultivation
   - Serum-free media
   - Suspension and adherent cell cultures

Mobilization Studies of Circulating VSELs



- **25-30% Increase in the number of circulating VSELs following**
  - **Intense physical stress (1hr. of running)**
  - **Ingestion of a nutriceutical (1 hr. post-ingestion)**
- **Transient effect**
  - **Cell numbers returns to baseline 2-3 hrs. after exercising**
  - **Cell numbers returns to base line after 4-6 hrs. after ingestion**
- **50% Decrease cell numbers**
  - **After 1 week of antibiotic chemotherapy**
  - **Rebound effect 72 hrs. post-therapy**

PROPRIETARY FORMULA WILL DRAMATICALLY INCREASE NUMBERS

Stem Cell Characteristics

Precursor Cell Characteristics

	ProgCs	GLLSCs	ELSCs	VSELs
Size	Variable	10-20 μm	6-8 μm	≤ 2 μm
Cryopreserved	Liquid N_2	-70ºC	-80ºC	-80ºC
SF-Medium	Quiescent	Quiescent	Quiescent	Quiescent
Commitment	Lineage-Sp	GL-Specific	Uncommitted	Uncommitted
Telomerase	Negative	Positive	Positive	Positive
Lifespan	Hayflick's	Extended	Extended	Extended
Confluence	Contact inhib	Contact inhib	Non-contact inhib	Non-contact inhib
BF Activities	Proliferation	Proliferation	Proliferation	Proliferation
Progression	Accel Expr	No effect	No effect	No effect
Induction	No effect	Lin Com-Prog	Lin Com-GLLSCs	ELSCs
Antibodies	Cell Specific	GLL-Specific	Embryonic	Embryonic
CD Markers	Cell Specific	CD10, CD13, CD90, MHC-I	CD10, CD66e	CD66e
Cells Formed	Lineage-Sp	GLL-Specific	Ectodermal, Mesodermal, Endodermal	Ectodermal, Mesodermal, Endodermal, Gametes

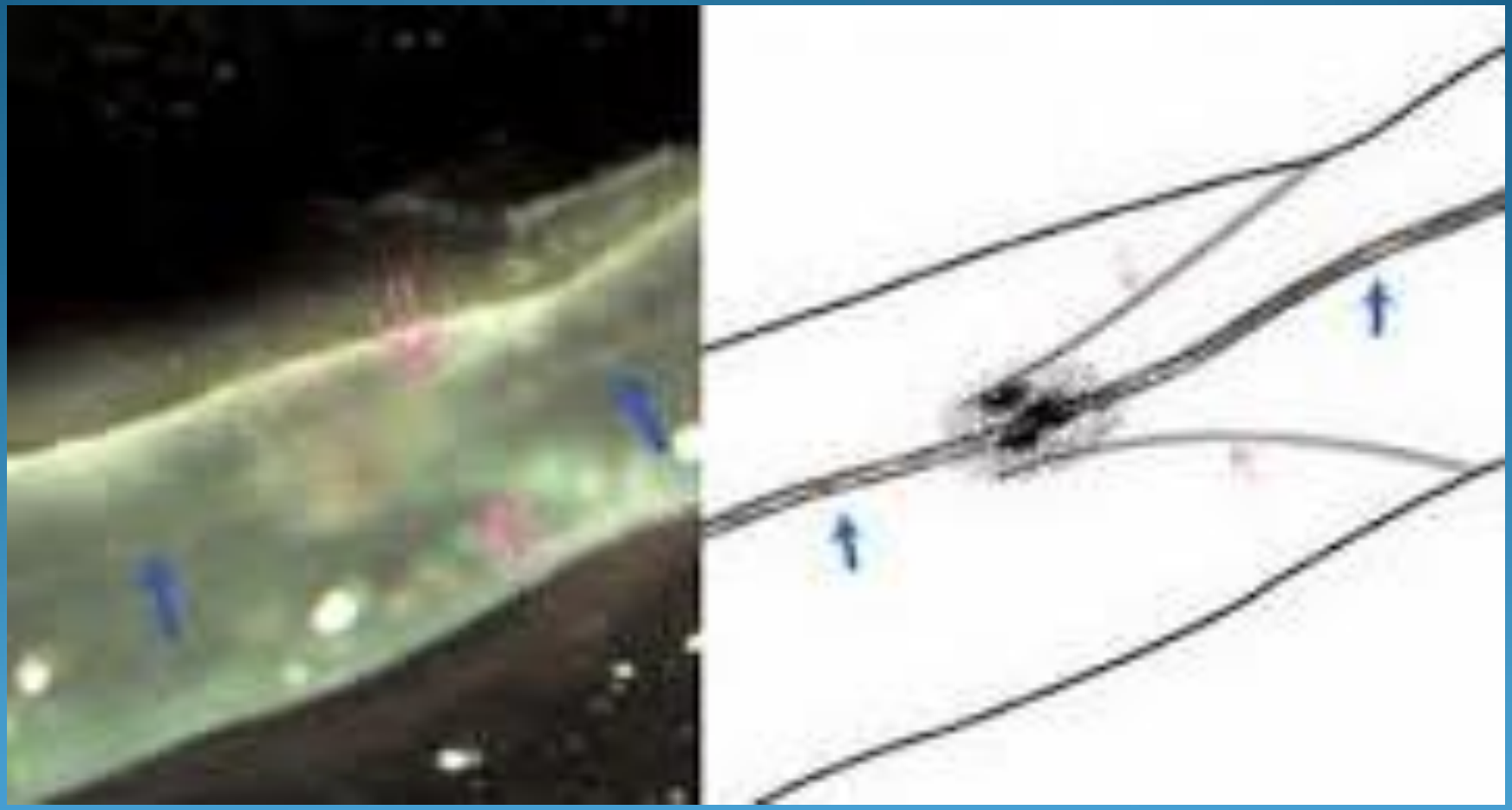
GLLSCs = GLL-EctoSCs, GLL-MesoSCs, & GLL-EndoSCs

In vivo implantation: adult stem cells remain quiescent or incorporate during tissue repair

ACUPUNCTURE AND STEM CELLS

1. There are threadlike channels that correspond to traditional acupuncture meridians.
2. These channels are called Bonghan Channels.
3. Bonghan Channels contain Hyaluronic acid and chromosomal material highly reactive to stem cell antibody stains.
4. When isolated the chromosomal material grew into cells of all three germ layers.
5. Acupuncture seems to stimulate these channels and thus the stem cells within the channels

BONGHAN CHANNELS

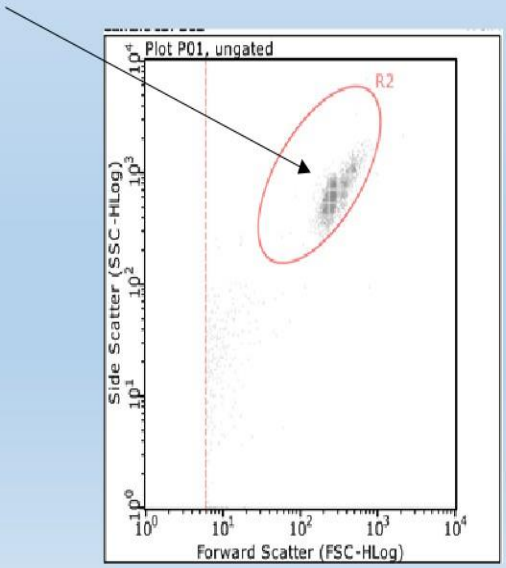


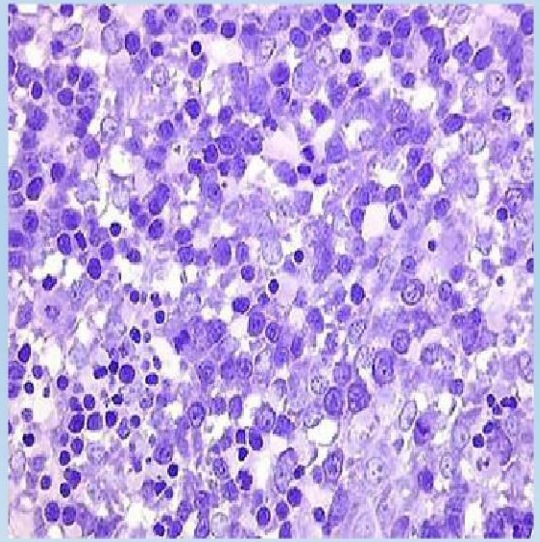
- **Pluripotency allows the cells to evolve to all tissue lineages of the three primary germ layers.**

- **With their potential for unlimited expansion, pluripotent cells are a potential source for regenerative medicine and tissue replacement after injury or disease.**

- **Through various analyses it has been determined that the cells in question are roughly 3µm in size and express stem cell markers such as Sox2, Oct4 and CD90 as well as other interesting markers such as PTHR1**

3-3.4µm size bead



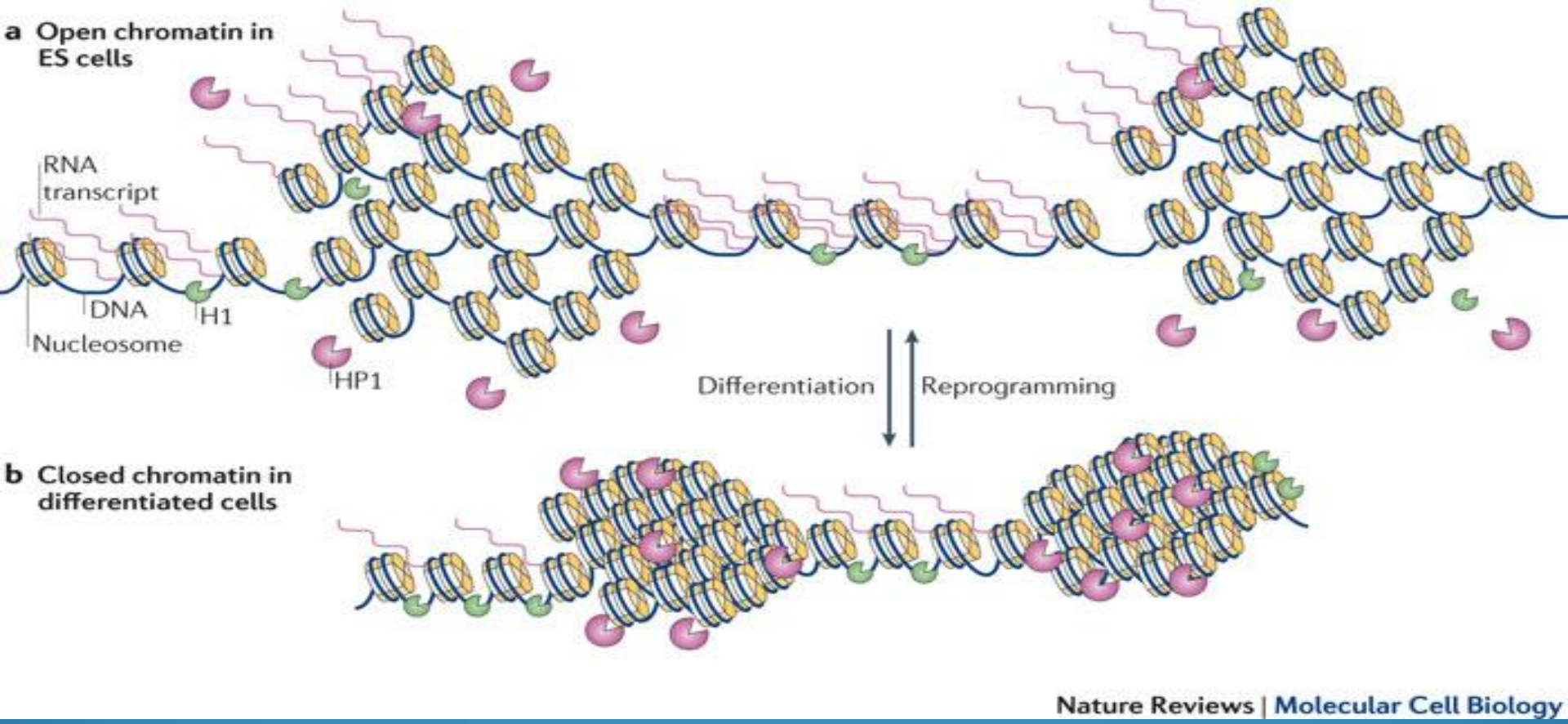


Yamanaka Factors



1. Through various analysis and experimental method we have been able to distinguish a cell population that exhibits multiple stem cell surface markers. That the cells express multiple markers at the same time. More importantly that they express PTH Receptors.
2. The cells are negative for markers of CD45 and CD200
3. Furthermore, we have been able to isolate these cells simply and efficiently.
4. These cells are exciting as they appear to be pluripotent and exhibit different differential markers at different time points as they start to mature. The cells have been turned, in vitro, into all three lineages (mesoderm, ectoderm and endoderm).
5. The cells proliferate and differentiate with intermittent doses of PTH or PTHrP both in vitro and in vivo.
6. Thus far, the cells isolated hold huge potential for various realms in regenerative medicine.

WHAT ARE SOME OF THE EFFECTS OBSERVED WITH THESE CELLS?



The structure of chromatin differs between undifferentiated embryonic stem (ES) cells (a) and differentiated cells (b) in several ways. Chromatin structure becomes more condensed upon differentiation and more open upon reprogramming. In ES cells, chromatin is globally decondensed; there are fewer heterochromatin foci and they are larger and more dispersed compared with those of differentiated cells. Architectural chromatin proteins, represented here by the histone H1 and heterochromatin protein 1 (HP1), are loosely bound to chromatin in ES cells and are bound more tightly to chromatin in differentiated cells.

PARATHYROID (PTH) AND PARATHYROID RELATED PROTEIN (PTHrP)

1. This now seems to be a novel disease modifying therapy for osteoarthritis which holds great clinical potential.
2. The effect on the V cells may be profound!!
3. **THE TRICK IS WHEN, HOW, AND WHAT DOSAGE TO USE**

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Author Manuscript
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Teriparatide, a Chondro-Regenerative Therapy for Injury-Induced Osteoarthritis

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Abstract

There is no disease-modifying therapy for osteoarthritis, a degenerative joint disease that is projected to afflict more than 67 million individuals in the US alone by 2030. As disease pathogenesis is associated with inappropriate articular chondrocyte maturation resembling that seen during normal endochondral ossification, pathways that govern the maturation of these cells are candidate therapeutic targets. It is well established that parathyroid hormone (PTH) induces matrix synthesis and suppresses maturation of chondrocytes via the type 1 PTH receptor. We have found that the PTH receptor is up-regulated in articular chondrocytes following meniscal injury and during osteoarthritis in humans and in a mouse model of injury-induced knee osteoarthritis. Thus, we hypothesized that recombinant human PTH(1-34) (teriparatide) would inhibit aberrant chondrocyte maturation and associated articular cartilage degeneration. To test this, we administered systemic teriparatide (Forteo), an FDA-approved treatment for osteoporosis, either immediately after or 8 weeks after meniscal/ligamentous injury in mice. Knee joints were harvested at 4, 8, or 12 weeks post-injury to examine the effects of teriparatide on cartilage degeneration and articular chondrocyte maturation. Confirming successful systemic delivery of the drug, micro-computed tomography revealed increased bone volume within joints from teriparatide-treated mice compared to saline-treated controls. Immediate systemic administration of teriparatide increased proteoglycan content and inhibited articular cartilage degeneration, whereas delayed treatment beginning 8 weeks post-injury induced a regenerative effect. The chondro-protective and chondro-regenerative effects of teriparatide correlated with decreased levels of type × collagen, Runx2, matrix metalloproteinase-13 and the c-terminal aggrecan cleavage product NITEGE. These preclinical findings provide proof-of-concept that teriparatide (Forteo) may be useful for decelerating cartilage degeneration and inducing matrix regeneration in osteoarthritis patients.

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†Both authors contributed equally to this work.

Author contributions: Study design and experimental planning: E.R.S., M.J.H., R.A.M., D.C., E.M.S., S.V.B., R.J.O., H.A., J.E.P., R.N.R., and M.J.Z. Execution of experiments and data collection: E.R.S., M.J.H., Y.T., R.N.R., and M.J.Z. Data analysis and interpretation: E.R.S., M.J.H., R.A.M., E.M.S., R.J.O., H.A., J.E.P., R.N.R., and M.J.Z. Preparation of the manuscript: E.R.S., R.N.R., and M.J.Z.

Competing interests: S.V.B. is a consultant for Eli Lilly; E.R.S., H.A., S.V.B., R.J.O., J.E.P., R.N.R. and M.J.Z. declare U.S. Provisional Patent Application No. 61/104,942 entitled "Protecting and repairing cartilage and musculoskeletal soft tissues" related to this work. The other authors declare no competing interests.

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Am J Physiol Heart Circ Physiol 298: H1466–H1471, 2010.
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Comparison of parathyroid hormone and G-CSF treatment after myocardial infarction on perfusion and stem cell homing

Bruno C. Huber,[1]* Rebekka Fischer,[1]* Stefan Brunner,[1] Michael Groebner,[1] Christoph Rischpler,[1] Alexander Segeth,[1] Marc M. Zaruba,[1] Tim Wollenweber,[2] Marcus Hacker,[2] and Wolfgang-Michael Franz[1]

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Huber BC, Fischer R, Brunner S, Groebner M, Rischpler C, Segeth A, Zaruba MM, Wollenweber T, Hacker M, Franz WM. Comparison of parathyroid hormone and G-CSF treatment after myocardial infarction on perfusion and stem cell homing. *Am J Physiol Heart Circ Physiol* 298: H1466–H1471, 2010. First published March 5, 2010; doi:10.1152/ajpheart.00033.2010.—Mobilization of stem cells by granulocyte colony-stimulating factor (G-CSF) was shown to have protective effects after myocardial infarction (MI); however, clinical trials failed to be effective. In search for alternative cytokines, parathyroid hormone (PTH) was recently shown to promote cardiac repair by enhanced neovascularization and cell survival. To compare the impact of the two cytokines G-CSF and PTH on myocardial perfusion, mice were noninvasively and repetitively investigated by pinhole single-photon emission computed tomography (SPECT) after MI. Mobilization and homing of bone marrow-derived stem cells (BMCs) was analyzed by fluorescence-activated cell sorter (FACS) analysis. Mice (C57BL/6J) were infarcted by left anterior descending artery ligation. PTH (80 μg/kg) and G-CSF (100 μg/kg) were injected for 5 days. Perfusion defects were determined by ^{99m}Tc-sestamibi SPECT at *days 6* and *30* after MI. The number of BMCs characterized by Lin$^-$/Sca-1$^+$/c-kit$^+$ cells in peripheral blood and heart was analyzed by FACS. Both G-CSF and PTH treatment resulted in an augmented mobilization of BMCs in the peripheral blood. Contrary to G-CSF and controls, PTH and the combination showed significant migration of BMCs in ischemic myocardium associated with a significant reduction of perfusion defects from *day 6* to *day 30*. A combination of both cytokines had no additional effects on migration and perfusion. In our preclinical model, SPECT analyses revealed the functional potential of PTH reducing size of infarction together with an enhanced homing of BMCs to the myocardium in contrast to G-CSF. A combination of both cytokines did not improve the functional outcome, suggesting clinical applications of PTH in ischemic heart diseases.

regenerative medicine; myocardial infarction; remodeling; cytokines; parathyroid hormone

IN RECENT YEARS, regeneration of damaged myocardial tissue has been attempted using stem and progenitor cells (1, 9, 17). Mobilization of bone marrow-derived stem cells (BMCs) using growth factors such as granulocyte macrophage colony-stimulating factor (G-CSF) offers an alternative to the direct injection of stem cells (10). Circulating mobilized stem cells can be recruited from the blood pool in the damaged myocardial tissue, and this homing behavior is mentioned to play a pivotal role for myocardial regeneration (3). In contrast to preclinical studies showing beneficial effects of G-CSF on myocardial function (8, 16, 18), larger clinical trials failed to demonstrate superiority of G-CSF over placebo treatment when given additionally after myocardial infarction (MI) (11, 28).

In search of alternative cytokines to G-CSF, a promising agent promoting cardiac repair is parathyroid hormone (PTH). PTH is a peptide hormone secreted from the parathyroid glands that mainly acts on bone and kidney cells and is involved in systemic calcium regulation (22). Besides its classical functions, PTH was also shown to act on cardiovascular cells. Upon PTH/PTH-related peptide receptor type I activation, PTH effects an increase of cAMP production leading to a decreased calcium influx resulting in vasodilatation (20). Moreover, it was recently shown that PTH treatment increased proliferation of bone marrow stem cells and facilitated homing to lethally irradiated recipients (7). In patients with primary hyperparathyroidism, we were able to show an increased number of circulating hematopoietic stem cells in the peripheral blood (5). In addition, PTH administration effectively induced stem cell mobilization related to an endogenous release of G-CSF without depletion of the bone marrow (6). Moreover, PTH treatment after MI in mice improved cardiac function associated with increased mobilization and homing of CD45$^+$/CD34$^+$ stem cells (21, 26). One problem of initially conducted small-animal trials was the lack of noninvasive, repetitive analyses because mice had to be killed for hemodynamic measurements by catheterization and histological analyses (8). Therefore, in the present study, we used the innovative pinhole single-photon emission computed tomography (SPECT) technique, which has recently been validated for myocardial defect size measurement in mice by comparison with histology and allows noninvasive, repetitive, quantitative, and especially intraindividual evaluations of infarct size (25). Because PTH-mediated stem cell mobilization is partly dependent on G-CSF (6), we focused our work on the impact of the two cytokines G-CSF and PTH alone and in combination on change of perfusion defect size after MI and analyzed effects of G-CSF, PTH, and the combination of both on mobilization and homing of BMCs.

MATERIALS AND METHODS

Animal model and administration of PTH/G-CSF. MI was induced in 8- to 12-wk-old male C57BL/6 mice by surgical occlusion of the left anterior descending artery (LAD) through a left anterolateral approach as described previously (26). As shown in Fig. 1, mice were divided into the following groups: *1*) intraperitoneal administration of saline (0.9% NaCl) daily for 5 days, *2*) administration of G-CSF daily for 5 days [100 μg·kg^{-1}·day^{-1} ip; Amgen Biologicals (8)], *3*) administration of PTH daily for 5 days [80 μg·kg^{-1}·day^{-1} ip; Bachem (6, 26)], and *4*) administration of G-CSF (100 μg·kg^{-1}·day^{-1} ip) and PTH (80 μg·kg^{-1}·day^{-1} ip) daily for 5 days. Because

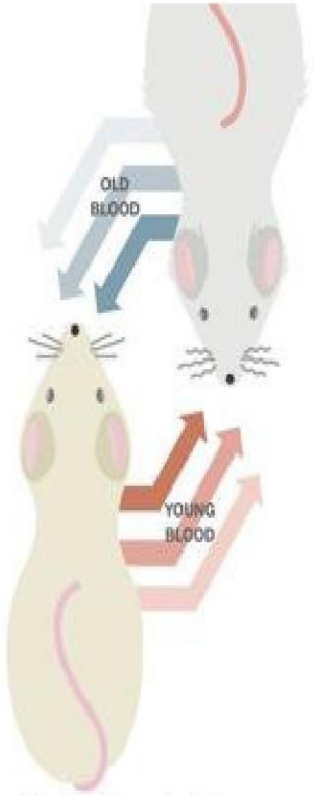
* B. C. Huber and R. Fischer contributed equally to this work.
Address for reprint requests and other correspondence: W.-M. Franz, Dept. of Internal Medicine I, Ludwig-Maximilians-Univ., Campus Grosshadern, Marchioninistr. 15, D-81377 Munich, Germany (e-mail: Wolfgang.Franz@med.uni-muenchen.de).

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Youth serum for real?

This year, in work with profound implications for aging, researchers showed that blood or blood components from a young mouse can rejuvenate an old mouse's muscles and brain. If the results hold up in people—an idea already in testing—factors in young blood could offer the antidote to aging that humanity has sought as far back as Juan Ponce de León's quest for the Fountain of Youth.

These findings grew out of strange-sounding experiments dating back 150 years, in which researchers sew together the skins of two mice to join their circulation. In the early 2000s, the approach was revived to study stem cells. Researchers found that when they connected the circulation of young and old mice, the muscle stem cells in the old mice were better able to regenerate muscle.



Old mice thrive when their circulatory systems are linked with those of younger mice, for reasons not yet clear.

Work published in 2014 strengthened the evidence that something in young blood can reverse multiple signs of aging. One group studied a factor isolated from young mouse blood called GDF11, which had already been shown to rejuvenate the heart. They found that it can also boost the muscle strength and endurance of an old mouse and spur neuron growth in the brain. Another team reported that young blood, or even cell-free blood plasma, bolsters an aging mouse's spatial memory.

Now, in the first clinical trial, 18 middle-aged and elderly Alzheimer's patients are receiving injections of plasma donated by young adults. By this time next year, we may know if young blood can fight one of the most feared diseases of aging.

–Jocelyn Kaiser

THE NEW ERA OF AGE REVERSAL

1. Intravenous Very Small Embryonic Like Stem Cells two times per year
2. Propriety oral cytokine formulas taken sublingually on a daily basis
3. Tailored supplement program which will stimulate stem cell numbers, telemerase activity and well being
4. Possible transfusion of V cells between young and old relatives with the same blood type--- HAS BEEN DONE ALREADY !!!

OFFICE STEM CELLS

1) HEMATOPOIETIC STEM CELLS
2) BONE MARROW STEM CELLS
3) FAT STEM CELLS
4) VERY SMALL EMBRYONIC LIKE STEM CELLS

MESENCHYMAL STEM CELLS

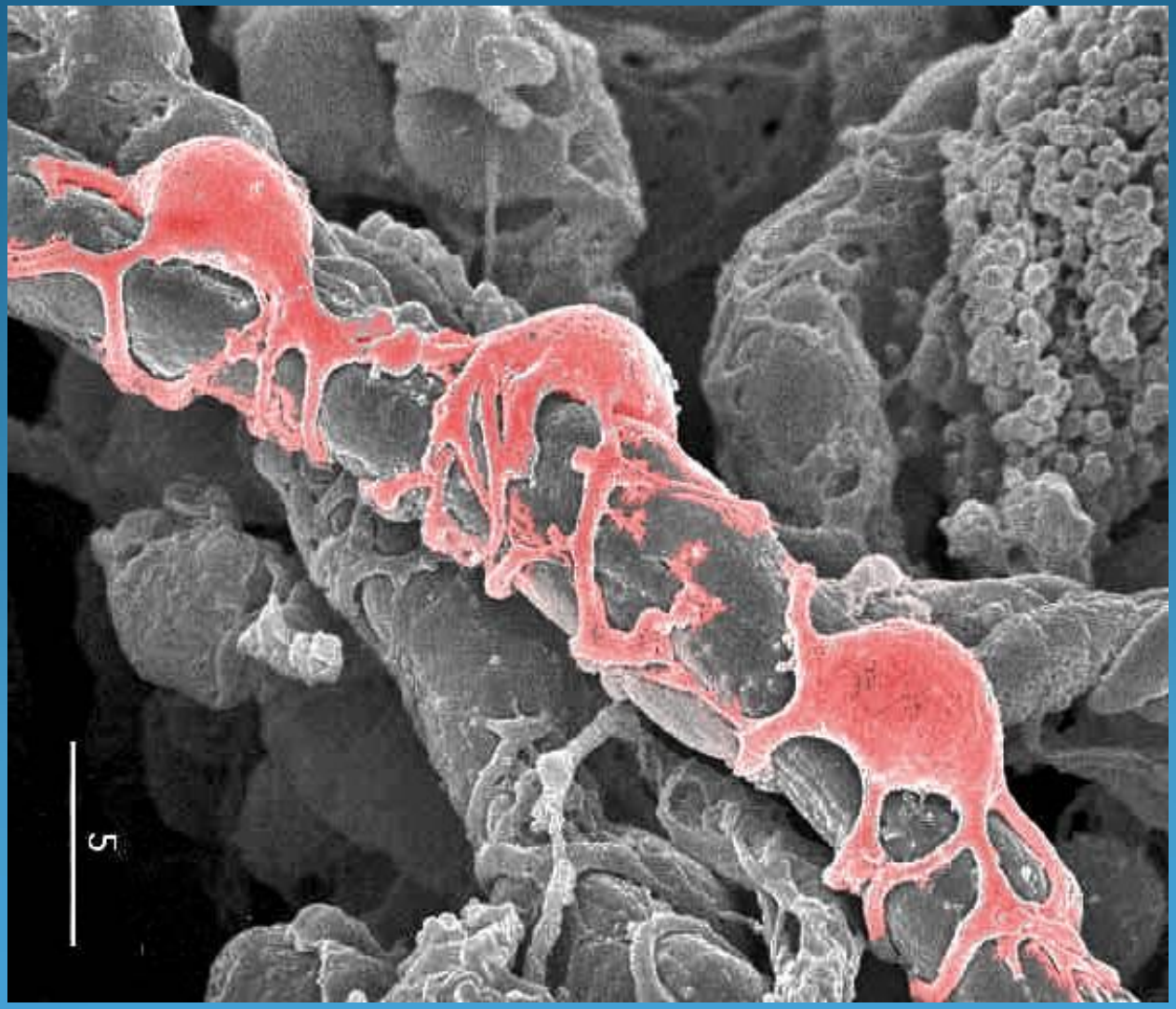
1. These are stem cells that help repair muscle, bone, cartilage, or tendons.
2. These are commonly called adult stem cells.
3. These stem cells are autologous meaning that they are from the same patient therefore no risk of genetic disease transmission.
4. **NOT THE OMNIPOTENT CELL AS ONCE THOUGHT**
5. **WITH CURRENT THINKING THEY MAY NOT EVEN BE CONSIDERED STEM CELLS**

MESENCHYMAL STEM CELLS

1. First is the realization that this class of cells can be isolated from almost every tissue in the human body. The central connecting aspect to explain this fact is that all of these tissues are vascularized and that every blood vessel in the body has mesenchymal cells in abluminal locations. These perivascular cells can be summarily called Pericytes.
2. MSCs are being used therapeutically because they undergo homing to sites of inflammation or tissue injury and they ***secrete massive levels of bioactive agents that are both immunomodulatory and trophic***
3. ***MSCs = medicinal signaling cells***

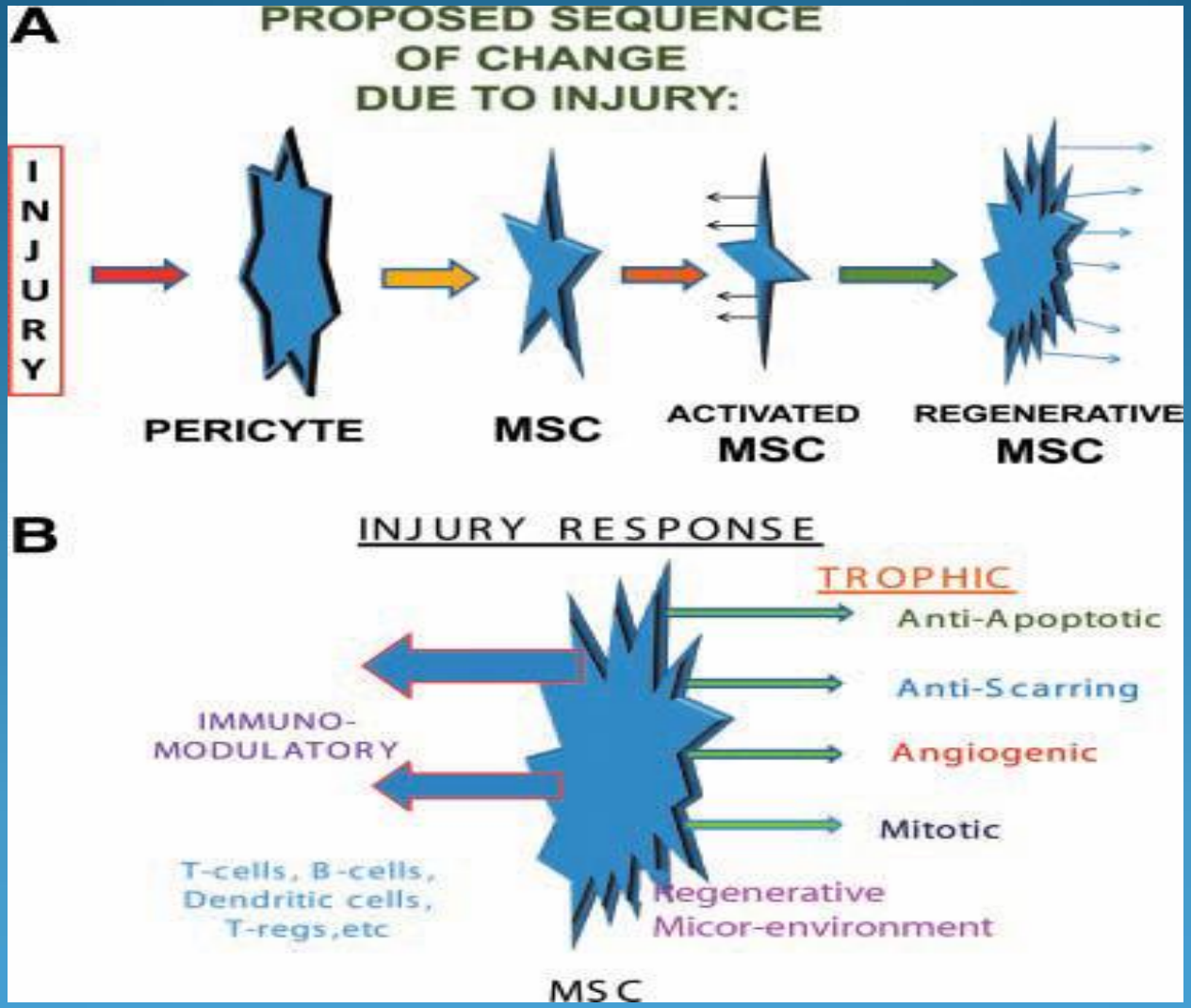
Pericytes: cells on capillaries and micro vessels.

ALL MSCs are PERICYTES!



modified by *BRUNO PEAULT* from http://www.geocities.co.jp/HeartLand-Suzuran/9389/kekkan

Injury response of pericytes



ANY RISKS WITH MESENCHYMAL STEM CELLS?

1. Since these cells are the patients' own there are minimal risks to the patient.
2. As of 2014 there are over 18,000 studies on this cell line.
3. FDA states it is ok to use these cells as long as they are put back into the same patient and they are minimally manipulated.

HOW ABOUT GROWING THESE MESENCHYMAL CELLS IN THE LAB?

1. There are studies that suggest that manipulating these cells outside the body such as culturing them can diminish their effectiveness.
2. Possibility exists that culturing the cells might lead to tumors. Probably effects telomeres.
3. Culturing the cells misses a host of other cells that are crucial in the overall repair process
4. **FDA CONSIDERS THESE CULTURED CELLS A DRUG AS PER RECENT COURT RULING!!**
5. **You are missing the "soup" of bone marrow aspirate .**

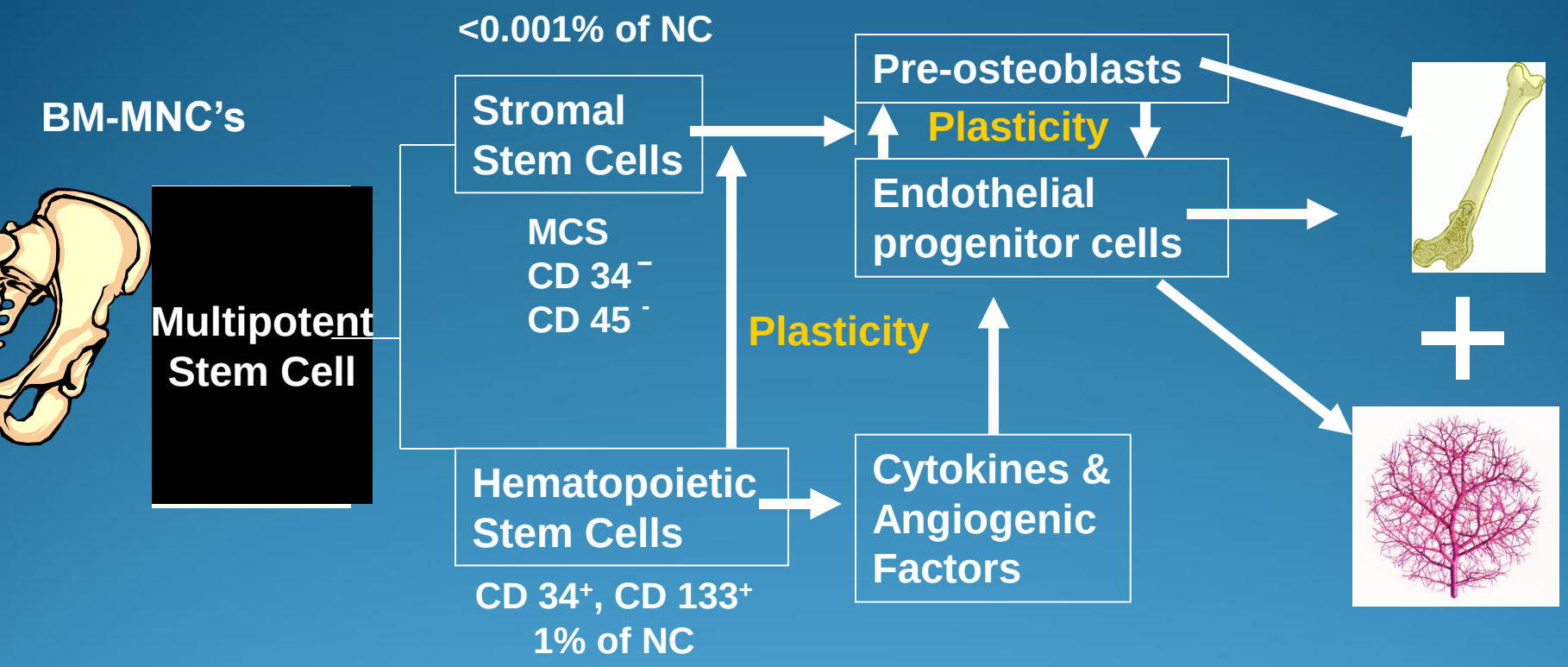
Hematopoietic Stem Cells

THE REAL WORKERS

Hematopoietic stem cells

1. These are the cells that form blood products such as white and red blood cells.
2. They help establish a blood supply where there previously had not been one.
3. They have the ability to turn into other type of stem cells by **the principle of plasticity**

Stem Cell Mechanism of Action



- **Both the stromal and hematopoietic stem cells work in concert to achieve tissue regeneration.**
- The presence of hematopoietic stems cells augment the limited number of available stromal cells

ADIPOSE (FAT TISSUE) TISSUE

ADIPOSE TISSUE IS A TREASURE TROVE OF STEM CELLS!!

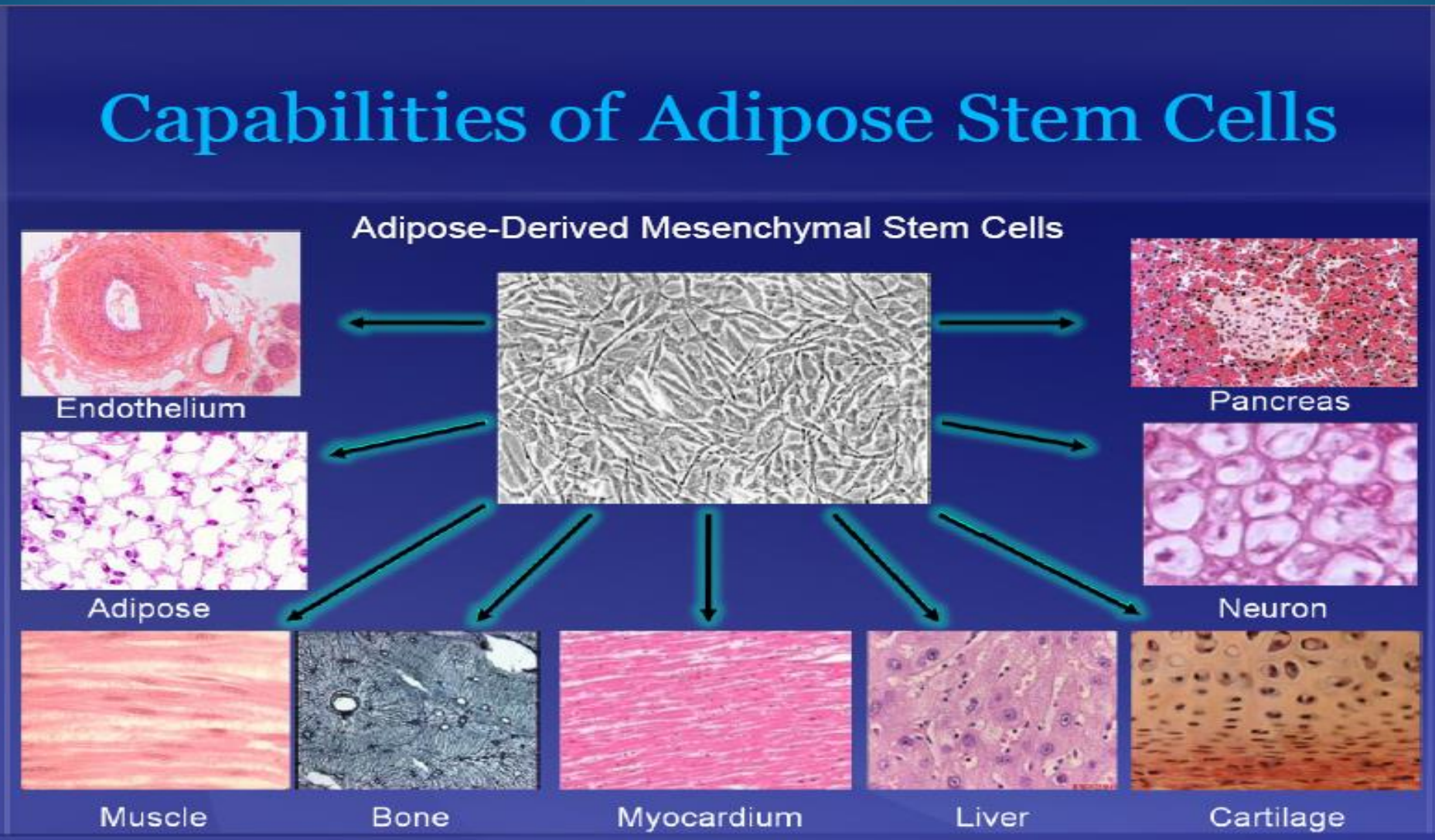


Fat is a "High Density" Source of Stem Cells

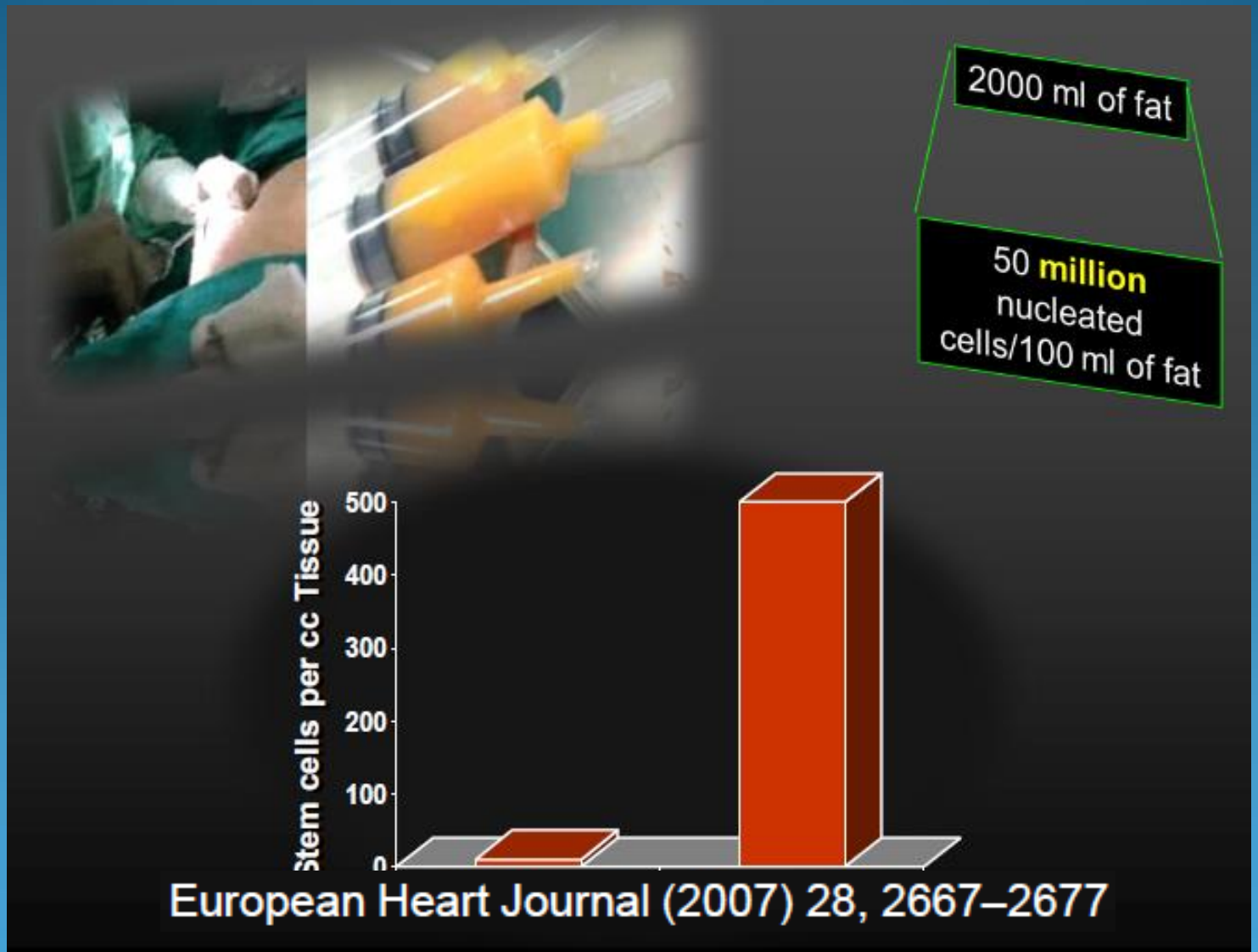
Tissue/Source of SCs	Stem Cell Density
Heart	1 out of 40,000 cells
Bone marrow	1 out of 100,000 cells *
Adipose tissue	1 out of 100 cells

* In old age

ADIPOSE STEM CELLS



FAT VS BONE MARROW



Current methods of obtaining Adipose stem cells

1. CYTORI SYSTEM
2. TISSUE GENESIS SYSTEM
3. ULTRASONIC CAVITATION
4. SIMPLE LIPOSUCTION TECHNIQUE WITH DOUBLE SPIN CENTRIFUGATION
5. SIMPLE LIPOSUCTION COMBINED WITH STEM CELL EXTRACTION TEHNIQUE

Cytori Celution,Ultrasonic Cavitation and Tissue Genesis Systems

Cost is high for most office based practices. The system prepares the graft for injection. Not much work for the lab tech. **ALL ARE GRAY AREA WITH FDA!!!**

SIMPLE LIPOSUCTION TECHNIQUE

1) THIS SYSTEM IS SIMPLE, COST EFFECTIVE, SAFE, AND REQUIRES MINIMAL LEARNING AND TIME INVESTMENT .
2) THE TOTAL COST FOR THIS SYSTEM IS APPROX. $10-15

FAT STEM CELL (SVF) ISOLATION

THIS IS A PROCESS THAT UTILIZES CELL WASHINGS, CENTRIFUGATION, AND ENZYMATIC DIGESTION. THIS PROCESS EXTRACTS THE STEM CELLS FROM THE FAT PRODUCING 100-150 MILLION STEM PER CASE. TYPICALLY 60 CC. OF FAT TISSUE PRODUCES 1-2 CC. OF SVF OR FAT STEM CELLS. **GRAY AREA WITH FDA!!**

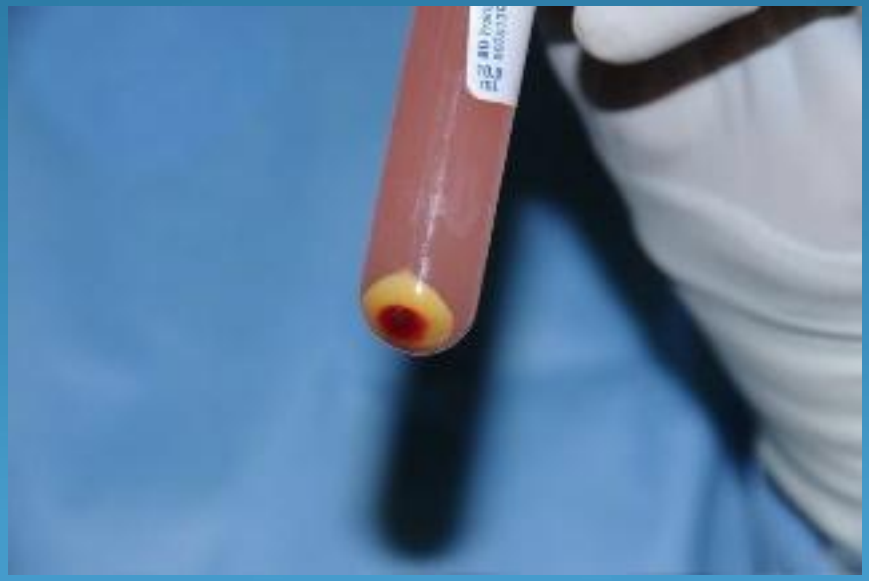
SVF POTENTIAL DRAWBACKS

1. May be throwing away the baby with the bath water. You are potentially excluding some of the most potent Regenerative Cells found in fat, namely the MUSE Cells.
2. The structural niche is irreparably damaged which dramatically reduces the viability of the cells.
3. The in vitro ADSC differentiation is not readily reproducible in the in vivo microenvironment, and therefore, after implantation, ADSCs would often fail to establish the intended cell population.

FDA THINKING ON SVF

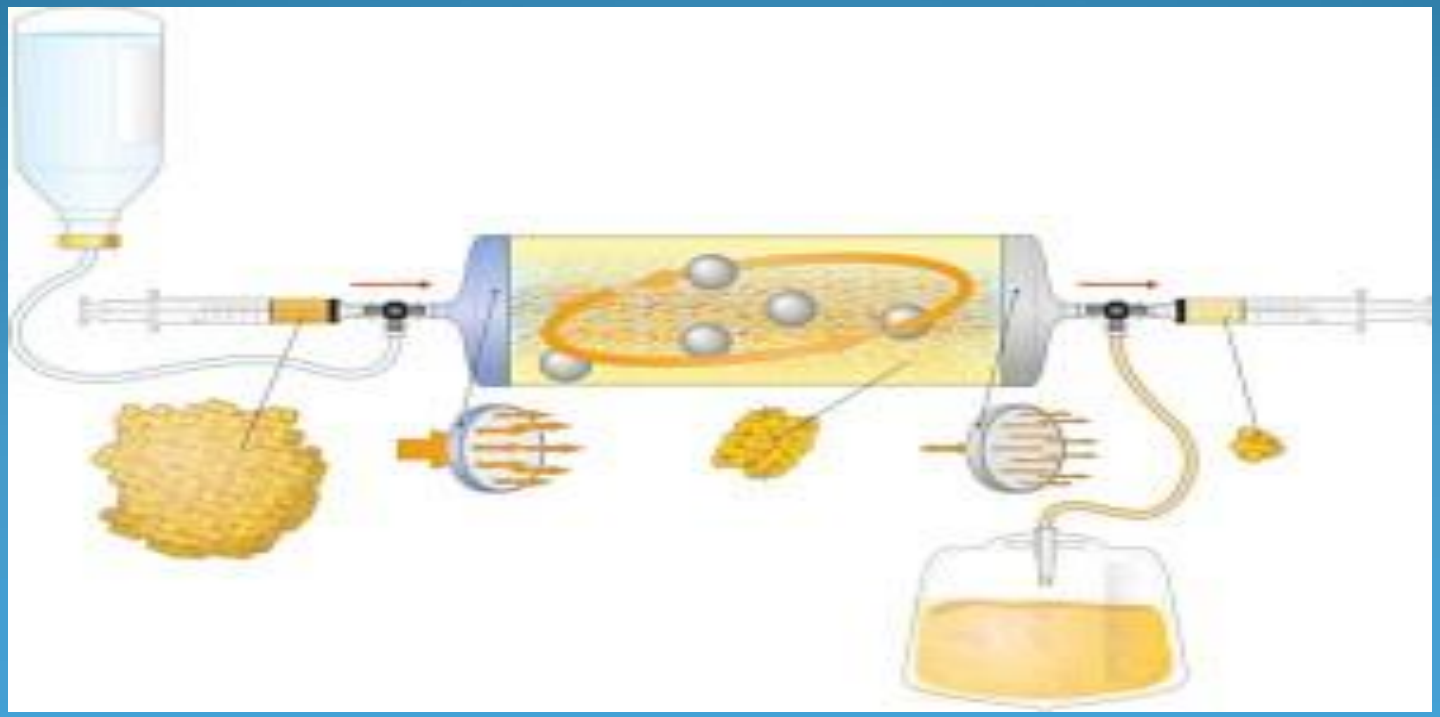
1. **The picture slowly is becoming more clear going from GRAY to BLACK**
2. **Recent Guidance (Dec. 2014) ruling "**A manufacturer ***recovers adipose tissue by tumescent liposuction*** and ***processes*** the adipose ***tissue to isolate cellular components, commonly referred to as stromal vascular fraction***, which is considered a potential source of adipose-derived stromal/stem cells. **The HCT/P generally is considered more than minimally manipulated** because the processing breaks down and eliminates the structural components that provide cushioning and support, thereby altering the original relevant characteristics of the HCT/P relating to its utility for reconstruction, repair, or replacement".

RESULTING SVF

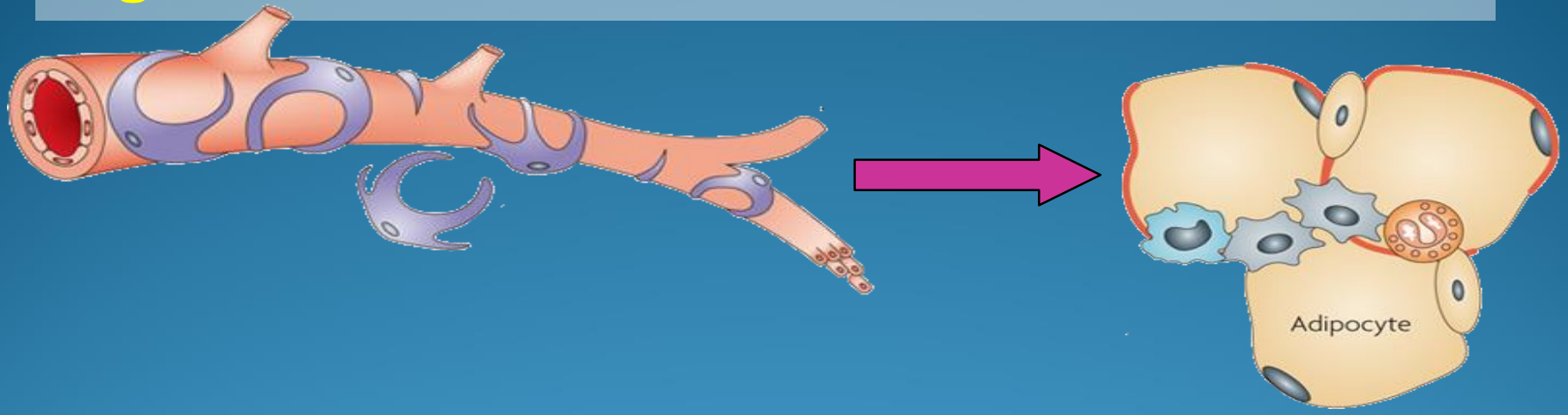


LIPOGEMS

A new non enzymatic method and device to obtain a fat tissue derivative highly enriched in pericyte-like elements by mild mechanical forces from human lipoaspirates.



Niches acts as natural scaffolds embedded in a vascular network where the stem cells naturally stay and teach tissues how to regenerate





OTHER PURPOSE OF FAT CELLS

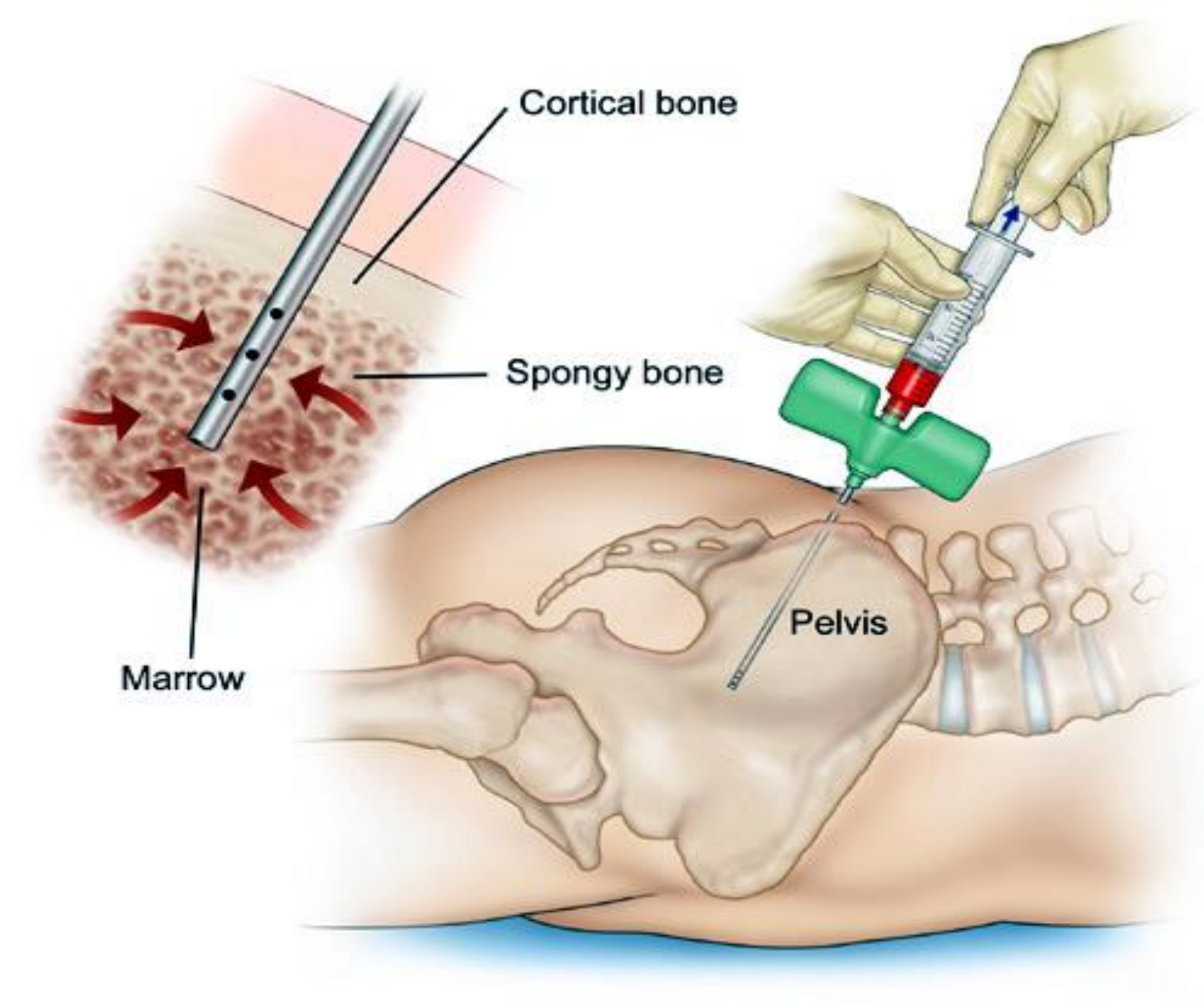
IN ADDITION TO ACTING AS A SOURCE OF STEM CELLS ***WE USE A FAT GRAFT IN JOINTS AND CERTAIN SOFT TISSUE INJURIES AS A SCAFFOLD***. THE FAT GRAFT ALLOWS STEM CELLS AND OTHER CELLS TO ADHERE TO IT AS A SCAFFOLD. IT ACTS AS A NATIVE 3-D BIOSCAFFOLD ENCOURAGING ADHESION AND PARACRINE FUNCTION ***FAT GRAFT FURTHER ACTIVATES SVF***

WHAT IS SIGNIFIANCE OF MUSE CELLS ?

1. These cells are **probably pluripotent** not multipotent like other adult stem cells.
2. These cells have a **very high survival rate upon transplantation** into other parts of the body.
3. Unlike embryonic cells they do not seem to form tumors!

BONE MARROW STEM CELLS

ONLY NECESSARY TO USE LOCAL ANESTHESIA TO ANESTHETIZE THE PERIOSTEUM

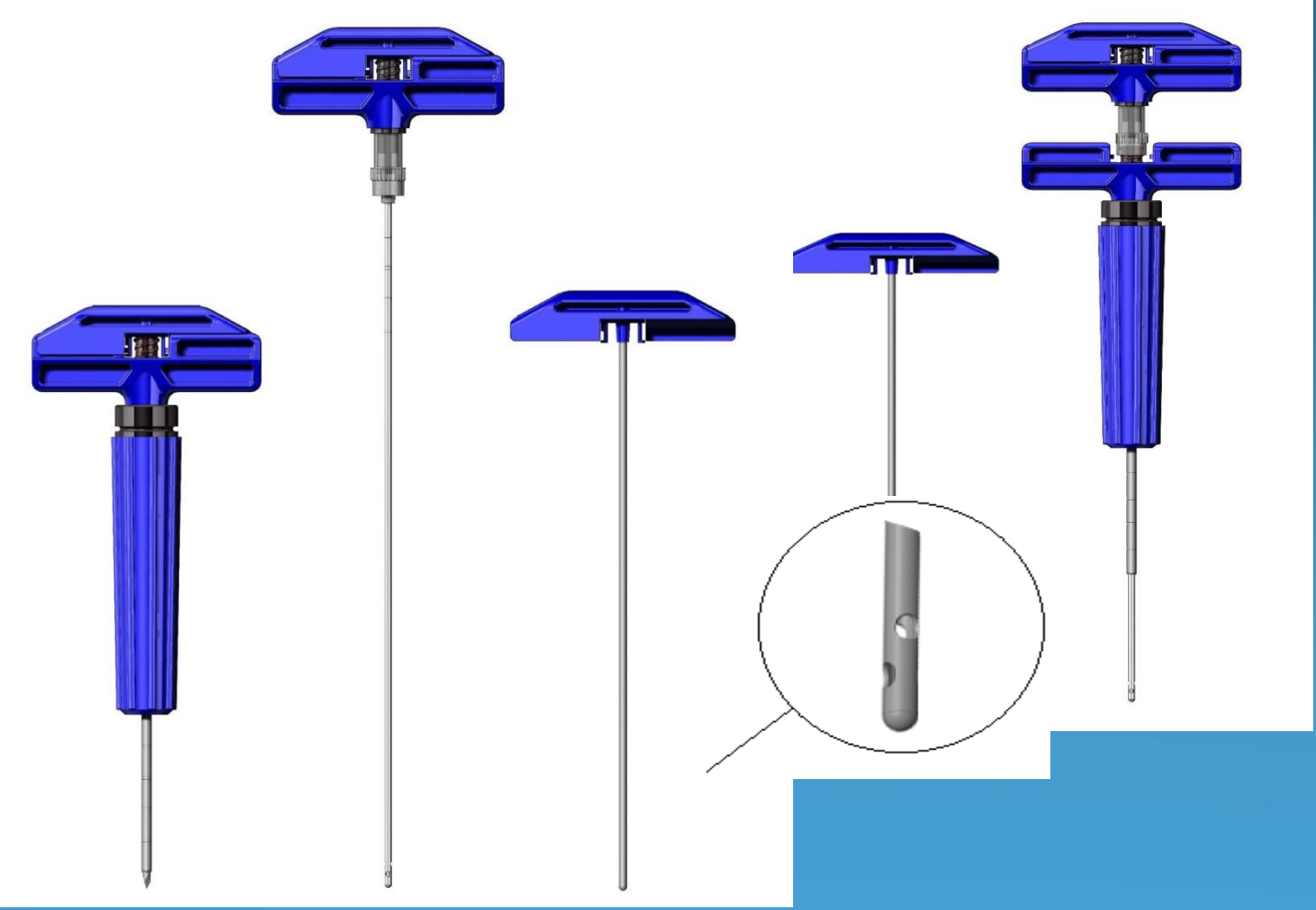

Cortical bone
Spongy bone
Marrow
Pelvis

MARROW CELLUTION NEEDLE A QUANTUM LEAP

1. **IT ALLOWS ONE CC SAMPLES TO BE TAKEN AT A TIME GIVING 10CCs OF FINISHED PRODUCT**
2. **Further, the single-step Marrow Cellution produced the same (as counted by $CD34^{+}$cells) or greater (as counted by fibroblast-like colony-forming units, CFU-f) stem/progenitor cell concentrations as a combination of traditional needles with the (BMAC) centrifuge-based cellular processing system**

Novel Design

Ranfac/Endocellutions



Dr C David B Harrell, PhD, Brt, OF, FRIPH, FAARM, DABRM

POINTERS FOR OBTAINING BONE MARROW STEM CELLS

1. WHEN DRAWING BONE MARROW ASPIRATE DO IT SLOWLY!!!!!!!!
2. REMEMBER THAT BONE MARROW ASPIRATE CONTAINS PRP
3. MANIPULATE NEEDLE BACK AND FORTH AND ROTATE AT THE SAME TIME
4. **MOST IMPORTANT ASPECT IS TO TRY TO INCREASE GEOGRAPHY OF ASPIRATION**

Aspiration Only Verses Aspiration and Centrifugation

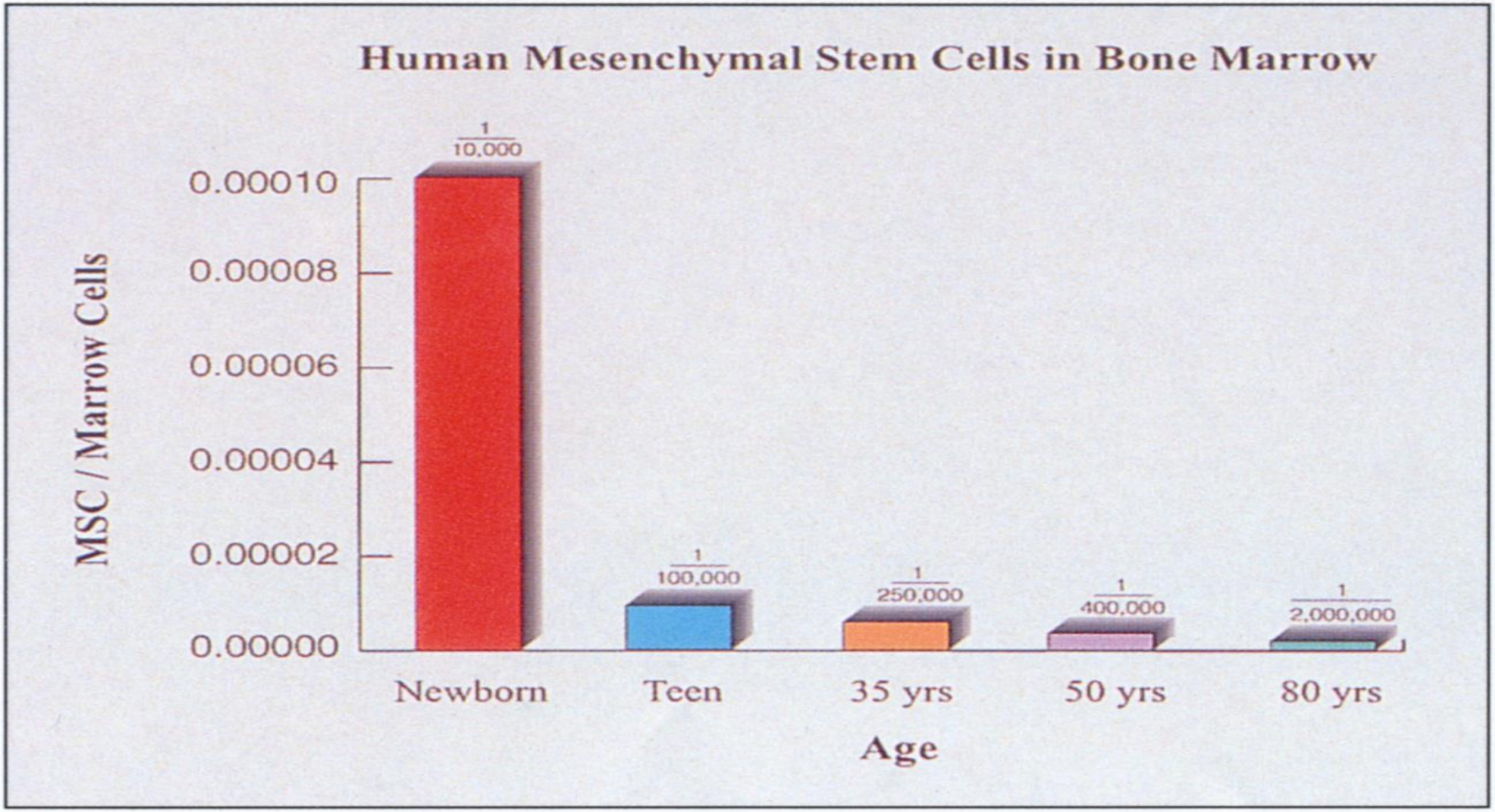
Aspiration Only Dr. JP		Aspiration & Centrifugation Lane et al HSS (1)	
CFU-f / mL	Total CFU-f	BMAC CFU-f / mL	Total CFU-f BMAC 7mL
1,199	11,990	1,014	7,100

Centrifuging Marrow:

- Does not increase the stem cell quality of the treating composition compared to a proper aspirate taken in small aliquots across a large geography
- Increases the number of contaminating peripheral blood nucleated cells
- Loses valuable cells in the 85% of the aspirate that is discarded

(1) Hegde V, Shonuga O, Ellis S, et al. A prospective comparison of 3 approved systems for autologous bone marrow concentration demonstrated nonequivalency in progenitor cell number and concentration. Journal of orthopaedic trauma 2014;28:591-8. Hospital for Special Surgery

FATHER TIME TAKES HIS TOLL ON MESENCHYMAL STEM CELLS



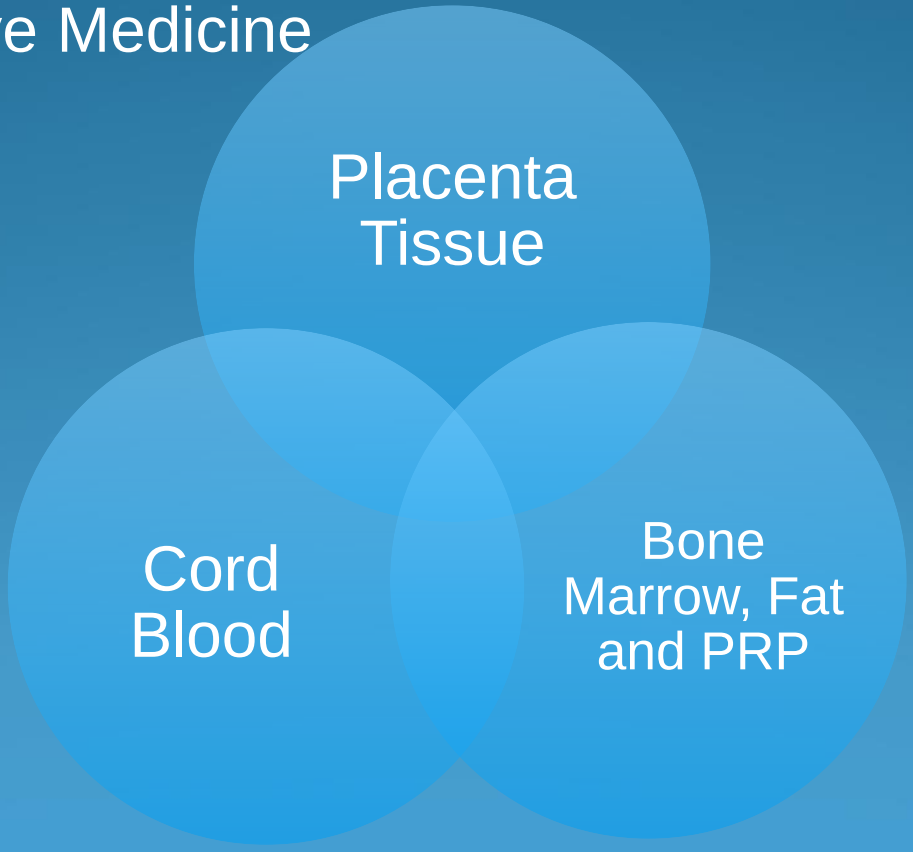
BONE MARROW VS FAT

These are both valuable sources of stem cells. THERE ARE SIGNIFICANT DIFFERENCES!!

1. Fat has more MSCs compared to Marrow. Fat has the advantage in this department.
2. Fat and Marrow have similar numbers of HSCs but those of Fat are short lived and seem to be different from the usual HSCs. Marrow has more effective HSCs and essentially greater numbers. Advantage Marrow
3. ***THE BOTTOM LINE IS TO USE BOTH!!***

Future Core Target Tissues

Combinations of Cells extracted and concentrated from these tissues hold the potential to revolutionize Regenerative Medicine



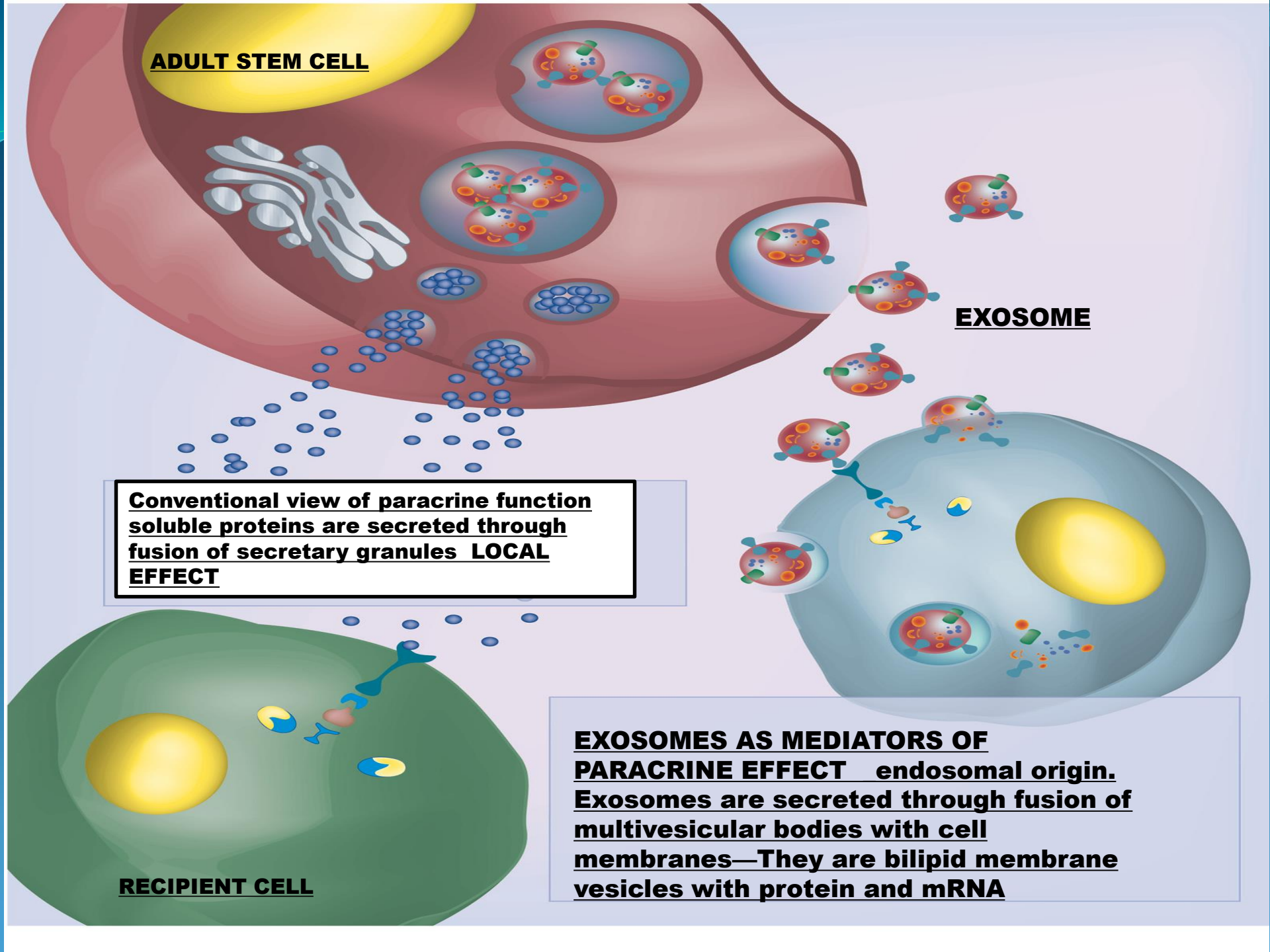
AMNIOTIC MEMBRANE COMPONENTS

1. These are Allografts that are made from human amniotic membrane tissue which consists of the amnion and chorion layers.

2. It has the whole package in that it contains, growth factors, interleukins (which contribute to the immuno-privileged properties), unique enzyme inhibitors (matrix metalloproteases), and an extracellular membrane containing many different types of collagen. **THEY DO NOT CONTAIN TRUE LIVING CELLS = ZOMBIE CELLS**

What are Exosomes ?

1. **Exosomes** are released from the cell when **multivesicular bodies** fuse with the **plasma membrane**
2. Scientists are actively researching the role that exosomes may play in **cell-to-cell signaling**, hypothesizing that because exosomes can merge with and release their contents into cells that are distant from their cell of origin, they may influence processes in the recipient cell


ADULT STEM CELL
EXOSOME
Conventional view of paracrine function soluble proteins are secreted through fusion of secretary granules LOCAL EFFECT
RECIPIENT CELL
EXOSOMES AS MEDIATORS OF PARACRINE EFFECT _endosomal origin. Exosomes are secreted through fusion of multivesicular bodies with cell membranes—They are bilipid membrane vesicles with protein and mRNA

SOURCES OF EXOSOMES

1. Saliva contains large numbers of exosomes. This enables many medicines to be rapidly absorbed sublingually (under the tongue).
2. We have a propriety method of concentrating Exosomes from the plasma

THE BOTTOM LINE FOR EXOSOMES

THINK OF THEM AS THE BODY'S FED EX SYSTEM

PHOTO MODULATION

PHOTO MODULATION SEEMS TO WORK ON BOTH PRP AND STEM CELLS. WE NOW HAVE THE NEW FIELD OF PHOTOCEUTICIALS WHICH ARE COMPOUNDS PRODUCED BY LIGHT ACTIVATION

“PHOTOCEUTICALS”

Photo activated PRP

1) PRP plus Autologous conditioned serum
2) "Healing and anti-inflammatory"
3) growth factors from platelets (healing)
4) IL1ra and IL2ra from WBCs (potent anti inflammatory)
5) beta-endorphin from WBCs (pain relieving)
6) pro-inflammatory cytokine receptor shedding from WBCs (anti-inflammatory)
7) similar to German process called Orthokine
8) May activate a primitive stem cell in blood
9) Increases Exosome production

ADISTEM LIGHT



SUMMARY OF DC STIMULATION

1. IT WILL INCREASE MICRO-CIRCULATION INCREASING VASCULAR PERMEABILITY AND ANGIOGENESIS
2. INCREASES PRODUCTION OF VEGF AND NITRIC OXIDE (NO)
3. INCREASES ATP PRODUCTION
4. INCREASES STEM CELL MOTILITY TO THE AREA


MICRO-Z MINI™

STEM CELL VS. PRP INJECTIONS

1) Stem cell injections more important in areas of **low oxygen content** (bone marrow is a low oxygen environment) such as a severely arthritic joint or disc.
2) Stem cells alone in an area will remain inactive unless they are in an environment of platelets whose growth factors activate the stem cells

WHAT MUST BE AVOIDED

1. NSAIDS USE DOES NOT MATTER THEREFORE IT IS NOT A CONCERN
2. MINIMAL ALCOHOL INTAKE. ALCOHOL WILL DIMINISH STEM CELL OUTPUT FROM MARROW
3. INACTIVITY
4. COUMADIN OK AND ASPIRIN OK IF NEEDED FOR HEART PROBLEMS
5. PLAVIX DOES NOT SEEM TO BE A PROBLEM
6. CORTISONE SHOULD BE AVOIDED

WHAT OTHER MATERIALS ARE INJECTED OR USED IN STEM CELL THERAPY?

HYPERBARIC OXYGEN

A NUMBER OF STUDIES SUGGEST HYPERBARIC OXYGEN **WILL MOBILIZE STEM CELLS IN THE BODY MAKING THEM AVAILABLE FOR REPAIR.** THIS APPEARS TO INCREASE NITRIC OXIDE PRODUCTION WHICH DIRECTLY INCREASES STEM CELL PRODUCTION AND RELEASE.

HYPERBARIC OXYGEN

Study by S. Thom et al (Univ of Penn) showed that hyperbaric oxygen will cause rapid mobilization of stem/progenitor cells in humans. ***The mobilization is thought to be caused by a nitric oxide (NO) dependent mechanism.*** Stem cell activation occurs via release of **Stem Cell active Cytokine Ckit ligand (SCF).** Over a course of 20 treatments the CD34+ cells increased eightfold.

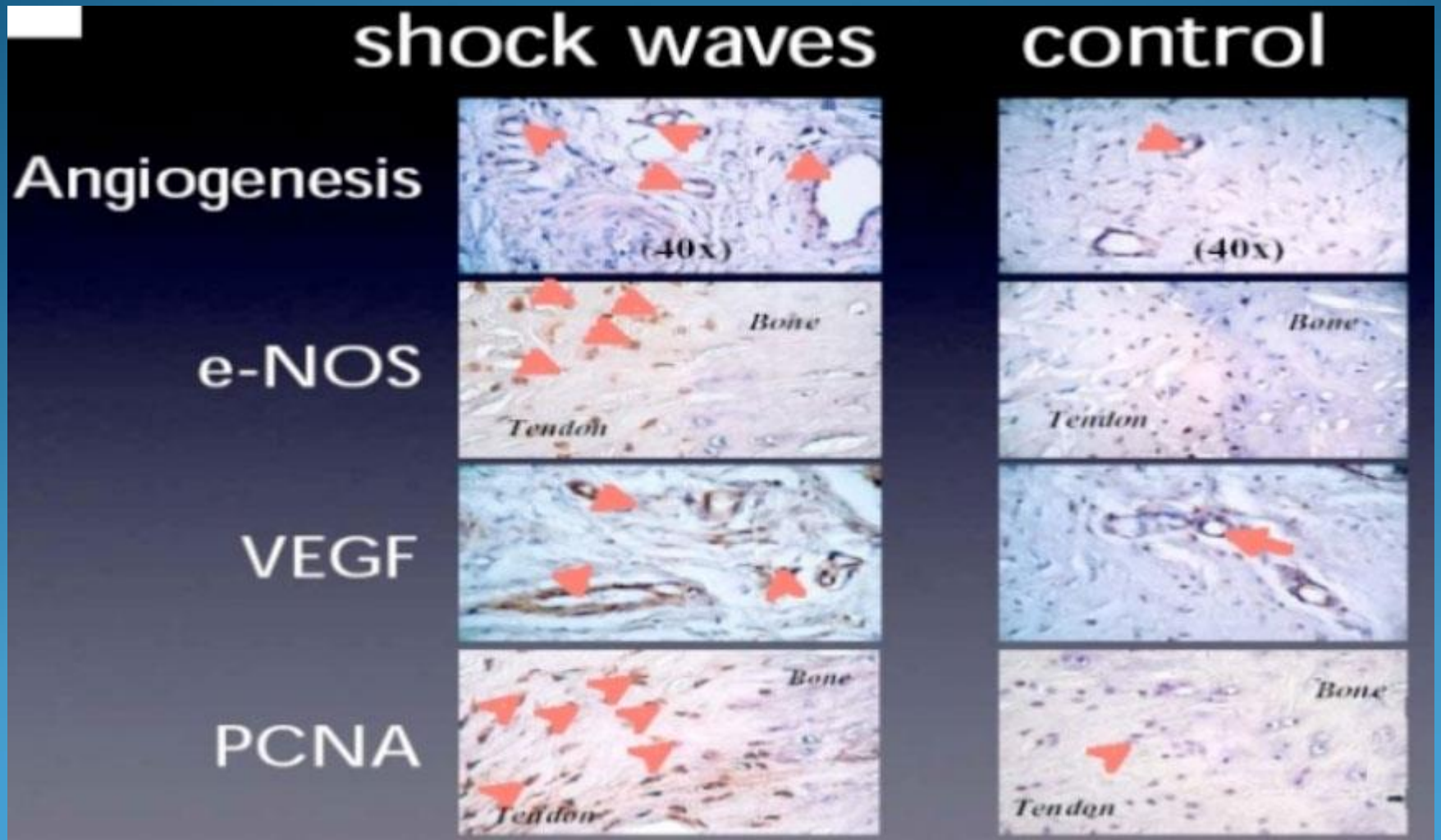
NITRIC OXIDE (NO)

NITRIC OXIDE PRODUCTION MAY BE PART OF THE HOLY GRAIL OF STEM CELL THERAPY IN THAT IT HELPS PRODUCE LARGE NUMBERS OF HEMATOPOETIC CELLS FROM THE MARROW.

EXTRACORPOREAL SHOCK WAVE THERAPY (ESWT)

1. **ESWT is a high power sound wave that causes mechanical stimulation of cells, resulting in increased expression of cytokines and growth factors.**
2. **ESWT applied to an area of chronic inflammation may enable acute inflammatory mediators to be released, facilitating appropriate progression of healing. It will allow Stem Cells to Hom to the area.**

EXTRACORPOREAL SHOCKWAVE THERAPY



low-intensity extracorporeal shockwave therapy (LI-ESWT)

- NO is non-enzymatically formed by shock wave.

  Gotte et al. FEBS Lett 2002.

- Enhance the expression of VEGF and its receptor Flt-1

  Nurzynska et al. Ultrasound Med Biol 2008, Nishida et al. Circulation 2004

- Neovascularization was confirmed by the angiogenic markers including VEGF and eNOS expressions and endothelial cell proliferation determined by PCNA expression.

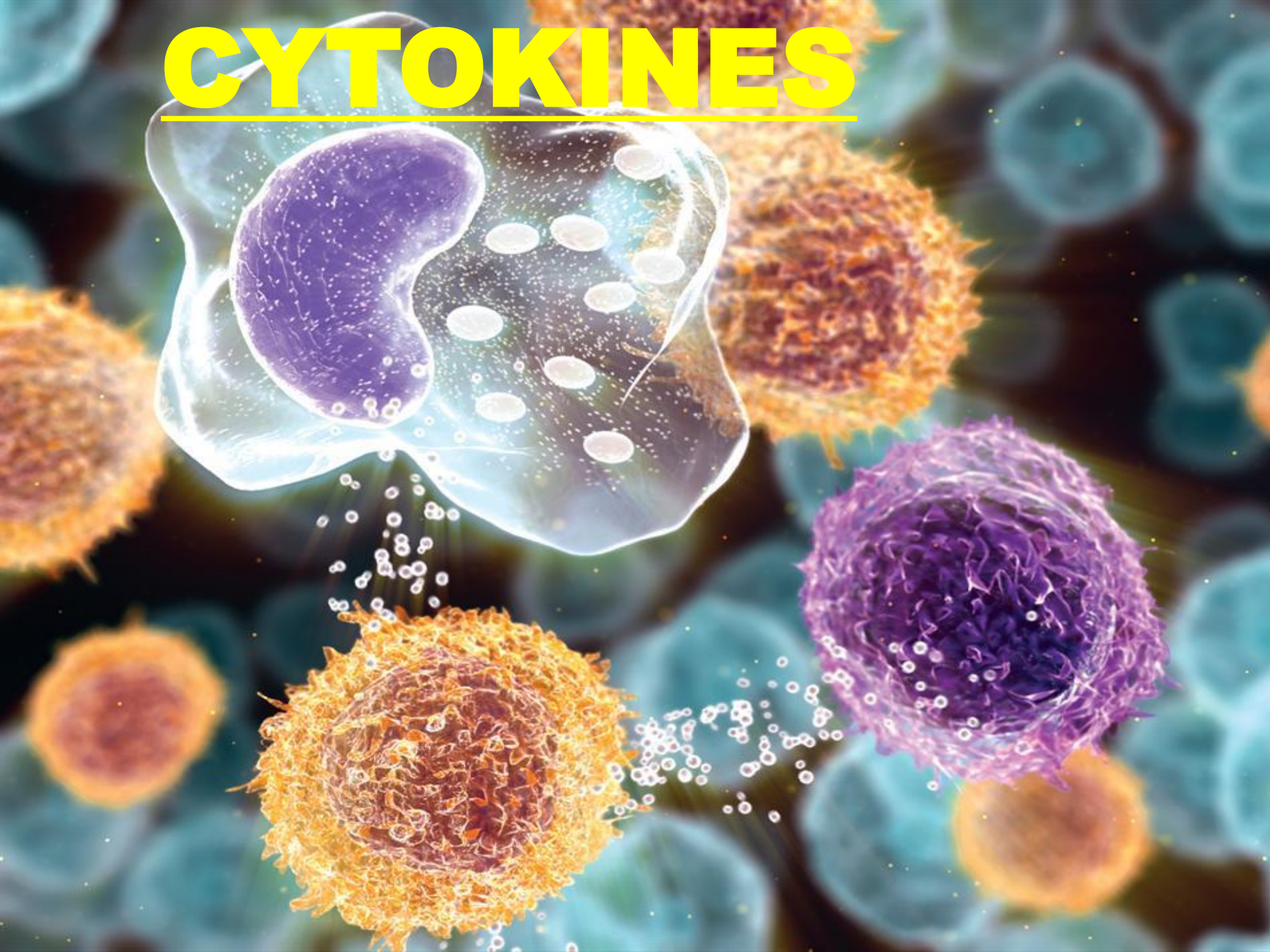
  Wang CJ et al., J Orthop Res 2003

Discovered at weSRCH.com
Where Technology = Opportunity

Biologic Effects of ESWT

1. Change of cell membrane permeability
2. Release of neurotransmitters
3. Antibacterial effects
4. Release of **NO (nitric oxide)**
5. Release of growth factors (VEGF, eNOS, BMP-2, PCNA)
6. Induction of vessel growth
7. Stem cell migration and differentiation

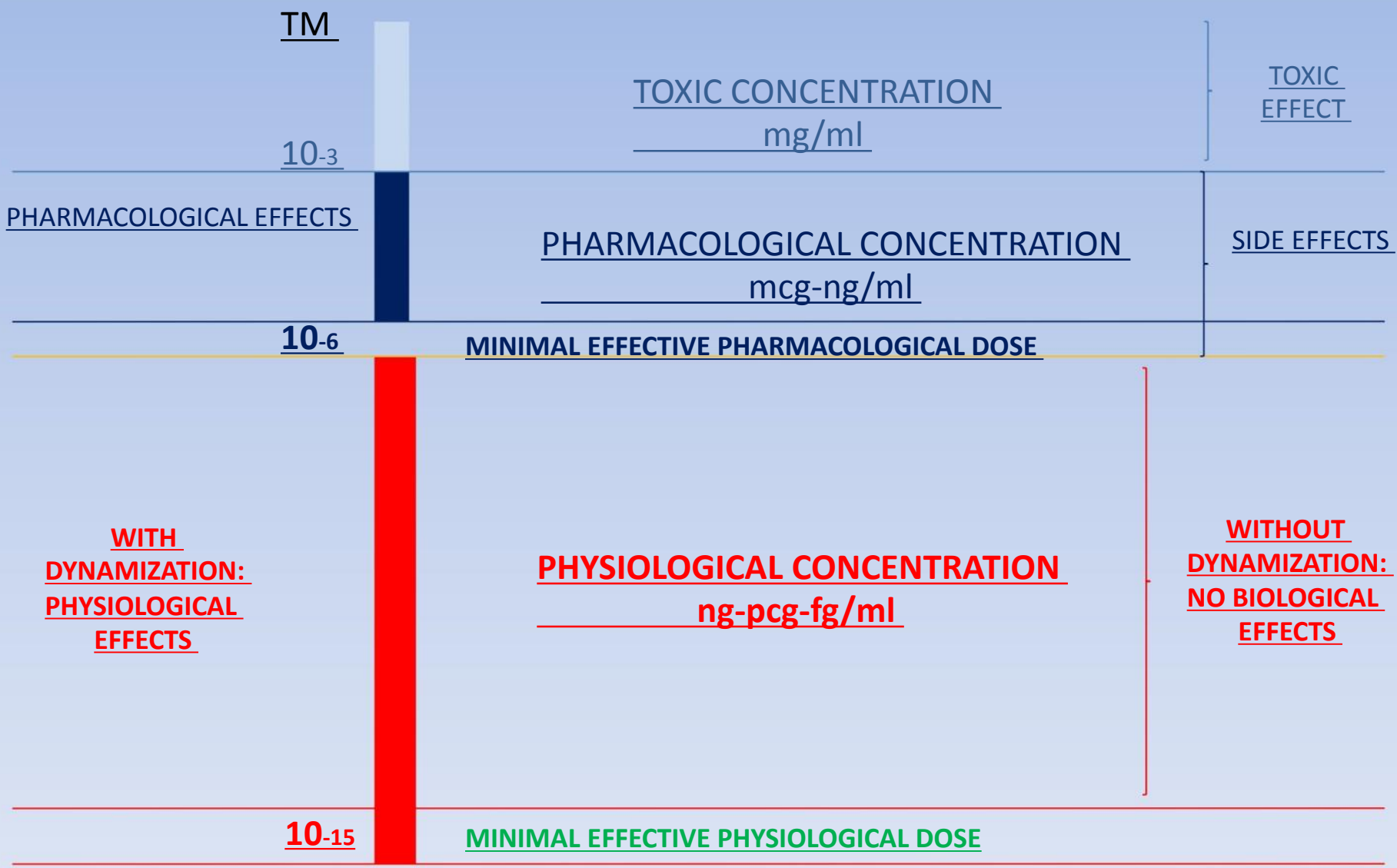
CYTOKINES



CYTOKINES

EVERY EFFECTIVE PRODUCT IN MEDICINE IS RELATED TO ITS RELATIONSHIP WITH CYTOKINES AND THEIR PATHWAYS. ALL DISEASES ARE RESULTS OF A CYTOKINE IMBALANCE

EFFECTS OF DIFFERENT DOSES OF CYTOKINES



DEFINITIONS

g (gram)= 1

10^{-1} = 0.1

10^{-2} = 0.01

mg (milligram)= 10^{-3} = 0.001

µg (microgram)= 10^{-6} = 0.000001

ng (nanogram)= 10^{-9} = 0.000000001

pcg (picogram)= 10^{-12} = 0.000000000001

fg (fentogram)= 10^{-15} = 0.000000000000001

DISEASE

HYPER-CONCENTRATION

10^{-6}

picogramms/milliliter

HEALTH

4CH - D6

PHYSIOLOGICAL CONCENTRATION

nanogramms/milliliter

10^{-15}

HYPO-CONCENTRATION

DISEASE

ORAL CYTOKINES

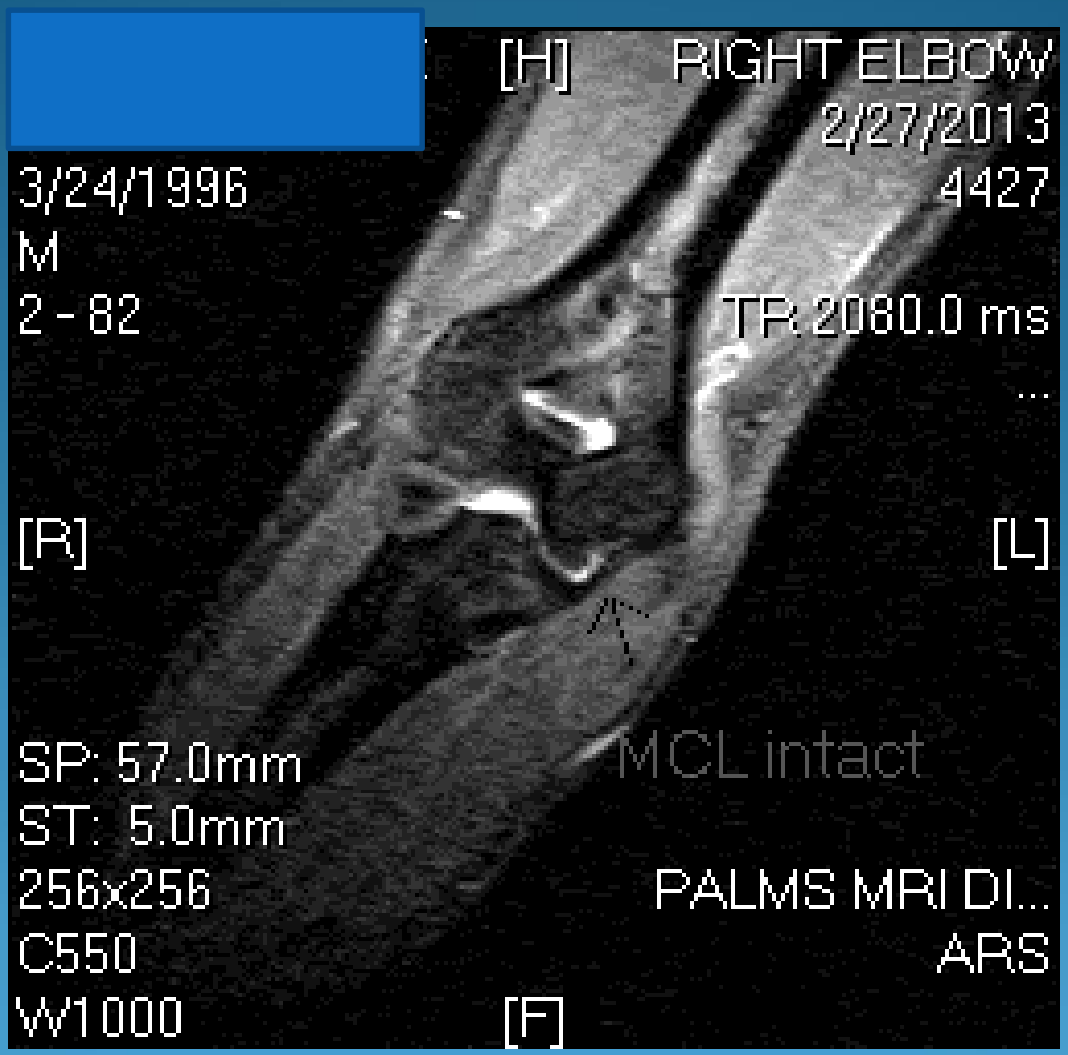
The same cytokines that are found in PRP injections and stem cells can now be delivered in an oral fashion (sublingually) on a daily basis. The benefit is that they are given daily and have a relatively uniform delivery to the injured area. There are various regimens depending upon the problem and area being treated. **A POTENTIAL QUANTUM LEAP IN ALL REGENERATIVE MEDICINE**

complete resolution of avn





Pre Injection



Post Injection

THANK YOU

