

Tissue Regeneration and Wound Healing

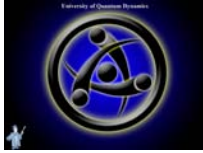
[Tissue Regeneration Without Scaffolding]

Prof. Stephen B. Palmer, MD, PhD & Prof. Hayk Arakelyan, MD, PhD

2/19/2010

ABSTRACT

Introduction to direct acting modalities of complete tissue regeneration without inflammation cascade, cytokine cascade or the use of external equipment or scaffolding and without the utilization of stem cells from other sources; de-differentiation and exclusion of bio-film as part of the processes occurring naturally in what is normally termed the second stage of wound healing and regeneration occurring initially; as well as references to other promising technologies along the same or different lines explaining its application to similar lines of thought.



Tissue Regeneration and Wound Healing



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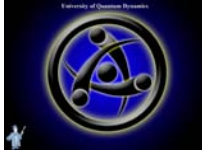
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[Without Scaffolding Modalities]

By:

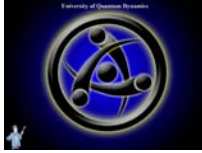
Stephen B. Palmer, M.D.*, Ph.D. ; Hayk Arakelyan, M.D., Ph.D.**

Most modalities either utilize some sort of scaffolding, electrical or photonic stimulation, or stem cells from various sources outside of the injured body; the scaffolding issue, in this author's opinion was a stepping stone into regenerative medicine and tissue engineering, but has been replaced by two very viable alternatives, both will be presented here along with histories of some promising aspects of the craft.

A good deal of research to date has been in the single tissue aspects from Adipose cells to Spinal Cord injuries and everything in between; current literature on regenerative medicine is literally covering all levels of research and springing up new institutes^{1,2} specializing in various aspects of regenerative medicine, but though the studies continue, few practice modalities are appearing at the clinical level. The trick is first apply it in emergency cases, and then try to understand the individual modalities of action that one is witnessing, rather than to try explain every aspect of the modality of action before applying the particular medical craft to an injured or ill patient. Waiting for them to die while you are in a research stage violates your oath as a healer, your obligation to the patient, and your personal integrity. The FDA only requires safety and efficacy!

¹ UCSF-Institute for Regenerative Medicine <http://irm.ucsf.edu/index.aspx>

² (McGowan Institute of Regenerative Medicine)



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The subject of nerve and cardiac cells not being able to regenerate is nonsense and has been disproven in many cases both in practice and in research papers. Let us start first with the so called impossible or neuronal axon damage of the Central Nervous System [CNS] outside of the cerebral cage and include the spinal cord; From UCSF excerpts:

Axon Regeneration in the Central Nervous system

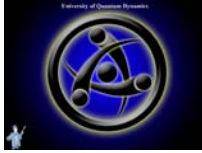
Many forms of CNS damage cut axons. Where axons can regenerate, as in peripheral nerves, they can bring back function. However in the CNS axon regeneration fails. This is the main reason why paralysis and loss of sensation is permanent in conditions such as spinal cord injury. If CNS axons could be made to regenerate some recovery from spinal cord injury and other conditions would be possible.

Axon regeneration in the CNS fails for two reasons. First because the environment surrounding CNS lesions is inhibitory to axon growth, and second because most CNS axons only mount a feeble regeneration response after they are cut.

Proteoglycans and the glial scar

The lab has been involved in finding out why the glial scar that develops after CNS injuries is inhibitory to axon regeneration. This scar contains astrocytes, meningeal cells, oligodendrocyte precursors and microglia/macrophages. The major inhibitory molecules are chondroitin sulphate proteoglycans, particularly NG2, neurocan, versican, brevican and phosphacan. Of these NG2, neurocan, versican and phosphacan are made by oligodendrocyte precursors that are recruited to CNS injuries in large numbers. Neurocan, phosphacan and brevican are made by astrocytes.

Proteoglycans have a protein core to which are attached highly charged sulfated glycosaminoglycan (sugar) chains. If the sugar chains are digested away with chondroitinase much of the inhibitory activity of the proteoglycans is lost.



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Digesting proteoglycans with chondroitinase promotes axon regeneration in the damaged brain and spinal cord. The lab has shown that axon regeneration can be induced in the nigrostriatal pathway, and has collaborated with the McMahon lab to show axon regeneration and return of function in the spinal cord.

The enzymes that synthesize the glycosaminoglycan chains of proteoglycans are potential therapeutic targets. The lab has studied the enzymes that perform sulfation, since preventing sulfation takes away much of the inhibition from proteoglycans. Following injury there is a specific upregulation of 6-sulfated glycosaminoglycan, accompanied by increased expression of the 6-sulfotransferase enzyme.^{3,4}

Intrinsic regenerative ability of axons

While axons in peripheral nerve regenerate well, those in the CNS regenerate poorly. This is not only due to the inhibitory molecules of the CNS. Many CNS axons mount little or no regenerative response after they are cut, in contrast to peripheral axons which make a vigorous effort to re-grow. In general embryonic axons regenerate more vigorously than adult axons, and there are also some axons, such as Purkinje cell axons, that show no regenerative response. CNS and PNS axons also differ in their ability to re-grow if they are cut in a tissue culture dish. The lab has been collaborating with the Holt lab to show that successful regeneration requires that axons are able to synthesize new proteins near the cut, and that axons that have a high regenerative potential have larger amounts of protein synthesis machinery in the axon than axons with low regenerative potential.^{5,6}

Perhaps they are leaving out other factors, or that their narrow band of research leads them in diverging causes and not allowing for the body's own communication to specialized cells, or action at a distance, or similar communications when presented with an injury and an organo-

³ (Morgenstern DA, 2002)

⁴ (Bradbury EJ, 2002).

⁵ (Campbell DS, Chemotropic responses of retinal growth cones mediated by rapid protein synthesis and degradation, 2001)

⁶ (Rossi F, 2001)



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metallic compound that allows for dedifferentiation and translation to primitive cells that can then initiate complete regeneration through the body's own resources rather than adding additional material or other compounds.

A similar study completed by Francesca Properzi and James W. Fawcett at the Brain Repair Center at Cambridge University in the UK entitled “Proteoglycans and Brain Repair” and again looking at possible indicators of function and refer to the works of 20 authors proving their points, yet still fail to see the base functionality of and capabilities of the body's own mechanisms for self repair given the proper environment of bio-film elimination and base stimulus products available in the organo-metallic compounds. There will be more on this subject after a thorough review of current technologies.

Decubital Wounds to the Spinal Cord

R.Šavrin and A.Cör from Slovenia⁷ use electrical stimulation of pressure sores in spinal cord injuries with a broad efficacy, but generally limited to Decubital wounds, but illustrating by their research that the entire body, specifically the CNS as well as PNS are easily stimulated by electrical, electromagnetic^{8, 9, 10, 11, 12} and photonic or acoustic external systems,^{13, 14, 15, 16} but could as easily be stimulated by internal charges created by the organo-metallic compounds, such as the copper-potassium, copper-sodium, silver-copper, silver-potassium or silver-sodium ions in the solution, acting like a direct current battery

⁷ Institute for Rehabilitation, Linhartova 51, 1000 Ljubljana, Slovenia and Institute of Histology, Faculty of Medicine, Korytkova 2, 1000, Ljubljana, Slovenia

⁸ (Sheffet A, 2000)

⁹ (Reger SI, 1999)

¹⁰ (Tro94)

¹¹ (Karba R, 1995)

¹² (Stefanovska A, 1993)

¹³ (Gardner SE, 1999)

¹⁴ (Trontelj K K. R., 1994)

¹⁵ (KR, 1985)

¹⁶ (Ken-Patton GP, 1988)



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using either the Ascorbate, citrate or interstitial body fluids as the ion [acid] source of electrical potential.

Other methods include the use of neurotransmitters and bone marrow cells as put forth by C.S. Paulose, et alia¹⁷ where they write: *“Spontaneous recovery after spinal cord injury is delayed by the limited ability of mammalian central nervous system to re-establish functional neural connections; re-myelinate, spread nerve fibers and replace lost cells. Spinal cord injury leads to sensory motor loss and disruption of autonomic nervous system. Most spinal cord injury victims also develop chronic pain conditions that severely reduce quality of life. Our results showed that the treatment using neurotransmitter’s combination and bone marrow cells of the same individual significantly improved the recovery from spinal cord injury. Pittenger et al.¹⁸ reported that mesenchymal stem cells derived from the bone marrow differentiated into osteocytes, chondrocytes and adipocytes. It is also reported that multipotent adult progenitor cells derived from the bone marrow, which comprise approximately 0.125% of the total marrow cells, are multipotent stem cells with the capacity to differentiate, under specific experimental conditions into several different types of cells including osteoblasts, adipocytes, chondrocytes, skeletal muscle fibres, cardiomyocytes, hepatocytes, neural cells and epithelial cells of the lung and intestinal tract. It has recently been reported that the bone marrow derived cells also have the potential to develop into neural lineages, such as neurons and astrocytes, both in vivo and in vitro. Bone marrow cells which are adherent in the culture of bone marrow aspirates, have already been used for the treatment of the injured spinal cord and brain. Recent studies indicated that transplantation of bone marrow cells by direct injection into the lesion might promote tissue repair in the injured spinal cord by reducing the size of the cavity at the lesion. The effects of transplanted bone marrow cells on tissue repair, as described above, suggest that some trophic factors might be released from bone marrow cells to promote the tissue repair.”*

This work clearly demonstrates that given the proper conditions the body is capable of producing the required types of cells to repair and regenerate any cell in the body that is needed to have a full recovery from any wound, even that of the spinal cord and CNS/PNS systems.

An alternative therapy utilizing a type of scaffolding developed by Kevin L. Reed¹⁹ has targeted CSI for 90% to 100% functional recovery when used with current SCI trauma stabilization and physical therapy protocols. It is a promising modality, but still falls short of self-propagated total regeneration and recovery. As he explains the modality:

¹⁷ (C.S. Paulose, 2009)

¹⁸ (Pittenger MF, 1999)

¹⁹ (Reed, 2009)



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SESCRC

1. **Conduit Structural Elements** made of electroactive polymer PP/gel composites and biodegradable PLGA that incorporate neurotropic factors and ECM molecules, formed by 3D prototyping machine using CADS files derived from axon fiber mapping with 11.7 Tesla MRI Scan axon fiber tract mapping.
2. **Electroactive Polypyrrole Polymer Fibers** that incorporate ECM molecule and NGF side chains, formed from pyrrole monomers that polymerize between selected nerve target electrodes within engineered voids inside the structural elements of the SESCRC.
3. **Nerve Stimulator Arrays/ polymer fiber formation electrodes** made with iridium and biodegradable polymers to form “sieve electrodes” that are formed at opposite ends of the SESCRC conduit.
4. **SESCRC structural element integration of Growth Factors, Neurotropic Factors and ECM molecules**, as gradients in electroactive polymers and in timed-release biodegradable polymers and as direct infusions in above and below the SESCRC insertion.
5. **Live transplant cell populations** including Schwann cells, oligodendrocytes, embryonic stem cells and mixed nerve cells and axons extended through the SESCRC Conduit from the proximal to the distal stumps of the damaged nerves to selectively regenerate the damaged nerve pathways. “

I was with him up until section 5 above where he uses transplantation including embryonic stem cells; with the addition of the organo-metallic compound, the body will make its own stem cells from blastotic cells of every type into those that are required for SCI repair as well as adjacent PNS damages that may have occurred during the SESCRC implantation surgery. But this device and its procedural modality is at least a step in the right direction, and is producing a product with immediate applications without the personal need to research it to death.

For additional current modalities of treatment and their known efficacies for SCI, please see Peter AC Lim and Adella M Tow Review Article²⁰ and summary of recent literature as of January 2007.

For most of the rest of this article, this author will follow the very good outline on tissue repair by Dr. Mohamed Sadequel Islam Talukder, MBBS, M. Phil. (Pathology) May 2002. While explaining the attributes of the organo-metallic compounds developed by this author and W. John Martin, M.D., Ph.D. in 2006. Articles have been published by this author and Dr. W. John Martin, (Palmer SB, Elimination of Gram Positive Pathogens and Tissue Regeneration, 2009) (Palmer SB A. H., 2010), (Arakelyan H, 2009) Dr. Frank Morales, and Dr. Hayk Arakelyan, concerning the

²⁰ (Tow, 2007)



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various modalities of action of the organo-metallic solutions. This article is a culmination of those articles and the overall summary of the art of regenerative medicine, and proof of concept for the US and International Patents already filed.

Additional areas to be covered are burns, bone regeneration, smooth muscle [Heart] regeneration principals, and cancer dedifferentiation to normal cells, antagonist virals that exist in the body which are pathogenic without causing immediate inflammation; such as human adapted animal virus strains that the human immune system does not recognize as toxic, such as Simian Cytomegalovirus and Herpes Complex Virus group, Lyme's Disease and Parvo Virus.

Tissue Repair, Cellular Growth and Wound Healing

Injurious stimuli trigger the activation of genes that are involved in cell replication.

Normal repair of tissues involves two distinct processes:

- 1) Regeneration and**
- 2) Fibroplasia or fibrosis – or forming scar tissue**

With the citric based organo-metallic one will understand in this writing that fibrosis does not occur if it is used as rapidly as possible by first responders.

Regeneration denotes replacement of injured cells by cells of the same types, sometimes with no residual trace of the previous injury.

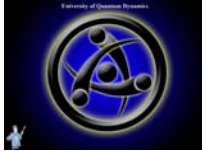
Fibroplasia denotes replacement by connective tissue, which leaves a permanent scar.

Repair of tissue involves the following mechanisms:

- _ Cell migration**
- _ Cell proliferation**
- _ Cell differentiation**
- _ Cell-matrix interactions**

And one more that is not in the normal sequence, with the solution, dedifferentiation, and translational differentiation, followed by proliferation in the injured area.

Cell-matrix interaction is important for repair. The orderly regeneration of the epithelial tissue of the skin and viscera requires the basement membrane (BM). This specialized



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extracellular matrix (ECM) functions as an extracellular scaffold for accurate regeneration of pre-existing structures. Maintenance of BM integrity provides for the specificity of the cell type and popularity and influence cell migration and growth.

In adult tissue, the size of a population of cells is determined by the rates of cell proliferation, differentiation, and death by apoptosis. Increased cell number may result from either increased proliferation or decreased cell death.

The impact of differentiation depends on the circumstance under which it occurs.

Replication of differentiated cells occurs in certain adult tissues; for example, after partial hepatectomy, liver cell division continues until the signals for such division is abrogated.

Apoptosis is induced by a variety of physiologic stimuli and is controlled by a number of genes.

Cell proliferation can be stimulated by injury, cell death and mechanical deformation of tissues.

Cell replication is controlled largely by chemical factors in the microenvironment, which either stimulate or inhibit cell proliferation.

The cells of the body are divided into three groups on the basis of their proliferative capacity and their relationship to the cell cycles. The cell growth cycle consists of G_1 (presynthetic), S (DNA synthesis), G_2 (premitotic), and M (mitotic) phases.

Most mature tissue composed of

- _ Labile cells or continuously dividing cells
- _ Stable cells or quiescent cells
- _ Permanent cells or nondividing cells

Labile cells follow the cell cycle from one mitosis to the next and continue to proliferate throughout the life, replacing cells that are continuously being destroyed. Examples are stratified squamous surface epithelium of skin, oral cavity, vagina and cervix; lining mucosa of all the excretory ducts of the glands; the columnar epithelium of the gastrointestinal tract and uterus; the transitional epithelium of the urinary tract and cells of the bone marrow and hematopoietic tissues. Regeneration is derived from a population of stem cells, which have unlimited capacity to proliferate and whose progeny may undergo various streams of differentiation.



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Stable cells normally demonstrate a low level of replication can undergo rapid division in response to stimuli and are thus capable of reconstitute the tissue of origin. They are considered to be in G_0 but can be stimulated into G_1 . Examples are parenchymal cells of virtually all the glandular organs, such as the liver, kidneys, and pancreas; mesenchymal cells such as fibroblasts and smooth muscle; and endothelial cells.

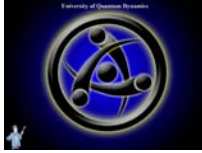
The underlying supporting stroma of the parenchymal cells – particularly the BM – is necessary for organized regeneration, forming a scaffold for the replicating parenchymal cells.

Permanent cells have left the cell cycle and cannot undergo mitotic division in postnatal life. Examples are most nerve cells and cardiac and skeletal muscle cells. Neuron destroyed in central nervous system is thought to be permanently lost. They are replaced by supportive elements, glial cells. Skeletal muscle cells have some proliferative capacity. Regeneration appears to occur from transformation of the satellite cells attached to the endomysial sheaths. Regenerative capacity of cardiac muscle is limited, and most large injuries to the heart are followed by connective tissue scarring. Up until now, with the use of both the Citric and Ascorbate based solutions, direct application to the neural axons/neurons and heart muscle can perform the same function of dedifferentiation to primitive cells back to the pre-natal stage from lipids and collagen found around the heart and neurons; Then normal differentiation and proliferation.

Molecular Events in Cell Growth

Molecular events in cell growth are complex and involve an increasing array of intercellular pathways and molecules. It is important because aberration in such pathways may underlie development of cancer and abnormal cellular response in a variety of diseases. Dedifferentiation pathway using Ascorbate based organo-metallic's can block the peptide receptors of cancer cells, thus allowing normal growth cycles to be restored.

Growth factors induce cell proliferation by affecting the expression of proto-oncogene's, involved in normal growth control pathways. The expression of these genes is tightly regulated during normal growth and regeneration. Alteration in the structure of such proto-oncogene's can convert them into oncogenes, which contribute to uncontrolled cell



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growth characteristic of cancer. As mentioned this can be reversed with appropriate Ascorbate protocols.

There are three general schemes of intercellular signaling:

- _ Autocrine signaling
- _ Paracrine signaling
- _ Endocrine signaling

Autocrine signaling: Cells respond to the signaling substances that they themselves secrete. This type of signaling occurs in compensatory hyperplasia and in tumors. Tumor cells frequently overproduce growth factors that can stimulate their own growth and proliferation.

Paracrine signaling: A cell produces molecules that affect only in a target cell in close proximity. This is common in connective tissue repair of wound healing.

Endocrine signaling: Hormones are synthesized by cells of endocrine glands and sent on to target cells distant from their site of synthesis being usually carried by blood.

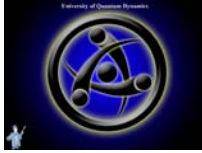
Cell growth is initiated by the binding of a signaling agent, most commonly a growth factor, to a specific receptor. Receptor proteins can be located on the cell surface of the target cells or found in either the cytoplasm or the nucleus. A receptor protein has binding specificity for particular ligands, and the resulting receptor ligand complex initiates a specific cellular response.

Three major classes of cell surface receptors are important for cell growth:

- 1) Receptors with intrinsic kinase activity
- 2) Receptors without intrinsic catalytic activity
- 3) G-protein linked receptors.

Receptors with intrinsic kinase activity

These types of receptors have an extracellular domain for ligand binding; a single transmembrane region; and a cytosolic domain. Many growth factors are dimeric proteins, contain two regions for receptor binding, and form stable receptor dimers by



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simultaneously binding two receptors. Dimerization of the receptor is followed by receptor autophosphorylation, creating binding sites for a series of cytosolic proteins. Such cytosolic proteins include (1) a series of adaptor proteins; (2) components of the phosphoinositide-3-kinase (PI-3-kinase) pathway; (3) phospholipase C- γ in the protein kinase C pathway; and (4) members of the SRC family of tyrosine kinase. Collectively, these four systems, in turn, generate a cascade of responses that ultimately signals irreversible commitment of the cell to enter S phase of the cell cycle.

Receptors without intrinsic catalytic activity

These types of receptors have an extracellular domain for ligand binding; a single transmembrane region; and a cytosolic domain, which directly associate with and activates one or more cytosolic protein tyrosine kinase, which, in turn, phosphorylate the receptor.

G-protein linked receptors

These receptors contain seven transmembrane loops and are frequently called seven-spanning receptors, are associated with a variety of important functions. For example, receptors for the inflammatory chemokines as well as the hormones epinephrine and glucagon are in this class. Ligand binding activates a signal transducing G protein complex, which in turn, activates an effector system that generates intracellular second messengers.

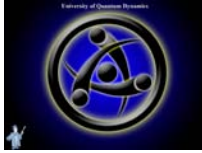
Signal Transduction System

_ Signal transduction is the process by which extracellular signals are detected and converted into intracellular signals, which, in turn, generates specific cellular responses.

_ Signal transduction systems are arranged as networks of sequential protein kinase; the most important ones involved in regulation of cell growth are the Mitogen activated protein kinase (MAP kinase), PI-3-kinase, inositol-lipid (IP3), cyclic adenosine monophosphate (cAMP), the Janus Kinase/Signal transducer and activators of transcription (JAK/STAT) signaling system, and the stress kinase system.

Mitogen-Activated Protein Kinase Pathway

_ It is particularly important in signaling by growth factors.



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_ Ligand binding by a receptor tyrosine kinase results in autophosphorylation of the receptor and binding of adaptor protein, which ultimately lead to activation of the Ras protein. Inactive Ras is in the guanosine diphosphate (GDP) binding form, which is converted by activation to the active GTP form, initiating a cascade of distal kinases, which culminate in change in gene expression.

_ Activation of Ras is counteracted by GAP (GTP activating protein), which switches Ras to the inactive GDP form.

_ The net result of this pathway is activation of a protein phosphorylation cascade, which amplifies the signal and stimulates quiescent cells to enter the growth cycle.

Phosphoinositide-3-kinase Pathway

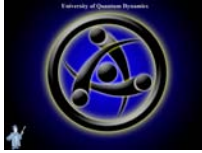
Phosphoinositide-3-kinase (PI-3-kinase) generates membrane-associated lipid mediators, which act as second messengers to recruit and activate a series of intracellular kinases. The activity of these kinases eventually leads to cellular responses that are correlated with cellular survival, such as phosphorylation of glycogen synthase kinase 3 and increased glycogen synthesis.

Inositol-Lipid Pathway

The IP₃ signaling system can be coupled to either tyrosine kinase or seven-spanning G protein-linked receptors causing activation of a G protein, which then activates phospholipase C_i. Phospholipase C_i cleaves PIP₂ to IP₃ and DAG. The IP₃ then diffuses in the cytoplasm and associate with IP₃-sensitive calcium channels in the membrane of the endoplasmic reticulum, causing release of calcium stores. DAG and calcium also activate protein kinase C, which then phosphorylates a variety of cellular components important in cell growth and metabolism.

Cyclic Adenosine Monophosphate Pathway

Binding of hormones to seven-spanning receptors is coupled through G proteins to activation of adenylate cyclase and generation of the second messenger cAMP. Elevated levels of cAMP activate protein kinase A, which, through a series of intermediate steps, stimulates expression of target genes.



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JAK/STAT Pathway

Members of the cytokine receptors super family lack intrinsic kinase activity. After ligand binding, the receptor associates with and activates one or more protein kinases present in the cytosol, designated Janus Kinases (JAKs). The JAKs phosphorylate the receptors as well as downstream proteins designated STATs (signal transducer and activators of transcription). In general JAK/STAT system mediates functional as opposed to proliferative responses.

In the case of organo-metallic solutions where normal protein systems are blocked from participating, and limit cytokine activity by binding the receptors, transcription is a functional fibroblastic lipid or fibroblastic collagen dedifferentiation of ECM and BM chain to primitive cell matrix that then proceeds along differentiation, proliferation and normal transcription routes while controlling cytokine production and proliferation, continues to block H1 and mast cell function. At this point the system enters intrinsic kinase activity in binding receptors, thus initiating normal transcription pathways.

Transcription Factors

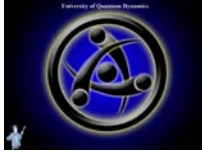
Transcription factors have a vital role in controlling cell growth. Transcription factors are phosphorylated by specific proximal kinases and such phosphorylation can change the subcellular localization of the transcription factor or its affinity for DNA, which alters gene expression.

Cell Cycle and the Regulation of Cell Division

Two types of molecular controls regulate the events leading to cell division: (1) a cascade of protein phosphorylation pathways involving a group of proteins called *cyclins* and (2) a set of *checkpoints* that monitor completion of the molecular events and, if necessary, delay progression to the next phase of the cycle.

Cyclins and Cyclin-dependent Kinases

The entry and progression of cells through the cell cycle are controlled by changes in the levels and activities of *cyclins*. Cyclins perform their functions by forming complexes with a group of constitutively expressed proteins called *cyclin-dependent kinases (CDKs)*. Different combinations of cyclins and CDKs are associated with each of the important transitions in the cell cycle. Cyclin β is synthesized when cell moves into G_2 and it binds to constitutive CDK1, creating the cyclin B/CDK1 complex, whose activity is necessary for cells to enter M phase. The complex is activated by phosphorylation; the active kinase then phosphorylates



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a variety of proteins involved in mitosis, DNA replication, and depolymerization of the nuclear lamina, and mitotic spindle formation. After mitotic division cyclins β are degraded by the *ubiquitinproteasome pathway*. In this pathway, proteins are first conjugated to the small protein cofactor ubiquitin, and the modified protein is specifically recognized and degraded within proteasome, a large multisubunit proteolytic complex.

The active CDK complexes are regulated by binding of CDK inhibitors, such as p21 and p27, as well as other kinases and phosphatases. The inhibitors control the cell cycle by balancing the activity of the CDKs. Checkpoints.

Checkpoints represent a second mode of cell cycle regulation and provide a surveillance mechanism for ensuring that certain transitions occur in the correct order and that important events are completed with fidelity. Checkpoints sense problems in DNA replication, DNA repair and chromosome segregation.

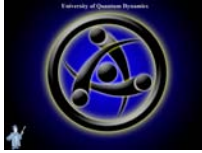
Activated checkpoints send signals to cell cycle machinery that arrest the cell cycle. By delaying progression through the cell cycle, checkpoints provide more times for repair and diminish the possibility of mutations. Checkpoints systems cause cell cycle arrest either by promoting inhibitory pathways or by inhibiting activating pathways.

Growth Inhibition

The other side of coin in cellular growth control is growth inhibition. The molecular mechanisms of growth inhibition are similar to those of growth stimulation and intertwine along their intercellular routes. A good example of a growth inhibitory signaling system involves the polypeptide growth factor transforming growth factor- β (TGF- β). TGF- β signals through cell surface receptors with serine/threonine kinase activity. The activated kinase phosphorylates its own cytoplasmic domain as well as substrate proteins. TGF- β inhibits cell cycle progression into S phase by affecting the function of both transcription factors and cell cycle control proteins.

Growth Factors

Some of the growth factors act on a variety of cell types, whereas others have effects on relatively specific targets. Growth factors also have effects on cell locomotion, contractility and differentiation.



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Some growth factors

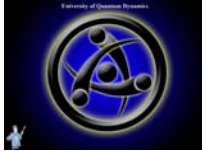
- _ **Epidermal growth factors**
 - o **EGF**
 - o **Transforming growth factors**
- _ **Platelet derived growth factors (PDGF)**
- _ **Fibroblast growth factor (FGF)**
- _ **Transforming growth factors β (TGF- β)**
- _ **Vascular endothelial growth factors (VEGF)**
- _ **Angiopoietins (Ang)**
- _ **Insulin like growth factors (IGF)**
- _ **Hepatocyte growth factors (HGF)**
- _ **Connective tissue growth factors (CTGF)**
- _ **Myeloid colony-stimulating growth factors (CSFs)**
- _ **Cytokines**
 - o **Interleukins**
 - o **Tumor necrosis factor (TNF)**
 - o **Interferon α , β**
- _ **Nerve growth factor (NGF)**

EGF/TGF- α :

- _ **Is mitogenic for a variety of epithelial cells and fibroblasts**
- _ **Widely distributed in tissue secretions and fluid**

PDGF:

- _ **Is stored in platelets and released on activation.**
- _ **Also be produced by a variety of cells, including activated macrophages, endothelial cells, smooth muscle cells and tumor cells.**



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_ Causes both migration and proliferation of fibroblasts, smooth muscle cells and monocytes and has other proinflammatory properties.

FGFs:

_ Produced by a variety of cells

_ Functions:

o New blood formation (angiogenesis) – has ability to induce all the steps necessary to new blood vessels formation.

o Wound repair – participate in macrophage, fibroblast, and endothelial cell migration in damaged tissue and migration of epithelium to form new epidermis.

o Development – play a role in skeletal muscle development and in lung maturation.

o Haemopoiesis – development of specific lineage of blood cells and development of bone marrow stroma.

VEGF:

_ Promotes angiogenesis in cancer, chronic inflammatory states, and healing wounds;

TGF- β :

_ Have both growth stimulatory and inhibitory function.

_ TGF- β is a growth inhibitor to most epithelial cells.

_ TGF- β also stimulates fibroblast chemotaxis and production of collagen and fibronectin by cells.

Cytokines:

_ Have growth promoting activities to a variety of cells Extracellular Matrix and Cell-Matrix Interactions

_ Cells grow, move and differentiate in intimate contact with the ECM.

_ The ECM is secreted locally and assembles into a network in spaces surrounding the cells.

_ the matrix proteins sequestered molecules to provide trigger to soft tissues or minerals to provide rigidity to skeletal tissues. It also provide reservoir for growth factors controlling cell proliferation.

_ Provides a substratum for cells to adhere, migrate, and proliferate and can directly influence the forms and function of cells.

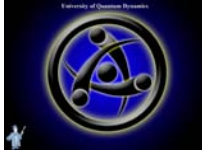
_ Degradation of ECM accompanies morphogenesis and wound healing as well tumor invasion and metastasis.

_ three groups of macromolecules are associated to form ECM:

o Fibrous structural protein – collagen and elastin.

o Adhesive glycoprotein – fibronectin and laminin

o Gel of proteoglycans and hyaluronan.



Tissue Regeneration and Wound Healing

These macromolecules assemble into two general organizations:

- _ **interstitial matrix**
- _ **Basal membrane (BM)**

Collagen:

- _ **Collagen is the common protein in the animal world.**
- _ **Collagens are composed of a triple helix of three polypeptide chains.**
- _ **about 30 chains form at least 14 distinct collagen types**

Major types of collagens:

Type I, bundles of fibers with high tensile strength distributed in the skin, bone, tendons and other organs.

Type II, thin fibrils and structural protein distributed in cartilage and vitreous humour.

Type III, thin pliable fibrils distributed in blood vessels, uterus and skin.

Type IV, Amorphous distributed in all Basement Membrane [BM].

Type V, Amorphous fine smooth fibers distributed in interstitial tissue and blood vessels.

Type VI, Amorphous fine rough fibers distributed in interstitial tissues.

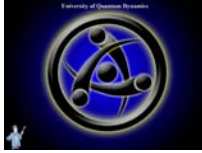
Type VII, Anchoring filaments distributed in the dermal and epidermal junctions.

Type VIII, Semi-Amorphous distributed in Endothelium-Descemet Membrane.

Type IX, Strong fibrous group for the maturation of cartilage, distributed primarily in cartilage.

Elastins, Fibrilins, and Elastic Fibres

- _ **Although tensile strength is provided by members of the collagen family, the ability of tissue to recoil is provided by elastic fibres.**
- _ **Central core of elastic fiber contains elastin, which is surrounded by a peripheral microfibrillar network called fibrilin.**



Tissue Regeneration and Wound Healing

Adhesive Glycoproteins and Integrins

- _ These protein link ECM components to one another and to cells.
- _ Important such proteins are:
 - o Laminin
 - o Fibronectin
 - o Integrin

Fibronectin:

- _ Primary role of fibronectin is to attach cells to a variety of matrices
- _ Produced by fibroblasts, endothelial cells, and other cells
- _ Associated with cell surface, BMs, and pericellular matrices
- _ binds to a number of ECM component including collagen, fibrin and proteoglycans and to cells

Laminin:

- _ Laminin is the most abundant glycoprotein in BMs.
- _ binds specific receptors on the cell surface with matrix components such as collagen type IV and heparin sulfate.

Integrins:

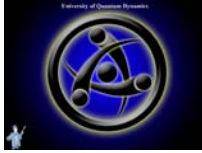
- _ Integrins are the major family of the cell surface receptors that modulate cellular attachment to the ECM
- _ It also mediates important cell-cell interactions involved in leukocytes adhesion
- _ Integrins are transmembrane glycoproteins

Matricellular Protein:

- _ These proteins do not function as structural components of the ECM but interact with matrix proteins; cell surface receptors or other molecules that interact, in turn, with cell surface

Proteoglycans and Hyaluronan:

- _ Common proteoglycans are heparin sulfate, chondroitin sulfate and dermatan sulfate
- _ they have diverse roles in regulating connective tissue structure and permeability
- _ Hyaluronan is found in the ECM of many cells
- _ Serves as ligand for core proteins such as cartilage link protein, aggrecan and versican



Tissue Regeneration and Wound Healing

Normal Repair by Connective Tissue (Fibrosis):

- **Persistent tissue destruction, with damage to both parenchymal cells and stromal framework is a hallmark of chronic inflammation**
- **Repair cannot be accomplished solely by regeneration of parenchymal cells. Repairing occurs by replacement of parenchymal cells by connective tissues, which in time produce fibrosis and scarring**
- **There are 4 components to this process:**
 - 1. Formation of new blood vessels (angiogenesis)**
 - 2. Migration and proliferation of fibroblast**
 - 3. Deposition of extracellular matrix**
 - 4. Maturation and organization of the fibrous tissue (remodeling)**

Granulation tissue:

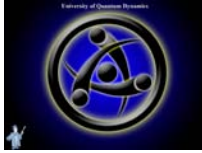
Repair begins early in inflammation. Sometimes as early as 24 hours after injury, if resolution has not occurred, fibroblasts and vascular endothelial cells begin proliferating to form granulation tissue by 3 to 4 days. It is the hallmark of healing. Granulation tissue is pink, soft and granular in appearance. Histologic features that are characteristic: the formation of new small blood vessels (angiogenesis) and proliferation of fibroblasts. The new blood vessels are leaky allowing passes of proteins and red cells into the extracellular space. Thus, new granulation tissue is often edematous.

Angiogenesis:

Four steps are needed in the development of new capillary vessels:

- 1) Proteolytic degradation of the basement membrane of the parent vessels to allow formation of a capillary sprout and subsequent cell migration**
- 2) Migration of endothelial cells toward the angiogenetic stimulus**
- 3) Proliferation of endothelial cells, just behind the leading front of migrating cells**
- 4) Maturation of endothelial cells, which includes inhibition of growth and remodeling into Capillary tubes**

Recruitment of periendothelial cells including pericytes and vascular smooth muscle cells, to support the endothelial tubes.



Tissue Regeneration and Wound Healing

All these steps are controlled by interactions among growth factors, vascular cells and the ECM.

Growth Factors and Receptors:

- _ Although many growth factors exhibit angiogenic activity, most evidence points to a special role for VEGF and the Angiopoietins in vasculogenesis.
- _ These factors are secreted by many mesenchymal and stromal cells, but their receptors are largely restricted to endothelium.
- _ VEGF expression is stimulated by certain cytokines and growth factors (e.g., TGF- β , PDGF, TGF- α) and tissue hypoxia.

Extracellular Matrix Proteins as Regulators of Angiogenesis:

- _ The key component of angiogenesis is the motility and directed migration of endothelial cells.
- _ These processes are controlled by several classes of proteins, including integrins, matricellular proteins and proteases

Fibrosis (Fibroplasia)

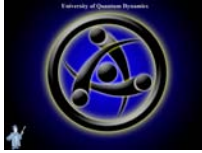
- _ Fibroplasia occurs within the granulation tissue framework of new blood vessels and loose ECM that initially form at the repair sites.

_ two processes are involved in fibrosis:

- _ Emigration and proliferation of fibroblasts
- _ Deposition of ECM by these cells

Fibroblasts Proliferation

- _ Granulation tissue contains numerous newly formed blood vessels.
- _ VEGF promotes angiogenesis but also is responsible for a marked increased in vascular permeability, leading to increased deposition of plasma protein in the ECM and provides a provisional stroma for fibroblasts in growth.
- _ Migration of fibroblasts to the site of injury and their subsequent proliferation are triggered by multiple growth factors (TGF- β , PDGF, EGF, and FGF) and fibrogenic cytokine (IL-1 and TNF- α).
- _ Macrophages are important constituent of granulation tissue, responsible for clearing extracellular debris, fibrin, and other foreign material; these all also elaborate TGF- β , PDGF and therefore promote fibroblast migration and proliferation.
- _ If the appropriate chemotactic stimuli are present, mast cells, eosinophils and lymphocytes may be increased in number. Each of these can contribute directly or indirectly to fibroblast migration and proliferation.



Tissue Regeneration and Wound Healing

_ TGF- β appears to be the most important growth factor involved in inflammatory fibrosis, because of the multitude of effect that favor fibrous tissue deposition. TGF- β causes fibroblasts migration and proliferation, increased synthesis of collagen and fibronectin, decreased degradation of ECM by metal proteases. TGF- β is also chemotactic for macrophages.

Extracellular Matrix Deposition

- _ When repair progresses, the number of proliferating endothelial cells and fibroblasts decreases.**
- _ Fibroblasts progressively become more synthetic and deposit increase amount of ECM.**
- _ Fibrillar collagens form a major portion of the connective tissue in repair sites and are important for the development of strength in healing wound.**
- _ Many of the same growth factors regulate fibroblast proliferation also stimulate ECM synthesis.**
- _ Net collagen accumulation depends not only on synthesis but also on collagen degradation.**
- _ Ultimately granulation tissue scaffolding is converted into a scar composed of spindle shaped fibroblasts, dense collagen, fragments of elastic tissue, and other ECM components.**
- _ When scar matures, vascular regression continues, eventually transforming the richly vascularized granulation tissue into a pale, avascular scar**

Tissue Remodeling

- _ remodeling of the connective tissue framework is an important feature of both chronic inflammation and wound repair**
- _ the net result of ECM synthesis versus degradation results in remodeling.**
- _ Degradation of collagen and other ECM proteins is achieved by a family of matrix metalloproteinase, which are dependent on zinc ions for their activity.**

- _ Metalloproteinase consists of:**
 - o Interstitial collagenase which cleaves the fibrillar collagen I, II, III**
 - o Gelatinase (or type IV collagenase), which degrade amorphous collagen and fibronectin.**
 - o Stromelysin, which act on a variety of ECM components including proteoglycans, laminin, fibronectin, and amorphous collagens.**
- _ Metalloproteinase's are produced by fibroblasts, macrophages, neutrophils, synovial cells and sometimes epithelial cells;**
- _ Secretion of metalloproteinase is induced by certain stimuli, including growth factors (PDGF, FGF), cytokines (IL-1, TNF-a), phagocytosis, and physical stress;**
- _ Metalloproteinase is inhibited by TGF-b and steroids.**



Tissue Regeneration and Wound Healing

Repair by Connective Tissue in presence of Organo-Metallic Compound

Taking this series step by step “turn to page 23” *“Persistent tissue destruction, with damage to both parenchymal cells and stromal framework is a hallmark of chronic inflammation”* The cause of the inflammatory response is extracellular debris and “Bio-Film”, which is cleared by either to solution form or gel form of the organo-metallic compound, generally a full irrigation of the wound with the solution followed by a gel impregnated moleskin patch; this does the following: 1) causes micro-organism “Bio-Film” lysing and apoptosis adding to the extracellular debris. 2) Clears the wound of most of the debris on the first irrigation, and the remainder on a full second flushing of the wound area. 3) Blocks mast cell, leukocyte and protein receptors preventing pain and the inflammation cascade. 4) Eliminates skin and dust mites as they do not survive long in the acidic solution of 3.2 to 4.6 pH. 5) Utilizes the ever present ECM, BM, Fibroblasts, Lipids and Collagen Types I, II, III, IV and V to begin the dedifferentiation and re-translation to primitive or pre-natal stem cells, which in turn follow the normal paths as outlined on pages 23, 24 and 25 with the exception of scarring sequences, which do not occur. The wound regenerates new cells from the deepest part of the wound back up to the surface epithelial cells and closes the wound when all other subcutaneous cells have been translated, proliferated, differentiated and complete their new growth cycles. During this time of between several hours to 2 days for most wounds, up to two weeks for very deep wounds or bone and nerve damage deep into the structural, muscle and connective tissue membranes or in serious burns where damage is extensive. Where this occurs, gel impregnated moleskin is used over the wound, or burn area to do the following: 1. Prevent “Bio-Film” from re-invasion of weakened cells; 2. Prevent external debris from entering the wound; 3. Provide additional signaling compound for the prevention of the inflammation cascade; 4) Provide additional energy components for the differentiation and regeneration pathway.

“Repair cannot be accomplished solely by regeneration of parenchymal cells. Repairing occurs by replacement of parenchymal cells by connective tissues, which in time produce fibrosis and scarring”; this is inaccurate when the compound is present. The connective tissues and fibrinogens are otherwise occupied in the dedifferentiation pathway, which will regenerate all required parenchymal cells and prevent scarring.



Tissue Regeneration and Wound Healing

ECM is used only for its growth factor characteristics and proliferation and not for deposition in the wound [Fibrosis].

Accelerated appearance of Granulation Tissue in as little as 15 minutes and up to three hours; normally small paper cuts and first degree burns are resolved within this time frame and granulation tissue never forms, second degree burns and wider or deeper wounds will present Granulation Tissue in minutes to six hours, but will not allow ECM to fill the space due to the blocking of mast cells and protein receptors, both Citrate and Ascorbate are enzyme enhancers and cell food, as are the cycle components Sodium and Potassium thus stimulating growth factors and specifically VEGF and PDGF and regulates fibrogenic cytokines to prevent excessive proliferation, specifically IL-1.

Looking at the structure of Cu-Ag-K Citrate as an electrolytically produced complex organo-metallic compound from NMR studies [Fig. 1] it is easy to see how the dimmers that are formed would have multiple roles apart from their original strait chain molecular structures.

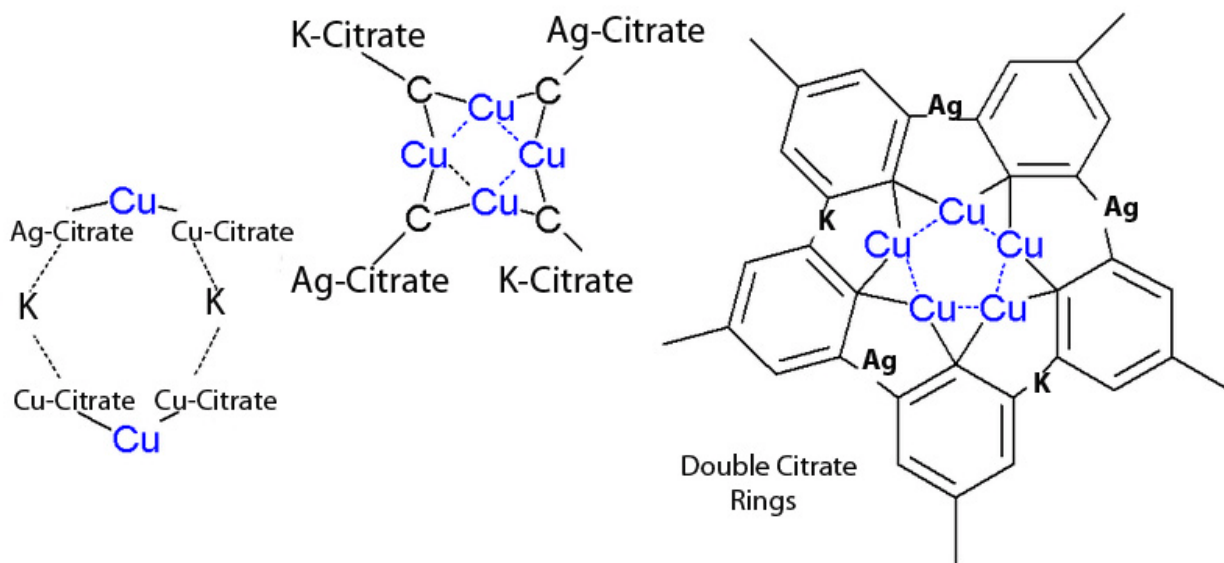


Figure 1

Left to right: First Dimmer, Second, and third. The third dimmer exhibits the doublet rings and an uncharacteristic expansion that is expressed from physical ionization which

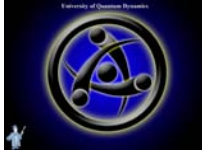


Tissue Regeneration and Wound Healing

produces larger atoms and in turn more complex molecules and compounds. It is assumed that the Ascorbate compound exhibits similar features.

Active fresh compound contains less than 15% straight chain form, while the third dimer comprises over 30% of the total, the first dimer comprising the bulk and the second dimer 10% of the active ingredients, free molecules of water, citric acid and potassium hydro-carbonate, potassium citrate make up the semi-active portions of the solution which would compose less than 3% of the total. Solution loses activity over time, generally 90 days, where the third dimer cleaves into dimmers one and two and a few straight chains; reactivation is a simple process, utilizing a vitreous non-conductive container such as a glass jar, placing a carbon anode and carbon cathode in the solution and charging it with a standard auto battery for half to one hour re-polymerizes the matrix to include almost the original 30+ percent of dimer three. The dedifferentiation dimer then is thought to be the third dimer, whereas the other two are receptor blockers, and the straight chain for delivery of copper, silver, potassium and citric acid to bio-film and individual proteins and to intercept iron-protease, replacing the iron which inactivates it causing cellular hypoxia which in turn activate VEGF and Angiogenesis, increase proliferation of fibroblasts and ECM enzymatic activity in the dedifferentiation pathway.

Granulated Tissue formed under the influence of this solution does not form leaky vascular beds, thus loss of proteins and blood cells does not occur, the normal fibril parts and ECM parts and fibroblast, dense collagen and fragments of elastic tissue that would normally be used to deposit in the bedding of the Granulated Tissue is used to completely remodel the vascular beds to useable capillary tubes. Thus normal degradation does not occur, fibrosis does not occur, and subsidence of the Granulated Tissue is replaced by specific remodeling.



Tissue Regeneration and Wound Healing

Normal Wound Healing

Processes of Wound Healing

- _ Induction of an acute inflammatory process by the initial injury
- _ Regeneration of parenchymal cells
- _ Migration and proliferation of both parenchymal and connective tissue cells
- _ Synthesis of connective tissue and parenchymal components
- _ Collagenization and acquisition of wound strength

Mechanism Involved in Wound Healing

- _ The mediators of acute inflammation
- _ The role of growth factors
- _ Cell-ECM interaction in cell migration, proliferation and differentiation
- _ The mechanism of angiogenesis and fibrosis

General Principles of Wound Healing

There are two types of wound healing

- _ Healing by first intension or primary union
- _ Healing by second intension

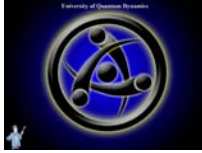
Healing by First Intension

- _ Healing occurs in a clean, uninfected surgical incision approximated by surgical suture
- _ The incision causes death of a limited number of epithelial cells and connective tissue cells as well as disruption of epithelial BM continuity. The narrow incisional space immediately fills with clotted blood containing fibrin and blood cells. Dehydration of surface of clot forms the well known scab that covers the wound
- _ Within 24 hour, neutrophils appear at the margins of the incision, moving toward the fibrin clot

Epidermis at its cut edges thickens as a result of mitotic activity of basal cells and within 24 to 48 hours, spurs of epithelial cells from the edge both migrate and grow along the cut margins of the dermis, depositing BM components as they move. They fuse in the midline beneath the surface scab, then producing a continuous but thin epithelial layer.

_ By day 3, the neutrophils have been largely replaced by macrophages. Granulation tissue progressively invades the incision space. Collagen fibers are present in margins of the incision, but at first these are vertically oriented and do not bridge the incision. Epithelial cell proliferation continues, thickening the epidermal-covering layer.

_ By day 5, the incisional space is filled with granulation tissue. Neovascularization is maximal; Collagen fibres are more abundant and begin to bridge the incision. The



Tissue Regeneration and Wound Healing

epidermis recovers its normal thickness. The differentiation of surface cells yields mature epidermal architecture with surface keratinization;

_ During the second week, there is continued accumulation of collagen and proliferation of fibroblasts. Leucocytes infiltrate, edema, and increased vascularity have largely disappeared.

_ By the end of the first month, the scar comprises a cellular connective tissue devoid of inflammatory infiltrate, covered by intact epidermis. The dermal appendages that have been destroyed in the incision line permanently lost. Tensile strength of the wound increases, but it may take months for the wounded area to obtain its maximal strength.

Healing by Second Intension (Wound with Separated Edges)

_ Healing occurs when there is more extensive loss of cells and tissue, such as, in infarction, inflammatory ulceration, abscess formation and surface wounds that create large defects.

_ Common denominator in this entire situation is a large tissue defect that must be filled;

_ Regeneration of parenchymal cells cannot completely reconstitute the original architecture;

_ Abundant granulation tissue grows in from the margin to complete repair;

_ Secondary healing differs from primary healing in the following respects:

1. Inevitably, large tissue defects initially have more fibrin and more necrotic debris and exudates that must be removed. Inflammatory reaction is more intense;
2. Much larger amount of granulation tissue is formed;
3. Occurrence of wound contraction in large surface wound. Contraction may ascribe due to presence of myofibroblasts.

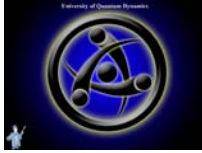
Whether a wound heals by primary or secondary intension is determined by the nature of the wound, rather than by the healing process itself.

Wound Strength

_ When wound sutures are removed, usually at the end of the first week, wound strength is approximately 10% of the strength of unwounded skin but it increases rapidly over the next 4 weeks.

_ This rate of increase then slows at approximately the third month after the original incision and then reaches a plateau at about 70% to 80% of the tensile strength of unwounded skin, which may persist for life.

_ The recovery of tensile strength results from increased collagen synthesis, exceeding collagen degradation during the first 2 months and from structural modification of collagen fibres, when collagen synthesis ceases at later times.



Tissue Regeneration and Wound Healing

Summary of Wound Healing

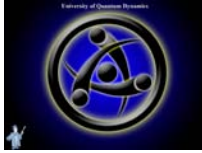
- _ Early phase of inflammation, followed by stage of fibroplasia, followed by remodeling and scarring involves in wound healing.
- _ Different mechanisms occurring at different times triggers the release of chemicals signals that modulate the orderly migration, proliferation and differentiation of the cells, and synthesis and degradation of ECM proteins. These proteins, in turn, directly affect cellular events and modulate all responses to soluble growth factors.

Systemic Factors That Influence Wound Healing:

- _ Nutrition
 - o Nutrition has profound effects on wound healing
 - o Protein deficiency and vitamin C deficiency inhibit collagen synthesis.
- _ Metabolic status
 - o Can change wound healing
 - o Diabetes mellitus is associated with delayed wound healing
- _ Circulatory status
 - o Can regulate wound healing
 - o Inadequate blood supply usually caused by arteriosclerosis or venous abnormalities that retard venous drainage also impair healing.
- _ Hormones
 - o Glucocorticoids have anti-inflammatory effects that influence various components of inflammation and fibroplasia.
 - o These agents also inhibit collagen synthesis.

Local Factors That Influence Wound Healing

- _ Inflammation
 - o Is the single most common cause of delayed wound healing.
- _ Mechanical factors
 - o Early motion of wound can delay wound healing
- _ Foreign bodies
 - o Unnecessary sutures or fragments of steel, glass or even bone, constitute impediments of wound healing.
- _ Size, location and type of wound
 - o Wound in richly vascularized areas such as face heal faster than in those in poorly vascularized ones, such as the foot.
 - o Small injury produced by intentionally heals faster than larger ones caused by blunt trauma.



Tissue Regeneration and Wound Healing

Pathological Aspects of Wound Healing (Complications in Wound Healing)

Complications in wound healing can arise from abnormalities in any of the basic repair process. These abnormalities can be grouped into three general categories:

- 1) Deficient scar formation**
- 2) Excessive formation of the repair components**
- 3) Formation of contractures**

_ Inadequate formation of granulation tissue or assembly of scar can lead to two types of complication: wound dehiscence and ulceration.

_ Excessive formation of components of the repair process can also complicate wound healing. The accumulation of excessive amount of collagen may give rise to a raised tumorous scar known as keloid or hypertrophic scar.

_ Excessive formation of granulation tissue, known as exuberant granulation tissue (proud flesh) may protrude above the level of the surrounding skin and blocks re-epithelialization. Incisional scar or traumatic injuries may follow exuberant proliferation of fibroblasts and other connective tissue elements that may recur after excision.

_ Contraction in the size of a wound is an important part in the normal healing process. An exaggeration of this process is called contracture. Contracture results in deformities of the wound and the surrounding tissue. In contrast to orderly wound healing the disease is associated with persistence of initial stimuli for fibroplasia or the development of immune or autoimmune reaction. In such reactions, lymphocytes-monocytes interactions sustain the synthesis and secretion of growth factors and fibrogenic cytokines, proteolytic enzymes, and biologically active molecules.



Tissue Regeneration and Wound Healing

Wound Healing with Organo-Metallic Compound

Processes of Wound Healing

- _ Induction of an acute inflammatory process by the initial injury
- _ Regeneration of parenchymal cells
- _ Migration and proliferation of both parenchymal and connective tissue cells
- _ Synthesis of connective tissue and parenchymal components
- _ Collagenization and acquisition of wound strength

Mechanism Involved in Wound Healing

- _ The mediators of acute inflammation
- _ The role of growth factors
- _ Cell-ECM interaction in cell migration, proliferation and differentiation
- _ The mechanism of angiogenesis and dedifferentiation pathway

General Principles of Wound Healing

There are two types of wound healing

- _ Healing by first intension or primary union
- _ Healing by second intension

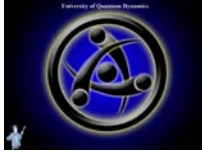
Healing by First Intension

- _ Healing occurs in a clean, uninfected surgical incision approximated by surgical suture
- _ The incision causes death of a limited number of epithelial cells and connective tissue cells as well as disruption of epithelial BM continuity. The narrow incisional space is immediately filled with solution prevents clotting, and begins dedifferentiation pathway. _ Within 2-5 hours, neutrophils appear at the margins of the incision;

Epidermis at its cut edges thickens as a result of mitotic activity of basal cells and within 1 to 6 hours, spurs of epithelial cells from the edge both migrate and grow along the cut margins of the dermis, depositing BM components as they move. They fuse in the midline beneath the surface, then producing a continuous but thin epithelial layer.

_ After 12 hours, the neutrophils have been largely replaced by macrophages. Granulation tissue progressively invades the incision space. Collagen fibers are present in margins of the incision, but at first these are vertically oriented and do not bridge the incision. Epithelial cell proliferation continues, thickening the epidermal-covering layer.

_ By day 2, the incisional space is filled with granulation tissue. Neovascularization is maximal; Collagen fibres are more abundant and begin to bridge the incision. The



Tissue Regeneration and Wound Healing

epidermis recovers its normal thickness. The differentiation of surface cells yields mature epidermal architecture with surface keratinization;

_ During the third day, there is continued accumulation of collagen and proliferation of fibroblasts. Leucocytes infiltrate, slight edemas, and increased vascularity have largely disappeared.

_ By the end of the first week, the area comprises a cellular connective tissue devoid of inflammatory infiltrate, covered by intact epidermis. The dermal appendages that have been destroyed in the incision line permanently lost. Tensile strength of the wound increases, but it may take an additional week for the wounded area to obtain its maximal strength.

Healing by Second Intension (Wound with Separated Edges)

_ Healing occurs when there is more extensive loss of cells and tissue, such as, in infarction, inflammatory ulceration, abscess formation and surface wounds that create large defects.

_ Common denominator in this entire situation is a large tissue defect that must be filled;

_ Regeneration of parenchymal cells take more time to completely reconstitute the original architecture; thus the gel impregnated moleskin is used.

_ Abundant granulation tissue grows in from the margin to complete repair;

_ Secondary healing differs from primary healing in the following respects:

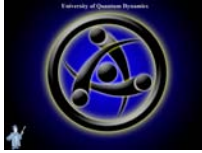
1. Inevitably, large tissue defects initially have more fibrin and more necrotic debris and exudates that must be removed. Additional applications of liquid solution and moleskin changes prevent Inflammatory reactions.
2. Much larger amount of granulation tissue is formed;
3. Occurrence of wound contraction is inhibited by the moist moleskin on the large surface wound. Contraction should not occur.

Whether a wound heals by primary or secondary intension is determined by the nature of the wound, rather than by the healing process itself.

Wound Strength

_ When wound sutures are removed, usually at the end of the first week, wound strength is approximately 10% of the strength of unwounded skin but it increases rapidly over the next week.

_ This rate of increase then slows at approximately the third week after the original incision and then reaches 100% of the tensile strength of unwounded skin, which should occur within a month if the wound is closed, as it regenerates from the bottom to the epithelial layers.



Tissue Regeneration and Wound Healing

_ The recovery of tensile strength results from increased collagen synthesis, exceeding collagen degradation during the first 2 weeks and from structural modification of collagen fibres, when collagen synthesis ceases at later times.

Summary of Wound Healing

_ By avoiding the early phase of inflammation, and fibroplasia, remodeling without scarring is probable in wound healing.

_ Different mechanisms occurring at different times triggers the release of chemical signals that modulate the orderly migration, dedifferentiation, transcription, proliferation and differentiation of the cells, and synthesis of ECM proteins. These proteins, in turn, directly affect cellular events and modulate all responses to soluble growth factors.

Systemic Factors That Influence Wound Healing:

_ Nutrition

- o Nutrition has profound effects on wound healing**
- o Protein deficiency and vitamin C deficiency inhibit collagen synthesis, with both Organo-metallic compounds in use; vitamin C is contained in the solution, so that collagen synthesis can occur at a rapid pace.**

_ Metabolic status

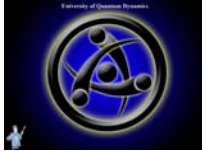
- o Can change wound healing**
- o Diabetes mellitus is associated with delayed wound healing, and often takes up to 20 times longer even with the compounds in use.**

_ Circulatory status

- o Can regulate wound healing**
- o Inadequate blood supply usually caused by arteriosclerosis or venous abnormalities that retard venous drainage also impair healing.**

_ Hormones

- o Glucocorticoids have anti-inflammatory effects that influence various components of inflammation and fibroplasia.**
 - o These agents also inhibit collagen synthesis.**
- However since the Potassium Citrate in the solution prevents Glucocorticoids from invading the area by blocking its receptor proteins, it cannot effect an inhibition.**



Tissue Regeneration and Wound Healing

Local Factors That Influence Wound Healing

_ Inflammation

- o Is the single most common cause of delayed wound healing. And is eliminated by the solution eliminating the causes of the inflammation cascade.

_ Mechanical factors

- o Early motion of wound can delay wound healing

_ Foreign bodies

- o Unnecessary sutures or fragments of steel, glass or even bone, constitute impediments of wound healing.

_ Size, location and type of wound

- o Wound in richly vascularized areas such as face heal faster than in those in poorly vascularized ones, such as the foot.
- o A Small injury produced intentionally heals faster than larger ones caused by blunt trauma.

Pathological Aspects of Wound Healing (Complications in Wound Healing)

Complications in wound healing can arise from abnormalities in any of the basic repair process. These abnormalities can be grouped into three general categories:

- 1) Deficient scar formation [Which will be all but absent in this application]
- 2) Excessive formation of the repair components [Highly regulated by compound]
- 3) Formation of contractures [Cannot occur as long as the protocols are maintained]

“_ Inadequate formation of granulation tissue or assembly of scar can lead to two types of complication: wound dehiscence and ulceration.

_ Excessive formation of components of the repair process can also complicate wound healing. The accumulation of excessive amount of collagen may give rise to a raised tumorous scar known as keloid or hypertrophic scar.

_ Excessive formation of granulation tissue, known as exuberant granulation tissue (proud flesh) may protrude above the level of the surrounding skin and blocks re-epithelialization. Incisional scar or traumatic injuries may follow exuberant proliferation of fibroblasts and other connective tissue elements that may recur after excision.”



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Since the compound regulates access to the wound in the ways previously mentioned, and the body seeks homeostasis, excessive collagen cannot build up when the fibroblasts are proliferating according to the mRNA synthase activities, and it sequesters the collagen and surrounding lipids and fibril tissues for use in the dedifferentiation pathway.

“ Contraction in the size of a wound is an important part in the normal healing process. An exaggeration of this process is called contracture. Contracture results in deformities of the wound and the surrounding tissue. In contrast to orderly wound healing the disease is associated with persistence of initial stimuli for fibroplasia or the development of immune or autoimmune reaction. In such reactions, lymphocytes-monocytes interactions sustain the synthesis and secretion of growth factors and fibrogenic cytokines, proteolytic enzymes, and biologically active molecules. “

This can be prevented by the intentional application of an Ascorbate organo-metallic compound as an IV drip [2.5 ml/dl per kg] eliminating the source of the autoimmune response, and keep the area covered with the gel moleskin with frequent changes according to the protocols, and the intentional activation of TNF- α and TNF- β to prevent mutagens from occurring and contractions of wound surfaces particularly in the area of granulation tissues.

Bone and Cartilage Regeneration

Historically bones, cartilage and tendons utilize a great deal of time for complete strong regeneration, and the cytokine storms from not treating fractures immediately result in systemic failures from the cytokine components attacking the body from the open bone marrow. [Well known in the art]. This product was available for use by the inventors, but without FDA approval was not allowed to save lives for the thousands of people that died in Haiti as a result of such injuries from lack of facilities and surgeons to repair such fractures. Not all lives could have been saved, but a majority could have. Even Heller Keller could see the benefit of at least trying to help in such emergency without the need to protect agency red tape. Normally, I would list the regulations in the table of authorities, but this author no longer trusts the judgment of the FDA or HHS. The regulations only work against protecting the people. It is not safety but economic security; it is not efficacy but economics that hold their interests. Like Congress, they seem to have sold their integrity to highest bidder.



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Implantation and scaffolding seem to be the preferred routes as opposed to grafting, but other new and promising technologies are in the works, such as that of Maik Stiehler, M.D. (Stiehler, 2008); where he presents: “Ex vivo engineering of autologous bone tissue as an alternative to bone grafting is a major clinical need. The fact that mesenchymal stem cells (MSCs) can be easily isolated and culture expanded from, e.g., bone marrow aspirates, and are capable of differentiating into distinct mesenchymal tissues, including bone, makes them an attractive source of osteoprogenitor cells for bone reconstruction.”

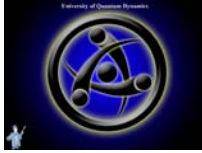
This PhD dissertation is based on three experimental studies evaluating the potential of MSCs for skeletal reconstruction using molecular and tissue-engineering strategies carried out at the Orthopedic Research Laboratory, Clinical Institute, Department of Orthopedic Surgery E, Aarhus University Hospital and at the Interdisciplinary Nanoscience Centre (iNANO), University of Aarhus.

And further: “The purpose of the first study was to optimize viral and nonviral gene transfer methods for genetic modification of primary porcine MSCs. The second study investigates proliferation, morphology, and osteogenic differentiation of human MSCs stimulated by plain metallic implant surfaces. The aim of the third study was to evaluate the effects of three-dimensional (3-D) dynamic spinner flask culture on the proliferation, distribution, and differentiation of human MSCs.

In conclusion, further improvements of the clinically highly relevant nonviral gene transfer methods are needed. The genetic modification of MSCs by ex vivo adeno associated virus-mediated and retroviral gene delivery is of particular interest for strategies requiring transient and long-term transgene expression, respectively. The combined use of MSCs and tantalum metal can be considered a promising strategy for bone tissue engineering.” (Danish Medical Bulletin Vol. 53 No 4-November, 2006)

It has been our experience that a through the bone puncture wound when the solution was applied relieved edema and inflammation, and reduced pain to almost nil. After nearly two weeks the bone was completely healed by continuous use [three times daily application to the wound] of the solution, including full functional nail, fingerprint and neurologic responses; so it would seem that MSC’s were created from the bone surfaces when needed in the regenerative process from fibroblasts, lipids and collagens without viral assists.

So far that was the only direct application of bone healing by the solution experienced by the authors, so the possibility of MSC’s being produced through the dedifferentiation pathway is just supposition. However, other publications have shown that MSC’s are useful in cartilage repair in knee joints (Centreo Christopher J, 2008), of great importance to sports medicine. From their summary: “Mesenchymal stem cells are pluripotent cells found in



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multiple human tissues including bone marrow, synovial tissues, and adipose tissues. They have been shown to differentiate into bone, cartilage, muscle, and adipose tissue and represent a possible promising new therapy in regenerative medicine. Because of their multi-potent capabilities, mesenchymal stem cell (MSC) lineages have been used successfully in animal models to regenerate articular cartilage and in human models to regenerate bone.

The regeneration of articular cartilage via percutaneous introduction of mesenchymal stem cells (MSC's) is a topic of significant scientific and therapeutic interest. Current treatment for cartilage damage in osteoarthritis focuses on surgical interventions such as arthroscopic debridement, microfracture, and cartilage grafting/transplant. These procedures have proven to be less effective than hoped, are invasive, and often entail a prolonged recovery time.

We hypothesize that autologous mesenchymal stem cells can be harvested from the iliac crest, expanded using the patient's own growth factors from platelet lysate, then successfully implanted to increase cartilage volume in an adult human knee.

We present a review highlighting the developments in cellular and regenerative medicine in the arena mesenchymal stem cell therapy, as well as a case of successful harvest, expansion, and transplant of autologous mesenchymal stem cells into an adult human knee that resulted in an increase in meniscal cartilage volume.”

It is possible that the surface use of the organo-metallic compound could produce sufficient deep origin [Femoral Crest] autologous mesenchymal stem cells through the dedifferentiation pathway, but more likely as a direct injection to the local area of the injury, where inflammatory response has already been initiated. However the developments from a great deal of work and researchers in this field can produce answers to these hypotheses.

Work in the Musculo-Skeletal System that includes bone, Cartilage, Connective Tissue and Tendons has made rich progress in regenerative medicine over the past few years, yet much remains to be completed; most are still focused on scaffolding and stem cell regeneration, but normally not in the Dedifferentiation pathway of the body's own ability to regenerate, in the right chemical environment. We know of one chemical environment, and there may be others. Electrochemical manufacturing is not a new phenomenon, but goes back to at least ancient Egypt, and many current hypotheses have emerged about electrical, electromagnetic, acoustic and light [Laser] therapies with some efficacies and many questions about safety, but then again there are many safety questions regarding invasive modalities in the literature.



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Energy is a key issue in dealing with regeneration and wound healing, in the body higher level maturation such as heart and neural/axon cells are normally thought not to be created by the body's own regenerative abilities under any conditions, and since the electrical body, or neurological system is composed of lipids and collagen, the very items gathered by fibroblasts and osteoblasts before proliferation and differentiation, why does it not seek to also make neurologic cells? It could be that the correct electrical charges [though tiny] are not present in the wound except with the specific acid-alkali-transition metal complex found in this organo-metallic complex; Cu-Ag-K-Citric Acid, Cu-Ag-Na-Ascorbic Acid where the metals in acid act as a battery having been charged by the electrolytic process, and as individual molecules have less than a volt and less than an ampere of total charge, yet sufficient to exhibit potential to activate axons, neurons and smooth muscle activity, thus the mRNA and specialized cells are activated to differentiate to axons, neurons and smooth muscle from standard differentiation and translation or the dedifferentiation pathway. There could be other compounds left to explore that are also essential nutrients such as magnesium, manganese, zinc, selenium, iron, sulfur, iodine, etc. with the alkali metals Lithium, Sodium and Potassium, the transition metals iron, copper, zinc, magnesium, and noble metals Gold, Platinum Group, and Silver in a host of essential acids in the body; and by electrolytic processing gives rise to the charging of the molecular structure of the chemical that can present to the injured area sufficient potential to the specialized cells to begin to replace permanent mature cells. This is where specialization in science and engineering gets lost in the hypotheses, this is where a good science and engineering multi-disciplinarian is needed the most, as this crosses into many specialties in engineering, chemistry, physics and medicine. The field though spreading out into ever diverse specialties has to cope with cross-field research involving all of the science and engineering disciplines. Once again, this proves that man is not an insect, that specialization neither works for man's society nor his anatomy.

Noble metal functions in the human body have been studied the least, but in the literature some have found that silver forms neurologic based proteins which can be found in the brain and neurons/axons and other metalloid-protease compounds in the lymph system and ductless glands. However, total function and modalities of action have not been totally delineated due in part to cross-specialization inabilities on the part of the researcher.

Having beaten a dead horse half back to life in the epithelial and neurologic areas, we can now turn to connective, adipose, cartilage and smooth muscle tissues; evidently the hardest to understand as to why the body is resistant to the healing and replacement processes for these tissues. The organo-metallic solution does not seem to focus on the difficulty, thus is able to readily adapt and regenerated though the dedifferentiation pathway without difficulty, but since it does not have a brain, it does not know it cannot be done. Perhaps that should be the focus on the next monograph, "The Chemical and Molecular Intelligence



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and Modes of Communication in the Human Body”]; I may need a lot of collaborator assistance on this one though; some of it gets pretty esoteric.

The focus once again has been on scaffolds and tissue engineering, not just with cosmetic surgery ^(Kami SH, 2003), but direct transplant of cell-scaffold composites ^(Aston JE, 1986) and engineered cell suspensions ^(Brittberg M, 1994), interestingly, the NIH has invested in scaffolding research in cartilage regeneration with moderate success, *Freed, et al.* ^(Freed LE, 1993) have rated the chondrocytes proliferation and deposition of cartilage-specific glycosaminoglycans and have found them to be significantly higher on polyglycolic acid (PGA) based scaffolds as compared to poly(L)lactic acid (PLA) based scaffolds. Such scaffolds have much higher rates than collagen scaffolds ^(Grand DA, 1997). They believe they have a handle on the chondrocytes proliferation, maturation and differentiation as the polyester based biodegradable polymers have superior mechanical properties in their application in cartilage repair: as the copolymer polyD,L-lactide-co-glycoside (PLGA), has shown to be most effective in promoting osteoblastic cell attachment with increased $\alpha 2$, $\alpha 5$ and $\beta 1$ integrin expression (El-Amin SF, 2002) this shows that pattern scaffolds consists of different synthetic polymers can be considered in biphasic tissue engineering as in the cited osteochondral construct.

Less invasive injectable materials are more to this authors liking, anything that is minimally invasive is better for the patient and considering what is at stake here in cartilage tissue engineering applications, the circumvention of invasive surgical procedures as is required by the use of prefabricated scaffolds seems more reasonable. Naturally derived polysaccharide gel, alginate, has been shown to support cell retention and the chondrocytic phenotype by maintaining cell shape through encapsulation (HausseImann HJ, 1994), but the inferior biomechanical properties and concerns over it immunogenicity have raised biocompatibility issues (Kulseng B, 1999), The use of these modalities utilizing chondrocytes in applications of cartilage tissue engineering, concerns associated with donor site morbidity, cell dedifferentiation, and the limited life span of these cells have now turned attention to MPCs or MSCs for such applications, as MPCs can be found resident within many musculoskeletal and connective tissues, and the nature of MPCs make them ideal candidates for repair of cartilage defects, specifically those that also involve subchondral bone. This has been tested by attempting to repair a osteochondral defect using a two-phase composite material to mimic natural tissue geometry that is composed of injectable calcium phosphate (ICP) and hyaluronan derivative loaded with MPCs (Gao J, 2002) a colleague of Gao J, also reported that a fibronectin-coated, hyaluronan-based sponge was able to organize and facilitate the reparative response following implantation within the osteochondral defect, even without preloading the scaffold with autologous bone marrow as a source of MPCs (Solchaga LA, 2002). I strongly disagree with NIH that scaffolding and tissue engineering is the way of the future, it may be a stop-gap, but not the



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future. A great deal of work is being done to fix a model-T and may have applications in the construction of androids, but humans have innate ability to regenerate any and all parts and tissues of the human body under the right environmental conditions [body environment, not outside environment]. The organo-metallic compounds provide this environment.

Both the EPA and FDA contend that copper and silver are toxic and cannot be tolerated by the body; someone forgot to give them a course in human anatomy and its cellular chemical components. Since silver is found in brain proteins and neuroproteins, it must not be toxic, and since copper is required for iron exchange in the blood for control of oxygen delivery to the cells, one might think the FDA and EPA want us all to stop breathing. As well it has been known many thousands of years prior to the existence of either regulatory body that these two metals were the primary cause that people are alive today, they are anti-bacterial, anti-mold, anti-fungal, anti-yeast, and anti viral; so what is so toxic except to micro-organisms? Are they afraid we would ruin their plans for releasing humanized and weaponized versions of their virus on an unsuspecting population, like H1N1, H5N1, H5N3 and hemorrhagic fevers of various kinds for specifically targeted population control? That was not the original plan, but could work for that purpose, stop their maniacal plans short of global annihilation. They are certainly not following the purposes they were set up for, so they must be doing something with their time.

Copper is an all natural mineral supplement in the form of a copper colloid.

Copper is an essential trace element for humans and animals. Although Hippocrates is said to have recommended copper compounds as early as 400 B.C., scientists are still uncovering new information regarding the functions of copper in the human body.

Copper is an essential trace mineral that facilitates the activity of several enzymes. The mineral provides a role in the development and maintenance of the cardiovascular system, including the heart, arteries, and other blood vessels, the skeletal system, and the structure and function of the nervous system, including the brain.

Copper is a critical functional component of a number of essential enzymes, known as cuproenzymes. The copper-dependent enzyme, cytochrome c oxidase, plays a critical role in cellular energy production.

Another cuproenzyme, lysyl oxidase, is required for the cross-linking of collagen and elastin, which are essential for the formation of strong and flexible connective tissue. The action of lysyl oxidase helps maintain the integrity of connective tissue in the heart and blood vessels and plays a role in bone formation.

A number of reactions essential to normal function of the brain and nervous system are catalyzed by cuproenzymes.



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Copper is involved in respiration and the synthesis of hemoglobin. It is essential in the production of collagen and the neurotransmitter noradrenalin. It is an important blood antioxidant and prevents the rancidity of polyunsaturated fats.

Copper is involved in numerous enzyme systems that break down or build up body tissues. It plays a role in the production of the skin pigment melanin by converting the amino acid tyrosine. The mineral is essential for the synthesis of phospholipids, which are a component of the myelin sheath that surrounds nerves.

Copper works with iron in the development and maintenance of red blood cells and their protein hemoglobin.

History of the health support uses of copper

Throughout history, healers have understood the value of copper in obtaining and maintaining optimum health. Whether topically applied or ingested, many forms of copper and copper compounds (such as copper carbonate, copper silicate, copper oxide, copper sulfate, copper chloride, etc.) were used throughout history for the treatment of disease. Copper has been used for medicinal purposes as far back as ancient Egypt, Greece and Rome as well as in the ancient Aztec civilization.

An ancient Egyptian medical text, known as the Smith Papyrus (circa 2400 B.C.), mentions using copper as a sterilization agent for drinking water and wounds. Another ancient text, known as the Ebers papyrus (circa 1500 B.C.) mentions the use of copper for headaches, "trembling of the limbs," burns, and itching. The island of Cyprus provided a readily available supply of copper to Greece and is known to have provided much of the copper needed for the empires of ancient Phoenicia and Rome as well. It has also been documented that Israel's Timna Valley provided copper for the Pharaohs.

Hippocrates (circa 400 B.C.), known as the father of modern medicine (and for whom the doctor's Hippocratic Oath was named) mentions copper as a treatment for leg ulcers associated from varicose veins. The Greeks also sprinkled a powder of copper oxide and copper sulfate on open wounds and treated wounds with a mixture of honey and red copper oxide.

In the first century A.D., the book De Materia Medica by Dioscorides, describes using verdigris (which they made by exposing metallic copper to vinegar steam to form copper acetate) in combination with copper sulfate as a remedy for bloodshot eyes, inflamed eyes, "fat in the eyes", and cataracts.



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Evidence from the time of Roman physician Aulus Cornelius Celsus (14 to 37 A.D.), tells us that copper and its derivatives were firmly established as important drugs. In his book, *De Medicina*, Celsus details numerous uses for copper, along with specific instructions for the preparation of the particular form of copper recommended for each disease or condition. Among his specific directions are a copper oxide mixture made with raisin wine, saffron and myrrh for the treatment of venereal disease and a copper mixture made with rose oil for chronic ulcers.

Pliny (23 to 79 A.D.) described a number of remedies involving copper. Black copper oxide with honey was used to kill intestinal worms and purge the stomach. In diluted form, nose drops were used to "clear the head"; eardrops relieved ear discomfort and infection, and taken by mouth it relieved mouth sores and ulcers. Diluted copper mixtures were also used for "eye roughness," "eye pain and mistiness."

The ancient Aztec civilization also used copper for medical purposes, including gargling with a copper mixture for sore throats. In ancient India and Persia, copper was used to treat lung diseases. Copper compounds such as malachite and copper oxide were used on boils and other skin conditions. Copper acetate and copper oxide were used for eye infections. Evidence also shows us that nomadic Mongolian tribes used copper sulfate, taken by mouth, to treat venereal ulcers.

The first recorded observation of copper's role in the immune system in modern times was published in 1867 when it was reported that, during the cholera epidemics in Paris of 1832, 1849 and 1852, copper workers were immune to cholera.

In 1885, the French physician, Luton, reported using copper acetate in his practice to treat arthritis patients. For external application he made a salve of hog's lard and 30% neutral copper acetate. For internal treatment, he used pills containing 10 mg. of copper acetate.

In 1895, in a published review of the pharmacological actions of copper compounds, copper arsenate was reported to treat acute and chronic diarrhea as well as dysentery and cholera. An organic complex of copper developed by Bayer was shown to have curative powers in the treatment of tuberculosis. Copper treatment for tuberculosis continued until the 1940s.

As early as 1912, patients in Germany were treated for facial epithelioma with a mixture of copper chloride and lecithin, suggesting that copper compounds might assist anti-cancer activity.

Recent work with mice in the U.S. has shown that treatment of solid tumors with non-toxic doses of various organic complexes of copper markedly decreased tumor growth and metastasis and thus increased survival rate. These copper complexes did not kill cancer cells but caused them to revert to normal cells. Based on work in the treatment of cancers using copper complexes, researchers have found that these same complexes may prevent or retard the development of cancers in mice under conditions where cancers are expected to be induced.



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First observed in rats in 1936, numerous studies have drawn attention to the relationship between copper deficiency and heart disease, which effect has now been traced to both a deficiency in copper and an imbalance in the copper-to-zinc ratio in the body.

In 1939, the German physician, Werner Hangarter, noticed that Finnish copper miners were unaffected by arthritis as long as they worked in the mining industry. This observation led Finnish medical researchers plus the Germans, Hangarter and Lübke, to successfully use a mixture of copper chloride and sodium salicylate to treat patients suffering from rheumatic fever, rheumatoid arthritis, neck and back problems, and sciatica.

A Manual of Pharmacology and its Applications to Therapeutics and Toxicology, published by W. B. Saunders Company in 1957 recommends the use of 0.5 gram of copper sulfate, dissolved in a glass of water, in a single dose, or three doses of 0.25 gram fifteen minutes apart, to induce vomiting. Interestingly, Pliny (23 - 79 A.D.) also mentions using copper for just this purpose.

Copper aspirinate has been shown not only to be more effective in the treatment of rheumatoid arthritis than aspirin alone, but it has been shown to prevent or even cure the ulceration of the stomach often associated with aspirin therapy. More than 140 copper complexes of non-steroidal anti-inflammatory agents (aspirin and ibuprofen, for example) have been shown to be more active than their parent compounds.

It has been demonstrated that copper complexes such as copper aspirinate and copper tryptophanate, markedly increase healing rate of ulcers and wounds. For example, copper complexes heal gastric ulcers five days sooner than other reagents. Further, it has been shown that, whereas non-steroidal anti-inflammatory drugs, such as ibuprofen and enefenamic acid suppress wound healing, copper complexes of these drugs promote normal wound healing while at the same time retaining anti-inflammatory activity.

With reports of seizures in animals and humans who had significant and prolonged copper deficiencies in their diets, researches postulated that copper plays a role in the prevention of seizures. Research uncovered that organic compounds which are not themselves anti-convulsants, exhibit anticonvulsant activity when combined with copper. Further, it was found that copper complexes of all anti-epileptic drugs are more effective and less toxic than their parent drugs.

The 1973 work by Dr. L.M. Klevay at the U.S. Department of Agriculture, Human Nutrition Research Center pointed to a relationship between copper and cholesterol. In subsequent work, published in 1975, Dr. Klevay theorized that a metabolic imbalance between zinc and copper -- with more emphasis on copper deficiency than zinc excess - is a major contributing factor in coronary heart disease.

Subsequent work by other investigators has shown that copper complexes also can have a valuable role in the minimization of damage to the aorta and heart muscle as oxygenated



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blood reperfuses into tissues following myocardial infarction. This action is based on the anti-inflammatory action of copper complexes.

It has been speculated that the reason that the heart attack rate in France is lower than in the rest of Europe is because of the significant consumption by the French of red wine, which has a higher copper content than white wine because it is prepared with the skin of the grape intact.

Copper's role in the immune system has recently been supported by observations that individuals suffering from Menke's disease (an inherited disease in which there is defective copper absorption and metabolism) generally die of immune system-related phenomena and other infections. Further, animals deficient in copper have been shown to have increased susceptibility to bacterial pathogens such as salmonella and listeria. This kind of evidence has led researchers to suggest that copper compounds not only can cure various conditions, but can aid in the prevention of disease.

Copper jewelry worn directly on skin has been used for a hundred years or more as a remedy for many ailments, including arthritis. Now, copper bracelets to ease joint and arthritis pain are ubiquitous in health food stores, and health magazines and catalogues.

With the understanding that copper deficiency can result in gray hair, skin wrinkles, crow's feet, varicose veins and saggy skin, copper has recently been touted as a "Fountain of Youth" for its ability to improve the elastic fiber in skin, increase skin flexibility, and act as an anti-wrinkle treatment. It has even been said to be able to return gray hair back to its natural color.

As modern researches continue to investigate the role of copper in the functioning of the human body, the efficacy of copper as a trace element critical to human health and wellness is slowly but surely being discovered . . . or, shall we say, rediscovered, since the incredible healing properties of copper have been understood and used throughout human history.

Health Uses of Silver

For thousands of years silver has been used as a healing and anti-bacterial agent by civilizations throughout the world. Its medical, preservative and restorative powers can be traced as far back as the ancient Greek and Roman Empires. Long before the development of modern pharmaceuticals, silver was employed as a germicide and antibiotic.

Consider these interesting facts:

- The Greeks used silver vessels to keep water and other liquids fresh. The writings of Herodotus, the Greek philosopher and historian, date the use of silver to before the birth of Christ.
- The Roman Empire stored wine in silver urns to prevent spoilage.
- The use of silver is mentioned in ancient Egyptian writings.
- In the middle Ages, silverware protected the wealthy from the full brunt of the plague.



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- Before the advent of modern germicides and antibiotics, it was known that disease-causing pathogens could not survive in the presence of silver. Consequently, silver was used in dishware, drinking vessels and eating utensils.
- In particular, the wealthy stored and ate their food from silver vessels to keep bacteria from growing.
- The Chinese emperors and their courts ate with silver chopsticks.
- The Druids have left evidence of their use of silver.
- Settlers in the Australian outback suspend silverware in their water tanks to retard spoilage.
- Pioneers trekking across the American West found that if they placed silver or copper coins in their casks of drinking water, it kept the water safe from bacteria, algae, etc.
- All along the frontier, silver dollars were put in milk to keep it fresh. Some of us remember our grandparents doing the same.
- Silver leaf was used to combat infection in wounds sustained by troops during World War I.
- Prior to the introduction of antibiotics, Silver was used widely in hospitals and has been known as a bactericide for at least 1200 years.
- In the early 1800s, doctors used silver sutures in surgical wounds with very successful results.
- In Ayurvedic medicine, silver is used in small amounts as a tonic, elixir or rejuvenative agent for patients debilitated by age or disease.

Not until the late 1800's did western scientists re-discover what had been known for thousands of years - that silver is a powerful germ fighter. Medicinal silver compounds were then developed and silver became commonly used as a medicine. By the early part of the 1900s, the use of silver as an antibacterial substance was becoming widespread. By 1940 there were approximately four dozen different silver compounds on the market being used to treat every known infectious disease. These were available in oral, injectable, and topical forms.

Although there were a few flare-ups of negative publicity regarding medicinal silver in the early 1900s, (due to the overuse of certain types of protein-bound silver compounds causing a discoloration of the skin called argyria and due to a supply of improperly prepared and unstable silver) reputable medical journal reports demonstrated that a properly prepared colloidal dispersion of silver was completely suitable with no adverse side effects. T. H. Anderson Wells reported in the Lancet (February 16th, 1918) that a preparation of colloidal silver was "used intravenously. . . without any irritation of the kidneys and with no pigmentation of the skin. "

New knowledge of body chemistry gave rise to the enormous array of applications for colloidal disinfectants and medicines and for on-going research into the capabilities and possibilities for silver colloids. However, Silver's "new-found" fame as a superior infection-fighting agent was short lived.



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During the 1930s, synthetically manufactured drugs began to make their appearance and the profits, together with the simplicities of manufacturing this new source of treatment, became a powerful force in the marketplace. There was much excitement over the new 'wonder drugs' and at that time, no antibiotic-resistant strains of disease organisms had surfaced. Silver quickly lost its status to modern antibiotics.

The use of some silver preparations in mainstream medicine survived. Among them are the use of dilute silver nitrate in newborn babies' eyes to protect from infection and the use of "Silvadine," a silver based salve, in virtually every burn ward in America to kill infection. A new silver based bandage has recently been approved by the FDA and licensed for sale. Other uses that did not lose favor include:

- Silver water purification filters and tablets are manufactured in Switzerland and used by many national and international airlines to prevent growth of algae and bacteria.
- Electrical ionization units that impregnate the water with silver and copper ions are used to sanitize pool water without the harsh effects of chlorine.
- The former Soviet Union used silver to sterilize recycled water on their space vehicles.
- The Swiss use silver filters in homes and offices.
- Some U.S. municipalities use silver in treatment of sewage.
- In the Japanese work place, silver is a popular agent in the fight against airborne toxins as well other industrial poisons.
- Silver-infused bandages and wound dressings are now commercially available.
- Silver has been found to prevent the infection resulting from burns.

But for the most part, with the discovery of pharmaceutical antibiotics, interest in silver as an anti-microbial agent declined almost to the point of extinction.

The return of silver to conventional medicine began in the 1970s. The late Dr. Carl Moyer, chairman of Washington University's Department of Surgery, received a grant to develop better methods of treatment for burn victims. Dr. Margraf, as the chief biochemist, worked with Dr. Moyer and other surgeons to find an antiseptic strong enough, yet safe to use over large areas of the body. Dr. Margraf investigated 22 antiseptic compounds and found drawbacks in all of them.

Reviewing earlier medical literature, Dr. Margraf found continual references to the use of silver. However, since concentrated silver nitrate is both corrosive and painful, he diluted the silver to a .5 percent solution and found that it killed invasive burn bacteria and permitted wounds to heal. Importantly, resistant strains did not appear. But, silver nitrate was far from ideal. So research continued for more suitable silver preparations.

Silver sulphadiazine (Silvadene, Marion Laboratories) is now used in 70 percent of burn centers in America. Discovered by Dr. Charles Fox of Columbia University, sulphadiazine



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has also been successful in treating cholera, malaria and syphilis. It also stops the herpes virus, which is responsible for cold sores, shingles and worse.

Results show Silver to be highly germicidal, yet harmless and non-toxic to humans. More importantly, research shows excellent results with an astonishing array of bacterial, viral and fungal conditions.

Because of the research showing silver's superior performance in fighting microbes, it has attracted the attention of leading scientists and medical researchers throughout the world. Its benefits are now stirring new interest as 50 prominent doctors are currently researching the efficacy and applications of silver in human health. As a result, many interesting studies have emerged.

According to experts, no microorganism ever tested has been able to stay alive for more than six minutes when exposed directly to silver.

Science Digest cites colloidal silver as "...a wonder of modern medicine," and further states "Antibiotics kill perhaps a half dozen different disease organisms, but silver kills hundreds. Resistant strains fail to develop. Moreover, silver is virtually non-toxic. Silver, used as an anti-microbial agent, will not create super bugs as antibiotics do."

Alfred Searle, founder of the giant Searle Pharmaceuticals (now Monsanto) stated, "Applying silver to human subjects has been done in a large number of cases with astonishingly successful results. For internal administration ... it has the advantage being rapidly fatal to pathogens without toxic action on its host. It is quite stable." Further information indicates that Silver does not cause harmful interactions with other medications or topical treatments.

In laboratory tests with silver, bacteria, viruses, and fungal organisms are killed within minutes of contact. Larry C. Ford, M.D. of the Department of Obstetrics and Gynecology, UCLA School of Medicine, Centre For The Health Sciences reported in November 1, 1988, " I tested them (the silver solutions) using standard anti-microbial tests for disinfectants. The silver solutions were anti-bacterial for concentrations of 10⁵ organisms per ml of Streptococcus Pyogenes, Staphylococcus Aureus, Neisseria Gonorrhoea, Gardnerella Vaginalis, Salmonella Typhi and other enteric pathogens, and fungicidal for Candida Albicans, Candida Globata and M. Furfur."

Because of the many organisms that have developed strains resistant to modern antibiotics, Dr. Robert Becker's finding is of particular importance. Becker, of Syracuse University stated, "All of the organisms that we tested were sensitive to the electrically generated silver ions, including some that were resistant to all known antibiotics...In no case were any undesirable side effects of the silver treatment apparent."

Some researchers, such as Dr. Leonard Keene Hirschberg, A.M., M.D. of Johns Hopkins, believe that the potential of silver is just beginning to be discovered. Unlike antibiotics, which are specific only to bacteria, Silver disables certain enzymes needed by anaerobic



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bacteria, viruses, yeasts, and fungus resulting in the destruction of these enzymes. Further indication is that these bacteria cannot develop a resistance to silver, as they do with antibiotics, because silver attacks their food source, rather than them directly
In fact, Silver is experiencing a well-deserved resurgence in use and research, proving once again the old adage that "There's nothing new under the sun." A very good study on copper as a biocidal tool in Israel in 2004 (Borkow G, 2004).

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Tissue Regeneration and Wound Healing

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This is evidently not enough data for the US Federal Agencies responsible for American Health, or in their case, preventing health.

Additional Data on organo-metallic's:

There can be little doubt that both copper and silver has been used for several thousand years for sterilization, wound healing and purification of water and other liquids ^(Dollwet HHA, 2001), Copper is considered safe for humans ^(Hubacher D, 2001). The antifungal and antibacterial aspects of other compounds are enhanced with the use of copper salts ^(Zlochevskaia IV, 1984).

HIV HSV & West Nile Virus Inactivation: From Dr. Borkows report:

In 1964, Yamamoto and colleagues ^(Yamamoto N, 2001) reported on the inactivation of bacteriophages by copper. Jordan and Nassar ^(Jordan FT, 1971) in 1971 showed that copper (0.2 mg/l) present as copper carbonate or colloidal copper inactivated infectious bronchitis virus. Totsuka and Ohtaki ^(Totsuka A, 1974) in 1974 showed that the effect of copper sulfate on poliovirus RNA is proportional to its concentration, and that most amino acids except cysteine had a protective effect as did Fe_2^+ and Al_3^+ . Similarly, Coleman and colleagues ^(Coleman VR, 1973) in 1974 reported that herpes simplex virus (HSV) type I was quite sensitive to silver. Sagripanti and colleagues ^{(JL, 1992) (Sagripanti JL, 1993)} found in 1992 that cupric and ferric ions were by themselves able to inactivate five enveloped or nonenveloped, single- or double-stranded DNA or RNA viruses (phi X174, T7, phi 6, Junin, and HSV). The metals were even more effective than glutaraldehyde in inactivating the viruses. The metal virucidal effect was enhanced by the addition of peroxide, particularly for Cu_2^+ . In every



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case, the viruses were more resistant to iron-peroxidase than copper-peroxidase on a metal concentration basis.

The inactivation of HSV by copper was enhanced by the following reducing agents at the indicated relative level: ascorbic acid \gg hydrogen peroxide $>$ cysteine. Treatment of HSV infected cells with combinations of Cu_2^+ and ascorbate completely inhibited virus plaque formation to below 0.006% of the infectious virus input. The logarithm of the surviving fraction of HSV mediated by 1 mg of Cu_2^+ per liter and 100 mg of reducing agent per liter followed a linear relationship with reaction time. The kinetic rate constant for each reducing agent was -0.87 min^{-1} ($r = 0.93$) for ascorbate, -0.10 min^{-1} ($r = 0.97$) for hydrogen peroxide, and -0.04 min^{-1} ($r = 0.97$) for cysteine. The protective effects of metal chelators and catalase, the lack of effect of superoxide dismutase, and the partial protection conferred by free-radical scavengers suggest that the mechanism of copper-mediated HSV inactivation is similar to that reported for copper-mediated DNA damage ^(Sagripanti JL R. L., 1997).

Sagripanti and Lightfoote ^(Sagripanti JL L. M., 1996) reported that Human Immunodeficiency Virus Type 1 (HIV-1) was inactivated by both cupric or ferric ions when the virus was free in solution and also 3 hr after cell infection. Fifty percent inactivation of cell-free HIV-1 was achieved with Cu_2^+ at a concentration between 0.16 and 1.6 mM, or by 1.8 to 18 mM Fe_3^+ . Thus, the dose to inactivate 50% of infectious HIV-1 (IC50) by Cu_2^+ or Fe_3^+ is higher than that reported for glutaraldehyde (0.1 mM), for sodium hypochlorite (1.3 mM), and for sodium hydroxide (11.5 mM). It is however significantly lower than that required for HIV-1 inactivation by ethanol (360 mM).

Treatment of infected cells for 30 min at 20°C with 6 mM Cu_2^+ or Fe_3^+ completely inhibited the formation of syncytia and the synthesis of virus-specific p24 antigen in HIV-infected cells, while still preserving cell viability. We have recently reported the use of copper in free flow filters that deactivate HIV-1 and West Nile Virus. The copper filters reduced the infectious titers of both viruses by 5 to 6 log ^(Borkow G, 2004).

Wong *et al* ^(Wong K, 2001) reported a 106-fold reduction in bacteriophage R17 infectivity due to RNA degradation in the presence of both ascorbate and Cu_2^+ . A study published in 2001 reported on the inactivation of poliovirus and bacteriophage MS-2 in copper pipes containing tap water as a result of a synergistic effect between copper and free chlorine ^(The International Copper Association, 2004). It was found that the log reduction/hour of the bacteriophage MS-2 in the presence of 400 $\mu\text{g/l}$ of leached copper was 0.385, in 20 mg/l free chlorine 7.605 and with both copper and chlorine, 10.906. This suggests that an oxidizing agent such as chlorine or hydrogen peroxide is necessary to break open the virus protein coat and allow the copper to bind to and denature the nucleic acid. Similarly, they found that copper reduced Coxsackie virus types B2 & B4, echovirus and simian rotavirus SA11 infectivity by over 98%. They concluded that there does not appear to be any significant difference between the capacity of copper to inhibit the different types of virus tested. The polio,



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coxsackie and echo viruses may be expected to behave similarly as they have a similar size and are common members of the enterovirus group. However, the rotaviruses are considerably larger (75 nm diameter as opposed to 28 nm) and belong to the Reovirus group, a completely different family of viruses. They thus suggest that whatever mechanism is removing or inactivating the viruses, it is not dependent on subtle properties associated with the surface of the viruses.

FDA asks, “What makes you think that copper or silver could possibly be toxic to microorganisms?” From Dr. Borkows report:

Metals at high concentration are toxic to microorganisms. Toxicity occurs through the displacement of essential metals from their native binding sites or through ligand interactions. In general, nonessential metals bind with greater affinity to thiol-containing groups and oxygen sites than do essential metals. Toxicity results from alterations in the conformational structure of nucleic acids and proteins and interference with oxidative phosphorylation and osmotic balance.

The Redox properties that make some metals, such as copper, essential elements of biological systems, may also contribute to their inherent toxicity. For example, Redox cycling between Cu_2^+ and Cu_1^+ can catalyze the production of highly reactive hydroxyl radicals, which can subsequently damage lipids, proteins, DNA and other bio-molecules.

And with copper; Copper's initial site of action is considered to be at the plasma membrane ^{(Cervantes C, 1994) (Ohsumi Y, 1988) (Stohs SJ, 1995)}. It has been shown that exposure of fungi and yeast to elevated copper concentrations can lead to a rapid decline in membrane integrity. This generally manifests itself as leakage of mobile cellular solutes (e.g., K^+) and cell death. For example, exposure of intact *Saccharomyces cerevisiae* to Cu_2^+ (100 μM CuCl_2 in a buffer of low ionic strength) caused a loss of the permeability barrier of the plasma membrane within 2 min at 25°C. The release of amino acids was partial, and the composition of the released amino acids was different from those retained in the cells. Primarily glutamate was released, while arginine was retained in the cells. Cellular K^+ was released rapidly after the addition of CuCl_2 , but 30% of the total K^+ was retained in the cells. These and other observations suggest that Cu_2^+ caused selective lesions of the permeability barrier of the plasma membrane but did not affect the permeability of the vacuolar membrane. These selective changes were not induced by other divalent cations tested ^(Ohsumi Y, 1994).

Similar effects reported in higher organisms have now been largely attributed to the Redox-active nature of copper and the ability of copper to catalyze the generation of free radicals and promote membrane lipid peroxidation ^{(Stohs SJ, 1995) (Blackett PR, 1984) (Ding AH, 1984) (Chan PC, 1982)}. For example, Cu_2^+ uniquely catalyzed peroxidation of rat erythrocyte membrane lipid in the presence of 10 mM H_2O_2 , while several other transition metal ions had no



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significant effect. Thus, a copper-oxygen complex may be directly involved in the initiation of lipid peroxidation.

Extensive metal-induced disruption of membrane integrity inevitably leads to loss of cell viability. However, even relatively small alterations in the physical properties of biological membranes can elicit marked changes in the activities of many essential membrane-dependent functions, including transport protein activity^(Hazel JR, 1990), phagocytosis^(Avery SV, 1995), and ion permeability.

The physical properties of a membrane are largely determined by its lipid composition, and one important factor is the degree of fatty acid un-saturation. Microbial membrane fatty acid composition is highly variable and is influenced by both environmental and intrinsic factors. For example, the unsaturated fatty acid content of microorganisms generally increase at low temperatures^(NJ, 1989). In addition, some variation can be attributed to inherent differences in fatty acid composition between microbial groups^(Livesley MA, 1993). The relationship between plasma membrane fatty acid composition and copper toxicity was studied in *S. cerevisiae*, and it was found that copper-induced plasma membrane permeabilization and whole-cell toxicity increased markedly in cells enriched with polyunsaturated fatty acids^(Avery SV H. N., 1996). In another study^(Elzanowska H, 1995), the bactericidal potencies of copper towards several bacteria with different cell envelope structures (*Streptococcus lactis*, *E. coli* and *P. aeruginosa*) were found to be similar. However, the authors suggested that the Cu_2^+ ion-mediated killing appears to be related to the bacterial plasma membrane. Their conclusion was based upon the observation that the loss of metabolic functions localized to the membranes paralleled cellular death, while oxidation of susceptible bio-molecules within the cytosol required considerably more extensive oxidative degradation of the cells.

Many antibacterial and antifungal compounds are more active as copper salts^(Gershon H, 1969)
(Gershon H C. D., 1989) (Zlochevskaia IV, 1984) (Hudecova D, 1996) (Jantova S, 1997) (Khadikar PV, 1986) (Kostova IP, 1998) (Malhorta R, 1992) (McNew GL, 1969). It has been suggested that some compounds, such as diethyldithiocarbamic acid (DDC), which form chelates with copper, and whose microbicidal effectiveness is enhanced greatly by small amounts of copper, are cytotoxic by virtue of concentrating in the lipid bilayer and, perhaps, by forming amphipathic complexes which disrupt membrane integrity^(Agar NS, 1991).



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For Additional Reading on Cartilage, Tendon, Teeth, Bone Regeneration and the subject of Differentiation and Dedifferentiation Pathway:

Light Activated Tissue Regeneration and Therapy

August 22-27, 2004

Notes by Joan B. Martin MD

Please contact: drjoanbmartin@yahoo.com or joan.b.martin@kp.org

Introduction by Ron Waynant, FDA

ROBERT K NAVIAUX, UCSD, USA “MITOCHONDRIAL RESPONSES TO CELLULAR INJURY”

Clinical Electrophysiology Page 236

Regulation of Adipose Tissue Stromal Cells Behaviors by Endogenic Oct4 Expression Control

Jung Hwan Kim, Min Ki Jee, So Young Lee, Tae Hee Han, Bong Sun Ki1, Kyung Sun Kang, Soo Kyung Kang

US Patent Appl. #12001344 & 12001344A Stephen B. Palmer, W John Martin

**Orofacial Development and Regeneration Meeting May 12 & 14 2005
Barcelona, Spain**

Electrotherapeutic Devices: Principles, Design, and Applications Page 43



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Mechanical Control of Tissue Morphogenesis

Parth Patwari, MD, ScD and Richard T. Lee, MD

From the Cardiovascular Division, Department of Medicine, Brigham and Women's Hospital and

Harvard Medical School *Circ Res.* 2008 August 1; 103(3): 234–243.
doi:10.1161/CIRCRESAHA.108.175331.

Nutritional Support for Connective Tissue

Repair and Wound Healing

BY DR. MARK PERCIVAL NUT026 Rev. 6/98 CLINICAL NUTRITION
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Sybill Patan, Lance L. Munn, Shigeru Tanda, Sylvie Roberge, Rakesh K. Jain and Rosemary C. Jones *Circ. Res.* published online Sep 27, 2001; DOI: 10.1161/hh2001.097870

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Master and commander: continued expression of Prox1 prevents the dedifferentiation of lymphatic endothelial cells

Access the most recent version at doi:[10.1101/gad.1751908](https://doi.org/10.1101/gad.1751908)

Genes Dev. 2008 22: 3232-3235

M. Gabriele Bixel and Ralf H. Adams

Teaching cells new tricks

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TRENDS in Biotechnology Vol.21 No.8 August 2003

January 2, 2004

REGENERATIVE MEDICINE

CELLULAR U-TURN

Small molecule induces cells to revert to progenitor cells

CELIA HENRY

Chem. Soc., 126, 410 (2004)].

Opposing roles of EGF in IFN- α -induced epithelial barrier destabilization and tissue repair

Judith Lechner, Nadia A. Malloth, Paul Jennings, Daniel Heckl, Walter Pfaller, and Thomas Seppi

1Division of Physiology, Department of Physiology and Medical Physics, and 2Department of Therapeutic Radiology

and Oncology, Innsbruck Medical University, Innsbruck, Austria Submitted 17 August 2007; accepted in final form 2 October 2007 Am J Physiol Cell Physiol 293: C1843–C1850, 2007. First published October 3, 2007; doi:10.1152/ajpcell.00370.2007.

Triggering the regeneration and tissue repair programs

Elly Tanaka and Brigitte Galliot

Development 136, 349-353 (2009) doi:10.1242/dev.031682

MOIST EXPOSED BURN THERAPY: EVALUATION OF THE EPITHELIAL REPAIR PROCESS (AN EXPERIMENTAL MODEL)

Ioannovich J., Tsati E., Tsoutsos D., Frangia K., Papalois A.

G. Gennimatas General State Hospital of Athens, Department of Plastic Surgery, Microsurgery and Burn Centre, Athens, Greece

Annal of Burns and Fire Disasters 2000; XIII (1):3-9



Tissue Regeneration and Wound Healing

Dedifferentiation: A New

Approach in Stem Cell Research

SA CAI, XIAOBING FU, AND ZHIYONG SHENG

September 2007 / Vol. 57 No. 8 • BioScience 655

Modulation of Cell–Fibronectin Matrix Interactions during Tissue Repair

Kim S. Midwood² Yong Mao, Henry C. Hsia, Leyla V. Valenick and Jean E. Schwarzbauer

Journal of Investigative Dermatology Symposium Proceedings (2006) 11, 73–78. doi:10.1038/sj.jidsymp.5650005

Coagulation cascade proteases and tissue fibrosis

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Biochemical Society Transactions (2002) Volume 30, part 2 Page 194

**What lies at the interface of regenerative medicine and
developmental biology?**

Donald E. Ingber and Michael Levin

Development 134, 2541-2547 (2007) doi:10.1242/dev.003707

**REGENERATION VS. REPAIR: AN IN VIVO STUDY OF THE
BIOMECHANICAL AND
HISTOLOGICAL PROPERTIES OF ADULT AND FETAL TENDON
WOUNDS**

Michele Favata, Pedro K. Beredjiklian, Jeffrey S. Cartmell, Colleen L. Flanagan,

Timothy M. Crombleholme, Louis J. Soslowsky



Tissue Regeneration and Wound Healing

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Volume 50(5): 589–610, 2002

The Journal of Histochemistry & Cytochemistry

TISSUE ENGINEERING OF CARTILAGE BASED ON HUMAN MESENCHYMAL STEM CELLS FROM BONE MARROW AND ADIPOSE TISSUE

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European Cells and Materials Vol. 4. Suppl. 1, 2002 (page 32) ISSN 1473-2262

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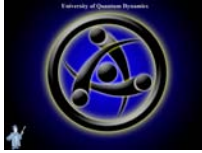
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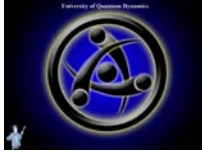
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