An Introduction to Tissue Engineering

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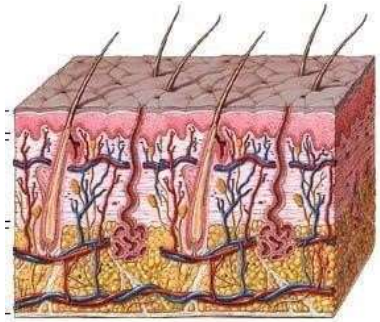
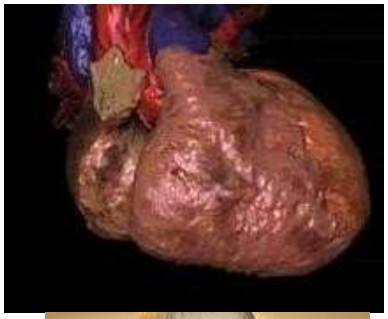
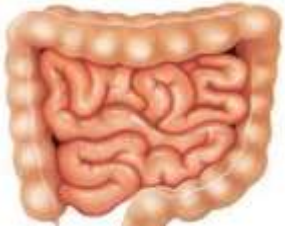
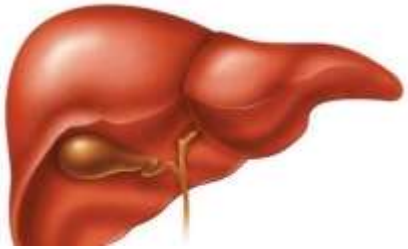
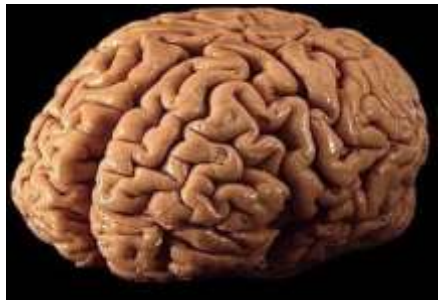
Tissue Engineering is...

*"an **interdisciplinary** field that applies the **principles of engineering and life sciences** towards the development of biological substitutes that **restore, maintain, or improve tissue function or a whole organ**"*

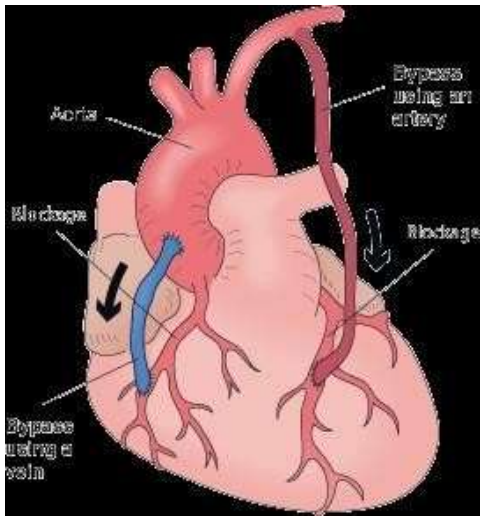
Langer and Vacanti, *Science* 1993

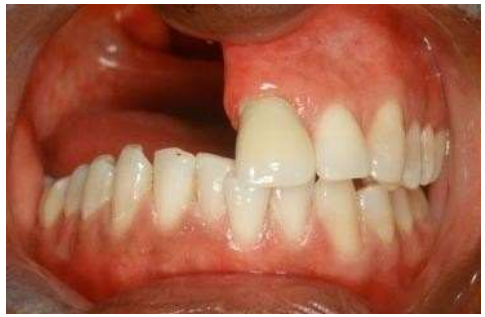
Regeneration in Humans

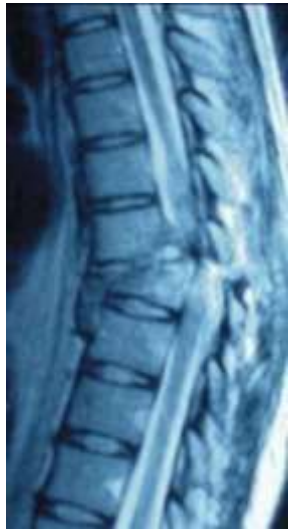
High	Moderate	Low
		
		
		

Clinical Needs

- Cardiovascular
  - Myocardial infarction
  - Stroke
- Bone
  - Non-union fractures
  - Tumor resections
- Nervous
  - Spinal Cord Injury
  - Degenerative diseases
- Skin
  - Burns
  - Ulcers

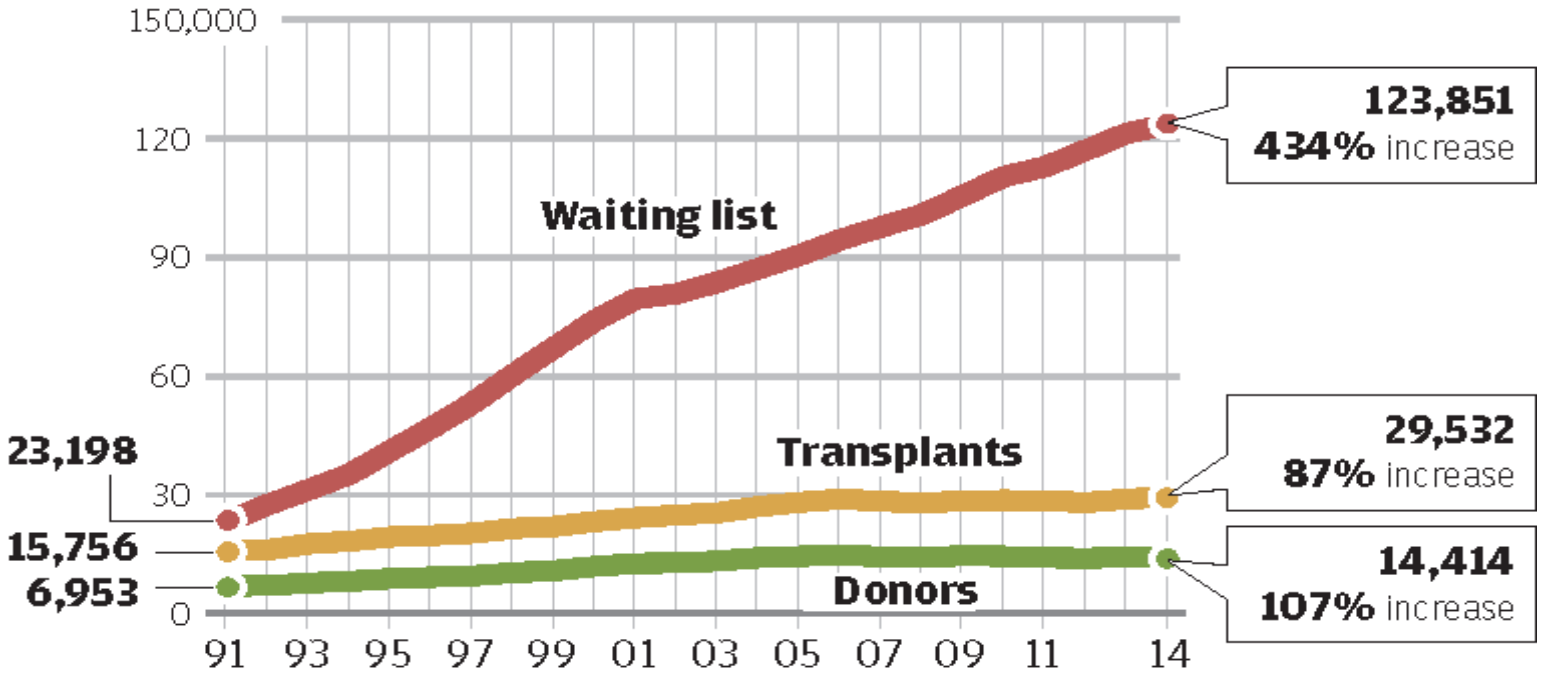






Need for organ transplants increasing

The waiting list for organ transplants continues to grow, but the supply of deceased and living donor organs has been stable in recent years.



Note: Waiting list figures include some patients temporarily considered inactive.

SOURCE: U.S. Department of Health and Human Services

State Journal

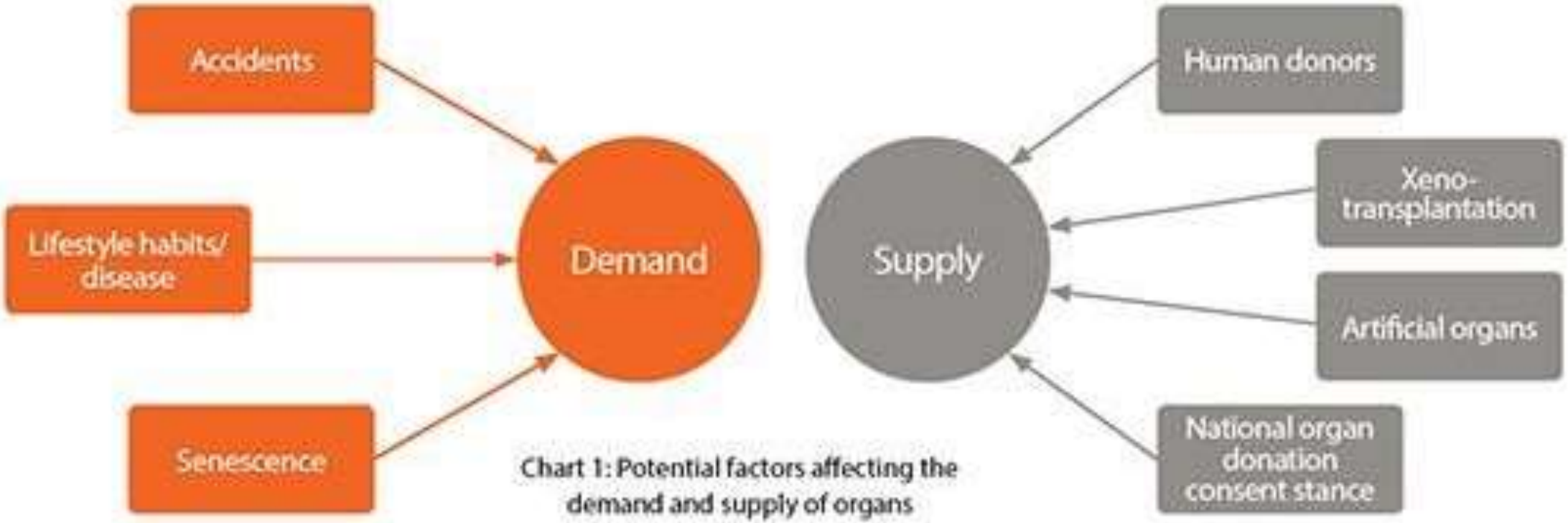
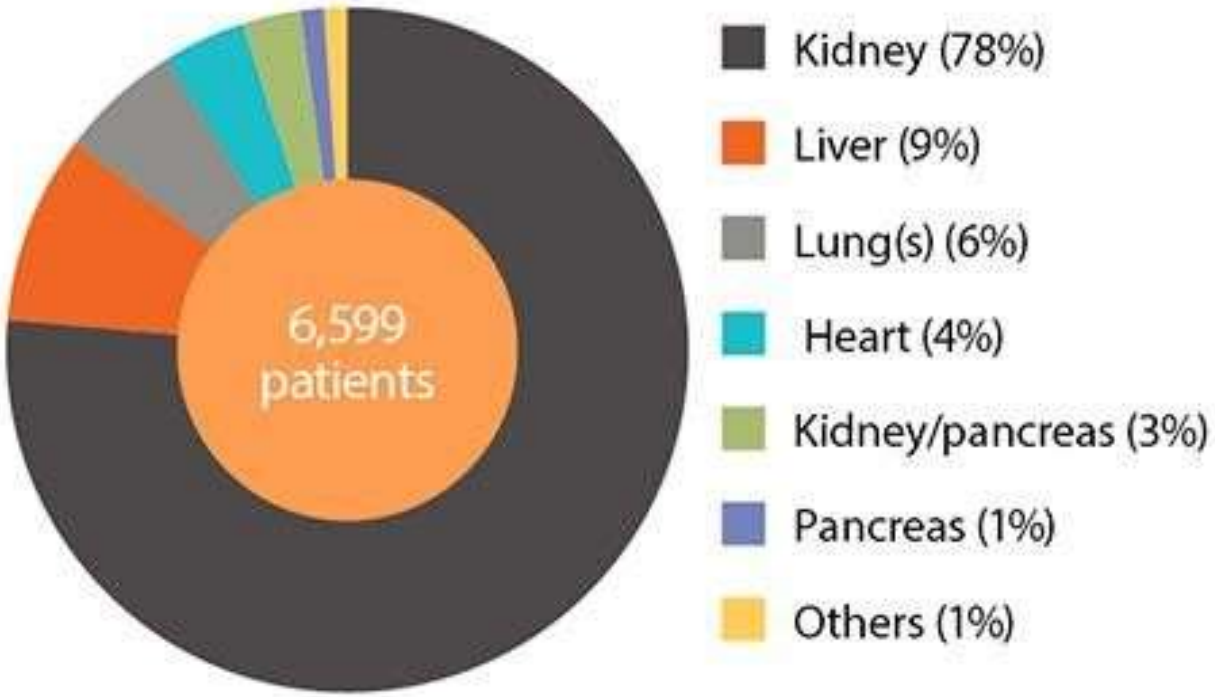


Chart 1: Potential factors affecting the demand and supply of organs

Organs required by patients on the NHS organ transplant waiting list



Source: NHS Blood and Transplant, Sept 2016

- Motivation:
  - 800 million surgical procedures p.a. (US) to repair tissue loss/organ failure
    - > USD $400billion p.a.
  - reconstructive surgery due to
    - trauma
    - congenital & degenerative diseases
    - cancer
    - cosmetics

- Tissue engineering (TE) is a rapidly growing scientific area that aims to create, regenerate, and/or replace tissues and organs by using combinations of cells, biomaterials, and/or biologically active molecules.

- TE intends to help the body to produce a material that resembles as much as possible the body's own native tissue.

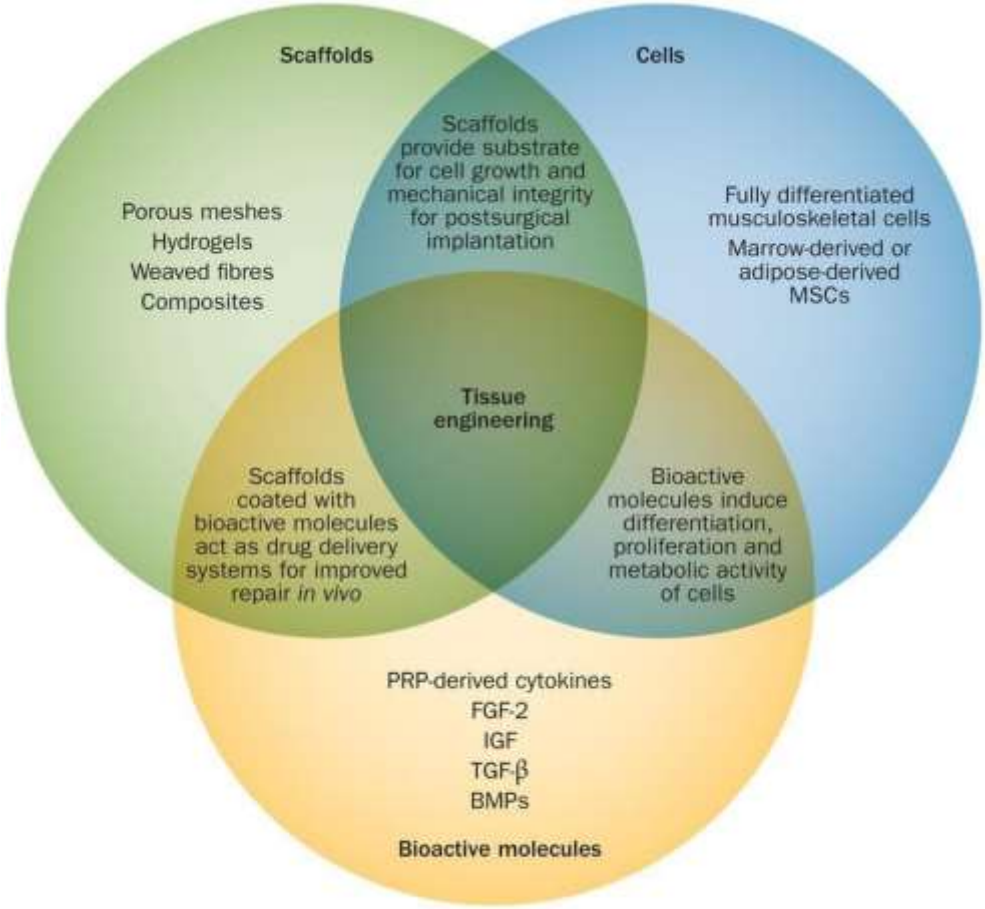
Tissue Engineering (TE)

- Scaffolds
  Biomaterials, which may be natural or artificially derived, providing a platform for cell function, adhesion and transplantation

- Cells
  Any class of cell, such as stem or mesenchymal cell

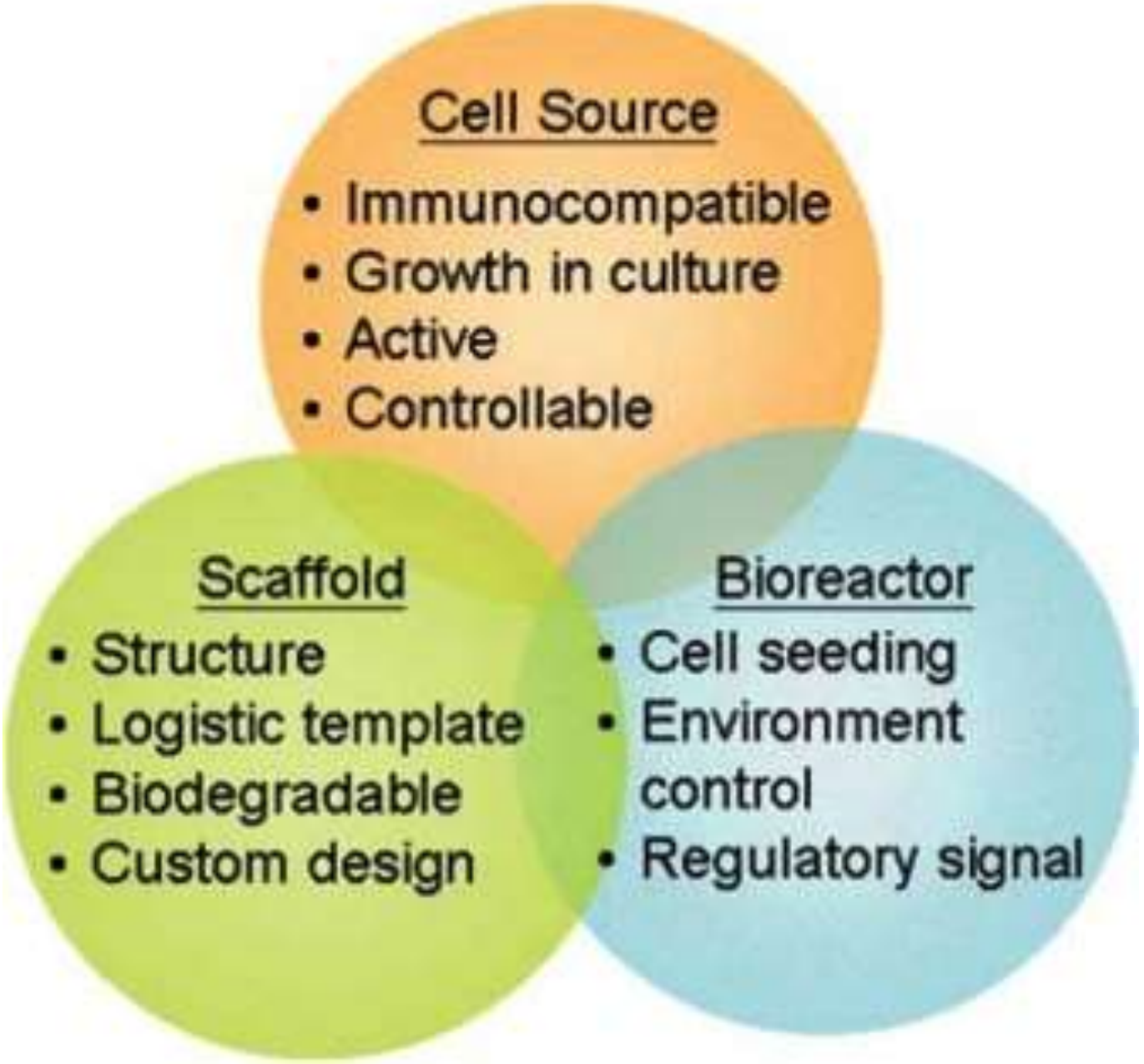
- Signals
  Proteins and growth factors driving the cellular functions of interest

- Bioreactor
  System that supports a biologically active environment (ex. Cell culture)



Nature Reviews | Rheumatology

Parameters in Tissue Engineering

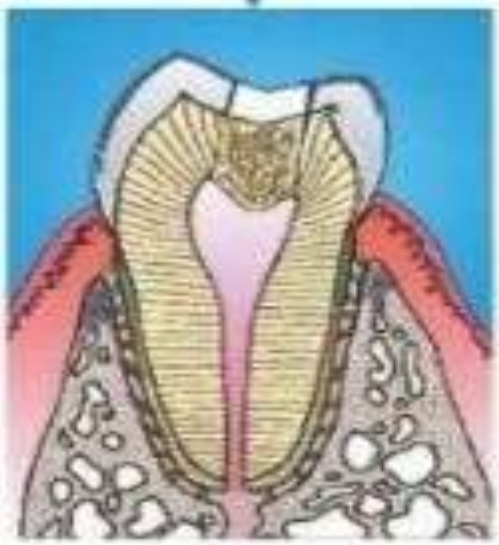


Scaffold

Collagen
Fibronectin
Fibrin
Hyaluronic acid
Proteoglycan
Foams, fibers
Gels and membranes

Cells

Adult
Embryonic
Marrow stroma
PDL stem
Dental pulp stem

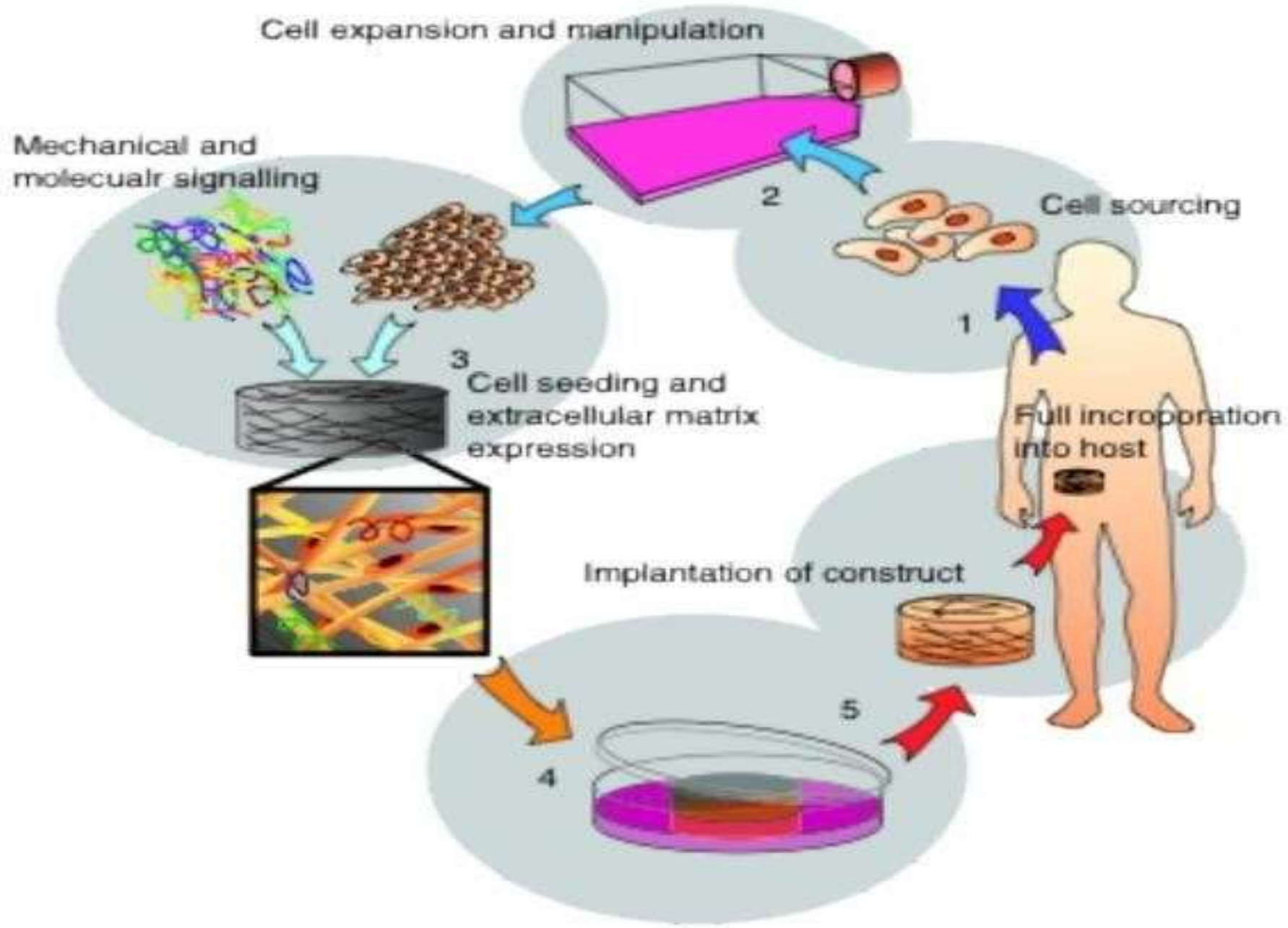


Regeneration

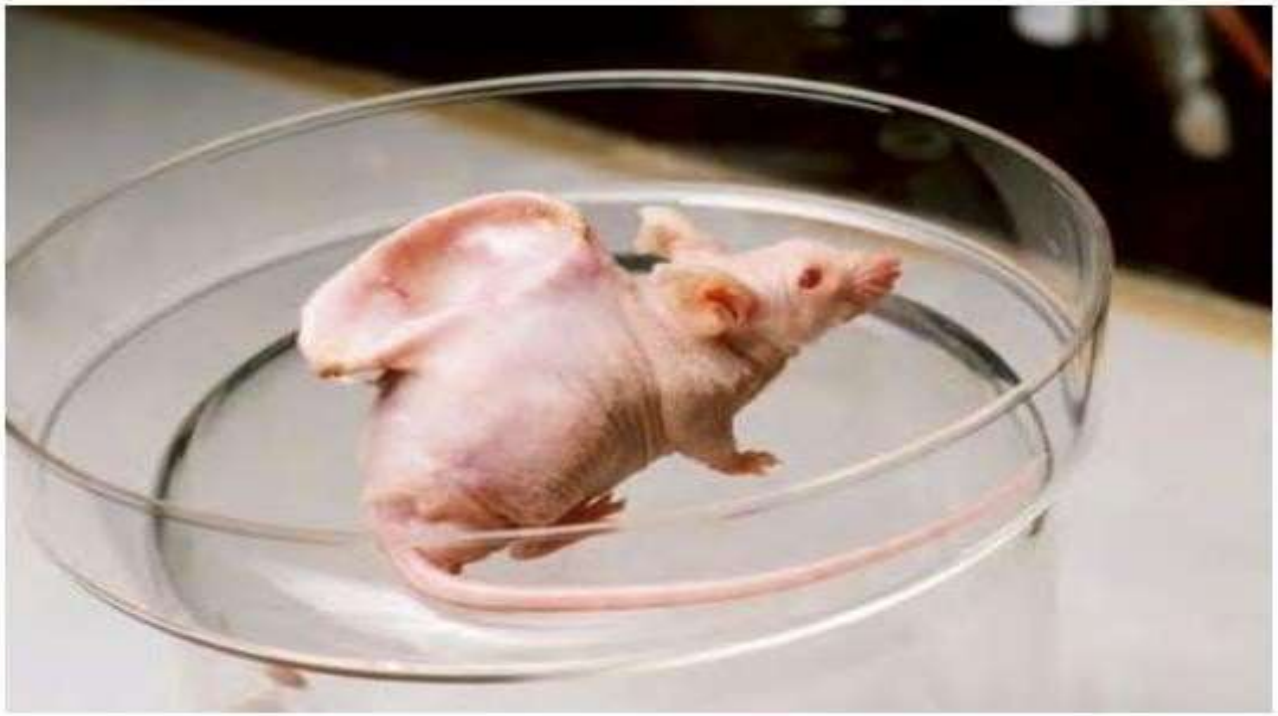
Alveolar bone
Periodontal ligament
Cementum
Dentin
Dental pulp
Enamel

Signals

TGFβ/BMPs
FGFs

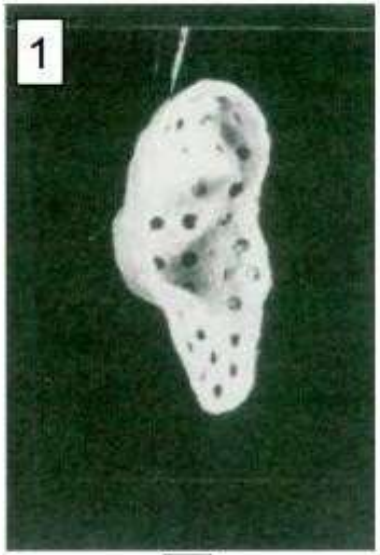

Cell expansion and manipulation
Mechanical and
molecualr signalling
2
Cell sourcing
1
3
Cell seeding and
extracellular matrix
expression
Full incropporation
into host
Implantation of construct
5
4

Classic Tissue Engineering: The Vacanti Mouse



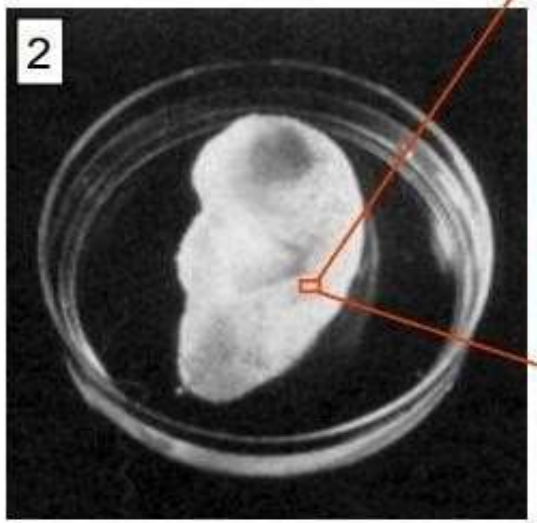
landmark study from 1997 that helped launched the field

Classic Tissue Engineering: The Vacanti Mouse



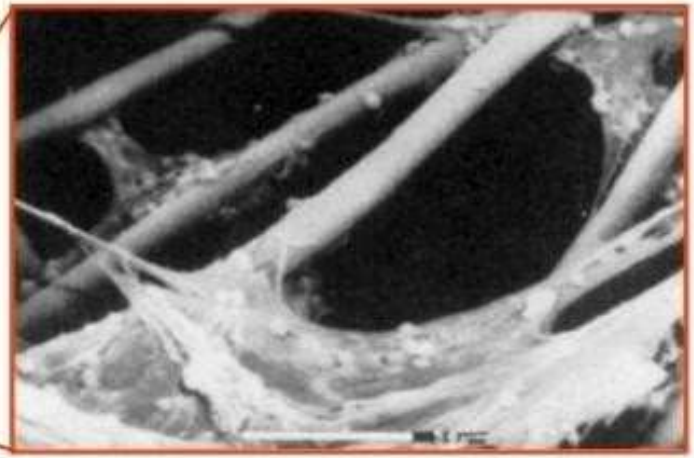
1

scaffold made from poly(glycolic acid) (PGA) and poly(lactic acid) (PLA) cast from plaster replica of an actual ear



2

scaffold seeded with chondrocytes and cultured for 1 week



SEM micrograph showing cells and ECM on scaffold

Classic Tissue Engineering: The Vacanti Mouse

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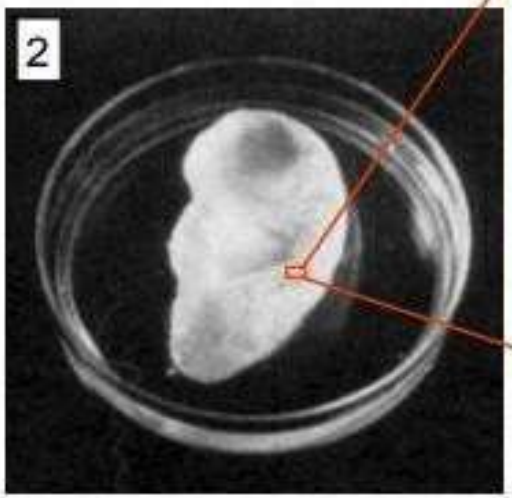
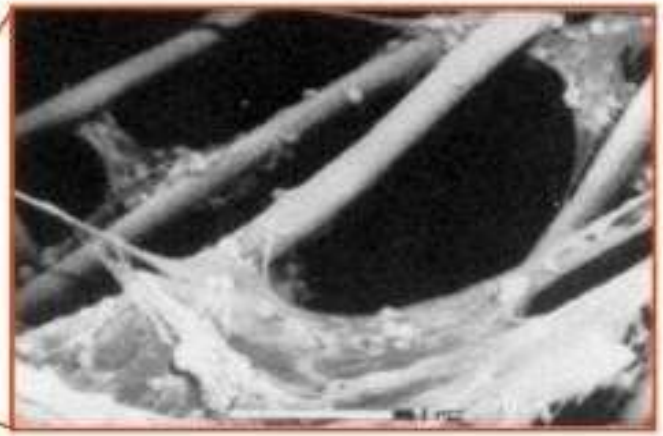


2

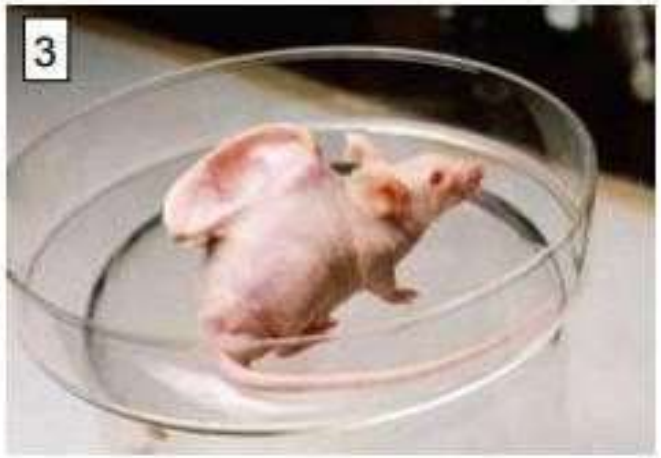
scaffold seeded with chondrocytes and cultured for 1 week



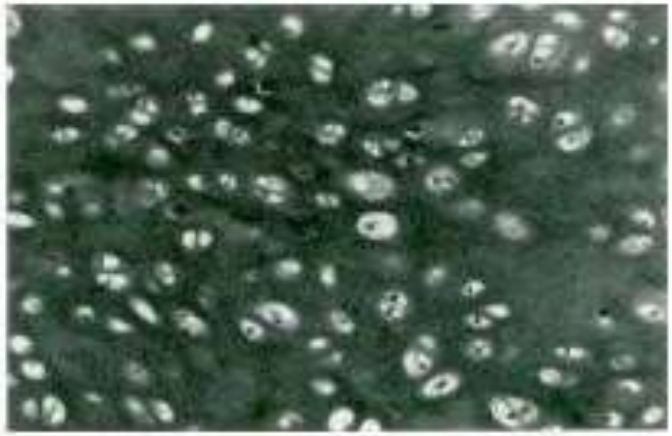
SEM micrograph showing cells and ECM on scaffold

3

implanted subcutaneously on the back of a mouse



The Vacanti Mouse set the tone for TE field



histology of construct at 6 weeks

- Extensive cartilage formation
- Anatomical shape could be maintained (with external stenting)

- conventional therapies for tissue loss:
  - non-biological implants
  - whole organ transplants
    - demand = 3x supply
  - autografts
    - expensive
    - donor site morbidity
  - xenografts/allografts
    - immunorejection
    - infectious diseases (e.g., HIV, hepatitis)
    - ethical?

➠ insufficient tissue available

- cells as therapeutic agents:
  - alternative to drugs/surgery/artificial implants
  - produce new cells/tissue
  - e.g., blood transfusion; bone marrow transplant, hematopoietic stem cell transplants

➠ Tissue Engineered Medical Products (TEMPs)

- Tissue Engineered Medical Products (TEMPs)
  - hybrid products of biological components with or without non-biological components **or**
  - products that induce a specific tissue response **or**
  - biological cells that have been significantly manipulated *in vitro*

➠ treat/replace critical tissue functions (i.e., not necessarily whole organ function)

A number of criteria must be satisfied in order to achieve effective, long-lasting repair of damaged tissues

- ✓An adequate number of cells must be produced to fill the defect
- ✓Cells must be able to diffentiate intodesired phenotypes
- ✓Cels must adopt appropriate 3-D structural support/scaffold and produce ECM

A number of criteria must be satisfied in order to achieve effective, long-lasting repair of damaged tissues

- ✓Produced cells must be structurally and mechanically complaint with native cells
- ✓Cels must successfully integrate with native cells and overcome the risk of immunologic rejection
- ✓There should be minimal associated biological risks

Types of cells

Cells are often categorized by their source:

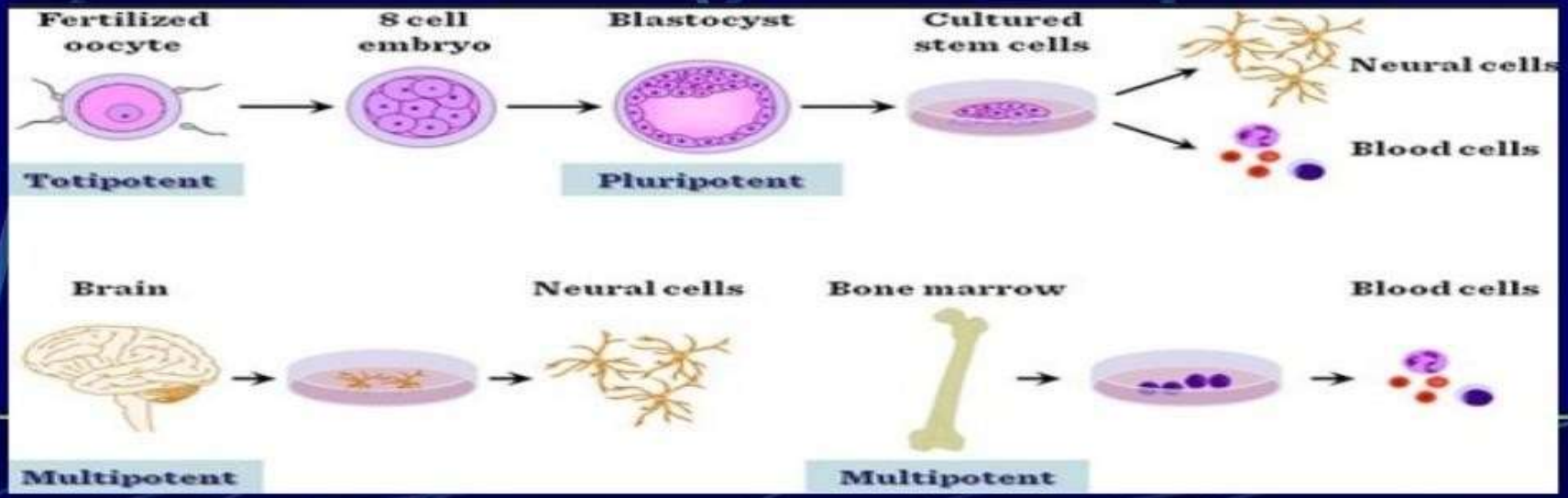
- **Autologous cells** are obtained from the same individual to which they will be reimplanted. Autologous cells have the fewest problems with rejection and pathogen transmission, however in some cases might not be available.
- **Allogeneic cells** come from the body of a donor of the same species. While there are some ethical constraints to the use of human cells for *in vitro* studies, the employment of dermal fibroblasts from human foreskin has been demonstrated to be immunologically safe and thus a viable choice for tissue engineering of skin.
- **Xenogenic cells** are these isolated from individuals of another species. In particular animal cells have been used quite extensively in experiments aimed at the construction of cardiovascular implants.

➢ **Isogenic cells** are isolated from genetically identical organisms, such as twins, clones, or highly inbred research animal models.

•*Primary* cells are from an organism.

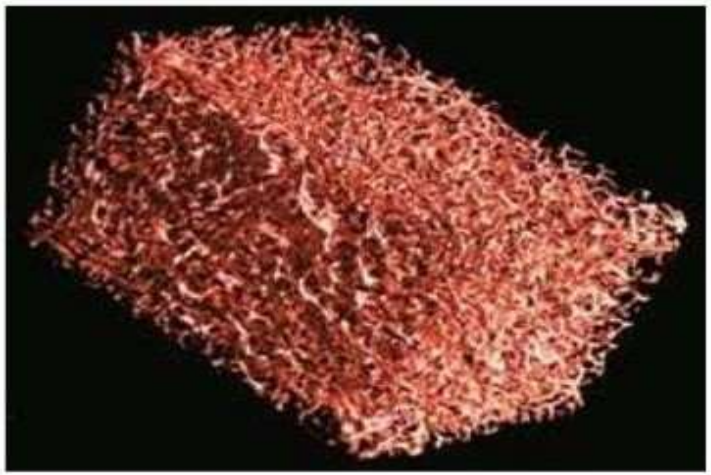
• *Secondary* cells are from a cell bank.

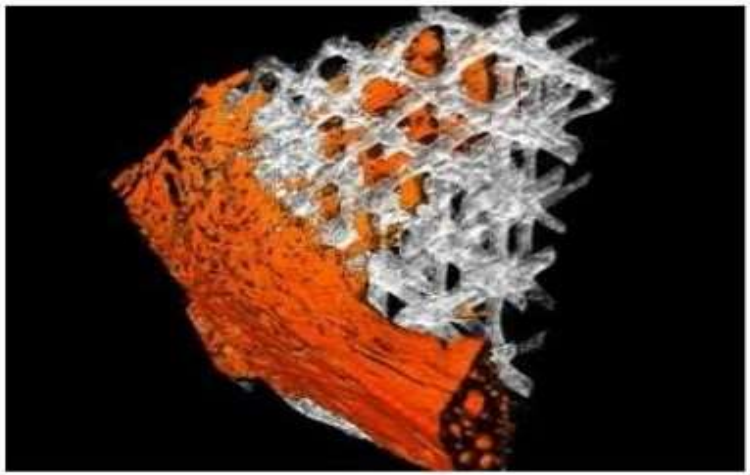
➢ **Stem cells** are undifferentiated cells with the ability to divide in culture and give rise to different forms of specialized cells. According to their source stem cells are divided multipotent, pluripotent& totipotent.



What are Scaffolds?

- Cells are often implanted or 'seeded' into an artificial structure capable of supporting 3-D tissue formation called **Scaffolds.**
- Scaffolds act as substrate for cellular growth, proliferation, and support for new tissue formation.





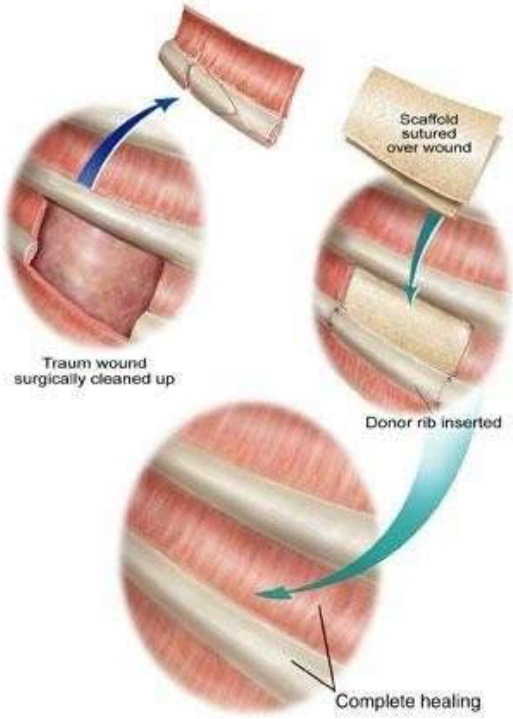
Scaffolds

•Allow cell attachment and migration

•Deliver and retain cells and biochemical factors

•Enable diffusion of vital cell nutrients

•Exert certain mechanical and biological influences to modify cell behaviour



Goals/Properties

Goal of TE is to combine cell, scaffold (artificial ECM) and bioreactor to design and fabricate tissues and organs.

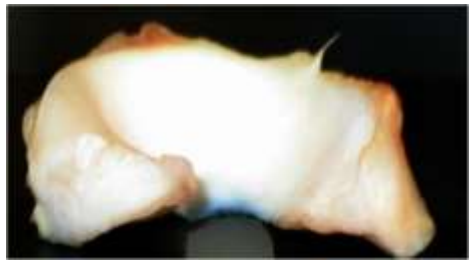
Design scaffold with maximum control over:
biocompatibility (chemical)
biodegradability (mechanical)

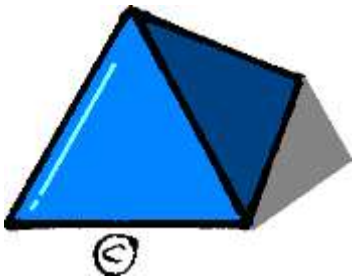
Scaffolds Properties

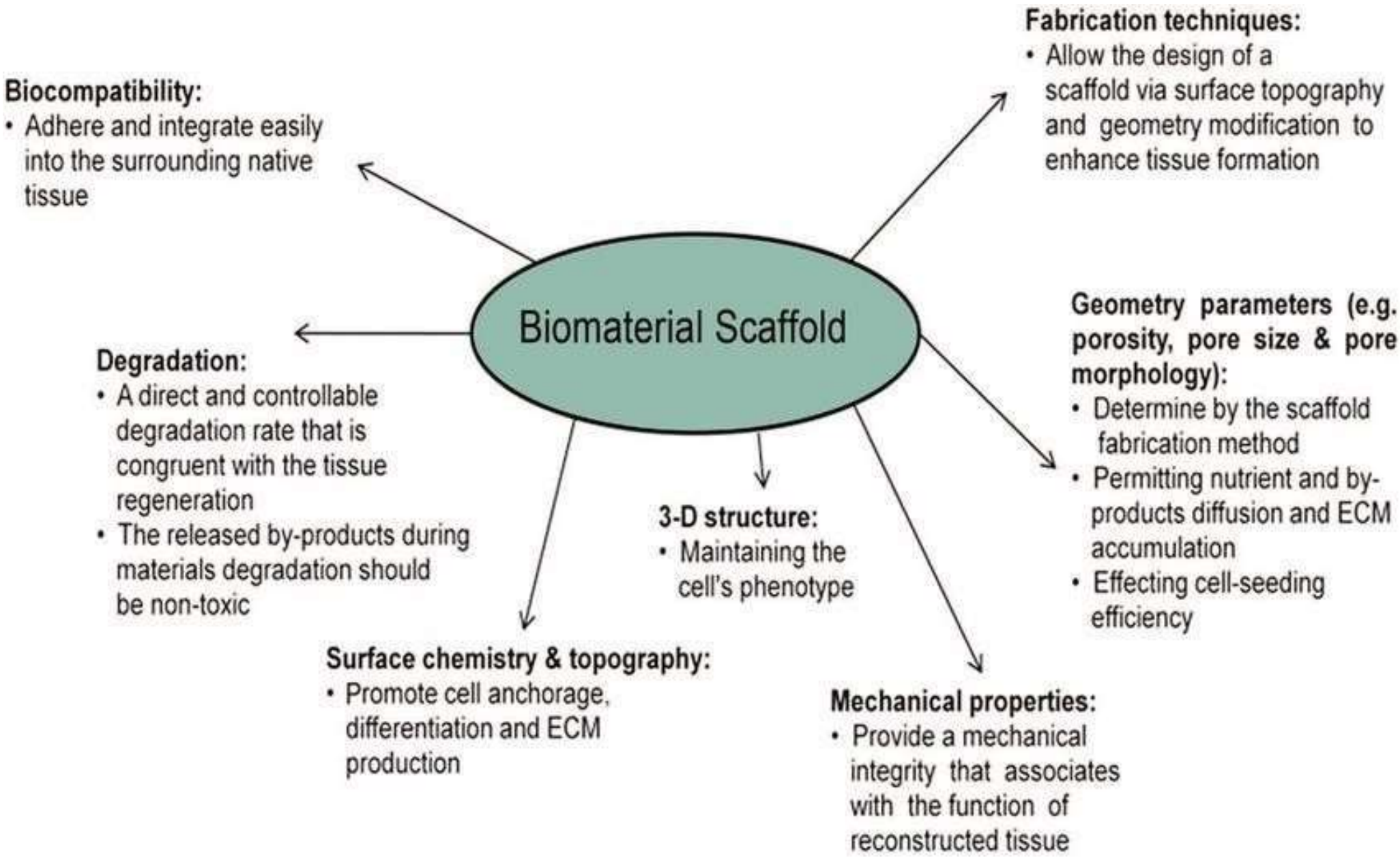
- Biocompatible
- Promote growth
- Maintain 3-D structure
- Non-immunogenic
- Support tissue and cell forces

What do we want in a scaffold?

- Biocompatiblity
- Biodegradablity
- Chemical and Mechanical Properties
- Proper architecture
- Non-Toxicity
- Porosity






Biomaterial Scaffold
Biocompatibility:
• Adhere and integrate easily into the surrounding native tissue
Fabrication techniques:
• Allow the design of a scaffold via surface topography and geometry modification to enhance tissue formation
Degradation:
• A direct and controllable degradation rate that is congruent with the tissue regeneration
• The released by-products during materials degradation should be non-toxic
Geometry parameters (e.g. porosity, pore size & pore morphology):
• Determine by the scaffold fabrication method
• Permitting nutrient and by-products diffusion and ECM accumulation
• Effecting cell-seeding efficiency
3-D structure:
• Maintaining the cell's phenotype
Surface chemistry & topography:
• Promote cell anchorage, differentiation and ECM production
Mechanical properties:
• Provide a mechanical integrity that associates with the function of reconstructed tissue

Biocompability

- The first criterion of any scaffold for tissue engineering is that it must be biocompatible, cells must

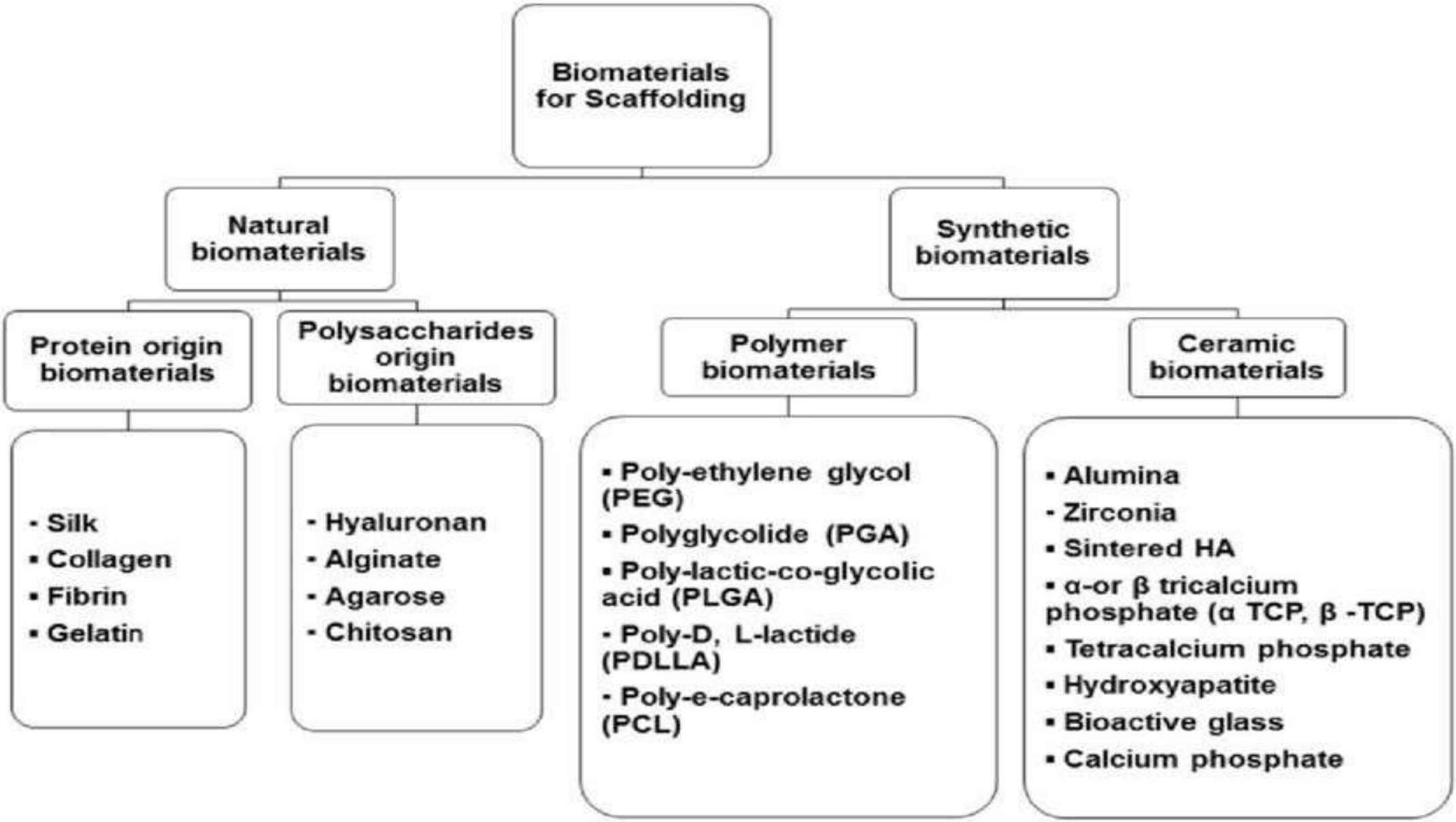
1. adhere,
2. function normally,
3. migrate onto the surface and eventually through the scaffold
4. begin to proliferate before laying down new matrix

- After implantation, the scaffold or tissue engineered construct elicit negligible immune reaction in order to prevent it causing such a severe inflammatory that it might reduce healing or cause rejection

Biodegradability

- The objective of tissue engineering is to allow body's own cells, overtime, to eventually replace the implanted scaffold tissue engineered construct.
- Scaffolds and constructs are not intended as permanent implants. The scaffold must then be biodegradable so as to allow cells to produce their own extracellular matrix

- The by-products of this degradation should also be non-toxic and able to exit body without interference with other organs.
- In order to allow degradation to occur in tandem with tissue formation, an inflammatory response combined with controlled infussion of cells such as macrophages is required


Biomaterials for Scaffolding
Natural biomaterials
Synthetic biomaterials
Protein origin biomaterials
Polysaccharides origin biomaterials
Polymer biomaterials
Ceramic biomaterials
- Silk
- Collagen
- Fibrin
- Gelatin
- Hyaluronan
- Alginate
- Agarose
- Chitosan
- Poly-ethylene glycol (PEG)
- Polyglycolide (PGA)
- Poly-lactic-co-glycolic acid (PLGA)
- Poly-D, L-lactide (PDLLA)
- Poly-e-caprolactone (PCL)
- Alumina
- Zirconia
- Sintered HA
- α-or β tricalcium phosphate (α TCP, β -TCP)
- Tetracalcium phosphate
- Hydroxyapatite
- Bioactive glass
- Calcium phosphate

Origin	Properties	Polymers	Applications
Natural polymers	Biocompatible, biodegradable, good in cell adhesion and proliferation properties Poor mechanical strength	Collagen, Hyaluronic acid, chitosan, gelatin, fibrin, silk and alginic acid etc.	Skin , cartilage, vessels, heart etc. tissue scaffold, Drug Delivery etc.
Synthetic polymers	Biocompatible Lack of cell recognition sites High mechanical strength	Poly(vinyl alcohol) (PVA), poly(lactic acid) (PLA), poly(ethylene-oxide) (PEO) and poly(caprolactone) (PCL) etc.	Skin, cartilage, tendon, bladder , liver tissue scaffold, Drug Delivery etc.
Composite	Biocompatible and biodegradable Good in cell adhesion and proliferation properties High mechanical strength	PCL/gelatin, PCL/chitosan PCL/gelatin/chitosan, Collagen/chitosan and Poly(lactic acid)/ tricalcium phosphate composite etc.	Cartilage, skin, nerve, bone, blood vessels tissue scaffold and drug delivery etc.
Ceramics	Bioinert, brittle and bioresorable, High resistance to wear Low toughness	Hydroxyapatite, Tricalcium phosphate (TCP) and Calcium metaphosphate etc.	Low- weight-bearing bone implants, Bone drug delivery, dental restoration etc.
Metals	Dense, too strong, Ductile, may corrode	Stainless steel Titanium Alumina etc.	Dental restoration, Load bearing bone implants etc.
Decellulai-zed matrix	Simple and economic for scaffolding Retains the original architecture of tissue which influences the more significantly cellular behavior	Collagen and elastin etc. from Cadaver Tissues	Urinary bladder, heart valves, nerves, liver tendon and ligament tissue etc.

Materials

- Synthetic
  - Mimic mechanical properties (strength, elongation and knot retention)
  - Mass production
  - Degradation between hosts is minimal
- Natural
  - Cell recognition
  - Biodegradable
  - Difficult degradation control between host

Why Nanotechnology?

- Biological components, such as DNA, involve nano-dimensionality, hence it has logically given rise to the interest in using nanomaterials for tissue engineering.
- Enables the development of new systems that mimic the complex, hierarchical structure of the native tissue.
- Nanomaterials have inherent high surface area-volume ratio
- Available polymeric porous scaffolds revealed insufficient stiffness and compressive strength

Ceramics

- Although not generally used for soft tissue regeneration, there has been widespread use of ceramic scaffolds such as hydroxyapatite (HA) and tri-calcium phosphate (TCP), for bone regeneration applications.
- Ceramic scaffolds are typically characterized by high mechanically stiffness, very low elasticity and a hard brittle surface.

Ceramics

- From a bone perspective, they exhibit excellent biocompability due to their chemical and structural similarity to the mineral phase of native bone
- The interactions of osteogenic cells with ceramics are important for bone regeneration as ceramics are known to enhance osteoblast differantiation and proliferation

Natural polymers vs. Synthetic Polymers

	Advantages	Disadvantages
Naturally derived polymers	• Biodegradable • Possess known cell-binding sites that support cell attachment and proliferation • Do not lead to immunogenic response • Do not involve the use of harsh chemicals during processing	• Poor mechanical strength • High speed of degradation • Limited ability to tailor for specific properties • Lack of control over the pore size and mechanical properties of the scaffold • Exist in finite supply • Expensive
Synthetic polymers	• Easily formed into desired scaffold architectures with relatively good mechanical strength • Controllable degradability by manipulating the crystallinity, molecular weight, and copolymer ratio • Exist in adequate supply	• Difficulty in 3-D fabrication (specifically, 3-D printing) • Uncontrollable shrinkage • Questionable cell–polymer interactions (lack cell recognition signals) • Possible local toxicity resulting from acidic degradation products

Synthetic Polymers

- Polyglycolic acid (PGA)
  - Highly crystalline, hydrophilic, byproduct is glycolic acid
- Polylactic acid (PLA)
  - Hydrophobic, lower melting temperature, byproduct is lactic acid
- Polydioxanone (PDO)
  - Highly crystalline
- Polycaprolactone (PCL)
  - Semi-crystalline properties, easily co-polymerized, byproduct caproic acid
- Blends
  - PGA-PLA
  - PGA-PCL
  - PLA-PCL
  - PDO-PCL

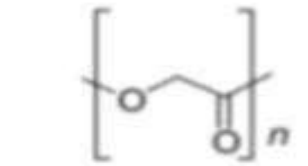
Poly(glycolic acid) (PGA)

Advantages:

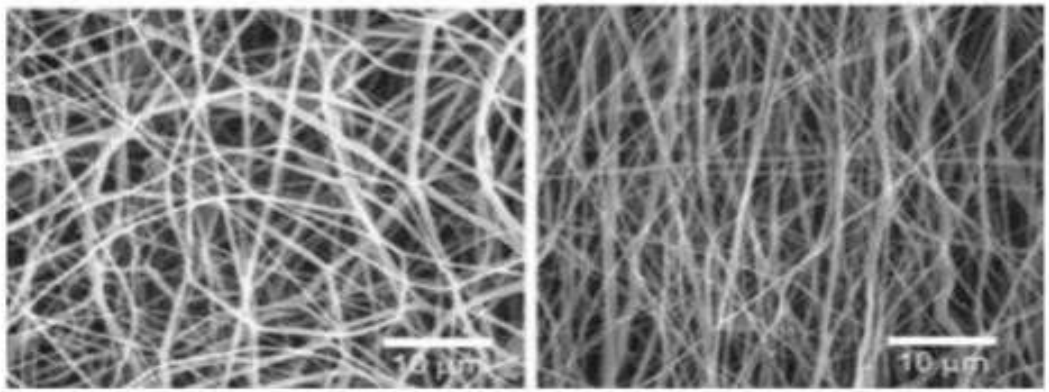
- Biocompatible & biodegradable
- bioabsorption (2-4 wks)
- electrospinning yields diameters ~ 200 nm
- Good choice for high strength and elasticity and fast degrading material

Disadvantages:

- fast degradation causes pH change
- Tissue may require buffering capacity



Poly(glycolic acid)



SEM showing the random fiber arrangement (left) and the aligned fiber orientation (right) (1600× magnification).

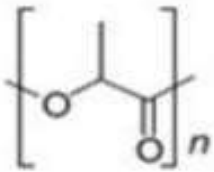
Poly(lactic acid) (PLA)

Advantages:

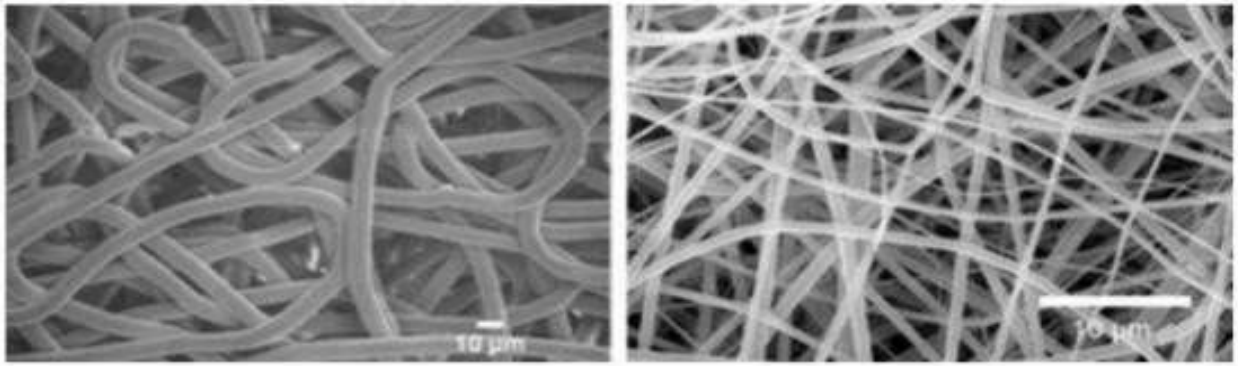
- Biocompatible & biodegradable
- bioabsorption (30 wks)
- Good choice for drug delivery do to predictable degradation

Disadvantages:

- Larger diameter fibers ~ microscale



Poly(L-lactic acid)



SEM showing the random oriented PLA from cholorform (left) and the randomly oriented PLA from HFP (right)

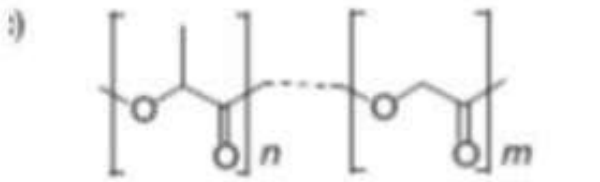
PGA + PLA blends (PLGA)

Group Tested copolymers of following ratios:

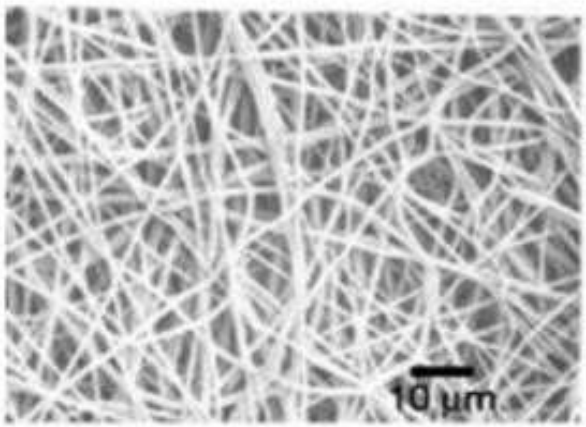
- 75%PLA–25%PGA
- 50%PLA–50%PGA,
- blended PLA and PGA together in HFP at ratios of 100:0, 75:25, 50:50, 25:75

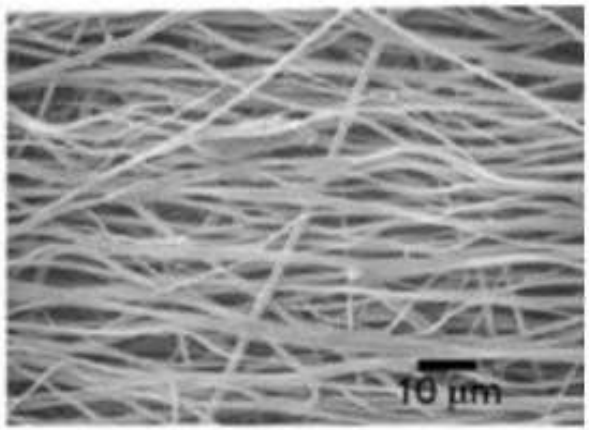
Group found:

- Hydrophilicity proportional to composition of copolymer
- Degradation rate proportional to composition of copolymer



Poly(DL-lactic acid-co-glycolic acid)

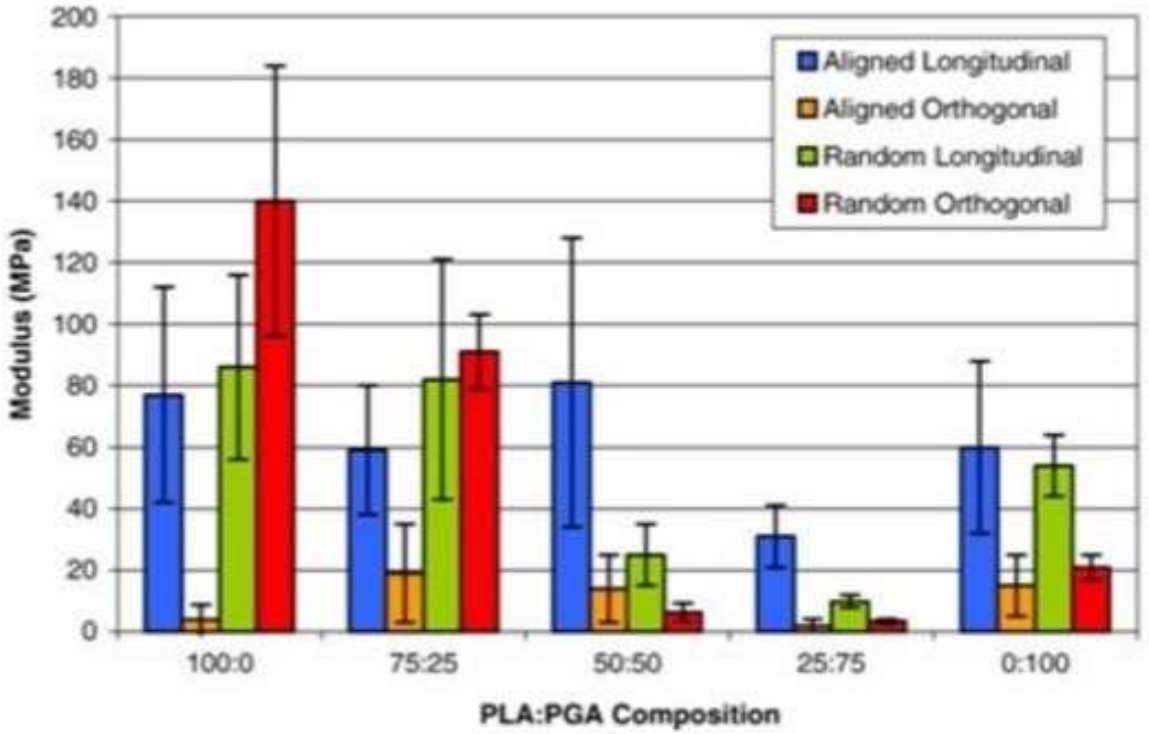




PLGA

Applications of PLGA:

- cardiac tissue in mice for tissue regeneration
- individual cardiomyocytes attachment at seeding
- scaffold loaded with antibiotics for wound healing



mechanical properties, such as tangential modulus, peak stress, and strain to failure, of these copolymers and blends appear to be controlled by the fiber/polymer composition

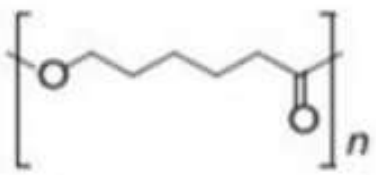
Polycaprolactone (PCL)

Advantages:

- Biocompatible & biodegradable
- Inexpensive
- Highly elastic
- Slow degradation (1 – 2 yrs)
- Good choice for Human mesenchymal stem cells (hMSCs) seeding to induce differentiation

Disadvantages:

- No shape retention (highly elastic)



Poly(caprolactone)

Applications:

- Bone tissue strengthening
- Cardiac grafts
- Collagen and cellular interaction
- Differentiation with MSC cells

PGA + PCL blend

Advantages:

- PGA – high stress tolerance
- PCL – highly elastic
- Optimum combination PCL/PGA ~ 1/3
- Longer degradation time ~ 3 months
  (PCL-2 yrs, PGA 2-4 wks)

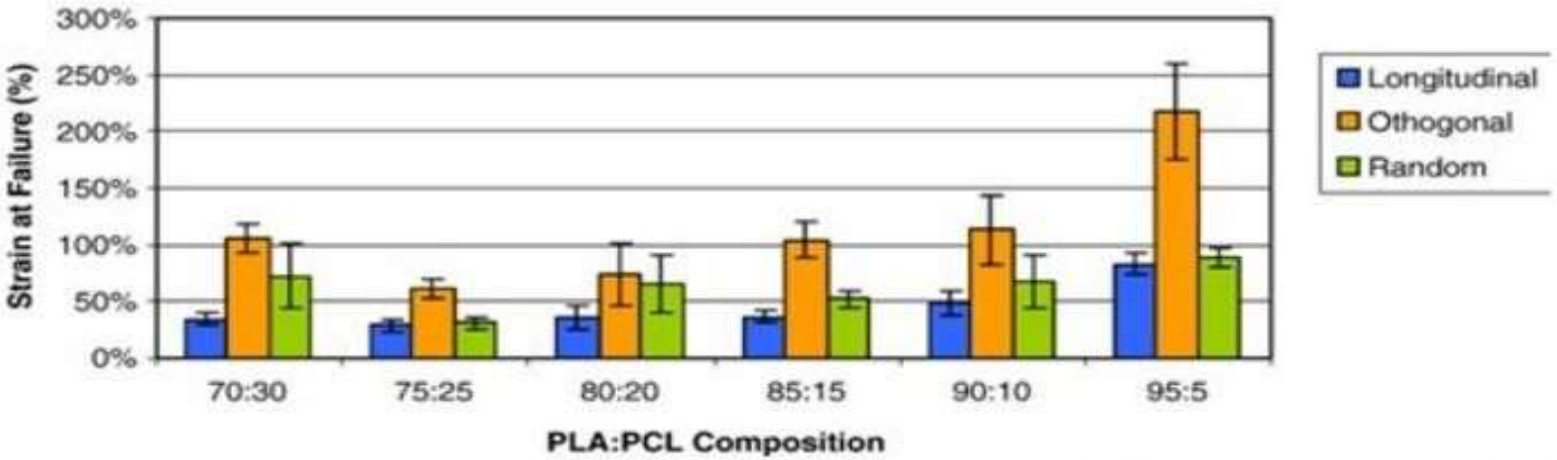
PLA + PCL blends

Advantages:

- Greater elasticity than PGA+PCL
- Similar tensile strength to PLA
- ~5% addition of PCL increased strain by 8 fold
- Overall best synthetic ECM for cardiac applications

Disadvantages:

- Decreasing PLA+PCL ratios decreases strain capacity, optimized at 95:5



Strain to failure of a tissue matrix a function of both composition (varying blend ratios of PLA and PCL) and fiber alignment

Natural

- Elastin
- Gelatin collagen
- Fibrillar collagen
- Collagen blends
- Fibrinogen

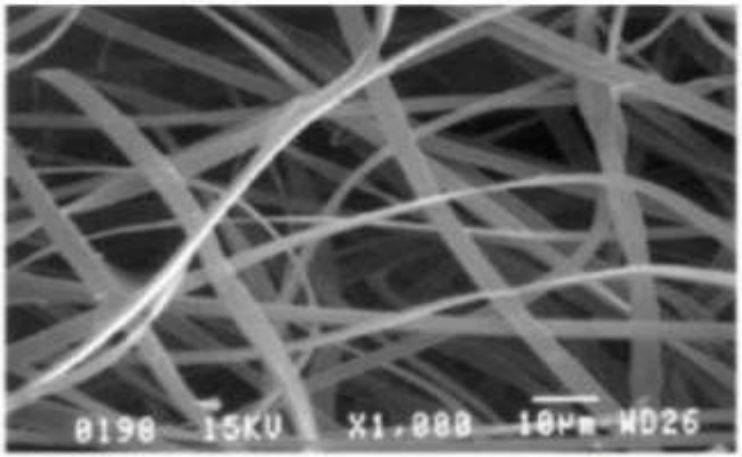
Elastin

Advantages:

- Linearly elastic biosolid
- Insoluble and hydrophobic
- Critical role in shape and energy recovery for organs

Disadvantages:

- Less elastic than native elastin
- Needs to be combined with PDO to increase tensile strength
- Fiber ~300 nm (not as small as PDO ~ 180 nm)
- Varying diameter



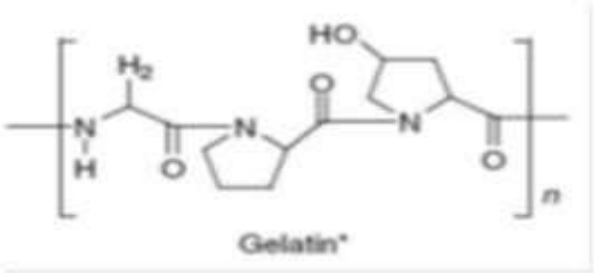
SEM of electrospun elastin scaffold at 250 mg/ml.

Collagen

Gelatin (denatured collagen)

Advantages:

- Biocamptibale and biodegradable
- Inexpensive



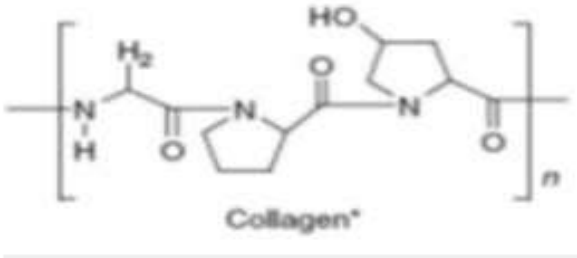
Gelatin*

Disadvantages:

- Quick to dissolve

Fibril-forming (Types I, II, III)

- Most abundant natural polymers in body
- Important role in ECM
- Type I: principal structure in ECM
- Type II: pore size and fiber diameter easily controlled
- Type III: still under investigation



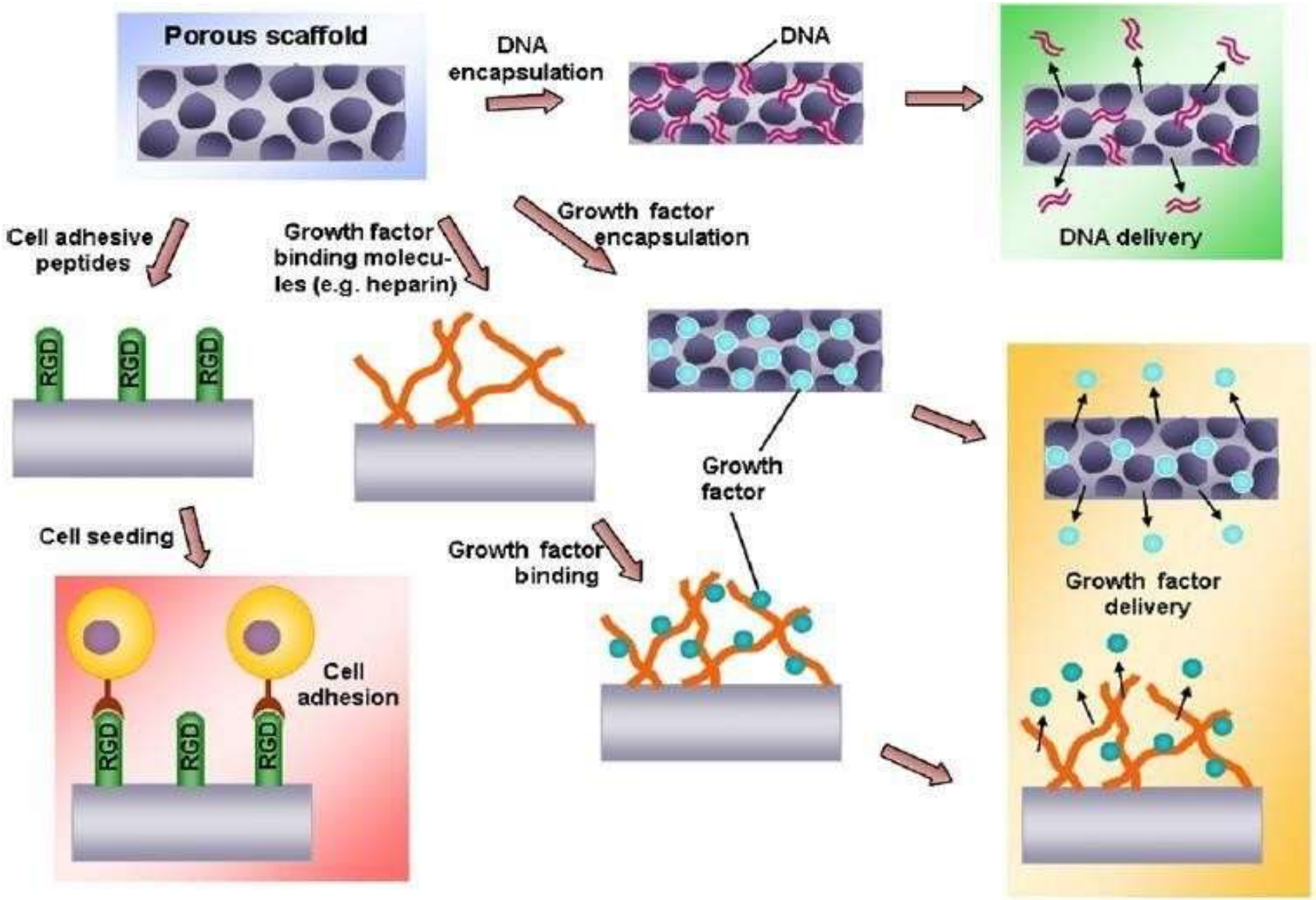
Collagen*

Metallic scaffolds

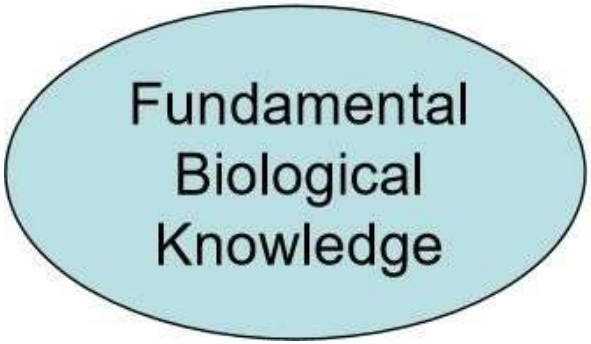
- **The main disadvantage** of metallic biomaterials is their **lack of biological recognition** on the material surface.
- To overcome this restraint, **surface coating or surface modification** presents a way to preserve the mechanical properties of established biocompatible metals improving the surface biocompatibility.
- **Another limitation of** the current metallic biomaterials is the possible release of **toxic metallic ions and/or particles through corrosion or wear** that lead to inflammatory cascades and allergic reactions, which reduce the biocompatibility and cause tissue loss.

Growth factor	Tissues	Effects
Bone morphogenetic protein (BMP) 2 and 7	Bone, cartilage	Osteoblast differentiation and migration Accelerated bone healing
Fibroblast growth factors 1, 2, and 18	Bone, muscle, blood vessel	Endothelial cell migration, proliferation, and survival Increased osteogenic differentiation of mesenchymal stromal cells
Insulin-like growth factor-1	Bone, cartilage, muscle	Osteoprogenitor cell proliferation and differentiation
Platelet-derived growth factor (PDGF)-AA and PDGF-BB	Bone, cartilage, blood vessel, muscle	Endothelial cell proliferation, migration, and growth Osteoblast replication *in vitro* Type 1 collagen synthesis
Parathyroid hormone and parathyroid hormone-related protein	Bone	Intermittent dosage → stimulation of osteoblasts → increased bone formation Continuous administration → bone resorption
Transforming growth factor-β3	Bone, cartilage	Bone-forming cell proliferation and differentiation Enhancement of *in vivo* hyaline cartilage formation Antiproliferative effect on epithelial cells
Vascular endothelial growth factor	Bone, blood vessel	Enhancement of vasculogenesis and angiogenesis (functionality of vasculature is concentration dependent) Reduction or increase in bone formation dependent on concentration when used in combinational with BMP-2 delivery

[a]*Adapted from Tang et al. (2016) and Gothard et al. (2014).*

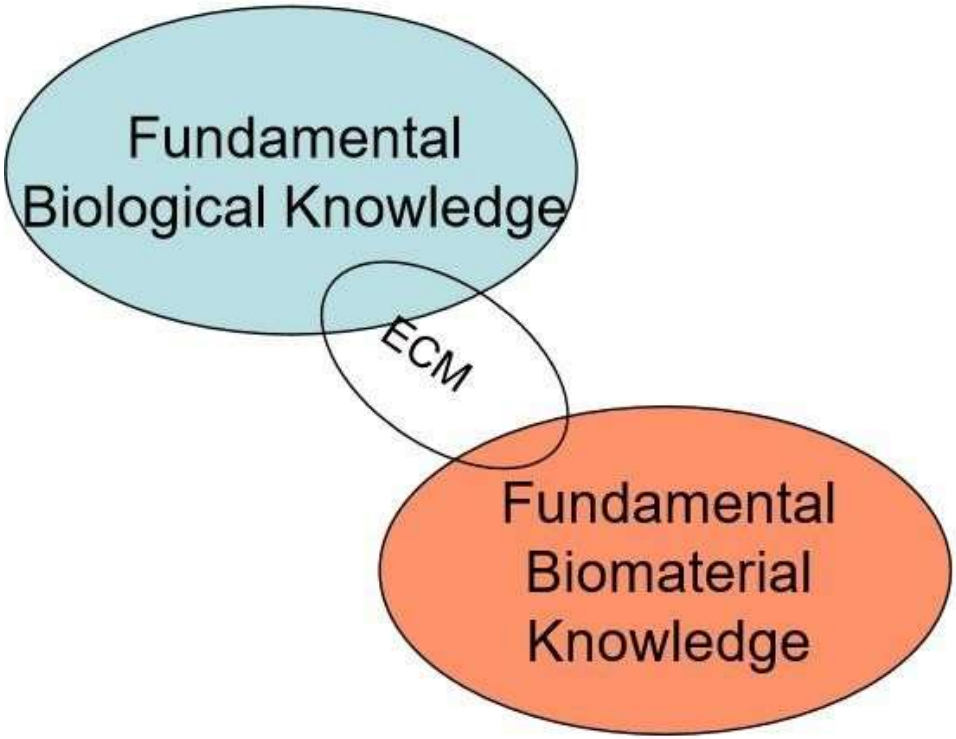

Porous scaffold
DNA encapsulation
DNA
DNA delivery
Cell adhesive peptides
Growth factor binding molecu-les (e.g. heparin)
Growth factor encapsulation
RGD
RGD
RGD
Growth factor
Cell seeding
Growth factor binding
Growth factor delivery
Cell adhesion
RGD
RGD
RGD

- course outline:



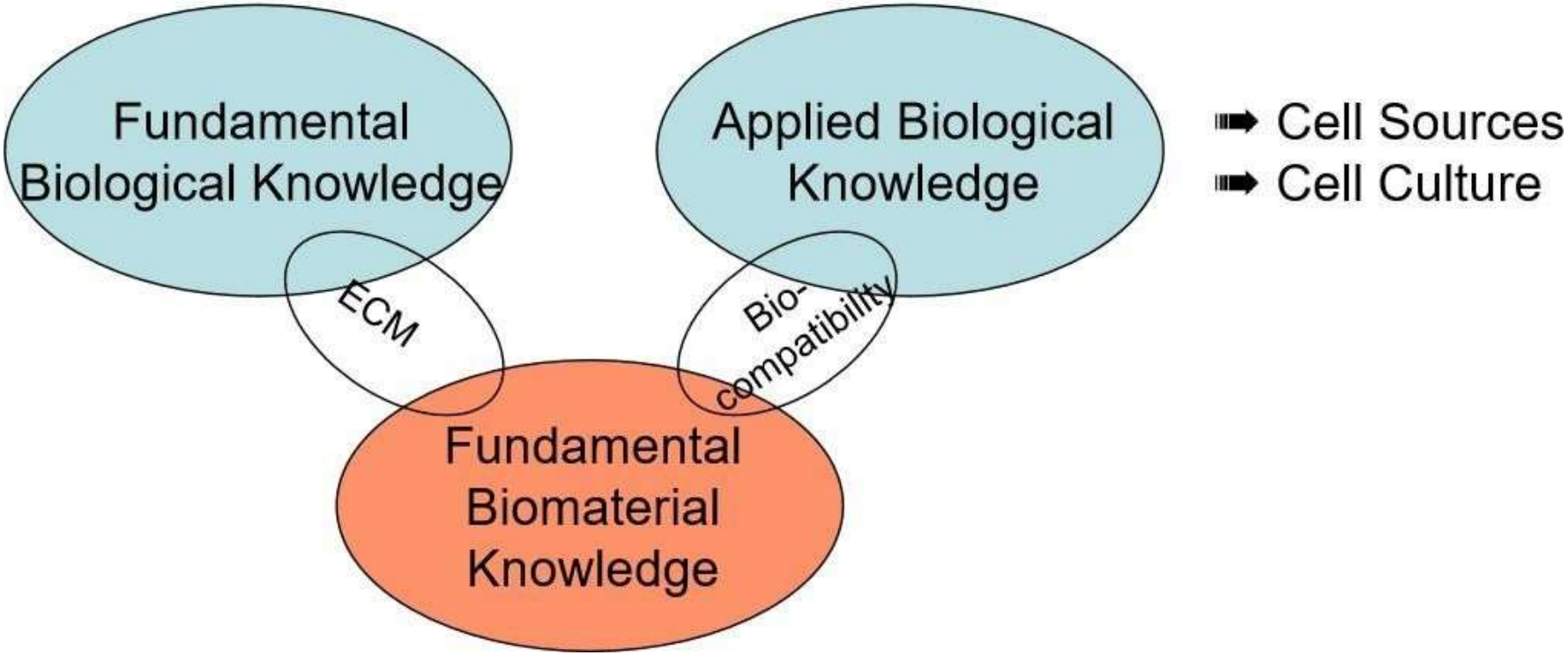
➠ Stem Cells
➠ Morphogenesis & Tissue Homeostasis
➠ Cellular Signalling/Cellular Processes

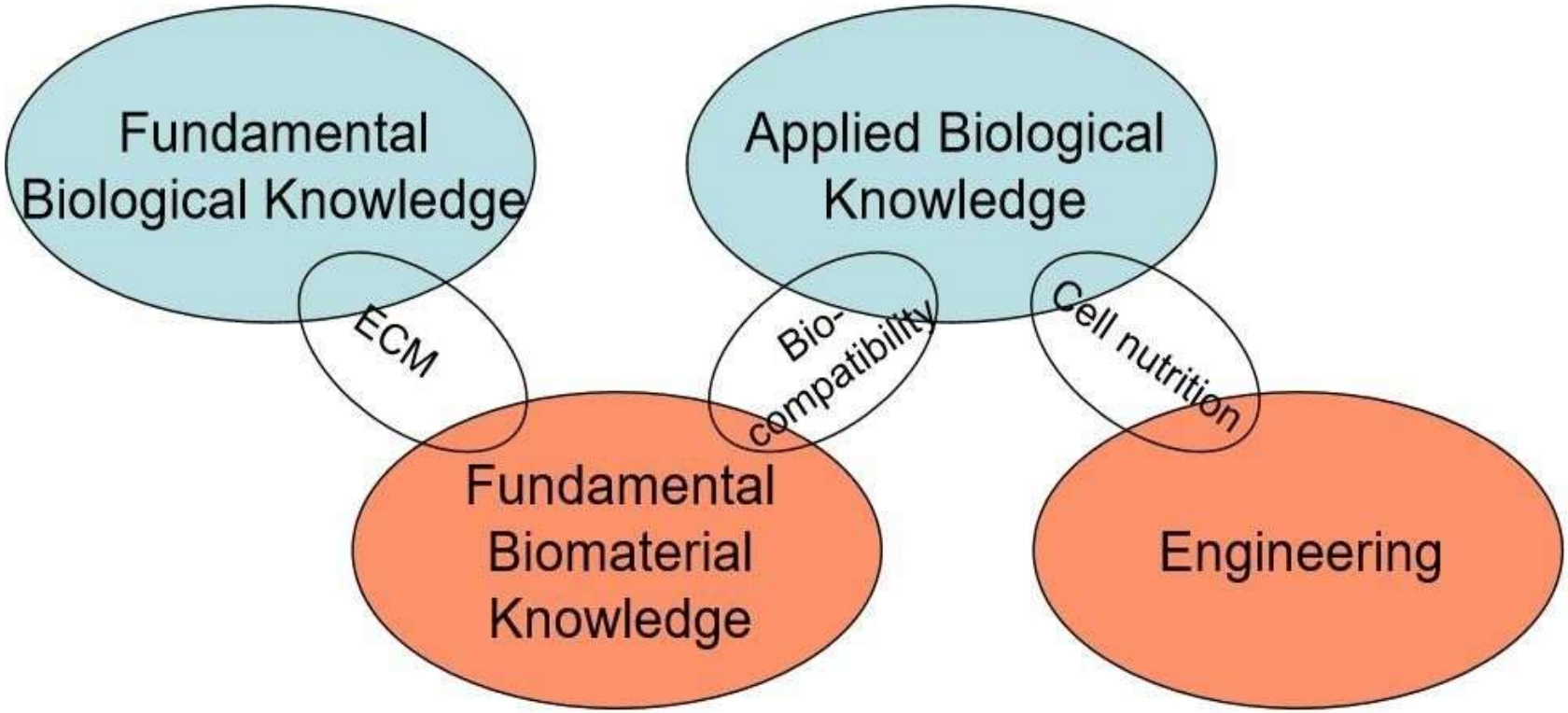
- course outline:



➠ Natural Polymers
➠ Degradable Polymers for TE
➠ Degradation of Bioceramics
➠ Physicochemical Properties of Synthetic Polymers and Matrices

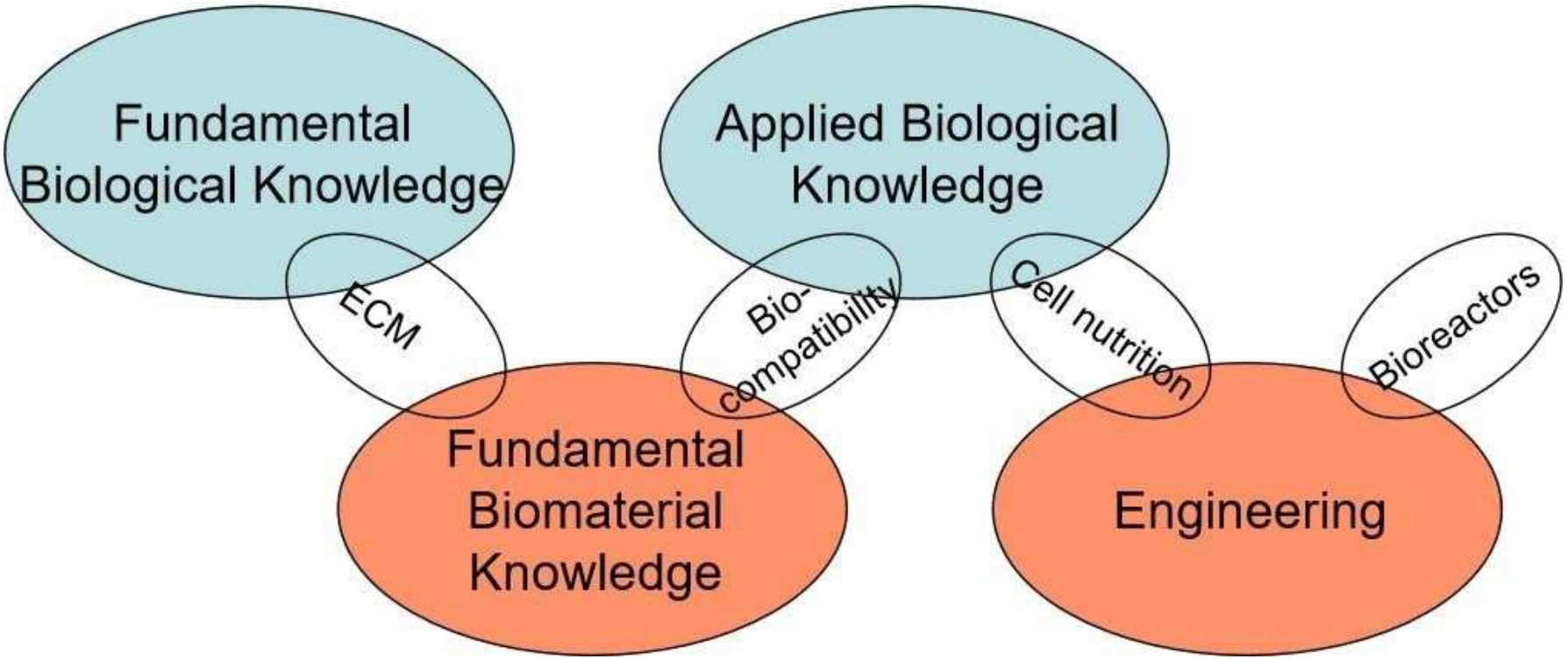
- course outline:





➠ Scaffold Design and Fabrication
➠ Controlled Release Strategies

- course outline:



- course outline: