Introduction on Tissue Engineering and Regenerative Medicine

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Current Life Science Topics

1. Fundamentals in Basic Biochemistry & Cell Biology
2. Introduction to Tissue Engineering & Regenerative Medicine
3. Developmental Tissue Reconstruction
4. Wound Healing & Regeneration
5. Natural Tissue Composition & Cell-ECM Interaction
6. Stem Cells & Cell-Based Therapy
7. Biomaterials
8. *Mid-Term Exam*
9. Mechano-transduction & Bioreactors
10. Discussions on Tissue Reconstruction
11. Regulation & Ethics
12. AI in Current Life Science
13. Machine Learning & Github
14. Deep Neural Network
15. Convolutional Neural Network
16. *Final Exam*

Study Materials

- Robert Langer & Joseph P. Vacanti 1993 Science
- Butler DL, Goldstein ST, & Guilak F 2000 J Biomech Eng
- Goldstein AS & Christ G 2009 Tissue Eng
- Atala A 2012 Science Translational Medicine

Tissue Engineering

[Robert Langer & Joseph P. Vacanti 1993 Science]

Current Status of Tissue Loss or Organ Failure

Every year, millions of American suffer tissue loss or end-stage organ failure.

Total national health care cost for these patients exceeds $400 billion/yr.

Approx. 8 million surgical procedures are performed annually.

40~90 million hospital days are required

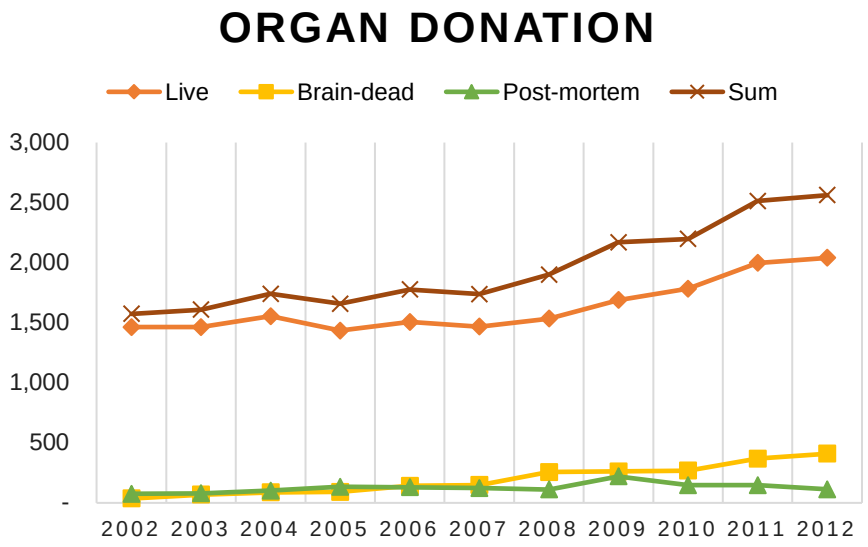
These are the number as of 1993. Current situation got worse due to the fact that it is rapidly becoming aging society.

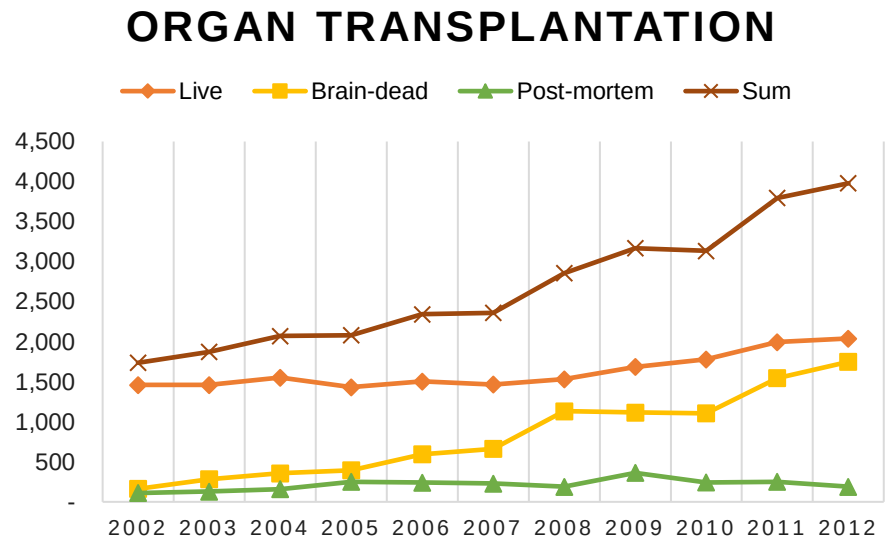
Indication	Procedures or patients per year
Skin	
Burns*	2,150,000
Pressure sores	1,500,000
Venous stasis ulcers	500,000
Diabetic ulcers	600,000
Neuromuscular disorders	200,000
Spinal cord and nerves	40,000
Bone	
Joint replacement	558,200
Bone graft	275,000
Internal fixation	480,000
Facial reconstruction	30,000
Cartilage	
Patella resurfacing	216,000
Chondromalacia patellae	103,400
Meniscal repair	250,000
Arthritis (knee)	149,900
Arthritis (hip)	219,300
Fingers and small joints	179,000
Osteochondritis dissecans	14,500
Tendon repair	33,000
Ligament repair	90,000
Blood vessels	
Heart	754,000
Large and small vessels	606,000
Liver	
Metabolic disorders	5,000
Liver cirrhosis	175,000
Liver cancer	25,000
Pancreas (diabetes)	728,000
Intestine	100,000
Kidney	600,000
Bladder	57,200
Ureter	30,000
Urethra	51,900
Hernia	290,000
Breast	261,000
Blood transfusions	18,000,000
Dental	10,000,000

*Approximately 150,000 of these individuals are hospitalized and 10,000 die annually.

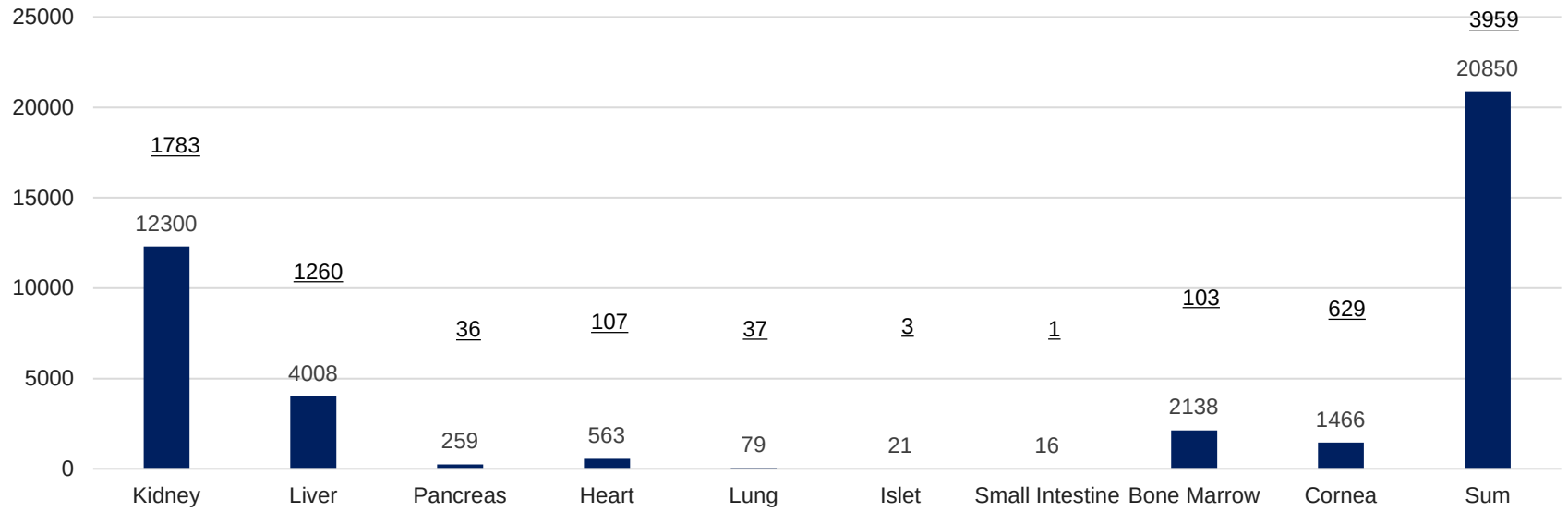
Organ Transplantation in Korea (2012)

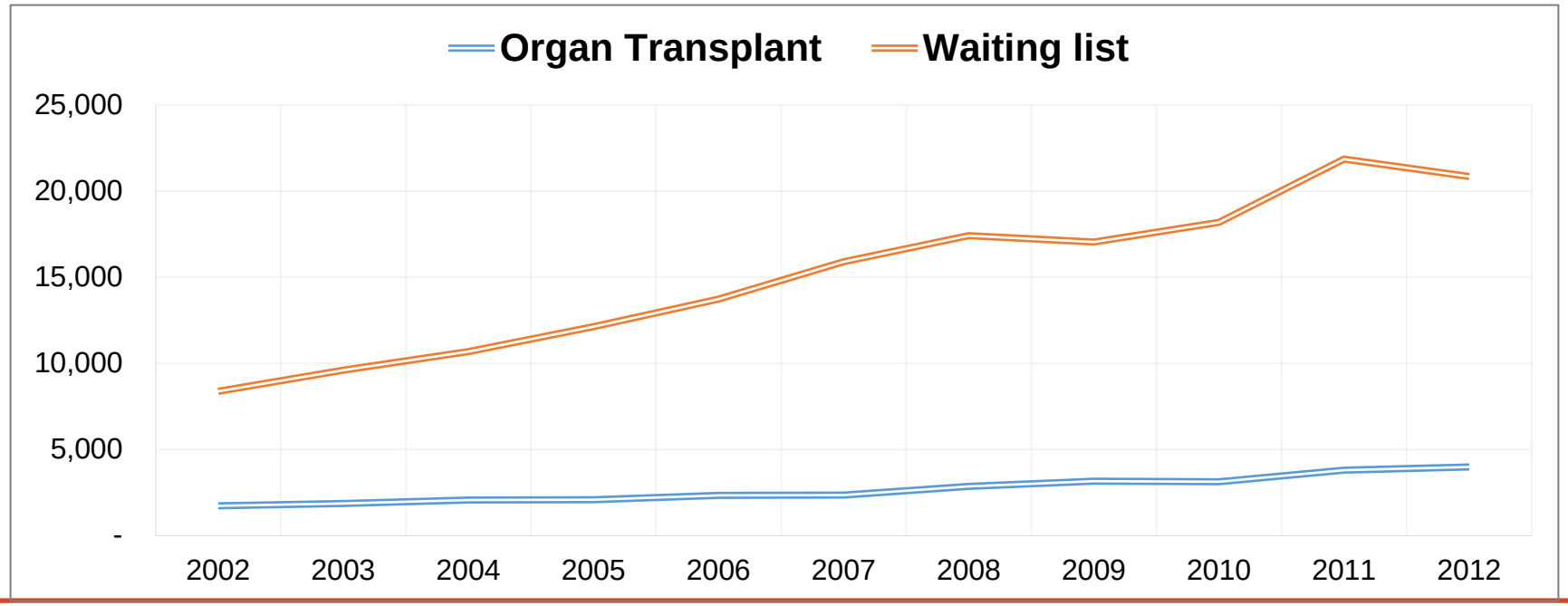
	Number of Donor	Total Transplant	Kidney	Liver	Pancreas	Heart	Lung	Islet	Small Intestine	BM	Cornea
Brain-dead	408	1747	766	363	34	107	37	3	1		436
NHBD	1	4	2								2
Post-mortem	112	191									191
Live	2040	2017	1015	897	2					103	
Sum	2561	3959	1783	1260	36	107	37	3	1	103	629





Transplant Waiting List (As of May 2013)





Conventional Treatment for Tissue Loss or Organ Failure

Imperfect solutions as follows;

Transplanting organs from one individual into another

- Severely limited by a critical donor shortage.
- Fewer than 3,000 donors are available annually for the approx. 30,000 patients who die from liver failure.
- Donor shortage worsen every year.

Performing surgical reconstruction

- Results in long-term problems
- Colon cancers often develop after surgical treatment of incontinence that directs urine into the colon.

Using mechanical devices (ex. Kidney dialyzer)

- Mechanical devices cannot perform all of the functions of a single organ
- Therefore cannot prevent progressive patient deterioration.

General Strategies of Tissue Engineering

Isolated cells or cell substitutes

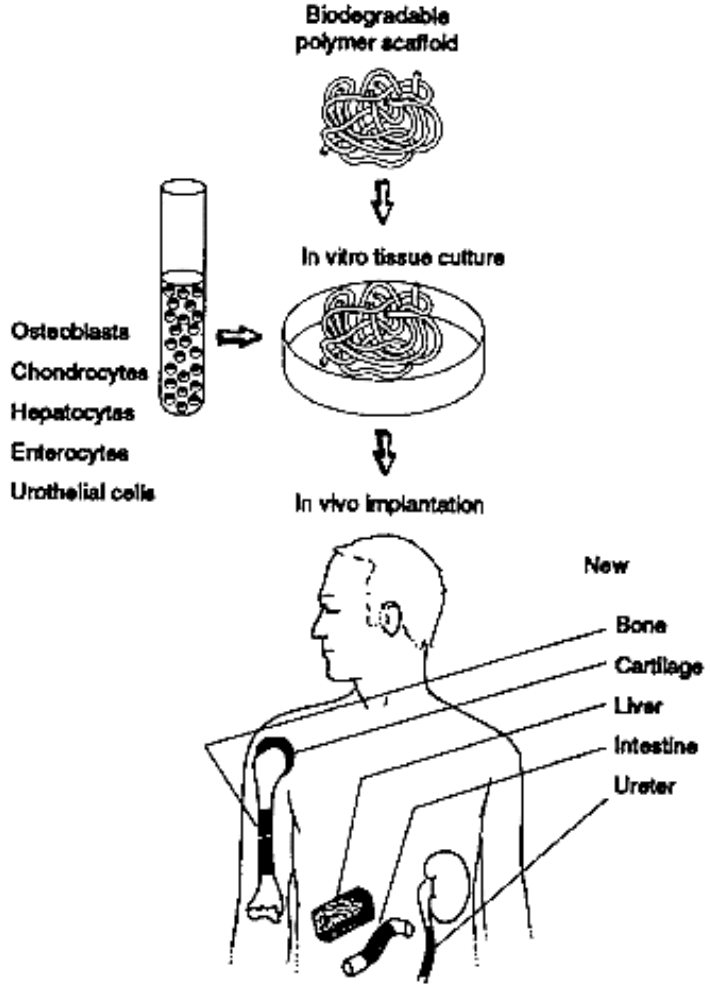
- Avoids complications of surgery
- Permits manipulation of cells before infusion
- Potential failure: failure of cells to maintain their function or immunological rejection

Tissue-inducing substances: bioactive factors

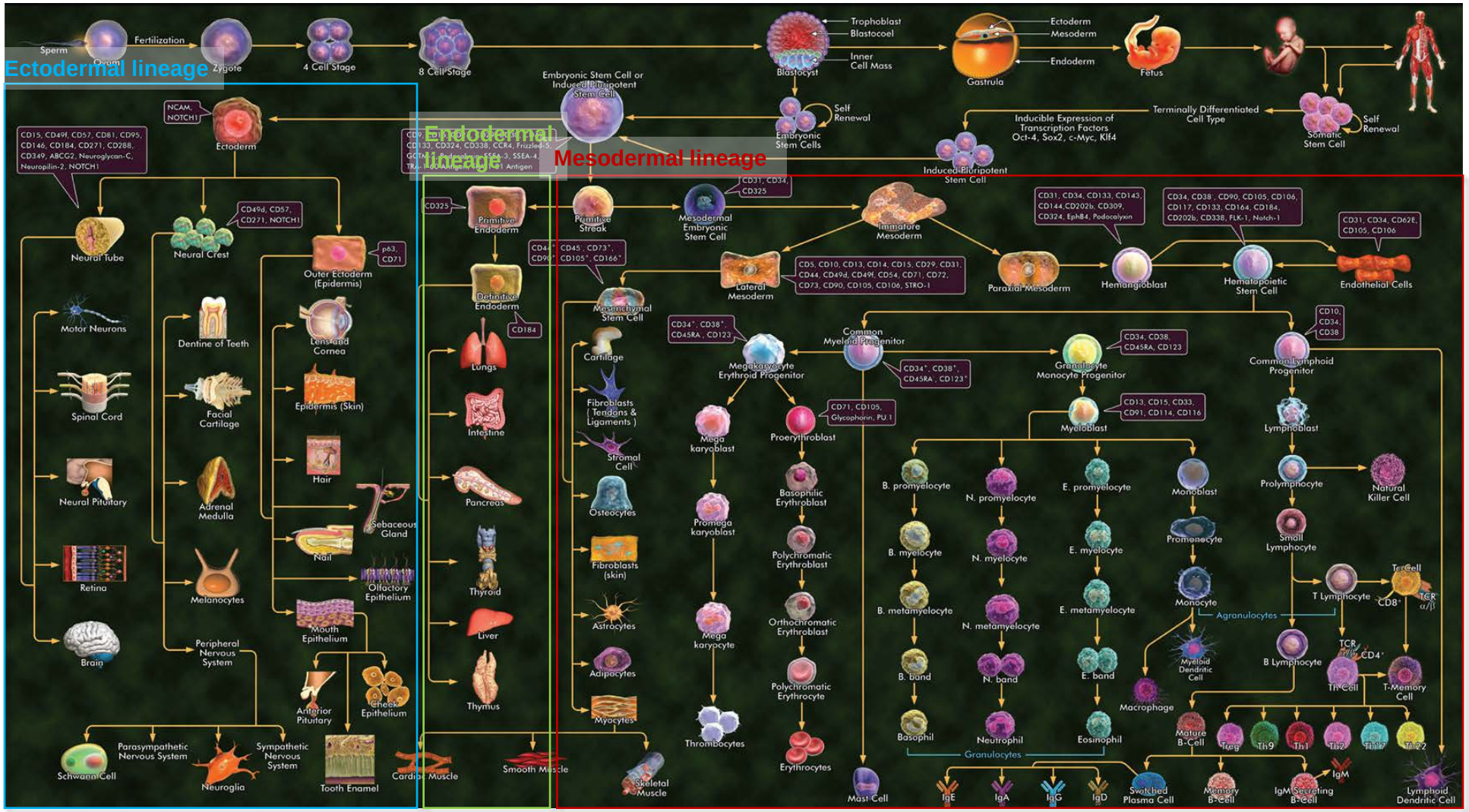
- This approach depends on purification and large scale production of appropriate signal molecules (GFs)

Cells placed on or within matrices: Scaffolds

- In Closed Systems: cells are isolated from the body by a membrane that allows permeation of nutrients and wastes but prevents large entities such as antibodies or immune cells from destroying the transplant
- In Open Systems: cells attached to matrices are implanted and become incorporated into the body
  - Natural polymer
  - Synthetic polymer



Tissue Formation



TE of Ectoderm - as of 1993

Nervous system -Parkinson´ s disease

- Loss of dopamine production by Dopaminergic neurons
- Transplantation of normal fetal dopamine-producing cells by stereotaxically guided injection into the brain
  -> induced significant reversal of debilitating symptoms

Cornea

- Current situation: more than 10 million people worldwide suffer from bilateral corneal blindness
- Transplant donors limited
- Risk of infectious agent transmission

Skin

- Current situation: 150,000 individuals are hospitalized and 10,000 die each year in the US because of burns
- Treatment needs: Burns, skin ulcers, deep wounds, and other injuries

TE of Endoderm - as of 1993

Liver

- Current situation: liver support systems (dialysis, charcoal hemo-perfusion, immobilized enzymes, exchange transfusion)
- Non of these can offer the full spectrum of functions performed by a health liver
- Liver replacement with isolated hepatocytes: Transplant hepatocytes encapsulated in microcapsules or hollow fibers -> produced albumin and other liver function markers

Pancreas

- Diabetes: characterized by pancreatic islet destruction, leading to loss of glucose control
- Current situation: over 728,000 new cases of diabetes are diagnosed and 150,000 Americans die from the disease each year; total yearly cost in the US is over $20 billion
- TE approaches focused on transplanting health pancreatic islets
- Tubular membrane containing islets with 50 kDa molecular mass cutoff (allow diffusion of glucose and insulin but blocked passage of Abs and lymphocytes)

Tubular structures

- Reconstruction of ureter, bladddar, urethra
- Extending the possibility: the concept of using tubular structures is being studied for other tissues such as the trachea, esophagus, intestine, and kidney

TE of Mesoderm - as of 1993

Cartilage

- Current situation: over 1 million surgical procedures in the US each year involve cartilage replacement
- Short coming of prostheses: result in adhesive breakdown at the host-prosthesis interface, no adaptation in response to environmental stress
- It is critical that cartilage transplanted be mechanically functional

Bone

- Over 1million operations annually involve bone repair
- Bone substitutes accelerate the bone ingrowth
    - Bone grafts: Materials limited, causes donor site morbidity, and contour irregularities
    - Allogenic bone: concerns on immune responses and pathogen transmission

Muscle

- Muscle injury, cardiac disease, disorders involving smooth muscle of the intestine or urinary tract, muscular dystrophy
- Heart disease: once patient become symptomatic, their life expectance is usually markedly shortened. This decline is generally attributed to the inability of cardiac cells to regenerate after injury.

Blood vessels and cells

- Difficult to develop vascular grafts of <5 mm i.d. because of biological reactions at the blood-material and tissue-material interface
- There are 18 million blood transfusions in the US annually.
- Problems: donor shortage, limited storage time, requirement for typing and cross-matching, infectious disease transmission -> Critical need for blood cell substitutes

Adipose tissue

Retina

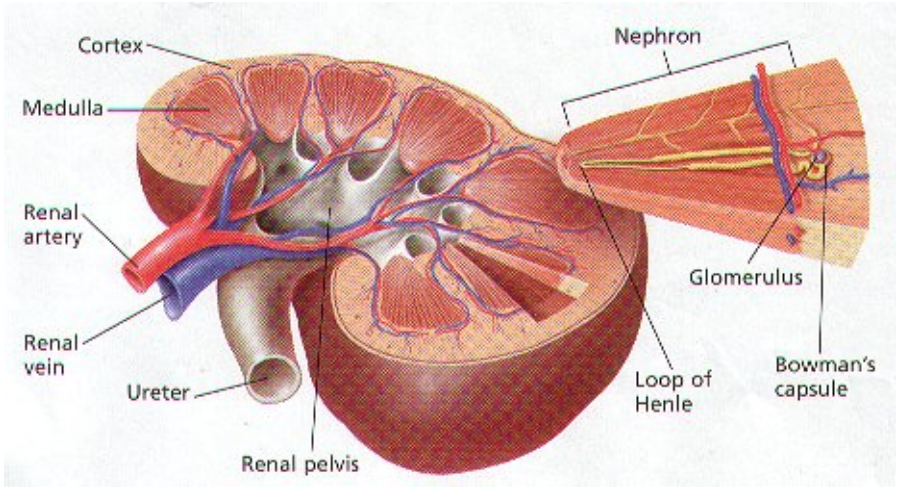
Kidney (complex tissue engineering)

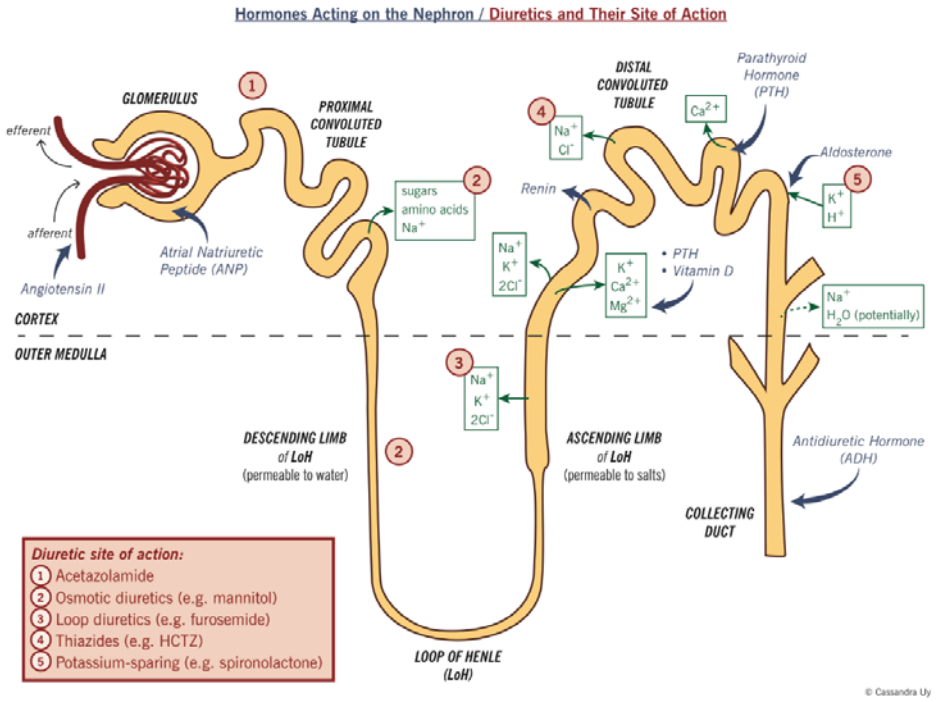
Reproductive system

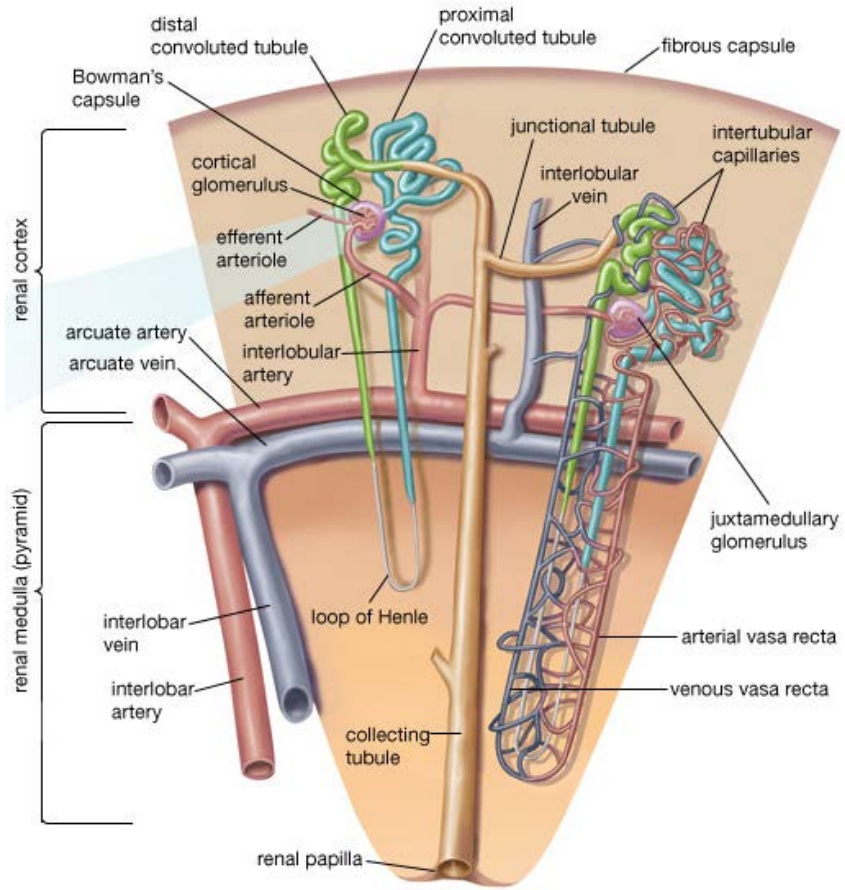
Tendon & ligament

Digit

Renal Function

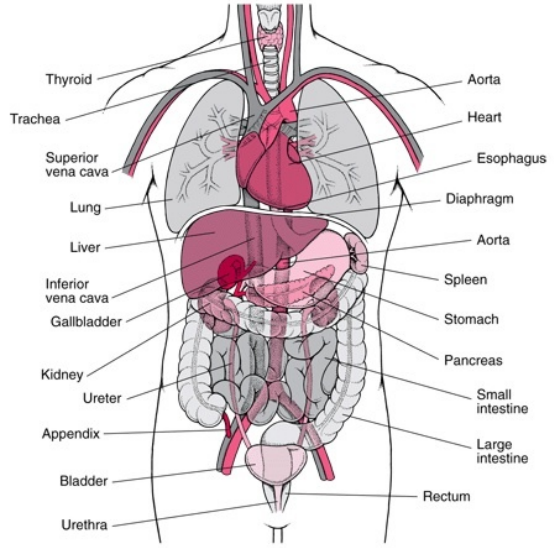


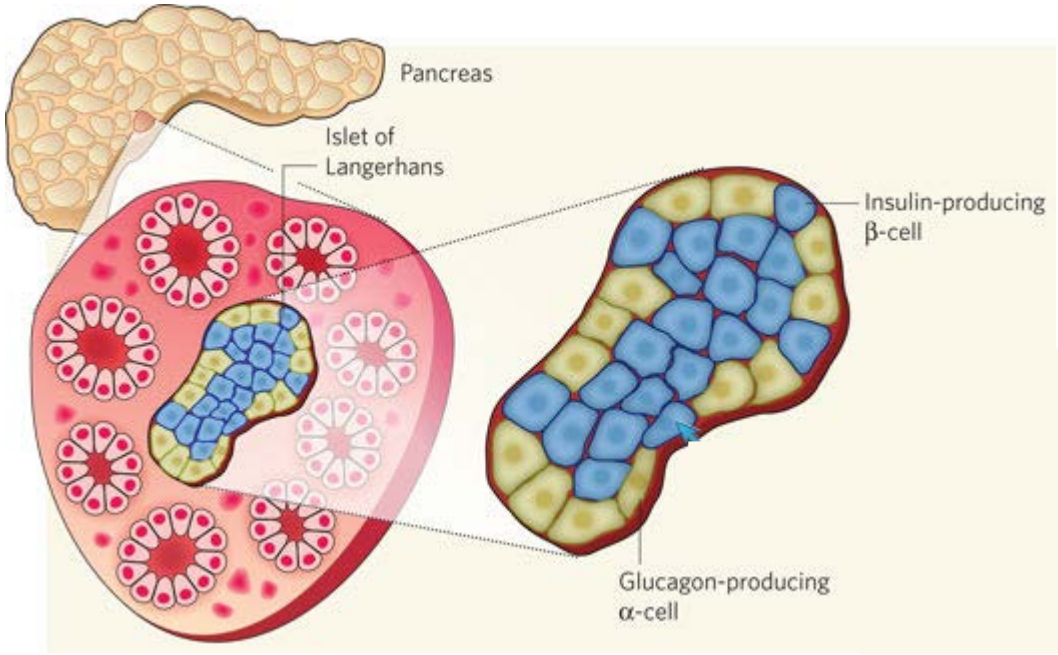




Hormones released from Kidney
1,25 Dihydroxyvitamin D
Erythropoietin
Renin

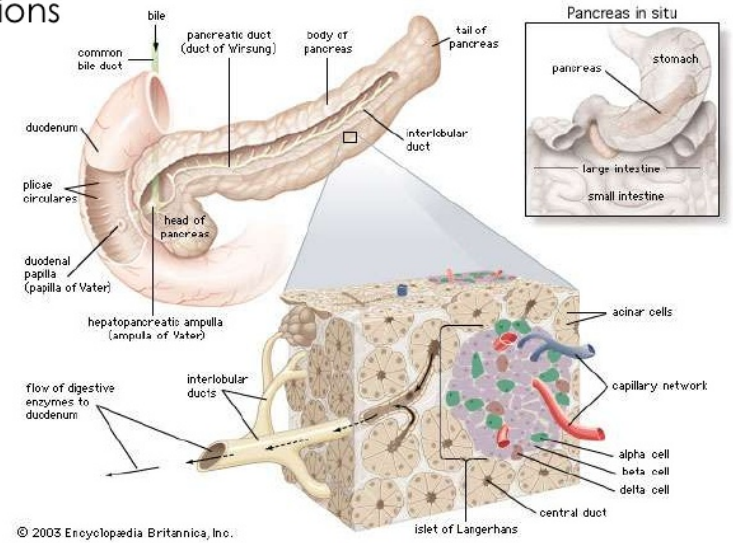
Pancreas





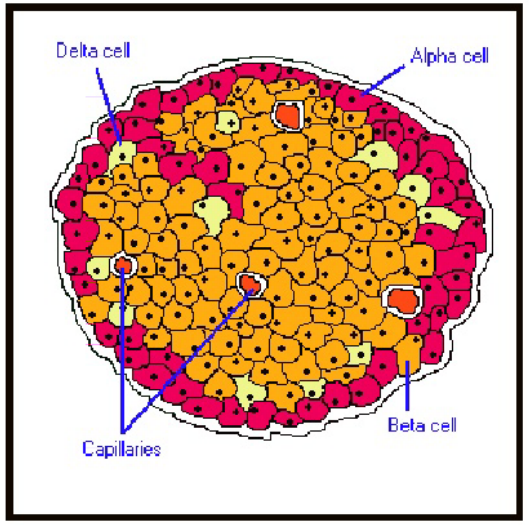
Pancreas

- Gland with both **exocrine** and **endocrine** functions



Histology- **Islets of Langerhans**

- Contain the cells that secrete:
- Glucagon - alpha cells, 20% of cells
- Insulin - beta cells, 70% of cells
- Somatostatin - delta cells, 5% of cells



Future Research - as of 1993

Cell source and cell preservation

- Ethical issues, safety issues and efficacy

Large-scale cell culture systems

Batch-to-batch variations or scale-up difficulties

- Biomaterials: synthetic polymers on the other hand, allow precise control over mw, degradation time, hydrophobicity, and other attributes may not interact with cells in a desired manner

Manufacturing highly porous structures - Reproducibility

Development of controlled-release systems

- Deliver molecules or GFs over long time periods

Develop methods of surface analysis for studying interfaces between cell and materials

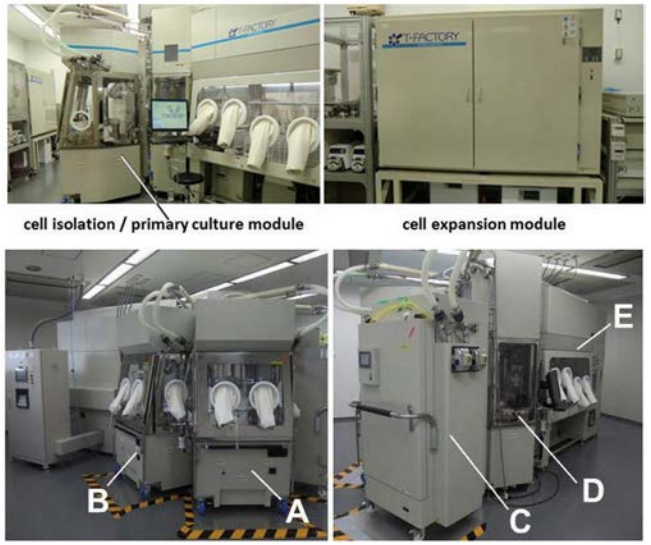
- Non-invasive detection on wound healing status

✓ **Cell technology – in vitro cell expansion, preservation induced pluripotency, & SCs for personalized medicine**

➢ **Large-scale cell culture system**

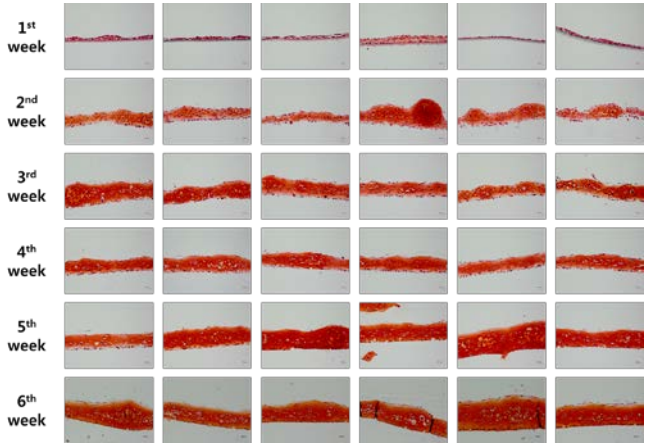
TAP Biosystems (UK)

CSTOF (Japan)



Fraunhofer (Germany)

❖ **Batch-to-batch variations, reproducibility**



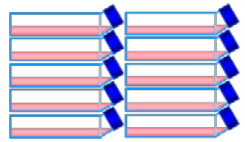
✓ **Controlled-release systems**

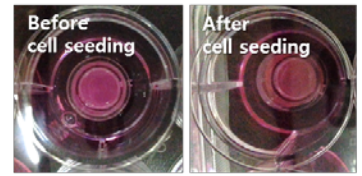
❖ **Non-invasive detection**

- **In vitro culture**
- **In vivo regeneration**

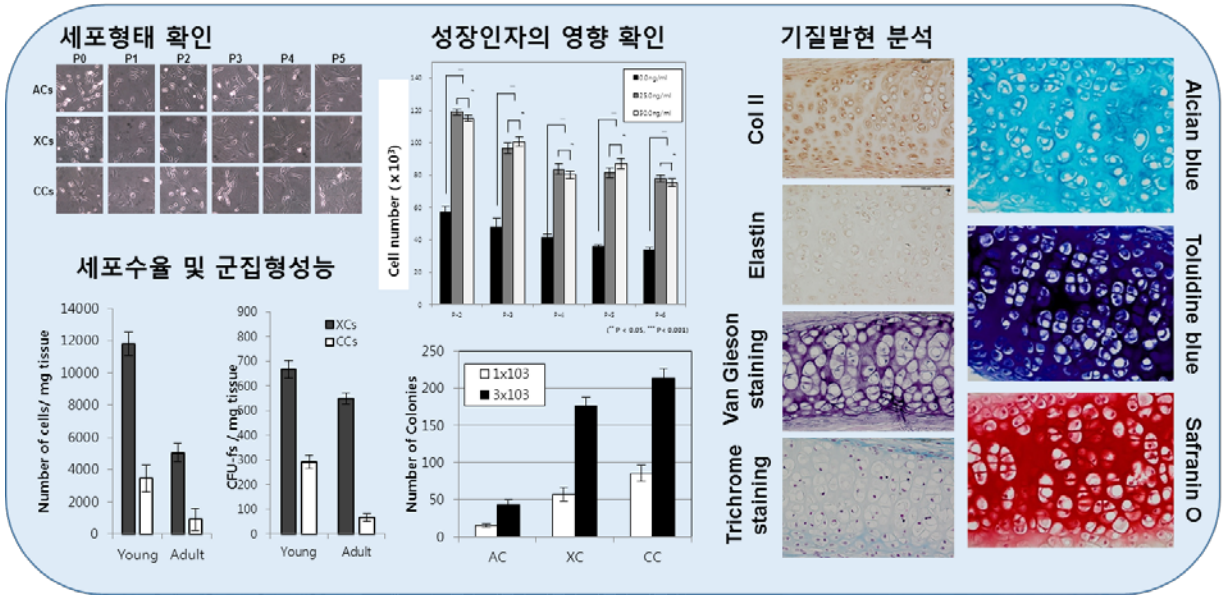
조직채취 → 1. 세포분리 → 2. 증식 → 3. 분화 → 4. 조직공학제제 품질확인

Samples	Initial Cell #/mg
Patient 1	2823.67 ± 851.41
Patient 2	1098.25 ± 443.45

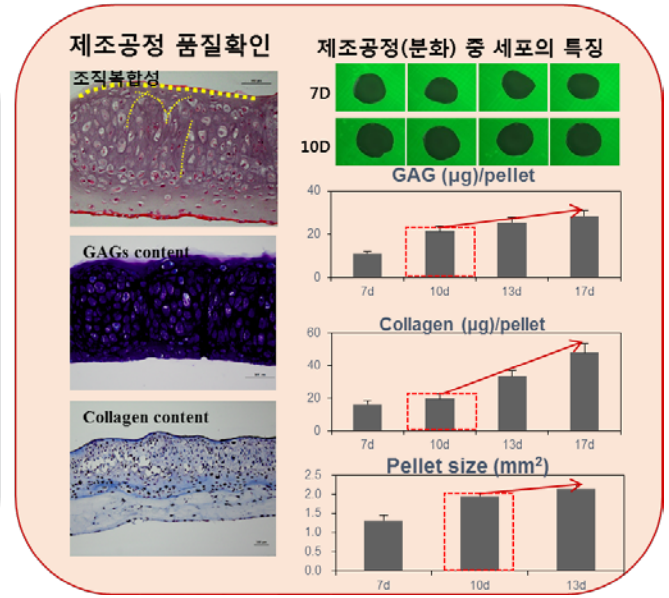




연구개발과정에서의 배양 조직 소모



품질평가 과정에서의 배양 조직 소모



Developing Artificial Tissue or Organ Takes Interdisciplinary work.

When biomaterials move from the lab to clinical use, the groups in different fields need to work together.

Engineers

- Think about formulas and numbers

Physicists

- Focus on explaining materials behavior

Biologists

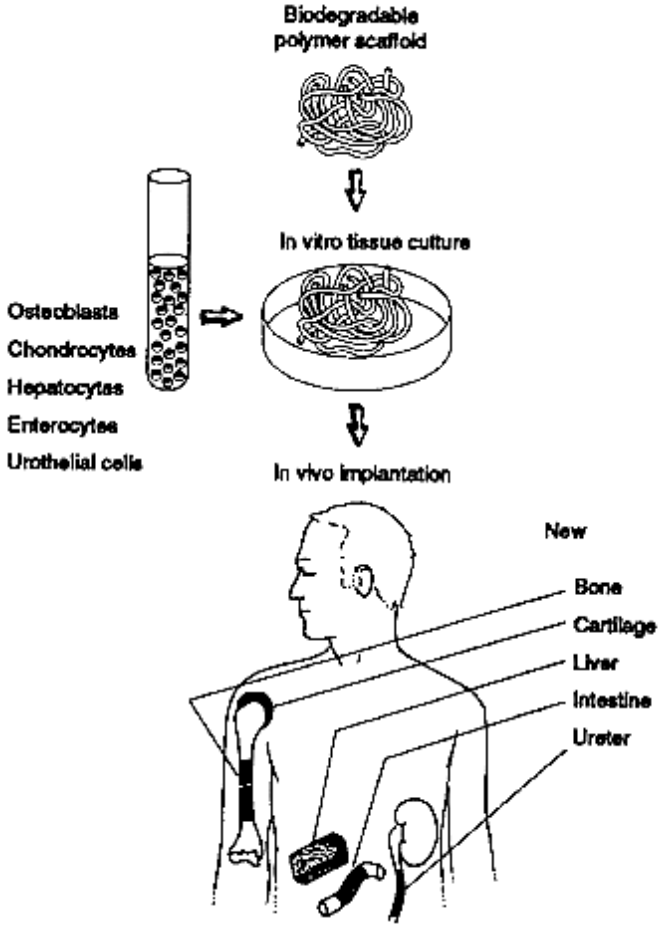
- Analyze complex cellular interactions

Surgeons

- Work with their hands

Industry & Regulation officer

- Commercialization aspect



- During all steps of translation, regulation and commercialization must be kept in mind.

Tissue engineering applies the principles of biology and engineering to the development of functional substitutes for damaged tissue.

- **Toward the development of biological substitutes that restore, maintain, or improve tissue function**

"Few areas of technology will require more interdisciplinary research than tissue engineering or have the potential to affect more positively the quality and length of life." -
Robert Langer and Joseph P. Vacanti 1993 Science

The field of TE is at the interface of bioengineering, materials science, chemistry, biology, and medicine, poised to meet these unmet clinical needs through the development of new technologies and refinement of existing ones.

Functional Tissue Engineering

[Butler DL, Goldstein ST, & Guilak F 2000 J Biomech Eng; Goldstein AS & Christ G 2009 Tissue Eng]

Challenges in Repairing Tissues that Serve a Predominantly Biomechanical Function

-To effectively repair or replace these load bearing structures

-An evolving discipline called “functional tissue engineering” (FTE) seeks to address these challenges

Unites States National Committee on Biomechanics (USNB), 1998

Concept of FTE with the following specific goals.

1. The importance of restoring function when engineering tissue constructs
2. Identifying the critical structural and mechanical requirements needed for each tissue-engineered construct
3. Incorporate functional criteria in design and manufacturing processes to optimize the overall success of engineered tissues

What constitutes "success" will be expected to differ among the tissues.

- Ex) Tissues or systems that are designed to prolong life may tolerate a lower margin for error than those that are designed to improve the quality of life.
- Therapies of replacement of regeneration of blood vessels or bone might be expected to last the lifetime of the individual, while replacement of cartilage may be considered successful if it delays total joint replacement for five to ten years.

Principles of Functional Tissue Engineering

1. In vivo stress and/or in vivo strain histories need to be measured in normal tissues for a variety of activities
2. The mechanical properties of the native tissues must be established for subfailure and failure conditions
3. A subset of these mechanical properties must be selected and prioritized
4. Standards must be set when evaluating the repairs/replacements after surgery so as to determine, "How good is good enough"?
5. The effects of physical factors on cellular activity must be determined in engineered tissues. How do physical factors influence cellular activity in bioreactors and can cell-matrix implants be mechanically stimulated before surgery to produce a better outcome?
   - Various physical factors have the capability to influence the biological activity of normal tissues - Positive or Negative?

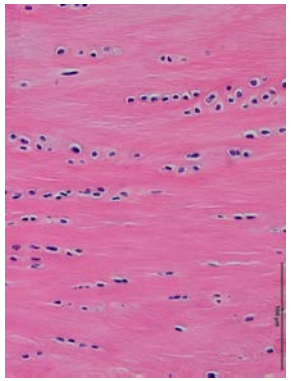
Tissue property in a material standpoint

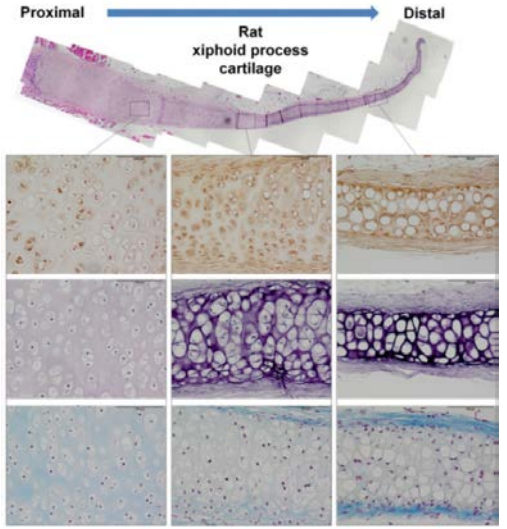
Anisotropy

Properties vary with direction

- **Tensile, compressive, shear moduli**
- **Permeability**
- **Failure stress and strain**
- **Fatigue life**

Rat fibro-cartilage

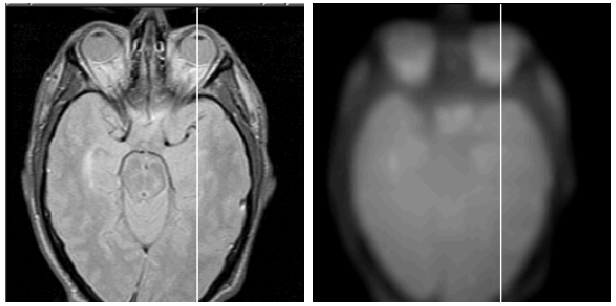




- Anisotropy in articular cartilage:
  - Highly non-linear mechanical properties such as strain-dependent moduli, strain-dependent hydraulic permeability, and a difference of nearly tow orders of magnitude in tensile and compressive moduli.
  - Particularly in tension, vary significantly with distance from the tissue surface and with site on the joint.

Inhomogeneity

- Properties vary with site
  - Potentially all properties
- Close relationship between structure - property



Geometry

- Morphology
- Microstructure
- Congruence

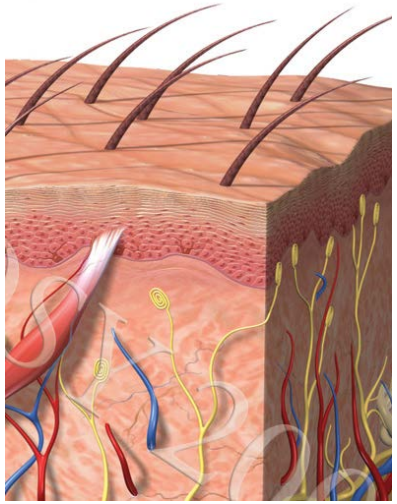
Bone microstructure

- Bone:
  - From a physiological, functional point of view, the integrity of a bone region is dominated by its gross geometry and apparent density.
  - Experimental data suggest that its preyield material properties are substantially dependent on its mineral (or mineral/matrix) content, and post-yield or failure behavior is more influenced by its glycoprotein matrix

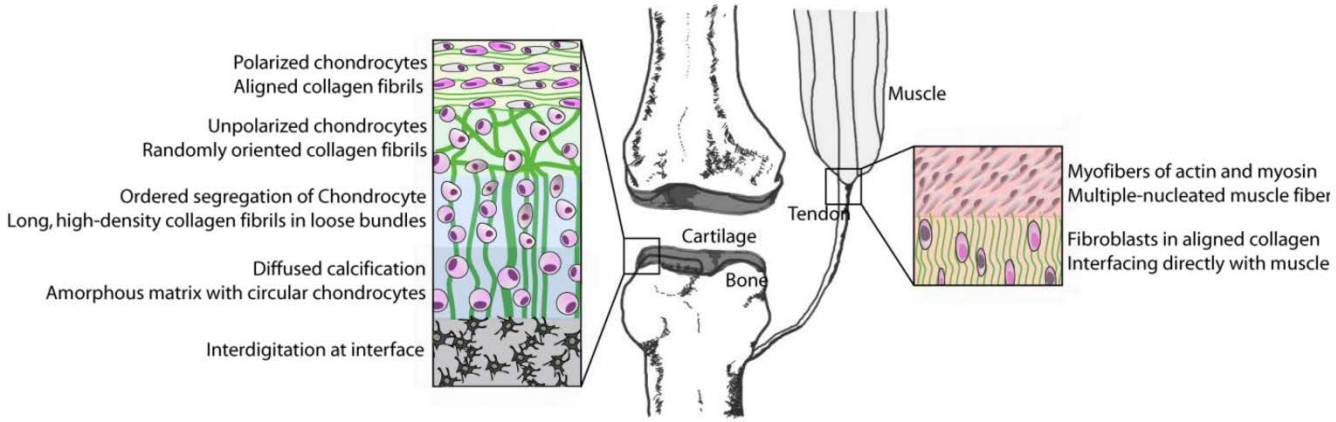
Nonlinearity

Stress-strain relationship is not linear

- **Tension-compression nonlinearity**
- **Nonlinear permeability**
- **Material nonlinearity**
- **Nonlinear viscoelasticity**
- **Coupling of normal and shear stress**



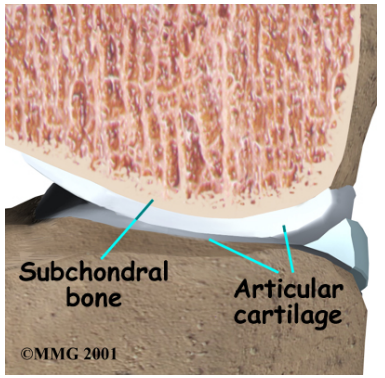
  - Articular cartilage:
    - Highly non-linear mechanical properties such as strain-dependent moduli, strain-dependent hydraulic permeability, and a difference of nearly two orders of magnitude in tensile and compressive moduli



Physicochemical-Mechanical Coupling

- **Residual stresses**
- **Swelling**
- **Electrokinetic effects**

Tribological Properties

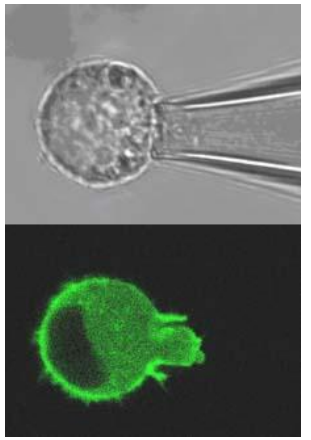


- Friction coefficient
- Wear properties
- Hardness

- Articular cartilage:
  - unique mechanical & tribological properties
  - Attributed to the complex structure and composition of ECM

Viscoelasticity

- Properties vary with time or rate of loading
  - Multiphasic or poroelastic
  - Energy dissipation
  - Intrinsic viscoelasticity
  - Fluid viscosity
- Viscoelasticity in cartilage results from frictional iterations between the solid and fluid phases



Choice of parameters depends on tissue in question

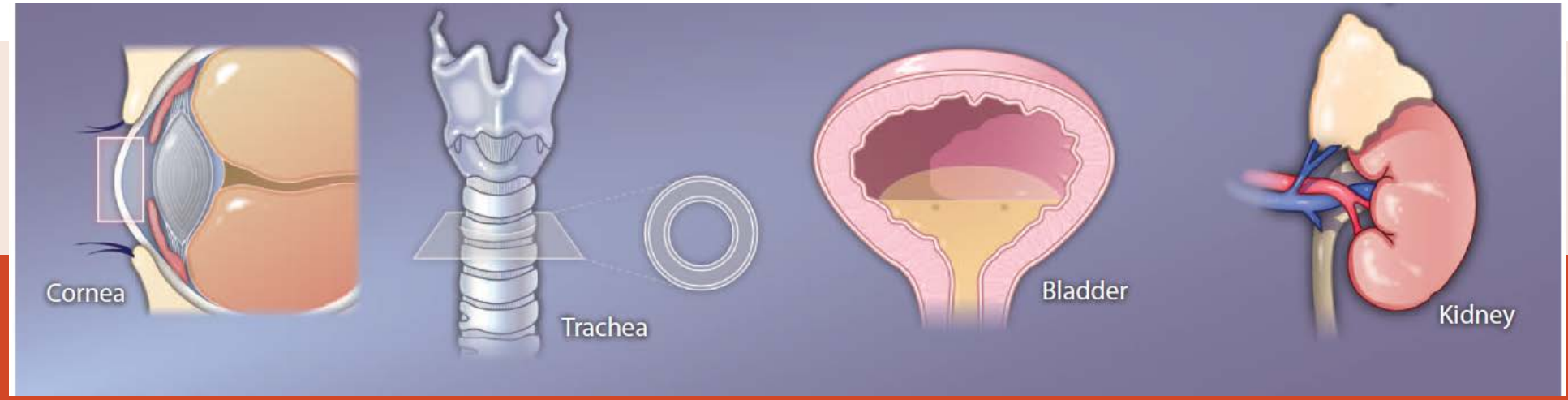
- **Articular cartilage:**
  - **compressive moduli**
  - **Hydraulic permeability**
  - **Complete integration between host and repair tissues**
  - **Precise control on structure and geometry of newly formed cartilage**
- **Bone**
  - **Ability of remodel**
  - **Adaptation to habitual physical demands**
    (Remodeling: Tissue response to external stimuli)
- **Tendon and ligament**
  - **Carry primarily tensile forces (only up to 10~40 percent of failure)**
  - **However, these tissues are also repeatedly loaded under a combination of load and displacement control.**
  - **Cyclic creep and stress relaxation parameters are probably equally important characteristics**

Important Unmet Need in Evaluation of Tissue Regeneration

The development of new methodologies that will allow assessment of the material or structural properties of engineered tissues in a non-invasive or minimally invasive manner

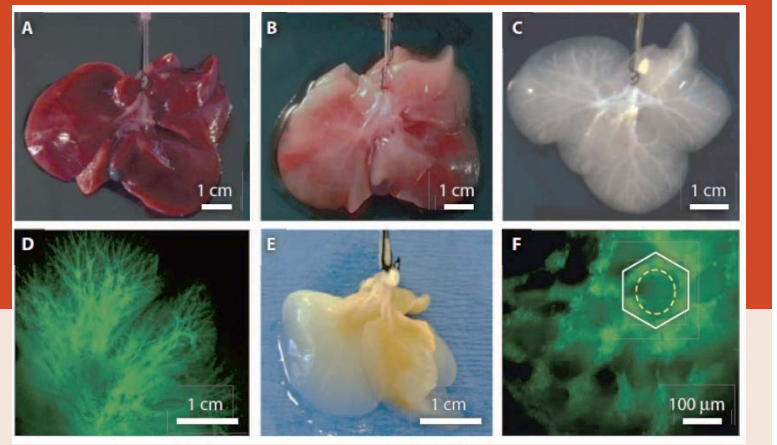
- Biological marker
- Biomechanical probes (arthroscopic)
- MRI
- CT
- Ultrasound
- DEXA

To summarize, there is clearly a need to establish both **functional criteria** as well as design **parameters for tissue regeneration**.



complex Tissue Engineering

[Atala A 2012 Science Translational Medicine]



Complex Tissue Engineering [Atala A 2012 Science Translational Medicine]

"On the applied side of biomaterials, a major unmet clinical need is engineered whole organs." - Lavine M et al., 2012 Science

It may be possible to combine cells and biomaterials into a structurally and functionally competent organ, but vascular networks are needed.

Engineering Complex Tissues

The complexity of many tissues and organs is a challenge to traditional engineering approaches.

Not all tissues are created equal, and all present unique challenges in tissue engineering

Increasing levels of tissue complexity
-> increased complexity in TE approaches
(-> increased complexity in regulation)

Current situation in TE: Basic TE strategies are universal

- **Combination of cells, biomaterials, & bioactive factors**
- **De novo growth in tissue culture or induction of tissue regeneration in vivo**

Scaffolds

Biomaterials

- Provide 3D structure and space for target tissue
- Can be tailored to support activity of cells
- Ideally should serve as a transient structure
- Category
    - Natural polymers
    - Synthetic polymers
    - Materials derived from native tissue

3D fabrication method

- 3D printing

Cells

Autologous cell populations

- Minimal risk of rejection

Limitation

- Difficulty in expanding cells to sufficient numbers
- Expansion of primary cells from some tissues and organs such as pancreas remains as challenge
- Cells derived from diseased tissue or organs may not yield a sufficient # of normal cells for clinical application

Possible alternative option

- Stem/progenitor cell populations

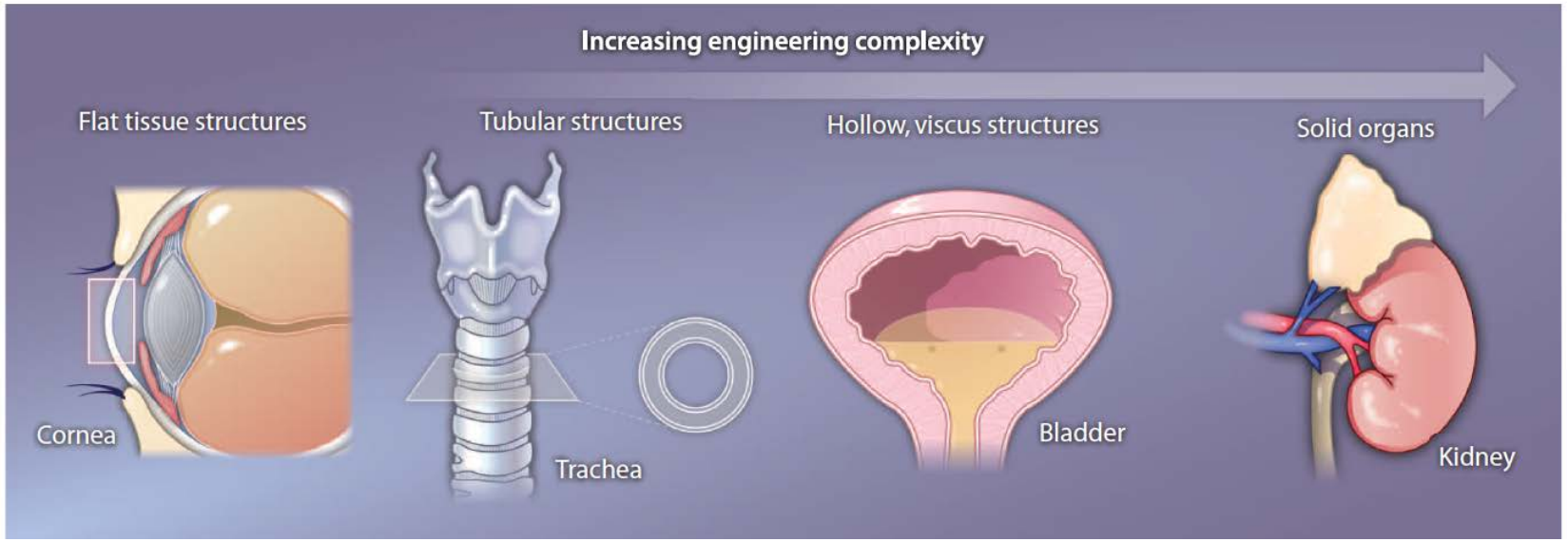
Creating Complex Organs

Common challenge

- To regenerate organs that have intricate 3D structures
- Integrating the regenerating tissue with surrounding tissues

Category of tissue complexity

- Flat
- Tubular
- Hollow, nontubular, viscous
- Complex solid organs

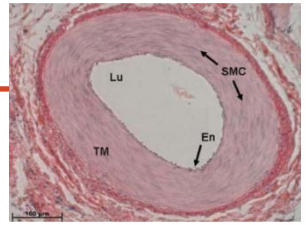


Flat structures

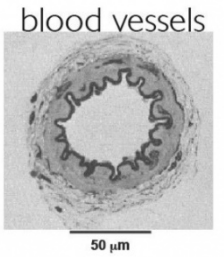
Simplest architectural subtype in the body

- Skin
  - Substantial loss of skin surface area are detrimental (Ex. Burn)
- Cornea
  - Cornea performs a fundamental function in the refraction of light for vision
  - Surgical transplantation of donor corneal tissue has long served as a clinical standard of treatment

Tubular Structures



Reinhardt et. al (2004)



Greensmith & Duling (1984)

Yang et al. (2007)

Tissues: Urethra, Trachea, or Esophagus

Consists of two different type of cells

- Inner layer of epithelial or endothelial cells (functional barrier)
- Outer layer of smooth muscle & connective tissue (Provide support)

Reported cases

- Urethral construct (PGA/PLGA + cells)
- Pulmonary artery transplant (collagen, synthetic polymer + cells)
- Acelluar vascular graft for longterm storage
- Tracha (decellularzed scaffolds)

Hollow, Viscous Structures

Tissues: Urinary bladder

Consist of two type of cells

- Inner layer: epithelial-type cells
- Outer layer: smooth muscle and/or connective tissue

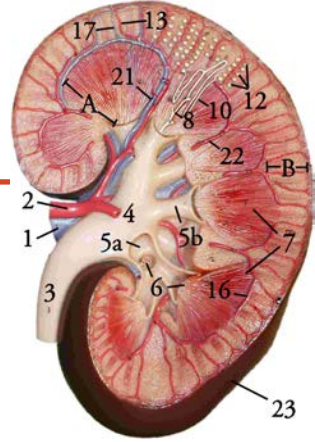
Additional functional parameters needed

- Higher metabolic requirements
- Complex intracelluar & inter organ interactions

Biofabrication process

- Scaffold seeded w/ at least two different type of cells
- Connecting issue!
- Reported case: regeneration of bladder (autologous cells + smooth muscle cells)
    - CT scan -> scaffold individualized for patient -> cells harvested -> seeded on collagen-PGA composite scaffold -> bioreactor incubation

Solid Organs



Highest levels of tissue complexity

- Kidney, pancrease, & liver

Traditional treatment

- Temporary supportive treatment by drugs, devices, or whole-organ transplantation

Challenge

- Incorporation of extensive vascular network
- Precise organization of multiple cell types

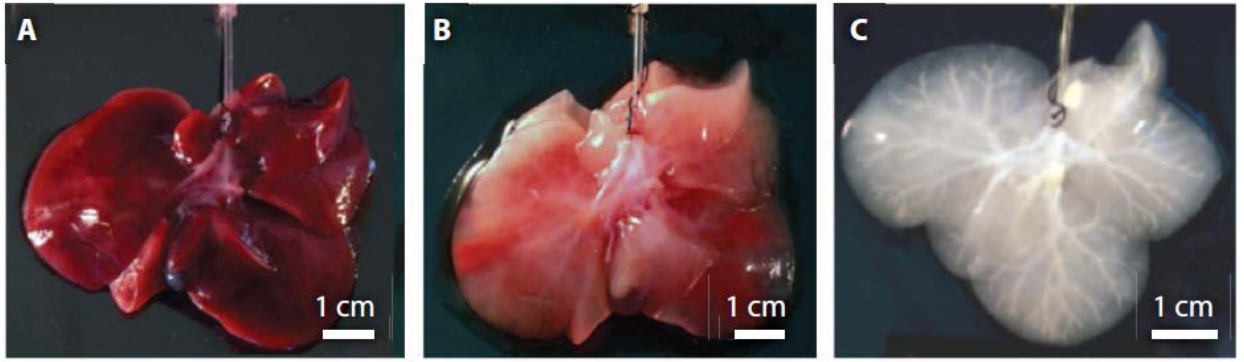
Biomaterials-based approaches

- Gradients of GFs
- Hybrid composite materials
- 3D printing methods

Decellularized Scaffold

Reported cases of decellularization -> recellularization

- **Reproductive organ (Rabbit)**
- **Heart (rodent model)**
  - **w/ walls, valves, & blood vessels**
  - **Perfused w/ harvested endothelial cells**
  - **Contractile function was observed**



- **Liver (mice, rabbits, ferrets, pigs)**
  - **Repopulated w/ human hepatocyte & umbilical vein endothelial cells**
  - **Albumin and urea secretion**
- **Pancreatic islet**
  - **cells seeded on a decellularized pancreas matrix**
  - **In vivo insulin secretion**

Limitation

- **Lack of available homologous scaffolding for solid organs**

Solution for limitation of decellularized scaffold

Xenotransplantation

- Decellularized donor scaffold from animal source + Autologous cells
- Promising results from evaluation on
    - Transmission of infectious agents
    - Rejection potential

Bioprinting

- Vascular network
- Functional paranchyma components

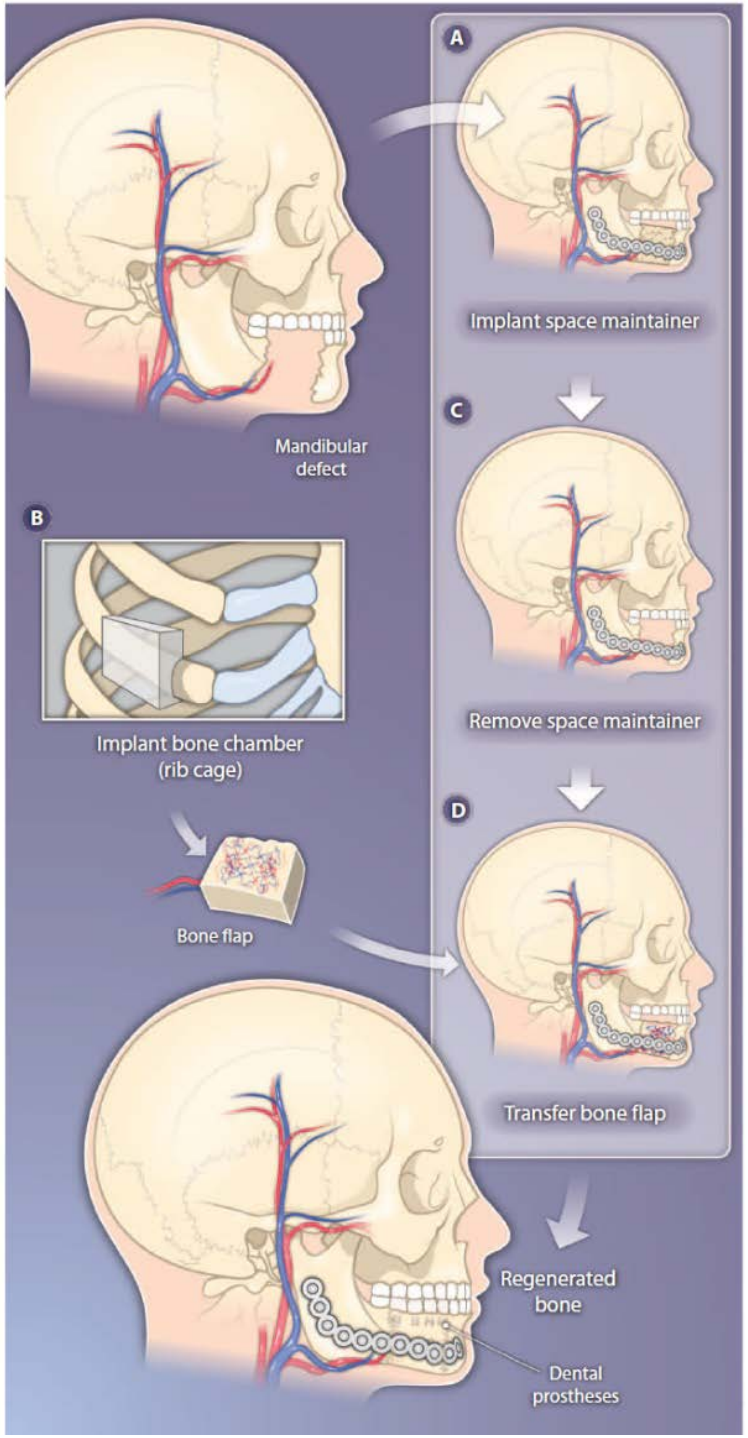
Clinical challenges

Case-specific structural customization

- General clinical challenges (scaffold for bone regeneration) -> The demands of a specific clinical application (scaffold for alveolar bone regeneration in tooth extraction sockets)

Aspects complicating translation

- Variability between patients
- Minimally invasive monitoring technology needed
- Variation between density of
  - Stem/progenitor cells
  - Regenerative capacity



Focused investigation needs to consider the clinical need and the commercial & regulatory pathways at the outset for…

- Clinical translation (“Bench to Bedside”)
- Market viability of the technology
- More tissue complexity -> more challenging in manufacturing
  - Cost of the product increase accordingly
  - Inter-lot consistency
  - Inconsistancy in cell populations
    - Thorough characterization
    - Establishing reproducible standard method of cell isolation and culture
  - Safety issue
- Regulatory considerations
  - Product complexity -> Regulatory complexity
  - Regulatory complexity and associated burden can be minimized through identification and pursuit of the simplest product sufficient to meet the desired clinical need
  - Interaction w/ relevant regulation body should be initiated at the initial stages of technology development

Commercialization

- Possible barrier and an added expense
  - Clinical ease of use
  - Sterility & purity issues
  - Scale up (dealing with batch-to-batch variation)
  - Quality control

EOD