ME 482 Experimental Tissue Mechanics

Professor A. Wagoner Johnson

Friday, 8/26/16

See Piazza for all handouts and some lecture notes
piazza.com/illinois/fall2016/me482/home

NOTE: many of the images used in the notes are selected based on their visual value, and are cited. The sources are not necessarily scholarly, and therefore these or ones like them might not be appropriate for use as references in your projects.

1

Lab Website should have all you need for the lab…

me482uiuc2016.wordpress.com

Sign-ups will be on Piazza. Times will generally be:

- Friday, 11-12
- Friday, 12-1
- Wednesday, late afternoons

- Lab is critical component of the course. You MUST attend ALL labs. If you miss, your grade will suffer.
- If you will be out of town on a Friday, you need to sign up for a Wednesday slot.
- It is not really possible to make up most labs bc so much is involved in getting everything set up and run.

2

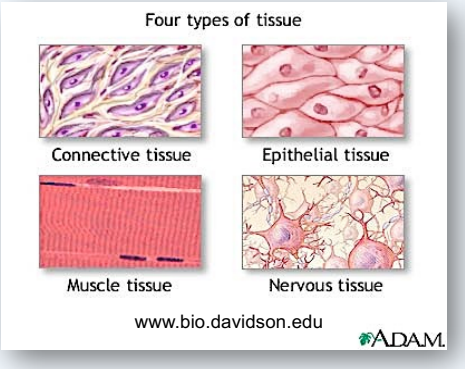
LAST TIME:
What are some challenges associated with characterization of mechanical properties of tissue?

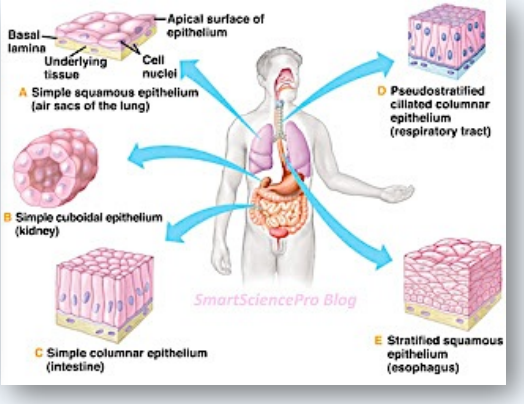
Many will become apparent throughout the course!

- Limited number of samples and difficult to obtain
- Structure and composition change during collection and preparation
- Inhomogeneous structure – what part do you test?
- Complex tissue – difficult to obtain fundamental physical parameters – what does modulus mean?
- Tissue often cannot be frozen or stored without altering structure and properties
- In vitro and in vivo measurements do not always correlate, sometimes impossible

Four types of tissue

- nerve transfers info from one location to another in the body…
- epithelial tissue
  - sheets of cells that line inner and outer surfaces of body
  - lots of cells, little ECM
  - serves as lining of lungs, stomach, intestines, blood vessels, skin etc

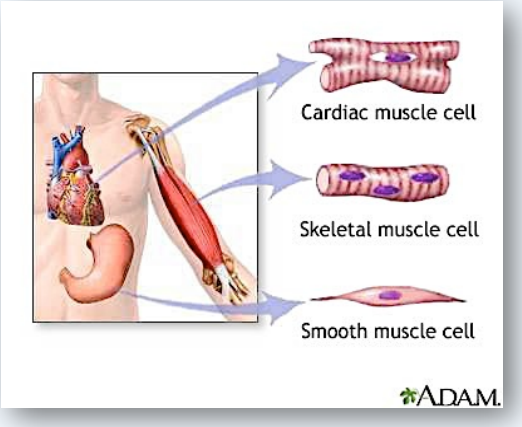




smartsciencepro.blogspot.com

Four types of tissue

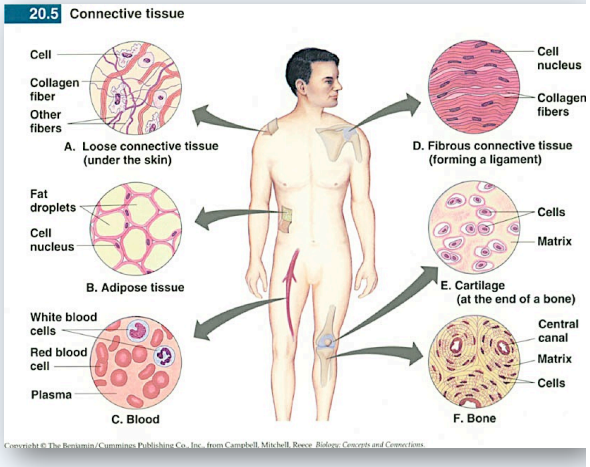
- muscle
  - consumes fuel (sugars and fats) to generate force thru contraction, resulting in mechanical work
  - Striated (skeletal) –
    - muscle that attaches to bone
  - smooth –
    - muscle that is in internal organs like bladder and digestive tract and larger vessels
  - cardiac –
    - perform the pumping function of the heart



www.healthcentral.com

Four types of tissue

- connective tissue
  - hold organs in place and provide structure to give shape.
  - Few cells, lots of ECM
  - cartilage, tendon, ligament, bone matrix, fatty tissue, skin, blood, lymph (latter two the fluid serves as the ECM)
  - local structural requirements dictate relative amounts and organization of the components (we will talk about in detail).



Think about the requirements of skin and tendon, as examples, and how any structures might be arranged within.

What are the main components of tissues?

The main components of the tissues:

- cells
- ECM (extracellular matrix)
- water/fluid (human body is about 60% water) in varying amounts depending on the tissue

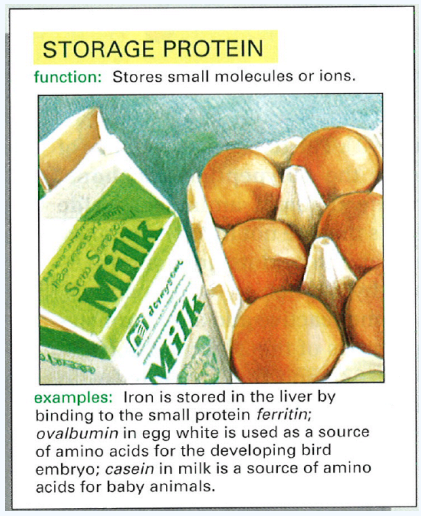
Most of dry mass of tissues and cells is protein – what is a protein?

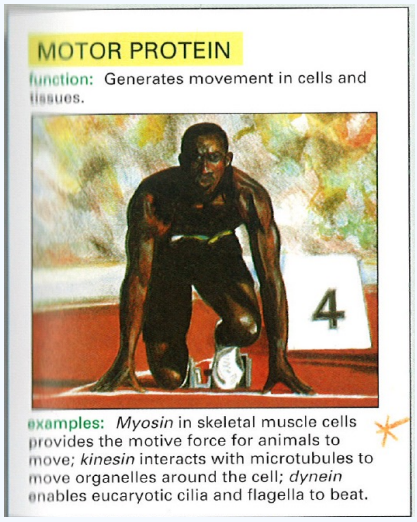
- **ECM**: **proteins**, polysaccharides (sugars), mineral, water, the arrangement and amount of which depends on the tissue
  - **Proteins are macromolecules that contain C, H, N, S and are composed of chains of amino acids.**
- **Cells:** mostly proteins, cell wall is lipid bilayer

The ECM carries load and is produced by the cells native to that tissue and largely dictates the properties of the tissue. The tensile strength of the ECM is owed to the collagen, the compressive strength to the other macromolecules.

- Tendon and ligament: flexible ECM
- Bone: hard and dense ECM
- Cartilage: shock absorbing ECM

Proteins have many functions, FYI (Alberts)

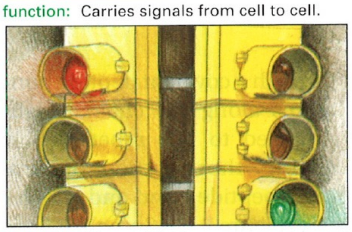




Proteins have many functions, FYI (Alberts)

SIGNALING PROTEIN

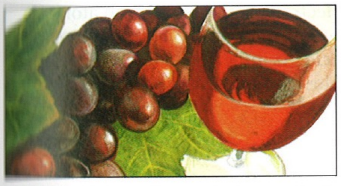
function: Carries signals from cell to cell.



examples: Many of the hormones and growth factors that coordinate physiological function in animals are proteins; *insulin*, for example, is a small protein that controls glucose levels in the blood; *netrin* attracts growing nerve cells in a specific direction in a developing embryo; *nerve growth factor (NGF)* stimulates some types of nerve cells to grow axons; *epidermal growth factor (EGF)* stimulates the growth and division of epithelial cells.

ENZYME

function: Catalysis of covalent bond breakage or formation.



examples: Living cells contain thousands of different enzymes, each of which catalyzes (speeds up) one particular reaction. Examples include: *tryptophan synthetase*—used to make the amino acid tryptophan; *pepsin*—degrades dietary proteins in the stomach; *ribulose bisphosphate carboxylase*—helps to convert carbon dioxide into sugars in plants; *DNA polymerase*—makes DNA; *protein kinase*—adds a phosphate group to a protein molecule.

Proteins have many functions, FYI (Alberts)

TRANSPORT PROTEIN

function: Carries small molecules or ions.

examples: In the bloodstream, *serum albumin* carries lipids, *hemoglobin* carries oxygen, and *transferrin* carries iron. Many proteins embedded in cell membranes transport ions or small molecules across the membrane. For example, the bacterial protein *bacteriorhodopsin* is a light-activated proton pump that transports H^+ ions out of the cell; the *glucose carrier* shuttles glucose into and out of liver cells; and a Ca^{2+} *pump* of muscle cells pumps the calcium ions needed to trigger muscle contraction into the endoplasmic reticulum, where they are stored.

SPECIAL PURPOSE PROTEIN

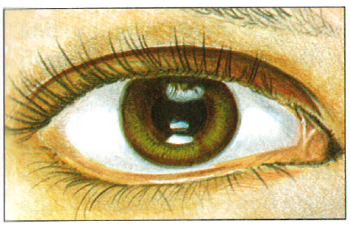
function: Highly variable.

examples: Organisms make many proteins with highly specialized properties. These molecules illustrate the amazing range of functions that proteins can perform. The *antifreeze proteins* of Arctic and Antarctic fishes protect their blood against freezing; *green fluorescent protein* from jellyfish emits a green light; *monellin*, a protein found in an African plant, has an intensely sweet taste; mussels and other marine organisms secrete *glue proteins* that attach them firmly to rocks, even when immersed in sea water.

Proteins have many functions, FYI (Alberts)

RECEPTOR PROTEIN

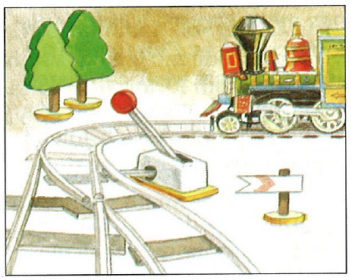
function: Used by cells to detect signals and transmit them to the cell's response machinery.



examples: *Rhodopsin* in the retina detects light; the *acetylcholine receptor* in the membrane of a muscle cell receives chemical signals released from a nerve ending; the *insulin receptor* allows a liver cell to respond to the hormone insulin by taking up glucose; the *adrenergic receptor* on heart muscle increases the rate of heartbeat when it binds adrenaline.

GENE REGULATORY PROTEIN

function: Binds to DNA to switch genes on or off.



examples: The *lactose repressor* in bacteria silences the gene for the enzymes that degrade the sugar lactose; many different *homeodomain proteins* act as genetic switches to control development in multicellular organisms, including humans.

The type of protein that is of primary interest here…

STRUCTURAL PROTEIN

function: Provides mechanical support to cells and tissues.

examples: Outside cells, *collagen* and *elastin* are common constituents of extracellular matrix and form fibers in tendons and ligaments. Inside cells, *tubulin* forms long, stiff microtubules and *actin* forms actin filaments that underlie and support the plasma membrane; *α-keratin* forms fibers that reinforce epithelial cells and is the major protein in hair and horn.

Provide <u>mechanical support</u> to cells & tissues.

Fibrous proteins meet structural and mechanical requirements of hair, horn, nails, muscle, epidermis, cytoskeletal elements, connective tissue

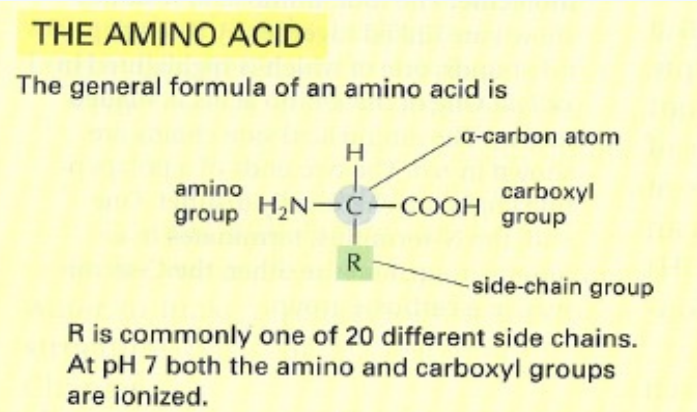
- **Keratin** is main component of hair skin, fingernails, toenails, hooves, scales, feathers, horns
- **Collagen** occurs in almost all tissues and is ¼ - 1/3 of total body protein
  - 40% in skin
  - 10-20% in bone and teeth
  - 7-8% in blood vessels
  - Also in tendon (highest %), cartilage, ligaments, internal organs, muscles, eyes, etc

Board

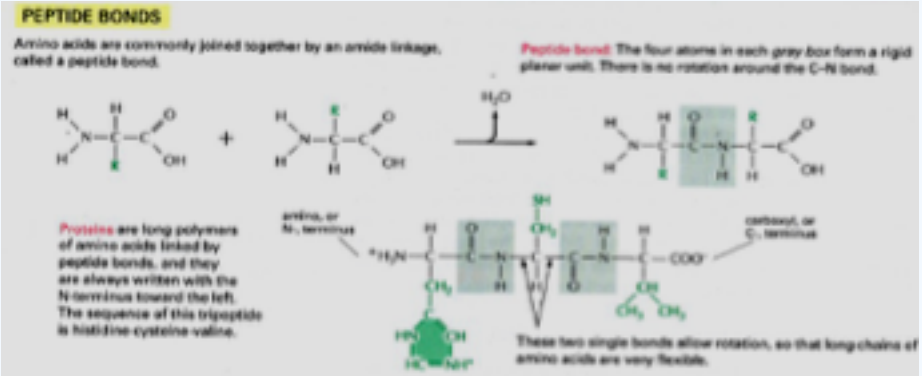
13

Amino Acids **consist of an amino group (N-terminus) and a carboxyl group (C-terminus). This gives it *structural* polarity.**

The side chain (R) distinguishes one from another.



Peptide **is a compound containing two or more amino acid residues joined by a** ***peptide bond*****.**



(Alberts)

There are only 20 different amino acids. These combine to form thousands and thousands of different proteins

AMINO ACID			SIDE CHAIN
Aspartic acid	Asp	D	negative
Glutamic acid	Glu	E	negative
Arginine	Arg	R	positive
Lysine	Lys	K	positive
Histidine	His	H	positive
Asparagine	Asn	N	uncharged polar
Glutamine	Gln	Q	uncharged polar
Serine	Ser	S	uncharged polar
Threonine	Thr	T	uncharged polar
Tyrosine	Tyr	Y	uncharged polar

POLAR AMINO ACIDS

AMINO ACID			SIDE CHAIN
Alanine	Ala	A	nonpolar
Glycine	Gly	G	nonpolar
Valine	Val	V	nonpolar
Leucine	Leu	L	nonpolar
Isoleucine	Ile	I	nonpolar
Proline	Pro	P	nonpolar
Phenylalanine	Phe	F	nonpolar
Methionine	Met	M	nonpolar
Tryptophan	Trp	W	nonpolar
Cysteine	Cys	C	nonpolar

NONPOLAR AMINO ACIDS

(Alberts)

Figure 5–3 The 20 different amino acids found in proteins. Both three-letter and one-letter abbreviations are listed. As shown, there are an equal number of polar and nonpolar side chains. For their atomic structures, see Panel 2–5 (pp. 62–63).

NOTE
- It is not necessary to know all 20 amino acids and their individual properties for this course.
- The purpose here is to show how the proteins of interest to tissues are built up and why they have specific properties, which lead to the properties of the tissues.

The order and combination of the amino acid (AA) sequence determines shape and structure of protein - and therefore its ???

- Sequences can be **10's or 100's or 1000's AA.**
- Many of the covalent bonds on the peptide backbone allow the chain to rotate freely, which **allows a protein to fold up in different ways.**
- The **folding depends on the weak non covalent bonds** that form (hydrogen bonds, ionic bond, van der Waals bonds), which depend primarily on the **combination of side groups in the sequence**.
- Common amino acids are grouped according to whether their side chains are acidic, basic, uncharged polar, or nonpolar (figs).
- Some are nonpolar, hydrophobic (doesn't like water), some are negatively or positively charged, some react, some don't.

Polar and nonpolar side chains and protein folding (Alberts)

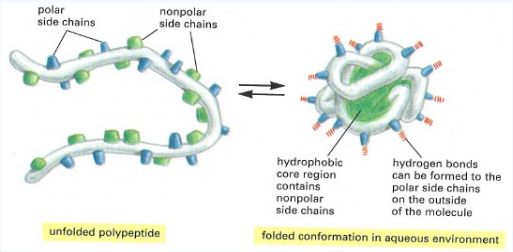
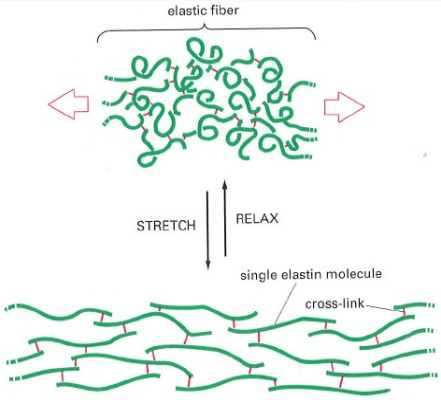


Figure 5–5 How a protein folds into a compact conformation. The polar amino acid side chains tend to gather on the outside of the protein, where they can interact with water; the nonpolar amino acid side chains are buried on the inside to form a highly packed hydrophobic core of atoms that are hidden from water. In this very schematic drawing, the protein contains only about 30 amino acids.

- The arrangement of the hydrophobic and hydrophilic side chains in a protein are important to the shape and properties of the protein.
- Hydrophobic molecules, or nonpolar side chains in amino acids, are forced together if they are near water in order to avoid the water.
- In non-polar proteins, the hydrophobic side chains group together and try to situate themselves at the interior of the protein, away from polar solutions such as water. Think of oil in water. It minimizes the area in contact with the water by forming a sphere (fig 5-5).
- In polar proteins, the hydrophilic portions arrange themselves to be on the outside of the protein, near water or other polar molecules.

Examples of Proteins: Elastin is a fibrous protein (Alberts)



Elastin polypeptide chains are cross-linked together to form rubberlike, elastic fibers. Each elastin molecule uncoils into a more extended conformation when the fiber is stretched and will recoil spontaneously as soon as the stretching force is relaxed.

Elastin is found in
- Arteries
- lungs
- Skin

What is common about these 3 tissues?

Elastin - continued

- **Elastin is a non-collagenous fibrous protein** (structural) in connective tissues of vertebrates.
- **Elastin is linear elastic** (what does that mean?)
- Just as collagen, it is **produced by the connective tissue cells - fibroblasts**.
- The elastin precursor, tropoelastin (soluble immature form of elastin), is synthesized intracellularly and then cross-linked outside of the cell. The **molecule is so stable and inert, that is can last the lifetime of the organism**.
- Made of relatively **short polypeptide chains that are covalently crosslinked** to make a material similar to rubber. The structure allows the elastin molecules for **uncoil and recoil under applied loads**.
- **Elastin enables tissues like arteries, veins, and lungs to undergo large deformations without permanent damage.**
- **Elastin is also found in skin**. It provides elasticity to tissue. In particular, it helps to keep skin from getting wrinkles. **Elastin is no longer synthesized after puberty.**

Numerous Diseases associated with changes in elastin (amount, composition, collagen to elastin ratio etc)

- **Emphysema, chronic obstructive pulmonary disease (COPD)**
  - *Elastase-released epidermal growth factor recruits epidermal growth factor receptor and extracellular signal-regulated kinases to down-regulate tropoelastin mRNA in lung fibroblasts.* DiCamillo, S.J., Carreras, I., Panchenko, M.V., Stone, P.J., Nugent, M.A., Foster, J.A., Panchenko, M.P. *J. Biol. Chem.* (2002)
- **Hypertension through small artery remodeling**
  - *Role of elastin in spontaneously hypertensive rat small mesenteric artery remodelling.* Briones, A.M., González, J.M., Somoza, B., Giraldo, J., Daly, C.J., Vila, E., González, M.C., McGrath, J.C., Arribas, S.M. *J. Physiol. (Lond.)* (2003)
- **Arterial aneurysms**
  - *Prevention of aneurysm development and rupture by local overexpression of plasminogen activator inhibitor-1.* Allaire, E., Hasenstab, D., Kenagy, R.D., Starcher, B., Clowes, M.M., Clowes, A.W. *Circulation* (1998)
- **Long term alcohol consumption (can result in degradation of vascular elastin components)**
  - *Long-term alcohol consumption increases matrix metalloproteinase-2 activity in rat aorta.* Partridge, C.R., Sampson, H.W., Forough, R. *Life Sci.* (1999)

Dermis and elastin

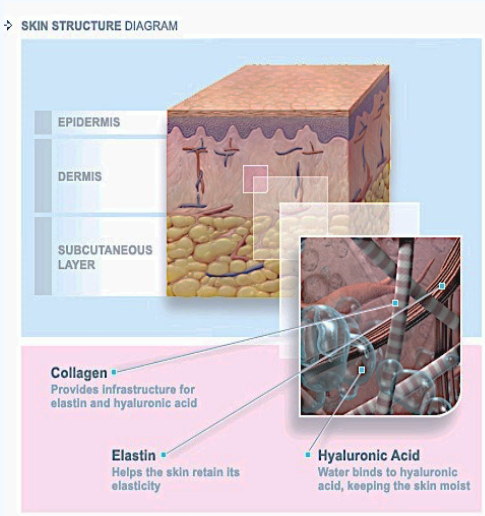
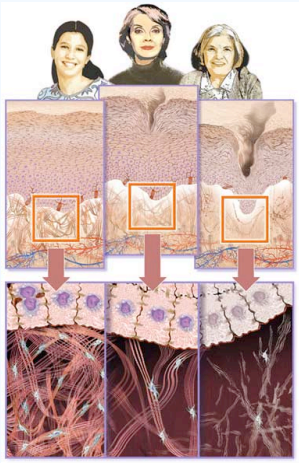


Image: www.agingwithbeauty.com/facialaging/skin-basics.aspx

Mechanisms of Ageing and Development
Vol 123,2002, pp 801–810,The Biology of Ageing
Image: www.skin-care-forum.basf.com



Skin ageing may result from

(i) Decreased ability to proliferate

(ii) Cells produce less matrix per cell

(iii) Increased degradation of the collagenous matrix from higher enzyme expression

The dermis (http://www.skin-science.com/_int/_en/topic/topic_sousrub.aspx?tc=SKIN_SCIENCE_ROOT^AN_ORGAN_REVEALED^THE_DERMIS&cur=THE_DERMIS)

- *80% water, plus elastin fibers and collagen floating in a glycoprotein gel, the dermis is the tissue supporting the skin.*
- In contrast with the epidermis, **the dermis is vascularized**, enabling it to not only provide energy and nutrition to the epidermis but also to play a primordial role in thermoregulation and in healing.
- The dermis is 10 to 40 times thicker than the epidermis. At the junction with the epidermis, its surface bristles with fibrous, vascular and nervous projections - the dermal papillae.
- *The fibroblasts are the main cells in the dermis. They are found only in very low numbers in the deep layers of the dermis.*
- *They are specialized in producing two types of protein fibres, collagen and **elastin fibres** constituent of the extra-cellular matrix.*
  - *Collagen fibres, 70% of the proteins in the dermis, gives dermis its resistance to strain and traction,*
  - **while elastin supplies its elastic properties.**

Elastin: A neglected essential of skin youth

http://www.smartskincare.com/skinbiology/skinbiology_elastin.html

Elastin and aging

- One simple test for skin aging - how long it takes for the skin to snap back after being pinch-pulled away. (You can try it on the back of your hand.) Young skin snaps back almost immediately. The old one takes up to several seconds. The reason for such difference is quantity and quality of elastin in the skin.
- The **amount of elastin in the skin usually peeks in adolescence or early adulthood and declines thereafter.**
- **Fibroblasts in older skin have a much reduced capacity to produce new elastin.**
- This deficiency does not appear to be a result of the loss of fibroblasts or mutations in elastin-encoding genes.
- More likely, age-related changes in the skin's biochemical environment shut down elastin production.
- Therefore, at least in theory, elastin production can be restored to its youthful levels with proper biochemical signals.
- **Can you boost elastin in your skin?**
- ……. as of the time of this writing, none is reliably proven to do so.

Elastin and chemical treatments etc (just for fun)

- http://www.dermalastyl.com/pages/index/

Elastin Prevents Wrinkles

Based on Dr. Burt Ensley's research on the phenomenon of Scarless Healing and Tissue Regeneration, Dermalastyl-*β*. contains Tropoelastin, a product that will:

1. Supplement elastin levels in the skin's surface
2. Reduce the appearance of existing wrinkles and help prevent the onset of new wrinkles

Cost just $89 for a two month supply

There is no other product sold today that contains human Tropoelastin. It is available to consumers only through this web site.

- DO YOU BELIEVE IT?

Photoageing (http://www.skin-science.com/_int/_en/topic/topic_sousrub.aspx?tc=SKIN_SCIENCE_ROOT^THE_SUN_BE_CAUTIOUS^GET_EXPOSED_AT_YOUR_OWN_RISK&cur=GET_EXPOSED_AT_YOUR_OWN_RISK)

UV and especially UVA rays bring about a premature ageing of the skin, known as cutaneous photoageing.

The harms caused to the skin resulting from this sun-induced ageing are different from and added to those of natural ageing. They involve changes in the skin clinically characterised by wrinkles, roughness, a lack of elasticity, depigmentation or hyperpigmentation marks and a variety of benign or malignant tumours. Although exposure to the sun enables the quality of the layer to be improved, this is only a short-term benefit. Over time, the rate of thickening diminishes, and ultimately the sun brings about epidermal atrophy. On the surface, the process of keratinocyte maturation is disturbed and corneocyte desquamation is delayed. Furthermore, disorganisation of melanocytes can result in the creation of pigmented marks.

Photoageing (http://www.skin-science.com/_int/_en/topic/topic_sousrub.aspx?tc=SKIN_SCIENCE_ROOT^THE_SUN_BE_CAUTIOUS^GET_EXPOSED_AT_YOUR_OWN_RISK&cur=GET_EXPOSED_AT_YOUR_OWN_RISK)

CONTINUED

In the event of *repeated exposure to the sun and UVA rays:*

- *fibroblast number diminishes.*
- *These cells become atrophied, gradually losing their capacity to produce collagen while at the same time their ability to digest it increases.*
- *The elastin fibres synthesised by the fibroblasts are also modified - produced in large quantities, they are unable to become attached to the collagen and gather in clusters.*
- These form small white lumps which can be seen beneath the surface of the skin surrounded by the network of blood vessels which also become visible, giving the skin a reddened aspect punctuated by white spots. The skin, which has now lost its elasticity, slackens and deep folds appear in the flesh. These are the symptoms of solar elastosis, which can take months or even years to appear.

Just because it is interesting…

NY Times Wednesday, September 2, 2009

Phys Ed: Does Ibuprofen Help or Hurt During Exercise?

By Gretchen Reynolds

….Moreover, Warden and other researchers have found that, in laboratory experiments on animal tissues, NSAIDs actually slowed the healing of injured muscles, tendons, ligament, and bones. "NSAIDs work by inhibiting the production of prostaglandins," substances that are involved in pain and also in the creation of collagen, Warden says. Collagen is the building block of most tissues. So fewer prostaglandins mean less collagen, "which inhibits the healing of tissue and bone injuries," Warden says, including the micro-tears and other trauma to muscles and tissues that can occur after any strenuous workout or race.

The painkillers also blunt the body's response to exercise at a deeper level. Normally, the stresses of exercise activate a particular molecular pathway that increases collagen, and leads, eventually, to creating denser bones and stronger tissues. If "you're taking ibuprofen before every workout, you lessen this training response," Warden says. Your bones don't thicken and your tissues don't strengthen as they should. They may be less able to withstand the next workout. In essence, the pills athletes take to reduce the chances that they'll feel sore may increase the odds that they'll wind up injured — and sore.