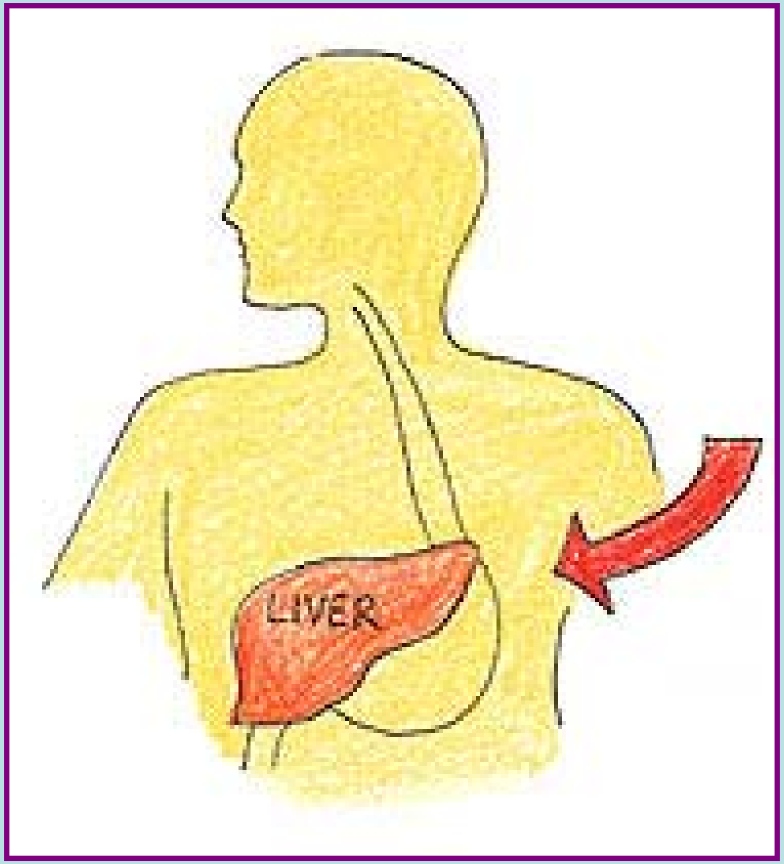
(Re-) Engineering the Liver



by Ravi Ramjit

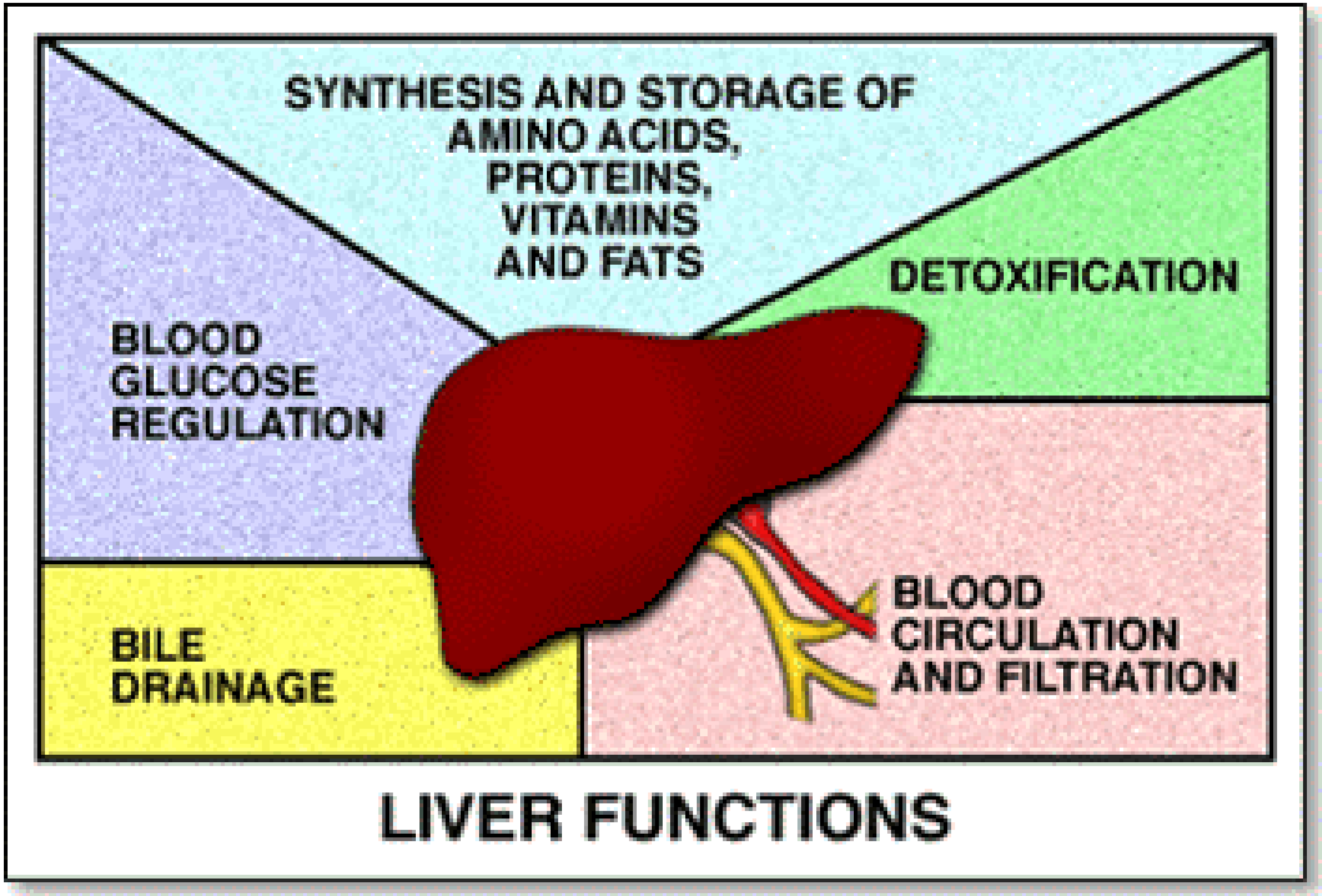
Outline of Talk

- Functions
- Structural Description
  - Surfaces
  - Lobes
  - Development
- Diseases
- Engineering Projects
- What has been done

Key Features and Functions of the Liver

- The liver is the largest gland in the body (approximately 1500 grams) and is located in the right upper quadrant of the abdomen.
- It is glossy in appearance and dark red in color from the rich supply of blood flowing through it.
- Approximately 25% of the cardiac output flows to the liver.
- It performs many important functions:
  - the uptake, storage, and disposal of nutrients (protein, glucose and fat), drugs and toxins
  - production of synthesis proteins critical for blood clotting and metabolism of substances produced by the body (Vitamins A, B, D, B-12, K).

Functions of the Liver



Functions of the Liver

- **1. Regulations, Synthesis, and Secretion.** Hepatocytes are metabolically active cells that serve many functions. For example, they take up glucose, minerals, and vitamins from portal and systemic blood and store them. In addition, hepatocytes can produce many important substances needed by the body, such as blood clotting factors, transporter proteins, cholesterol, and bile components. Finally, by regulating blood levels of substances such as cholesterol and glucose, the liver helps maintain body **homeostasis.**

- **a. Glucose.** The liver plays a major role in maintaining blood concentrations of glucose, by storing or releasing glucose as needed.
- **b. Proteins.** Most blood proteins (except for antibodies) are synthesized and secreted by the liver. One of the most abundant serum proteins is **albumin.** Impaired liver function that results in decreased amounts of serum albumin may lead to edema, swelling due to fluid accumulation in the tissues.
- The liver also produces most of the proteins responsible for blood clotting, called coagulation or clotting factors. If the blood cannot clot normally due to a decrease in the production of these factors, excessive bleeding may result.

Functions of the Liver

- **c. Bile.** Bile is a greenish fluid synthesized by hepatocytes and secreted into biliary ducts. It then leaves the liver to be temporarily stored in the gallbladder before emptying into the small intestine. The major components of bile include cholesterol, phospholipids, bilirubin (a metabolite of red blood cell hemoglobin), and bile salts. Importantly, bile salts act as "detergents" that aid in the digestion and absorption of dietary fats. Liver damage or obstruction of a bile duct (e.g., gallstone) can lead to **cholestasis,** (the blockage of bile flow, which causes the malabsorption of dietary fats), **steatorrhea** (foul-smelling diarrhea caused by non-absorbed fats), and jaundice.

- **d. Lipids.** Cholesterol, a type of lipid, is a substance found in cell membranes that helps maintain the physical integrity of cells. The liver synthesizes cholesterol, which is then packaged and distributed to the body to be sued or excreted into bile for removal from the body. Increased cholesterol concentrations in bile may predispose to gallstone formation.
- The liver also synthesizes lipoproteins, which are made up of cholesterol, triglycerides (containing fatty acids), phospholipids, and proteins. Lipoproteins circulate in the blood and shuttle cholesterol and fatty acids (an energy source) between the liver and body tissues. Most liver diseases do not significantly affect serum lipid levels, with the exception of cholestatic diseases, which may be associated with increased levels.

Functions of the Liver

- **2. Storage.** As mentioned above in the previous slide, the liver is designed to store important substances such as glucose (in the form of glycogen). The liver also stores fat-soluble vitamins (vitamins A, D, E and K), folate, vitamin B 12 , and minerals such as copper and iron. However, excessive accumulation of certain substances can be harmful. For example, patients with an inherited condition known as **Wilson's disease** cannot secrete copper into bile normally and usually have a low blood level of the copper-binding protein **ceruloplasmin.** Retained copper accumulates in the liver (leading to **cirrhosis** and in the central nervous system (resulting in neuropsychiatric symptoms).

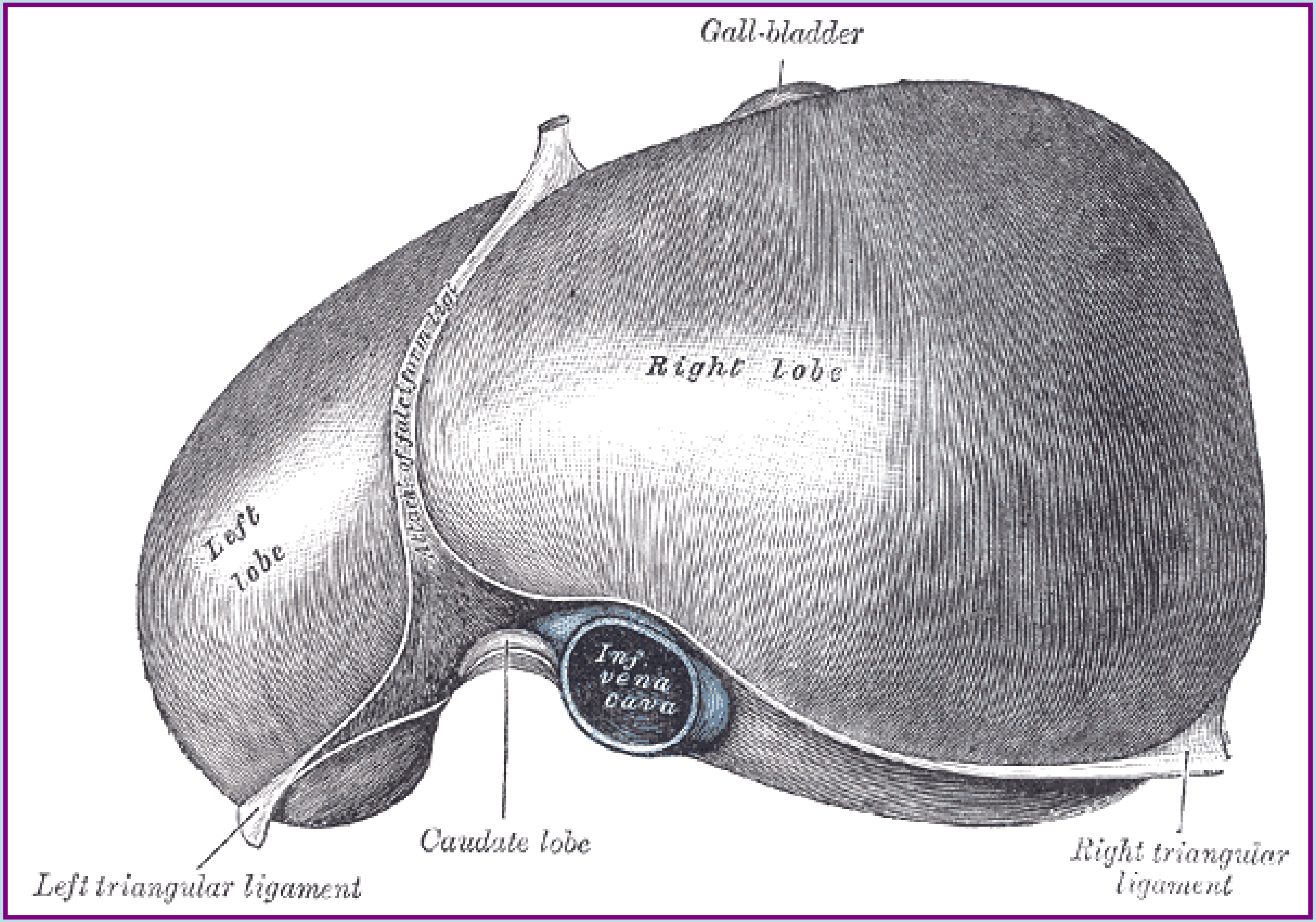
- **3. Purification, Transformation, and Clearance.** The liver removes harmful substances (such as ammonia and toxins) from the blood and then breaks them down or transforms them into less harmful compounds. In addition, the liver metabolizes most hormones and ingested drugs to either more or less active products.

- **a. Ammonia.** The liver converts ammonia to urea, which is excreted into the urine by the kidneys. In the presence of severe liver disease, ammonia accumulates in the blood because of both decreased blood clearance and decreased ability to form urea. Elevated ammonia levels can be toxic, especially to the brain, and may play a role in the development of hepatic **encephalopathy.**

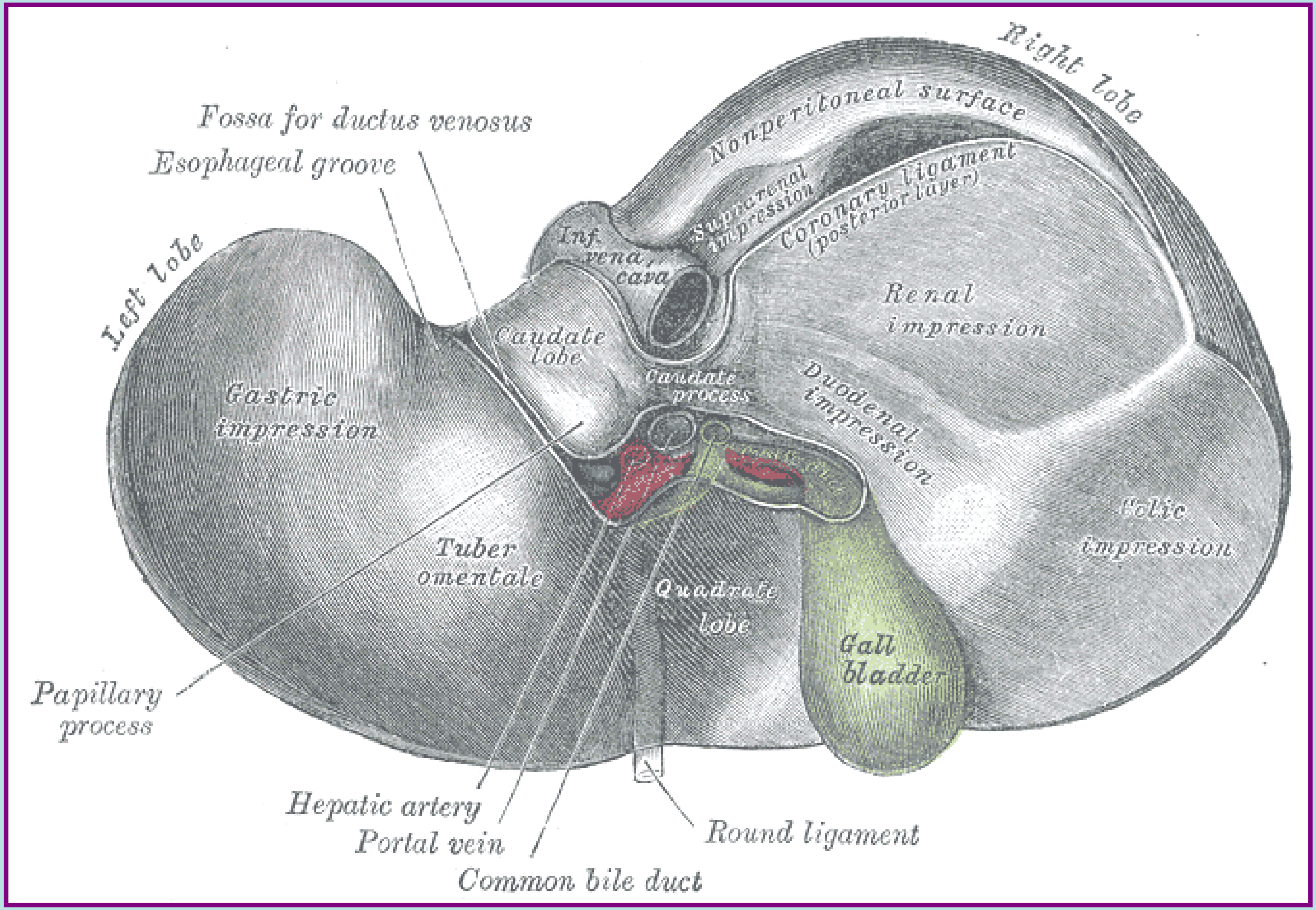
Functions of the Liver

- **b. Bilirubin.** Bilirubin is a yellow pigment formed as a breakdown product of red blood cell hemoglobin. The spleen, which destroys old red cells, releases "unconjugated" bilirubin into the blood, where it circulates in the blood bound to albumin. The liver efficiently takes up bilirubin and chemically modifies it to "conjugated," or water-solube, bilirubin that can be excreted into bile. Increased production or decreased clearance of bilirubin results in jaundice, a yellow pigmentation of the skin and eyes from bilirubin accumulation.
- **c. Hormones.** Since the liver plays important roles in hormonal modification and inactivation, chronic liver disease may cause hormonal imbalances. For example, the masculinizing hormone testosterone and the feminizing hormone estrogen are metabolized and inactivated by the liver. Men with cirrhosis, especially those who abuse alcohol, have increased circulating estrogens relative to testosterone derivatives, which may lead to body feminization.
- **d. Drugs.** Nearly all drugs are modified or degraded in the liver. In particular, oral drugs are absorbed by the gut and transported via the portal circulation to the liver. In the liver, drugs may undergo first-pass metabolism, a process in which they are modified, activated, or inactivated before they enter the systemic circulation, or they may be left unchanged.

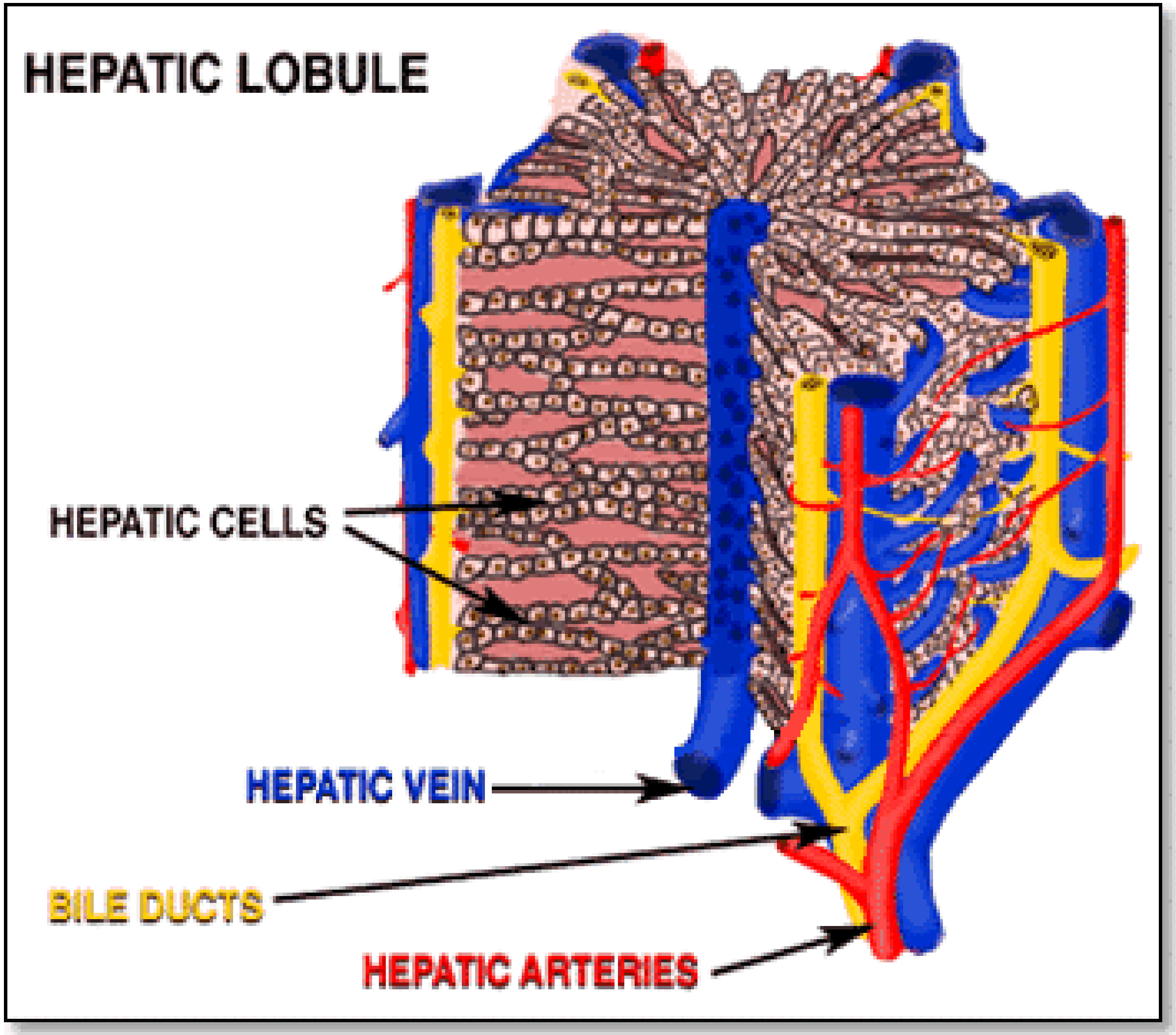
Superior Surface View



Inferior Surface

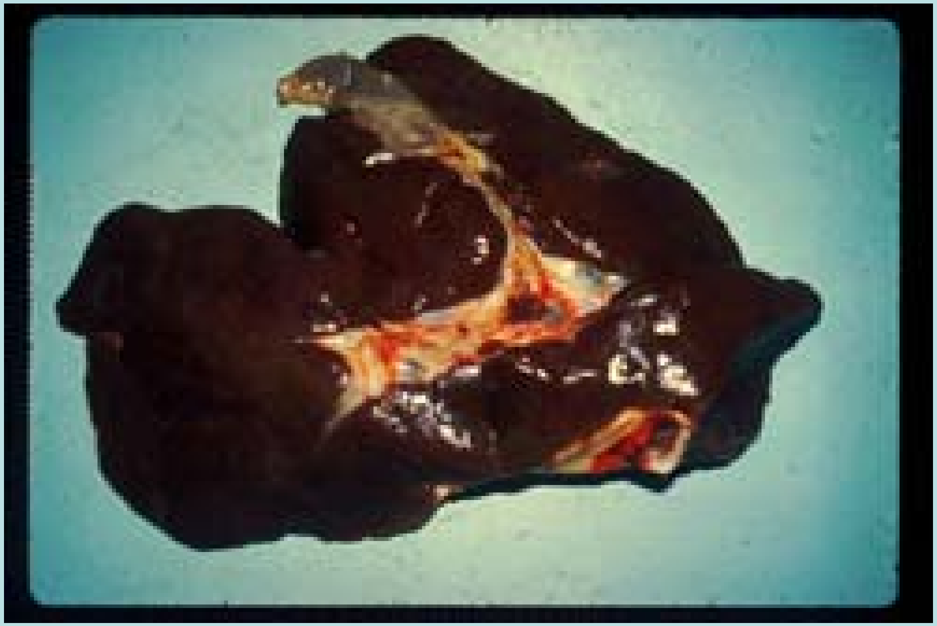


The Liver as a Network of Units



All the blood drains into the hepatic vein which then gets distribute throughout the rest of the body

Comparison of Healthy vs. Unhealthy Liver



Healthy liver

Ravi's liver (alcoholic)

ANY QUESTIONS?

Diseases of the Liver

- liver abscess
- liver cancer
- liver disease due to alcohol – consistent over consumption of alcohol
- cirrhosis – scarred liver due to alcohol abuse, and other types of liver disease
- amebic liver abscess
- autoimmune hepatitis
- biliary atresia
- coccidioidomycosis; disseminated
- delta agent (Hepatitis D)
- hemochromatosis
- Hepatitis A – inflammation of liver due to Hep A virus
- Hepatitis B
- Hepatitis C
- hepatocellular carcinoma
- primary biliary cirrhosis – inflammation of bile ducts leading to blockage of bile flow → damage
- pyogenic liver abscess
- Reye's syndrome
- sclerosing cholangitis
- Wilson disease
- drug induced hepatotoxicity
- fulminant or acute liver failure

Statistics

Why the need for liver engineering?

- ~30,000 people die each year in the U.S. from liver failure of some type.
- Less that 3,000 livers become available for transplant each year.
- 15% of these transplants do not take hold and are ineffective.

Initial Liver Engineering Ideas

- First types of tissues engineered were simple cartilage and skin.
  - Problem they faced in more complex systems involved growing the blood vessel networks.
  - In thicker tissues, nutrients are unable to get through the first few cell layers, which would be an essential liver function.

- Attempt was made via computer simulating blood network in liver.
- Tiny ‘livers’ are now grown.
  - Copy the blood vessel network of a real liver and using 3D computer modelling and machining to mimic its construction.
  - To copy the structure, they injected a liquid plastic into the blood vessels of a liver, wait for it to solidify and dissolve the liver tissue, leaving a solid cast of the organ's blood vessels.
  - They can then take "measure up" and feed data into a computer to create a 3D model of a liver's blood supply.
  - This model is then "sliced up" on the computer into horizontal layers, which can be used to make a silicon mould.

Initial Liver Engineering Ideas

- Biodegradable plastic is then poured into the mold to make enough slices that can be sandwiched together using pressure and heat, to create a **scaffold** (temporary platform suitable for growth onto) for a whole liver.
- The scaffold then has to be injected with at least seven types of cells that make up the solid part of the liver.
- A solution of endothelial cells, which normally line blood vessels, then has to be pumped into the empty channels in the frame where they stick to the walls.
- These can then be grown in a nutrient to form a network of blood vessels within the scaffold which again dissolves over a few months.
- If each step of this process works, it would result in a functioning liver.
- Blood vessel networks grown this way have so far been tested in rats, with no leakage or obstruction of the blood flow.
- Next step would be having all the correct cell types growing in their respective/natural positions.
- Work being done by Dr. Linda Griffith at MIT. (see printout)

Vivek Dixit, Ph.D. Professor of Medicine,
Head, Liver Support & Tissue Engineering Laboratory
Division of Digestive Diseases, Department of Medicine

- Studies cell surface interaction to create new liver tissue ("organoids") in vitro.
- Organoids are cell cultures, cultivated on specialized biodegradable polymer scaffoldings that can function as a biological substitute in organ replacement therapy.
- Studies on the adhesion and growth of hepatocytes on biomaterial support composed of a biodegradable polymer scaffold (modified chitosan) that they recently developed.
- Chitosan is a non-toxic available polymer that resembles glycoasaminoglycan, an important tissue building protein found in extracellular matrix proteins.
- Results so far indicate that
  - Hepatocytes rapidly de-differentiate into non-functioning liver cells when grown in an inappropriate cell culture environment
  - Transplanted hepatocytes had a limited survival when the transplantation site is not highly vascularized.

Tissue Engineering Department Harvard University

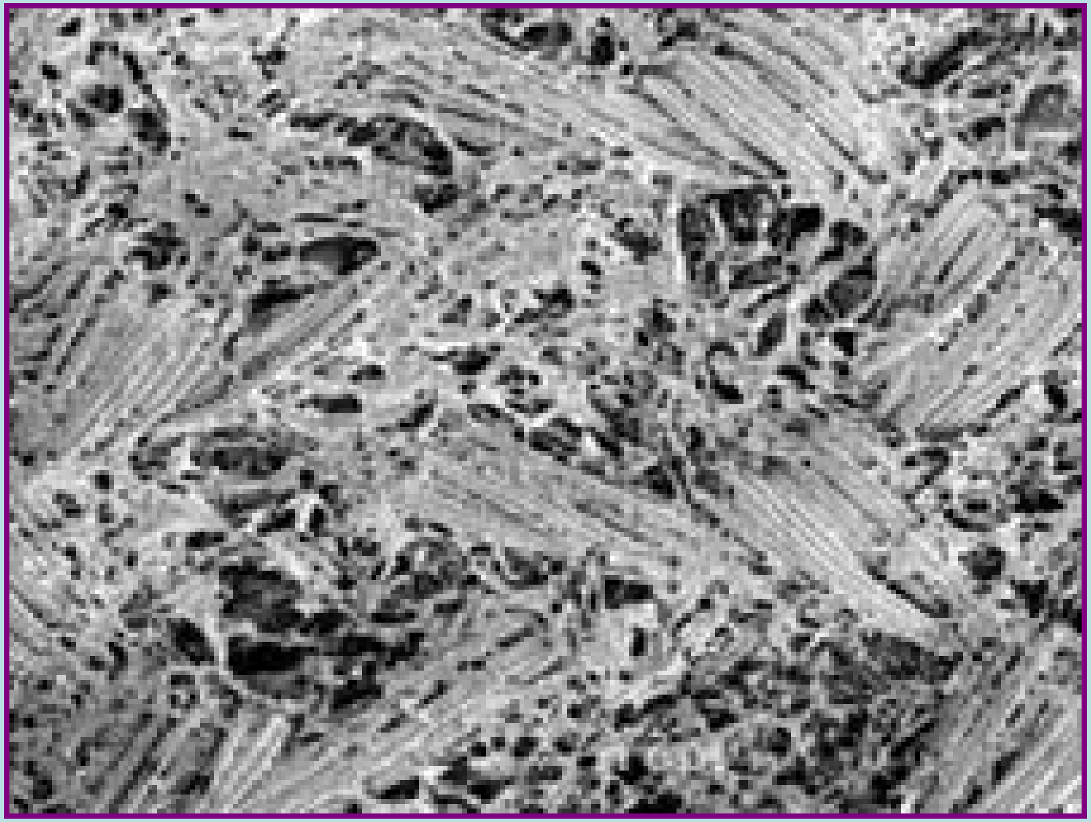
- Investigates liver engineering by using cell-laden polymer devices as a means of generating new tissue replacements.

  – a small number of healthy hepatocyte are harvested, cultured in vitro until they develop new tissue formation, and seeded onto a biodegradable polymer which serves as a structural scaffold upon which the cells can grow.

  – once implanted back into the patient, the scaffold will degrade, leaving behind a durable tissue structure that can provide a long-term solution for specific ailments by recreating the essential functions of the organ.

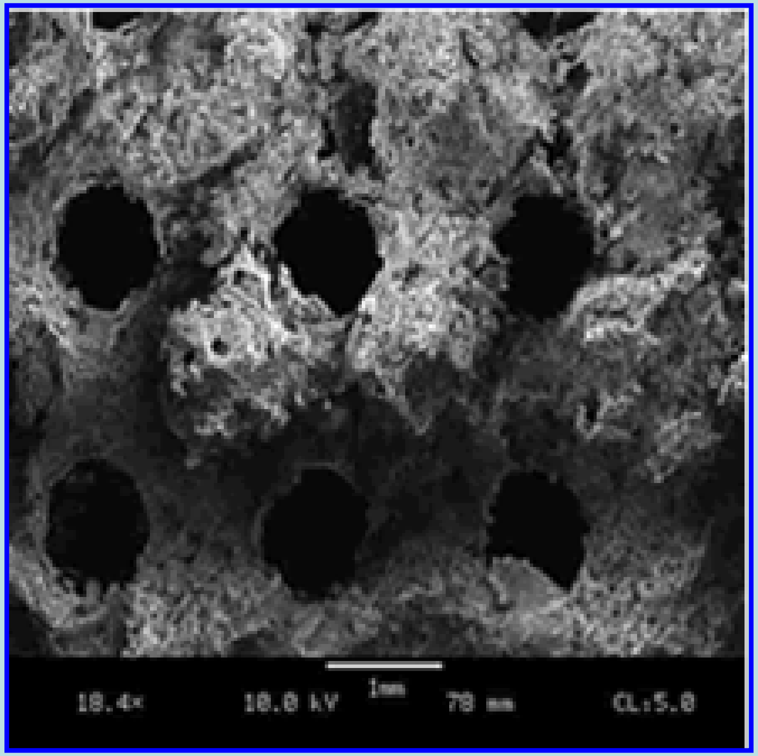
http://www.mgh.harvard.edu/depts/tissue/projects/projects/liver.html

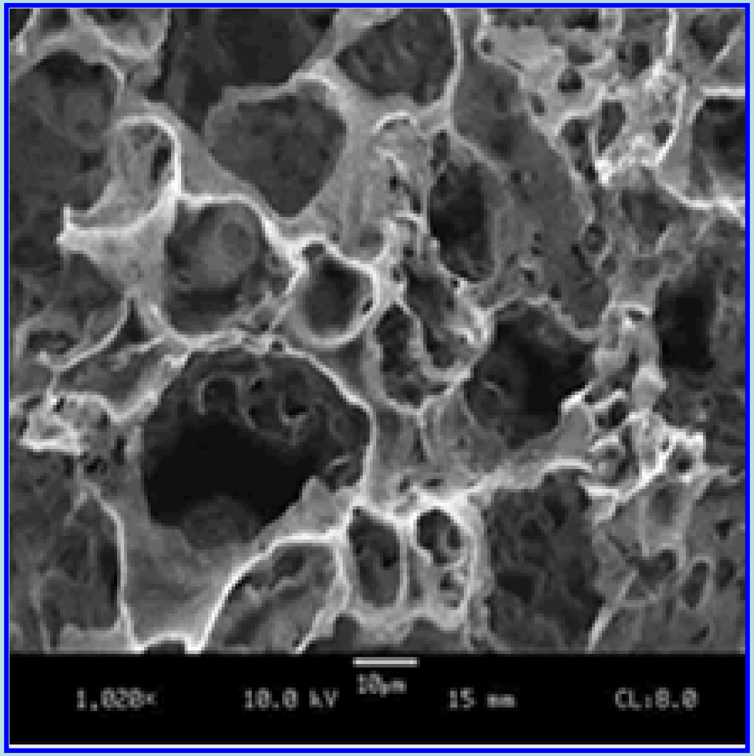
From Growing Skin on Scaffolds



“We can rebuild him,” David Adam, Nature, July 24, 2000

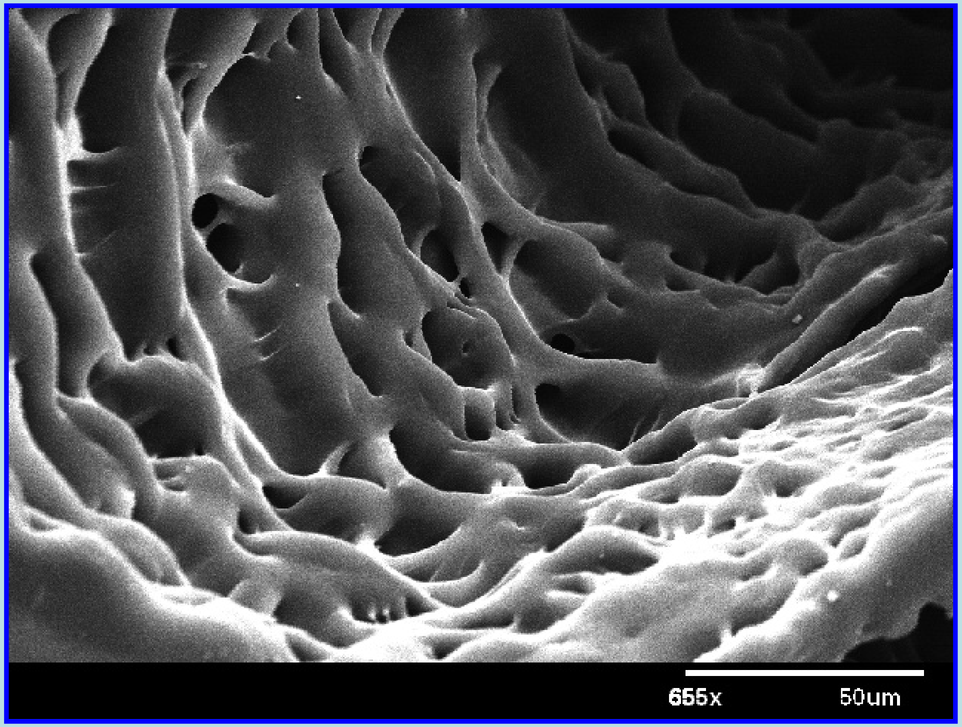
Scaffolds Which May Be Used



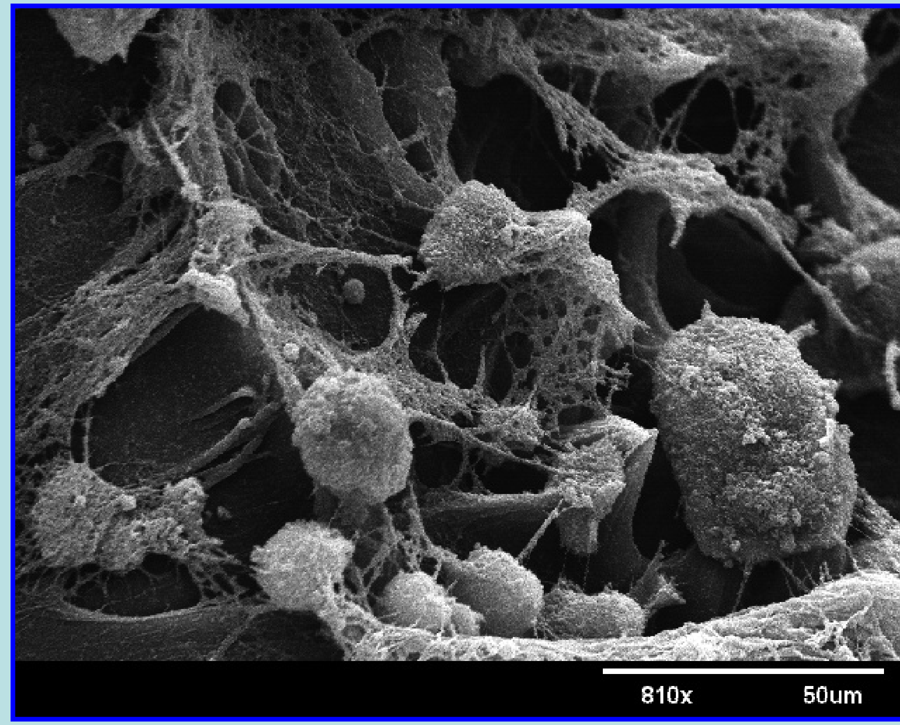


Example of scaffolds designed with local/global pore structures. (Left) A scanning electron micrograph showing both global interconnected designed pores (which may be fabricated in the range of > 250 microns) and local pores created by a variety of processes that range in size from 10-100 microns. (Right) a close-up scanning electron micrograph showing local feature sizes on the order of 10 microns

PLA Scaffolds by the Tissue Engineering Group at the Pharmacy School at the Univeristy of Nottingham



SEM of PLA (poly-lactic acid) scaffold used for liver regeneration



SEM of hepatocytes adhering to PLA scaffold.

Dr. Hanry Yu’s (P.I.) Group (Bio-artifical liver)

- Further developing the Singapore Bio-artificial Liver that is an extra-corporeal device (much like a kidney dialysis machine) that provides a life-saving solution to help patients with liver failure.

- Success in culturing cells with up to 30 folds higher level functions than in other culture systems, thereby, allowing less cells to be used in the device.testing of the Singapore Bio-artificial Liver in rat, rabbit and pig.

- Development of high-throughput system for microencapsulating cells.

- Preservation and storage of hepatocytes, microencapsulated hepatocytes, and bioreactors.

- Development of suitable hepatocyte source.

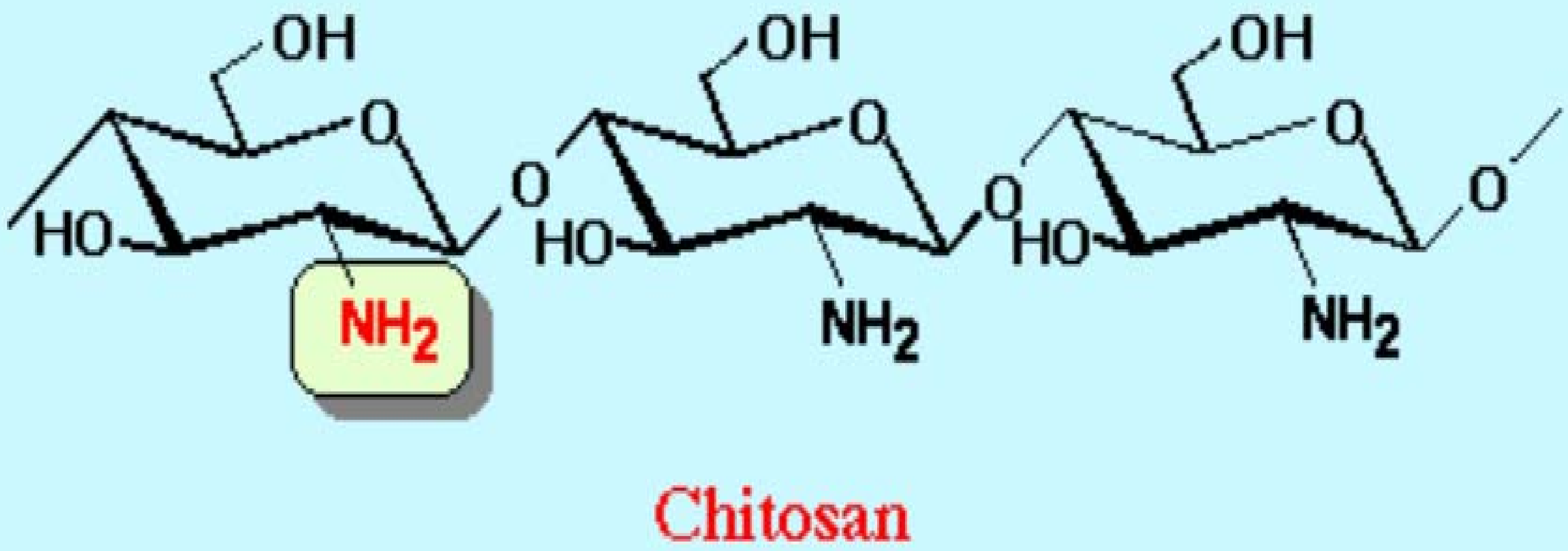
- Immune modulation to reduce rejection for the xenogenic (the supposed production of offspring markedly different from either parent) cells.

Other Research Groups

- **Tissue Engineering Research Center, Japan**
  - Works on engineering of liver and cardiovascular tissue, investigating the growth and function of hepatocytes and endothelial tissues, using porous biomaterials.

- **Incara Pharmaceuticals (sold liver and liver stem cell program to Vesta Therapeutics)**
  - developing a liver progenitor cell therapy as a treatment for liver failure.
  - can also assist in determining which genes are active at the various stages in a liver cell's maturation, providing genomic information for drug research.

Structure of Chitosan



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- PLA Scaffolds
  - http://www-personal.umich.edu/~scottho/BSD.HTML

Human Ear on Rat