Introduction in Tissue Engineering

Tissue engineering: is a multidisciplinary field bringing together experts from engineering, life sciences and medicine, utilizing the building blocks of cells, biomaterials and bioreactors for the development of 3-dimensional artificial tissue and organs which can be used to augment, repair and/or replace damaged and/or diseased tissue.

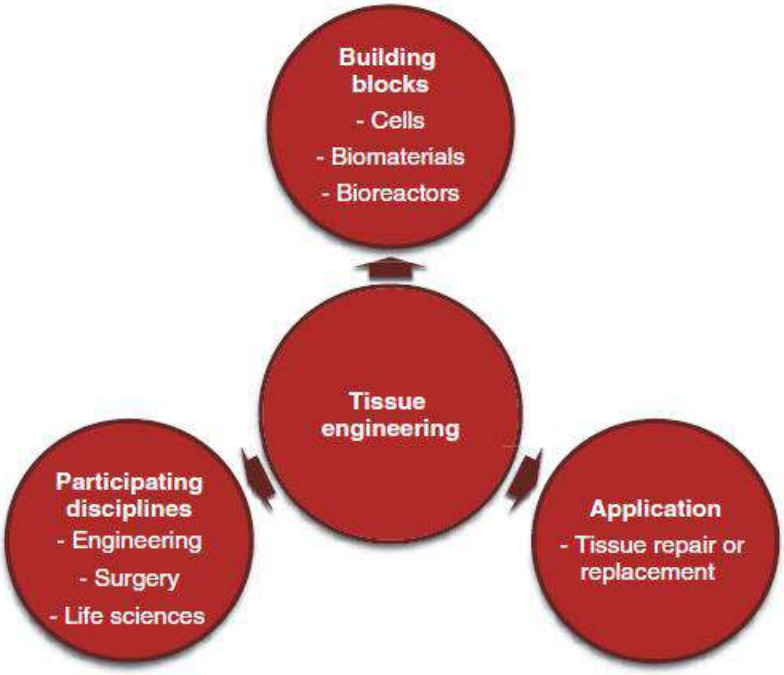
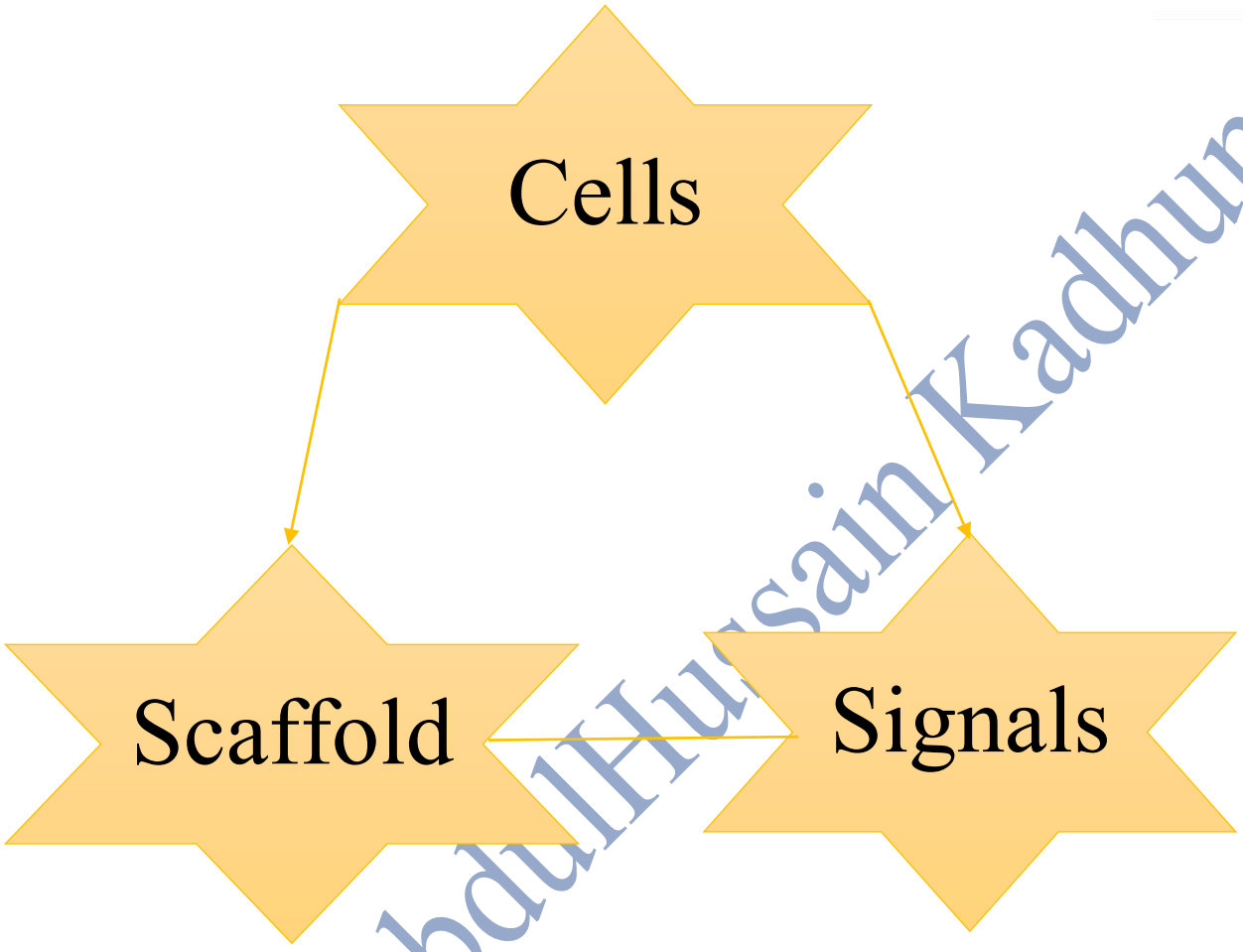


Figure (1): Definition of Tissue Engineering.

Tissue engineering require three things:



- The ***SIGNALS*** refer to molecular signaling molecules, also known as ***GROWTH FACTORS***.

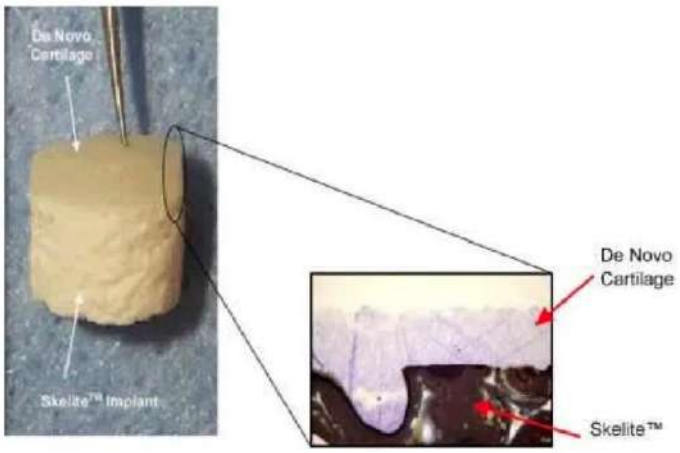


Figure (2): Scaffold.

Process for Tissue Fabrication:

Depending on the tissue system and the specific technology, the sequence of steps may also need to be changed. The eight-step process of bioengineering 3D artificial tissue involves: (Figure 2)

1- Cell sourcing—Cells provide the functional component of artificial tissue. Identification, isolation, purification, expansion, and characterization of a suitable cell source are important steps in cell sourcing.

Cell sources:

- **Primary cells:**

- Tissue biopsy; low cellular yield; potential age-related problems.

- **Passaged cells:**

- Cellular expansion of primary cells (can increase population by 100-1000 X).

- **Stem cells:**

Stem cells naturally exist in some tissues (especially those that rapidly proliferate or remodel)

- Un-differentiated cells.
- Self-renewable capability.
- Can differentiated into functional cell types.
- Ver rare.

There are two predominant lineages of stem cells:

1- Mesenchymal stem cells:

- Give rise to connective tissue (Bone; cartilage; etc.).
- Although found in some tissues; typically isolated from bone marrow.

2- Hematopoietic:

- Give rise to blood cells and lymphocytes.
- Isolated from bone marrow, blood (umbilical cord).

Sources of Cells:

- ***Autologous:*** From the person himself.
- ***Allogenic:*** From a body with the same species.
- ***Xenogenic:*** From a different species.
- ***Syngenic:*** From genetically identical people (Twins).
- ***Primary:*** From any organism.
- ***Secondary:*** From a cell bank.
- ***Stem cells:*** Undifferentiated cells.

Culturing of cells:

There are three types of cell cultures which are:

1- Monolayer (Adherent cells).

2- Suspension (Non-adherent cells).

3- Three dimensional (Scaffold or templates).

Figure (3): Types of Cell Culture.

2- **Biomaterial synthesis**—Biomaterials provide structural support during 3D tissue fabrication and serve the role provided by mammalian extracellular matrix. The choice of biomaterial depends on the specific tissue application; there are many different biomaterials to choose from, including polymers, metals and ceramics.

3- **Genetic manipulation**—Prior to scaffold cellularization, the genetic profile of the cells can be modified to increase the likelihood of cell survival or functional integration with the host. Specific genes can be manipulated to reduce apoptosis or increase the expression of specific integrins to increase cell-matrix interactions. In addition, functional genes can be upregulated, like myosin heavy chain for heart muscle, to increase the functional performance of 3D artificial tissue.

4- **Scaffold cellularization**—Scaffold cellularization refers to the process by which isolated cells are seeded within a 3D scaffold. An important variable during the scaffold cellularization process is coupling isolated cells with the scaffold to promote functional integration at the cell-cell and cell-material interface. The

cellularization strategy needs to be optimized to ensure uniformity in cell distribution throughout the scaffold.

5- **Sensor technology**—Sensors are necessary to monitor the overall health of the artificial tissue during the formation, development, and maturation stage of the tissue fabrication process. Monitoring of cell behavior, cell-cell interaction, cell-matrix interaction, and tissue formation and function is critical during the tissue fabrication process.

6- **Bioreactors for guidance**—During normal physiological function, mammalian tissue is exposed to a wide array of stimuli, which include electromechanical impulses, fluid stresses, and changes in the chemical environment based on changing concentrations of growth factors, hormones, and cytokines. These signals are important in maintaining tissue function.

7- **Vascularization**—Incorporation of blood vessels as an integrated component of the artificial tissue is a critical requirement and is required to support the metabolic activity of 3D artificial tissue.

8- ***In vivo* assessment**—Once functional 3D artificial tissue has been fabricated, the final step in the process is *in vivo* testing. In this case, the effectiveness of the tissue graft to repair, replace, and/or augment the function of damaged or diseased tissue is assessed.

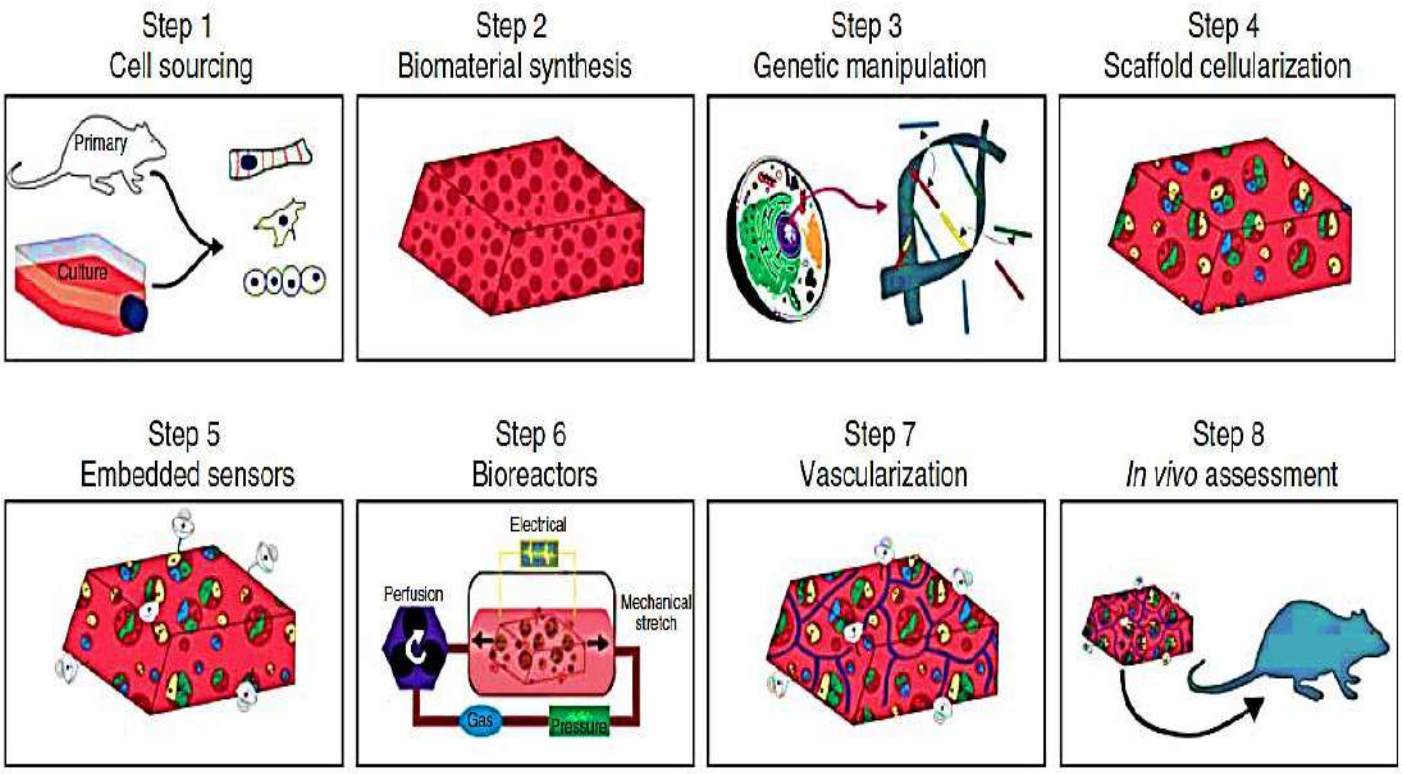


Figure (4): Process of fabricating 3D Artificial tissue and organ.

Scientific Challenges in Tissue Engineering

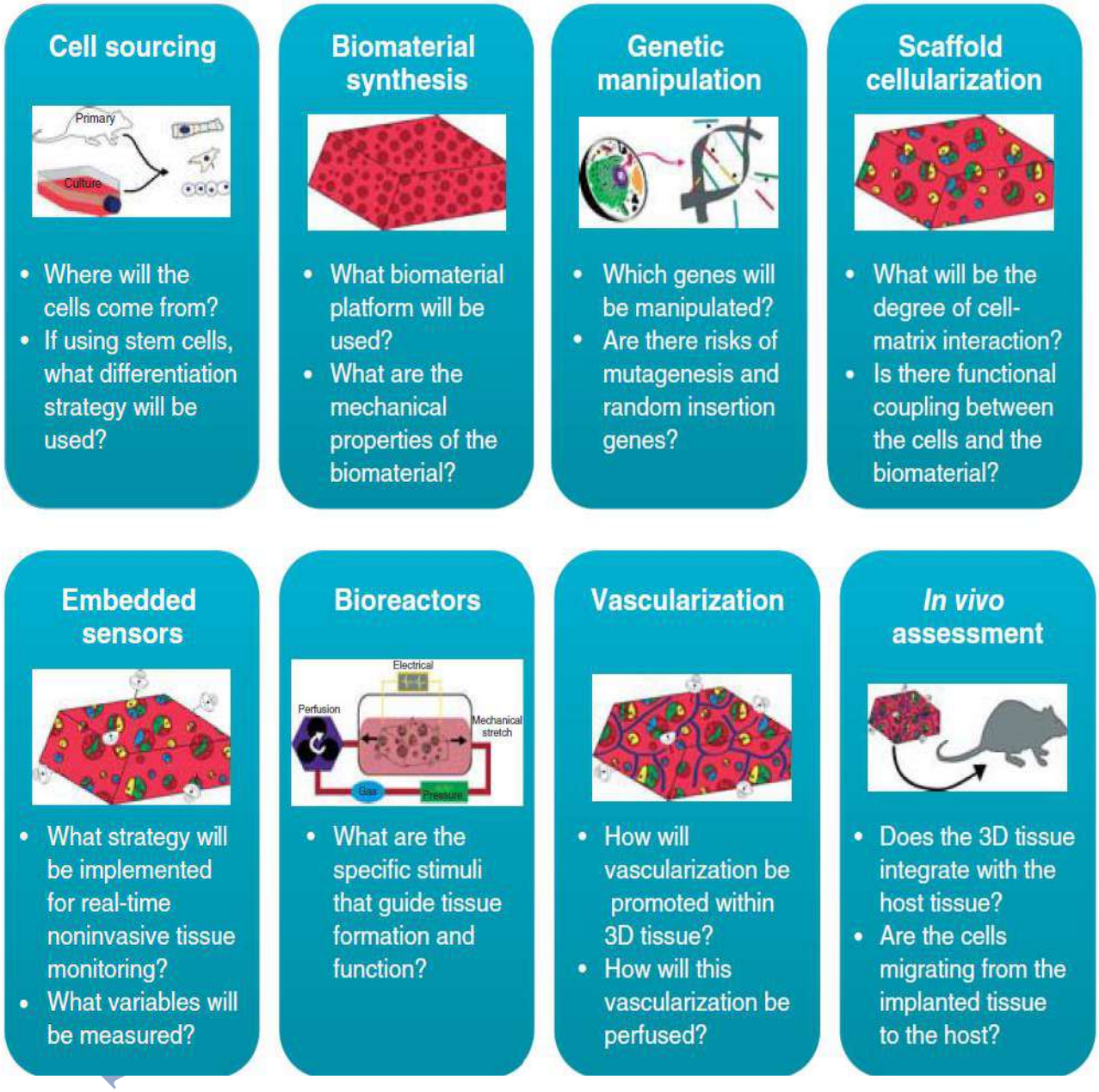


Figure (5): Scientific Challenges in Tissue Engineering.

Tissue Engineering and Tissue Fabrication

Tissue engineering should be viewed as a process of fabricating artificial tissue; in other words, tissue engineering equates to tissue fabrication. Like any fabrication process, tissue fabrication has inputs (cells and biomaterials) and an output (artificial tissue). At each step of the process, there are critical design variables and design constraints which need to be addressed, some of which are shown in the Figure (6).

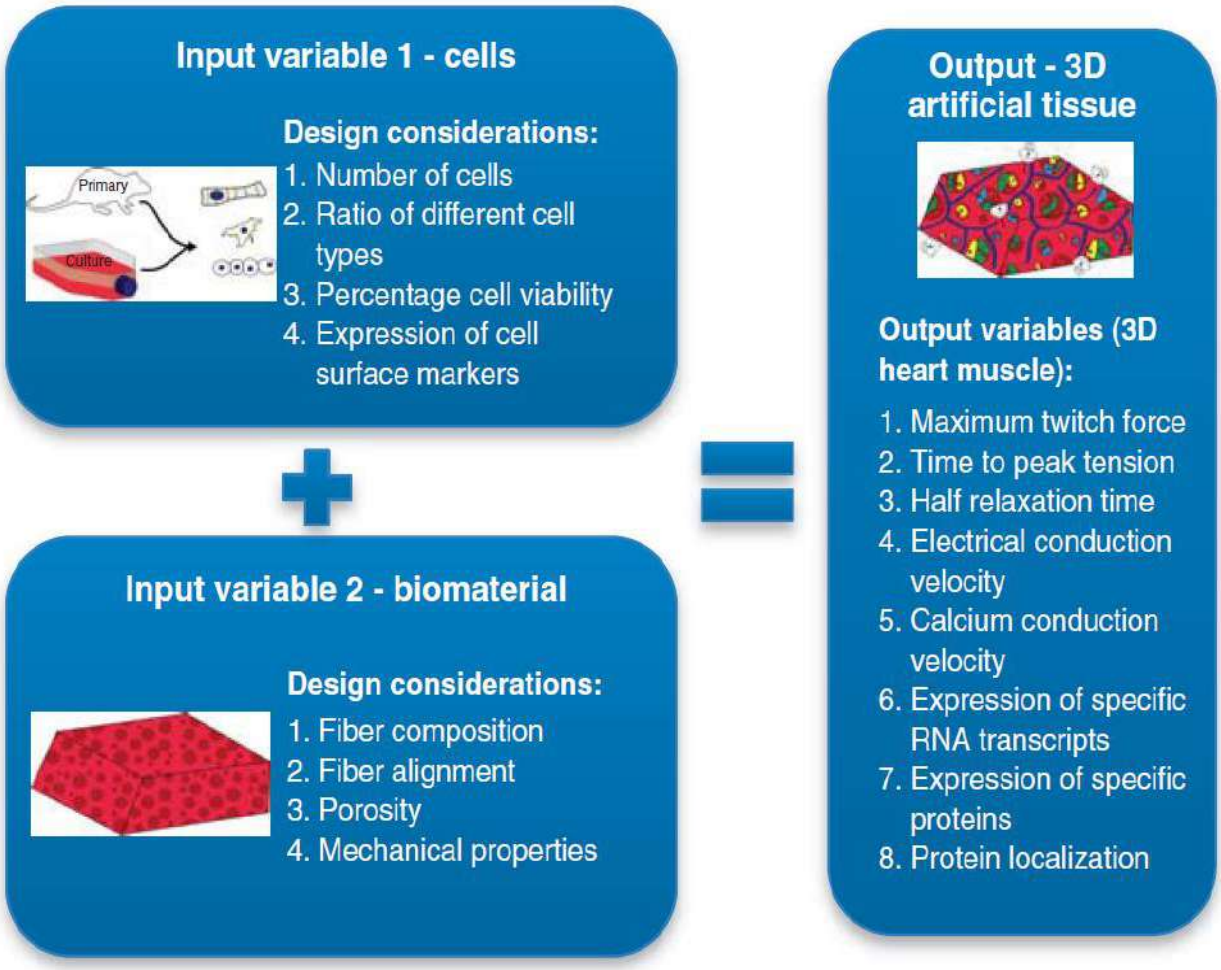


Figure (6): Tissue Engineering and Tissue Fabrication.

There are three steps in the tissue fabrication process that are considered to be the building blocks of artificial tissue: cells, biomaterials and bioreactors (Figure 7).

Cells are the functional components of artificial tissue; biomaterials are the structural components of artificial tissue while bioreactors provide guidance for tissue development and maturation. In the absence of any one of these three building blocks of tissue engineering, the functional performance of 3D artificial tissue will be significantly compromised.

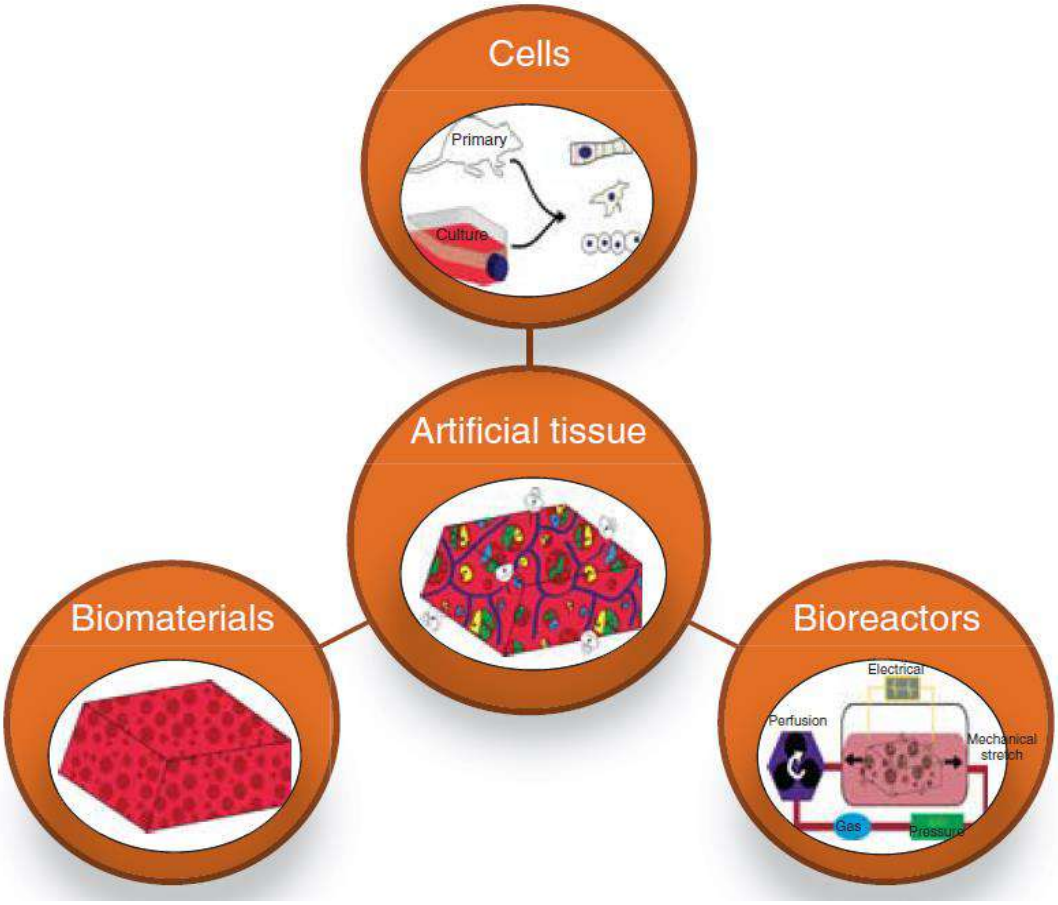


Figure (7): Building Blocks of Tissue Engineering—The building blocks of tissue engineering are cells, biomaterials and bioreactors.

Cells and Tissue Engineering

Cells: are the fundamental units of life and are one of the building blocks for tissue engineering. There are several aspects of cell biology that are important for the tissue fabrication process, and, as illustrated in Figure (8), cell biology, cell culture, cell transplantation, and stem cell engineering are important during the tissue fabrication process.

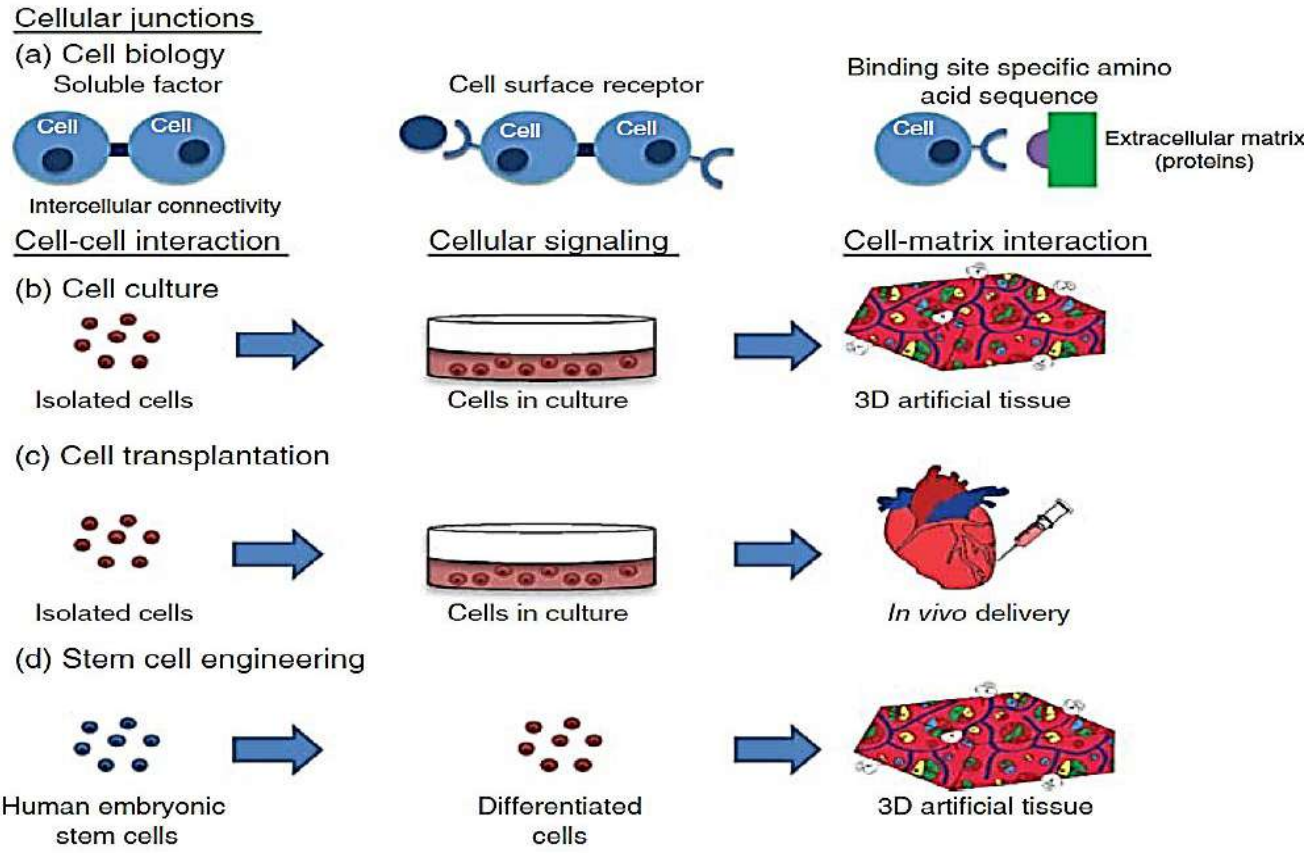


Figure (8): Cells and Tissue Engineering Cells are important for tissue engineering, and four important areas are (a) Cell Biology, (b) Cell Culture, (c) Cell Transplantation, and (d) Stem Cell Engineering.

Organ Engineering and Tissue Engineering

The term organ engineering refers to the design and fabrication of entire bioartificial organs and can be considered an extension of the field of tissue engineering.

Scaffolds

➢ The ***SCAFFOLD*** refers to the matrix with in the tissue model construct. An ideal scaffold should be:

(i) Three-dimensional and highly porous with an interconnected pore network for cell growth and flow transport of nutrients and metabolic waste;

(ii) Should have surface properties, which are optimized for the attachment, migration, proliferation, and differentiation of cell types of interest (depending on the targeted tissue);

(iii) Be biocompatible, not elicit an immune response;

(iv) Its Mechanical properties should match those of the tissue at the site of implantation and

(v) The scaffold structure should be easily and efficiently reproducible in various shapes and sizes.

Classical Scaffold Fabrication Techniques

There are many different methods developed in the 1990 and 2000s to fabricate porous scaffolds.

Four concept comprises the four essential components of a scaffold which are:

1- Form,

2- Function,

3- Formation, and

4- Fixation.

1- *Freeze Drying*

- Freeze drying or lyophilization, is based on the drying of polymeric solutions. It can be broken down into a three-step process:

i) Solution preparation,

ii) Casting or molding of the solution, and

iii) Freezing and drying at low pressure.

During the third step, the ice and the unfrozen water are extracted by sublimation and desorption, respectively.

- Freeze drying is capable of producing scaffolds with approximately 90% porosity
- Pore sizes ranging from 20 to 200 μm.
- Pore size is controlled by :
    1. Freeze rate,
    2. Polymer concentration, and
    3. Temperature.

2- Gas foaming

- Gas foaming of biodegradable polymers found its original application in the biomedical sciences in drug delivery applications during the 1980s.

1. It is a scaffold fabrication technique that permits solvent-free formation of porous materials through generation of gas bubbles within a polymer.
2. Molded polymers may be pressurized with a gas, typically CO_2, until the polymer is saturated.
3. The release of pressure results in nucleation and growth of the air bubbles up to 100 mm; however, interconnectivity is still limited and is often combined with particulate leaching to obtain improved interconnectivity between pores.

3- Decellularization

- Decellularized bone matrix (DBM) has been widely used in Bone tissue Engineering (BTE) as scaffolds and as bioinks for biofabrication, aiming to mimic the native bone microenvironment.
- Decellularization involves the removal of all cells from tissue, while retaining the native ECM composition and its architectural integrity, and its ability to promote cell growth and differentiation.

Processing techniques to obtain DBM include

1. Surfactants and enzymatic methods
2. Thermal shock,
3. Sonication, and
4. Hydrostatic pressure (i.e. Has the advantage of no harmful chemical usage and minimization of protein denaturation, thus a high level of ECM content can be preserved).

5- Use of nucleases and dehydrated alcohol for a complete removal of cellular remains.

4- Porogen leaching

Porogen leaching (Figure 9) was used in the early days of tissue engineering as it is one of the oldest polymer processing technologies to make porous products. Porogen leaching is a common approach to developing large, three-dimensional, and porous scaffolds. It is based on dispersing a template (particles, etc.) within a polymeric or monomeric solution, gelling or fixing the structure, and removal of the template to result in a porous morphology.

Particulate leaching is a technique used to create three-dimensional, porous scaffolds by mixing a polymer mixed with salt particles. The removal of salt results in a porous structure.

5- Phase separation

Phase separation has been employed for decades for producing hollow fiber membranes and porous structures (Figure 9). Thermally induced phase separation (TIPS), in particular, has produced a range of scaffolds and is based upon the reduction of polymer solubility when the temperature is lowered, or when the polymer is frozen out of solution (Figure 9). These two types of TIPS are termed (Liquid- liquid) and (solid – liquid) phase separation, respectively.

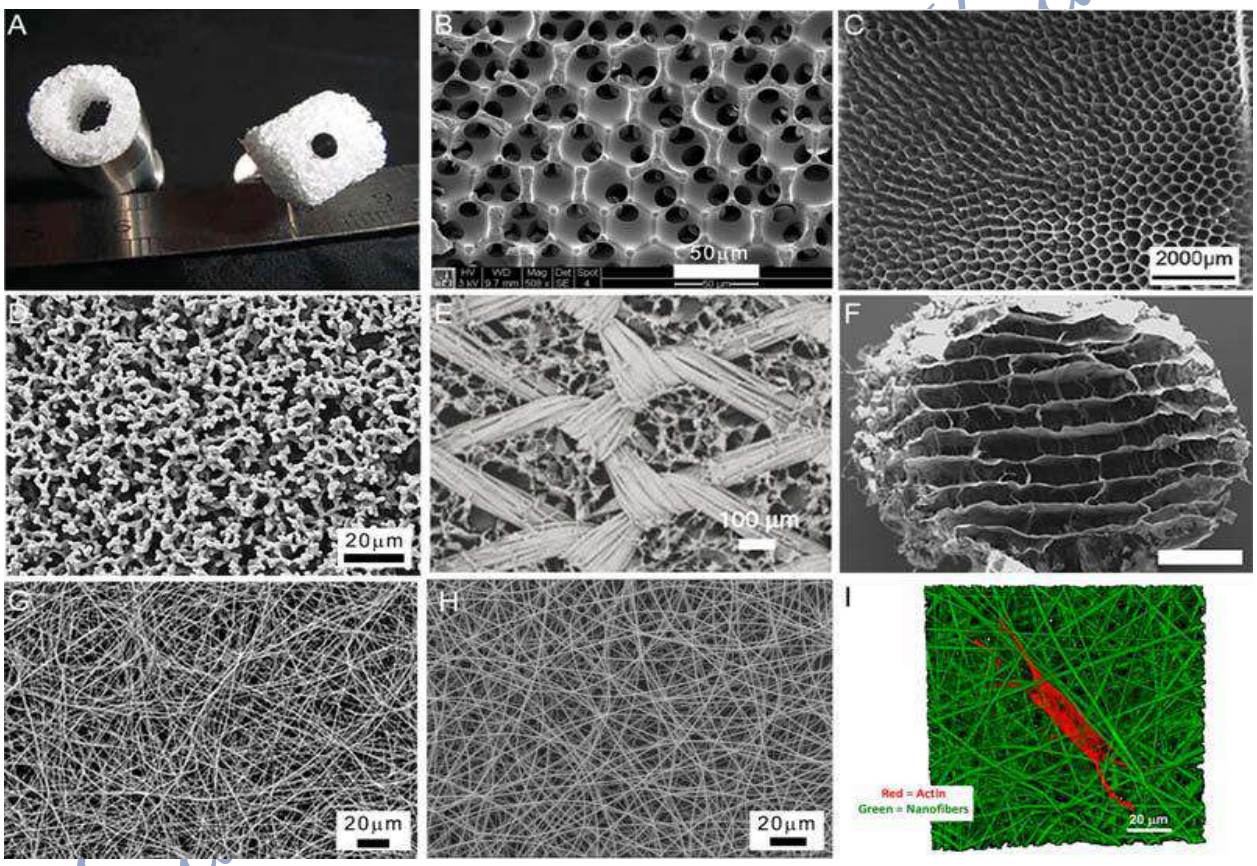


Figure (9): Selection of classical scaffold examples. (a) Photograph of salt porogen-leached PLGA, (b) SEM of a porogen-leached hydrogel (c) micromolded scaffolds used for spinal cord injury (d) randomly organized pore morphology of a scaffold made via polymerization-induced phase separation. (e) Braided yarns macropore architecture combined with a micropore filling collagen matrix. (f) Channeled pores made via ice templating. (g) A nonwoven fabricated via solution electrospinning, (h) melt electrospinning and (i) confocal microscope image of a cell growing on a solution electrospun membrane.

6- Electrospinning

A modern method for creating porous scaffolds composed of nano- and microscale biodegradable fibers employs electrostatic fiber spinning, or electrospinning, a technology derived from the electrostatic spraying of polymer coatings. Electrospinning involves a process in which a stream of an electrically-charged polymer in a viscous state or solution is drawn into fiber due to electrostatic forces. A basic electrospinning apparatus is comprised of four main parts:

i) Syringe pump,

ii) Power supply,

iii) Metallic needle to allow the electricity to move into the polymeric solution, and

iv) Metallic collector for fiber collection.

A scaffold is typically created by connecting the spinneret and fiber collector to opposite ended electrical terminals. The potential difference between terminals causes the material to be drawn out and deposited onto a collector, which facilitates the fabrication of fibers in the nano scale.

Electrospinning fabricates highly porous scaffolds of nonwoven and ultrafine fibers. Electrospinning is a tightly regulated process that supports fabrication of microfibers ranging in diameter from nanometers to micrometers (Figure 10). The process begins by:

1- Solubilizing a polymer in a solvent and placing the polymer solution in a syringe.

2- The polymer solution is held at the tip of the syringe by surface tension.

3- One electrode is placed in the polymer solution and a second electrode are placed in the collection device.

4- A high voltage power supply is used to propel the polymer solution out of the syringe by overcoming the surface tension that holds it in place.

5- Gradually increasing the voltage results in the formation of a Taylor cone, which refers to the attachment of the polymer solution to the tip of the syringe in the shape of a cone.

6- As the voltage is increased further, the fluid is ejected from the Taylor cone toward the collection device.

7- As the polymer travels toward the collection device, the solvent evaporates, resulting in the formation of fibers. The fibers are collected in a collection device that can be configured to support the formation of 3D scaffolds.

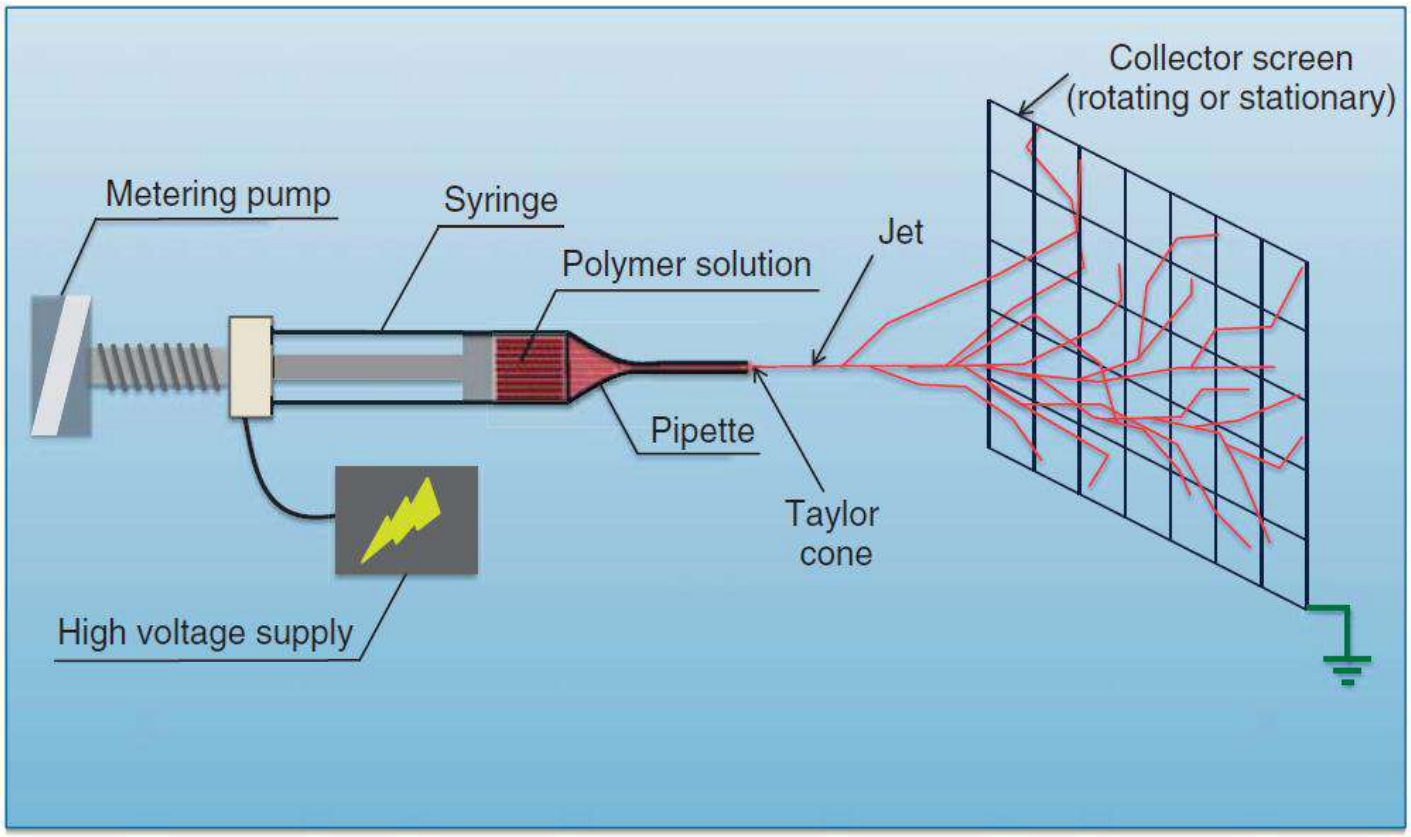


Figure (10): Electrospinning for Scaffold Fabrication.

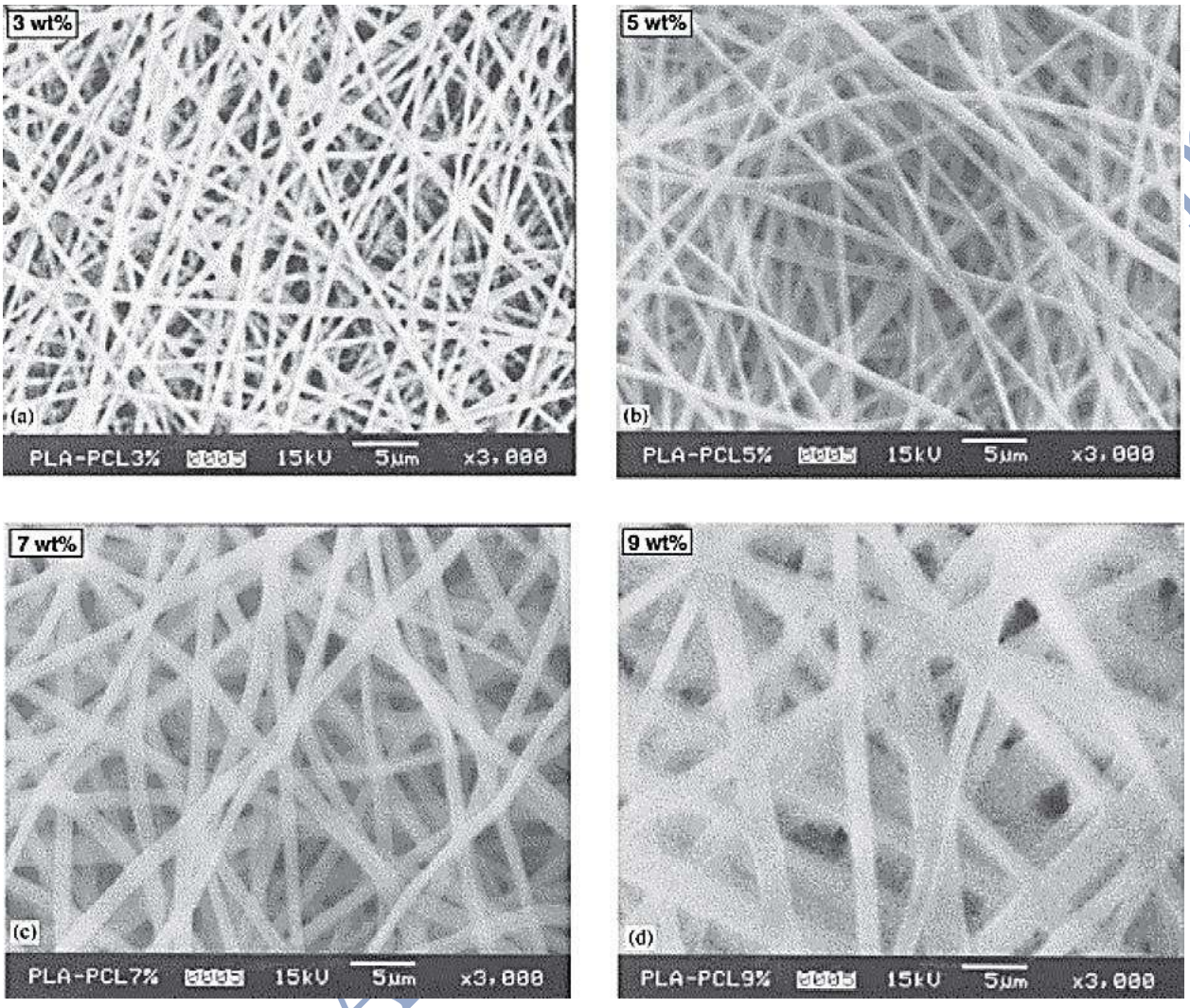


Figure (11): Scanning electron micrographs of (PLA-PCL) electrospun fibers at an applied voltage of 12 kV from different polymer concentrationsolutions: (A) 3 wt.%; (B) 5 wt.%; (C) 7 wt.%; (D) 9 wt.%.

7- Additive manufacturing

Additive manufacturing is the construction of a three-dimensional object from a computer-assisted design model or a digital 3D model. Rapid prototyping is a group of techniques used to quickly fabricate a scale model of a physical part or assembly using threedimensional data and computer-aided design.

In additive manufacturing (Figure 12), a starting material (powder, liquid, filament, etc.) is processed by a 3D printer, which deposits just the required amount of material in a layer-by layer approach to fabricate the object. The amount of residual material left over after additive manufacturing is significantly lower than that resulting from subtractive manufacturing. Finally, the printer starts depositing the material following the layer-by-layer sequence until the designed scaffold is fabricated. Additive manudacturing includes the folllowing processes:

A- Direct writing and extrusion of polymers

B- Stereolithography

C- Digital light processing

D- Digital light synthesis

E- Two-photon polymerization

F- Selective laser sintering

G- Melt electrowriting

A- Direct writing and extrusion of polymers

Melt extrusion of strands/filaments has been developed more recently toward establishment as additive manufacturing technology. A typical machine consists of a heated liquefier head attached to a carriage moving in the horizontal x-y plane. Polymer is forced through the liquefier/heating zone to a nozzle to fabricate the scaffold following a programmed path which is based on a CAD model and the slice parameters (Figure 12). Once a layer is built, the platform moves down one step in the z-direction to deposit the next layer. Parts are fabricated layer-by-layer with the layer thickness varying in proportion to the nozzle diameter chosen. This high temperature process is restricted to the use of thermoplastic materials with good melt viscosity properties; cells or other thermo-sensitive biological agents cannot be encapsulated into the scaffold during the fabrication process.

A design limitation of melt extrusion systems is that the pore size for the scaffolds may not be consistent in all three dimensions as the melt requires a surface to be deposited upon, there are challenges with overhanging structures. One technical limitation of mechanically extruding polymer melts is the large diameter of fibers produced: typically, above 100 mm.

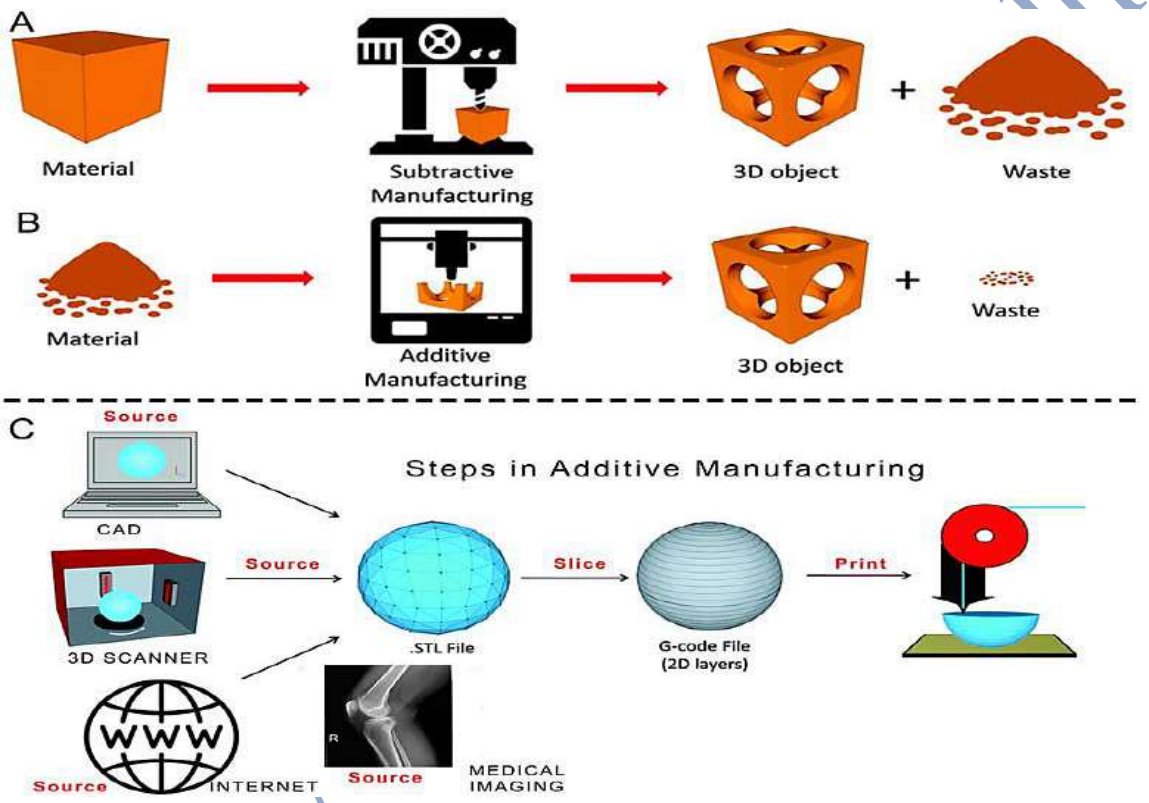


Figure (12): Schematics of demonstrating the differences between (a) subtractive manufacturing and (b) additive manufacturing. (c) shows a workflow of many additive manufacturing processes, using a surface tessellation language (STL) file created from different origins.

B- Stereolithography

Stereolithography (SLA) is based on the use of a focused UV laser which is vector scanned over the top of a liquid bath of a photopolymerizable material. The UV laser causes the bath to polymerize where the laser beam strikes the surface of

the bath, resulting in the creation of a first solid plastic layer at and just below the surface.

C- Digital light processing

3D printing systems based on digital light processing (DLP) involve use of light projection technology, developed by Texas Instruments in the 1980s, to polymerize materials for fabricating structures (Figure 13) in a layer-by-layer approach. The material being used should have adequate photosensitive properties for the process to function successfully. DLP printing has a micron scale resolution, and the printing process occurs without high temperature, pressure, or shear forces enabling mild printing conditions for cells.

D- Digital light synthesis

Digital light synthesis (DLS), also known as Continuous Liquid Interface Production (CLIP), is an additive manufacturing technology based on DLP but with a continuous layering approach. It is a photochemical process in which a series of UV images are continuously projected through an oxygen permeable membrane window on a liquid resin. The concept of continuous projecting the light through an oxygen permeable membrane is what makes DLS unique and differentiates it from DLP. This membrane creates a thin layer of liquid resin at the interface of the window and the printing part preventing the part from sticking to the window enabling easy postprocessing of the part fabricated.

Another unique feature of DLS is the use of heat to cure the parts to enhance the mechanical properties.

E- Two-photon polymerization

Two-photon polymerization (2PP) produces results the best resolved additively manufactured products, with nano-scale tolerances, and the ability to produce very small pores and highly detailed structures (Figure 13). Similar to that of SLA, a photo-curable resin is required, and currently only small objects can be produced. 3D photo grafting can produce functionalized regions within the hydrogel, while the opposite use of two-photon polymerization to weaken regions of a hydrogel matrix allows the control of cell migration.

F- Selective laser sintering

Selective laser sintering (SLS) also uses a focused laser beam, but to sinter areas of a loosely compacted powder. This method begins with a thin layer of powder spread evenly onto a flat surface with a roller mechanism and then raster-scanned with a high-power laser beam. The powder material that is struck by the laser beam is fused, while the other areas of powder remain dissociated.

Successive layers of powder are deposited and raster-scanned, one on top of another, until an entire part is complete (Figure 13). Each layer is sintered deeply enough to bond it to the preceding layer.

G- Melt electrowriting

A hybrid form between melt extrusion and electrospinning is the use of a voltage to maintain a continuous, thin, jet onto a collector. In this way, melt electrowriting (MEW) has no “whipping” compared to melt electrospinning and is more conducive to rapid solidification and better printing resolutions than its polymer solution counterpart or solution electrospinning. The thickest MEW scaffolds made to date are 7-mm thick and consist of 300 fiber layers.

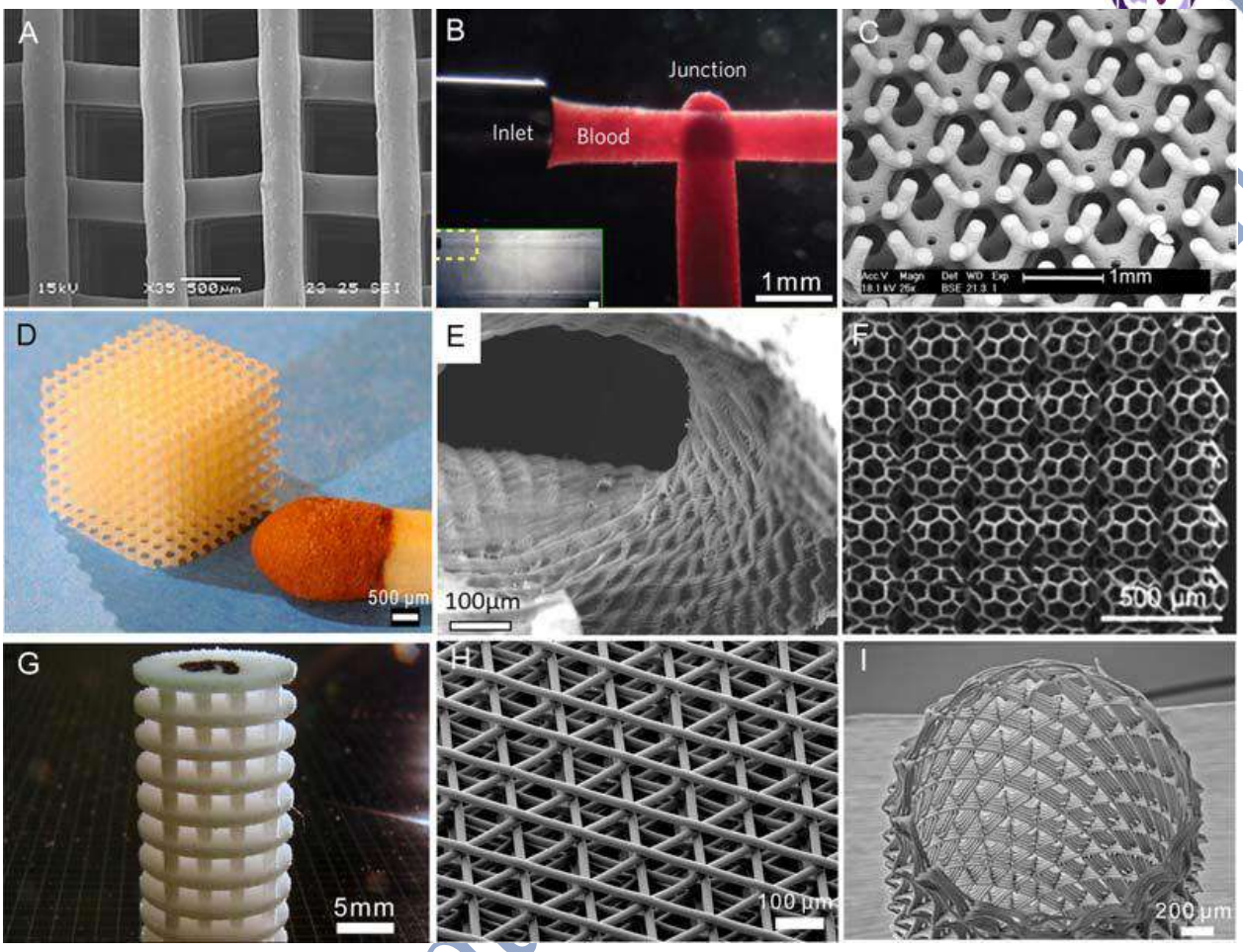


Figure (13): Additively manufactured scaffolds using (a) FDM, including (b) as a sacrificial template, Examples of SLA scaffolds at (c) high and (d) low magnification. (e) shows the morphology of a scaffold made using DLP. (f) Scaffolds made using Two-photon polymerization (2PP), (g) SLS and (h) melt electro written scaffold, as well as a (I) melt electro written tube.

Soft Tissue Engineering

In tissue engineering, "soft tissue" is a broad term that typically refers to all tissues except bone. This includes, but is not limited to:

1- Skin,
2- Musculoskeletal (Cartilage, Ligaments/Tendons, Muscle, Connective Tissues),
3- Adipose,
4- Cardiovascular,
5- Neural (Brain and Nerves),
6- Gastrointestinal,
7- Vasculature (Blood and Lymph Vessels),
8- Liver,
9- Lung,
10- Kidney,
11- Ocular tissues.

The design criteria for each of these tissue types is different, depending on the native anatomy, physiology, and the intended application, and the material requirements for each application also differ.

Properties of Soft Tissues

1- Soft tissues are highly flexible with stiffness values ranging from (0.1 kPa to 1 MPa), and the mechanical properties are related to the tissue function. For example, load-bearing tissues, such as cartilage and tendon, are much stiffer than non-load-bearing tissues, such as brain.

2- The mechanical properties of tissues are driven by the composition and organization of the extracellular matrix (ECM).

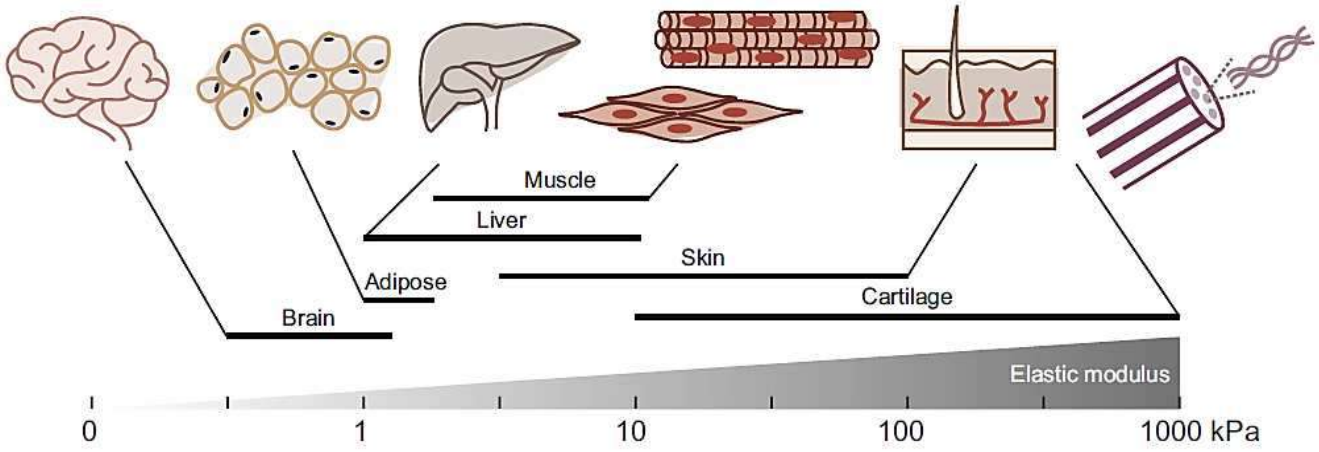


Figure (14): Range of elastic modulus of different types of soft tissues.

- The main components of soft tissue ECM are fibrous proteins (i.e., collagen, elastin), glycosaminoglycans (GAGs: i.e., hyaluronic acid, chondroitin sulfate), and proteoglycans (i.e., aggrecan, versican), though the relative concentration of each component and the structural organization depend on tissue type, age, and disease state. For example, the brain contains high concentrations of GAGs and lower amounts of collagens, whereas cartilage (specifically articular cartilage) is primarily composed of collagens, and proteoglycans are a secondary component.

Common Biomaterials Used for Soft Tissue Engineering

- Biomaterials for soft tissues can be fabricated from synthetic and/or natural polymers.
- Synthetic polymers have highly controlled, reproducible compositions and can usually be chemically modified. However, they lack intrinsic bioactivity and exhibit varying degrees of biocompatibility, with some synthesized or crosslinked using toxic reagents.
- On the other hand, natural biomaterials are generally biocompatible and chemically and physically similar to native tissue. However, naturally derived materials tend to have weaker mechanical properties, degrade more quickly compared to synthetic materials, can be challenging to modify in terms of biological and physical properties, and often have more variable compositions, depending on the source.

Synthetic Polymers

Poly(lactide-co-glycolide) (PLGA) is a Food and Drug Administration (FDA)-approved polymer widely used in tissue engineering and drug delivery. It is also one of the few synthetic polymers that is degradable, and has a tunable degradation rate. PLGA can be developed into a variety of different formats, including sponges, foams, fibers, hydrogels, microparticles, and nanoparticles.

Poly(ethylene glycol) (PEG) is another commonly used, FDA-approved synthetic biomaterial. It is nontoxic, bioinert, and can be functionalized to enable crosslinking or incorporation of bioactive components. The mechanical properties of PEG are suitable for soft tissue engineering, as they can be tailored to match the tissue of interest. Additionally, the polymer precursors to PEG-based hydrogels are cytocompatible, thus allowing for in situ gelation. Due to these advantages, PEG has

been utilized in many soft tissue-engineering applications, such as cartilage, adipose, neural, and cardiac tissue.

➡ Another FDA-approved synthetic polymer used in soft tissue applications are ***poly(ε-caprolactone) (PCL).*** This biocompatible and thermoresponsive polymer has relatively slow biodegradation and can be formed into films, hydrogels, and electrospun fibers. The elasticity, stability, and ease of processing of PCL make it an attractive material for tissue engineering. It is often used in combination with other polymers, including collagen, chitosan, and PEG, and has been applied in nerve, cardiovascular, skeletal muscle, ligament, bladder, ocular, skin, and cartilage tissue engineering.

Natural Polymers

Collagen and hyaluronic acid (HA) are two of the most commonly utilized natural polymers in tissue engineering. Both polymers are present in the ECM and are biodegradable and biocompatible.

➡ ***Collagen*** can be isolated natural sources, and contains epitopes for cell interactions. Collagens can be formulated into sponges, microparticles, fibers, and injectable hydrogels, allowing for use in a range of biomedical applications, including ocular, urogenital, skin, cardiovascular, and neural tissue engineering.

Collagen- based scaffolds are limited by their tendency to contract and rapidly degrade *in vivo*, necessitating modes to crosslink the protein to improve stability over time.

➡ ***Hyaluronic acid HA*** is a hydrophilic, naturally occurring polysaccharide that has a similar water content to soft tissues when formed into hydrogels. Although native HA is rapidly degraded in vivo through endogenous enzymes, it can be

chemically modified and crosslinked, thus altering the degradation profile, mechanical properties, and cellular interactions.

Adipose Tissue Engineering

Adipose tissue, commonly referred to as fat, is found throughout the body. The need for adipose tissue replacement arises when this soft tissue is lost or damaged due to:

1- Trauma (i.e., Burns),
2- Congenital Defects (i.e., Hemifacial Lipoatrophy),
3- Tumor Resections,
4- Elective Cosmetic procedures

- The most commonly used clinical treatments are soft tissue fillers for small defects, and prosthetics (e.g., silicone implants) or autologous adipose tissue transplants for larger defects.
- Significant limitations associated with current treatments are poor long-term stability and donor or recipient-site morbidity. Current material implants only temporarily augment or bulk the tissue. Due to these limitations, tissue-engineering principles are being used to develop new materials for the replacement of adipose tissue, focusing on the regeneration of fully functional tissue to provide a permanent solution.

Soft Tissue Engineering Applications: Adipose, Cardiovascular, Gastrointestinal, and Skin

➢ *Design Criteria for Adipose Tissue Engineering:*

The goal for adipose tissue engineering is to provide **volumetric persistence** while stimulating the **regeneration of functional adipose tissue**. To achieve this goal, a biomaterial system must be:

I. Biocompatible,
II. Provide appropriate mechanical properties and degradation profiles,
III. Facilitate and promote adipogenesis, and
IV. Promote vascularization.

Inducing adipogenesis and promoting vascularization are key to regenerating functional adipose tissue, especially in the case of large tissue defects.

Vascularization Strategy for Adipose Tissue Engineering:

1- Select a suitable growth factor:

Some common growth factors used for adipose tissue engineering include

1- Fibroblast growth factor-1 (FGF-1),
2- Basic fibroblast growth factor (bFGF),
3- Insulin-like growth factor-1 (IGF-1),
4- Vascular endothelial growth factor (VEGF).

2- Select a suitable biomaterial:

In choosing a biomaterial, it is critical to assess:

I. Toxicity,
II. Cytocompatibility, and

III. Inflammation, either from the bulk material or its degradation products, which can induce a foreign body response, cause tissue damage, and prevent functional regeneration.

3- ***Mechanical integrity and an appropriate degradation rate are also important parameters to consider:***

Adipose tissue is not subjected to mechanical loads unlike tissues, such as cartilage and bone; however, it can experience compressive forces from the surrounding tissues and can also be exposed to significant deformation during normal daily functions like sitting.

4- In addition, ***MATCHING THE NATIVE MECHANICS OF ADIPOSE TISSUE*** can facilitate human adipose-derived stem cell (hASC) differentiation into mature adipocytes.

5- As with most applications, ***DEGRADATION*** is a key factor in the success of the biomaterial; in adipose tissue engineering, the material must maintain volume while facilitating tissue regeneration.

6- Both ***in vitro*** and ***in vivo*** assessments can be performed to determine whether a particular biomaterial is suited as scaffolding for adipose tissue. For *in vivo* assays, biomaterials are commonly implanted or injected subcutaneously in small animal models (mice and rats). For *in vitro* and *in vivo* assays, a variety of analytical tools have been used to assess tissue regeneration, including:

I. Mechanics,
II. Biocompatibility/toxicity, and
III. Material degradation.

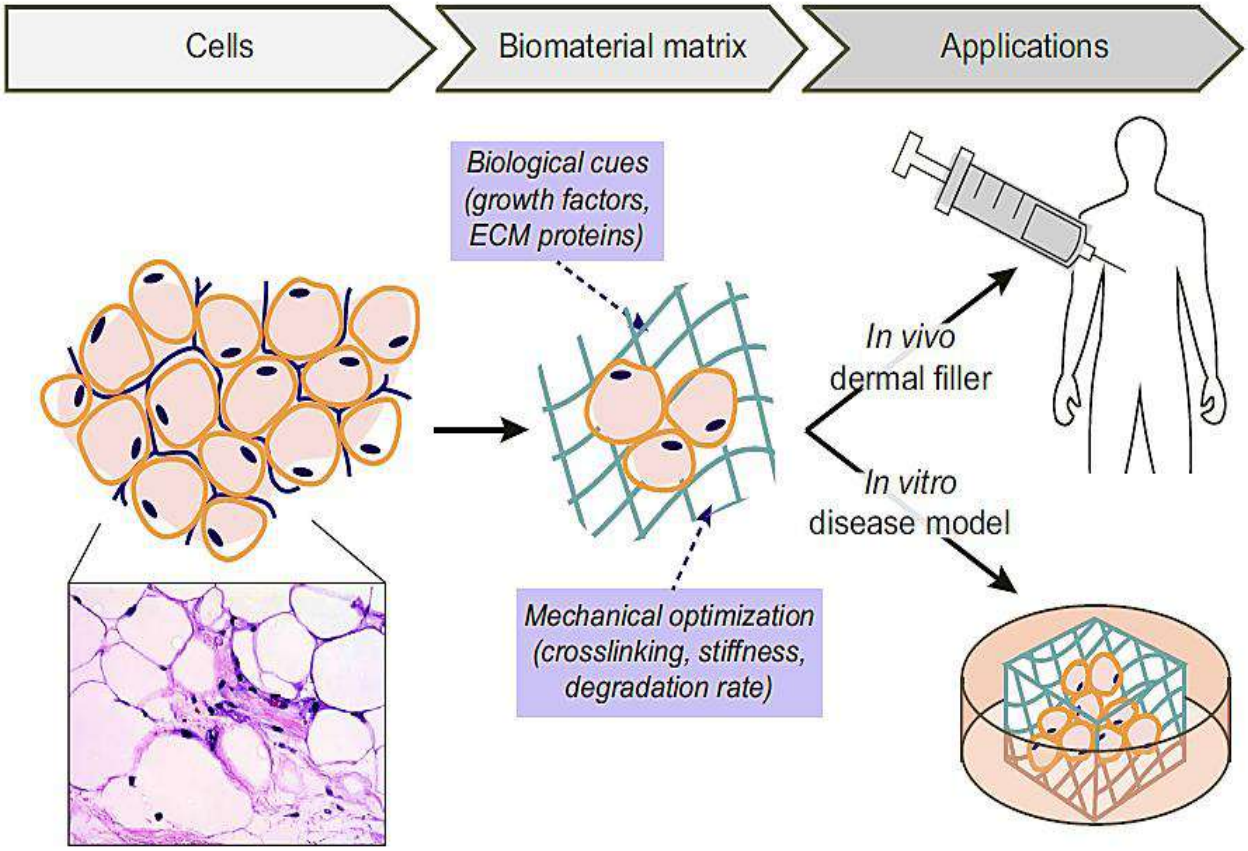


Figure (15): Principles of adipose tissue engineering.

Cardiovascular Tissue Engineering

Tissue engineering is of particular interest for the treatment of heart diseases, such as after myocardial infarction (MI), or due to congenital heart defects, where large portions of functional tissue are lost with very limited intrinsic regeneration ability.

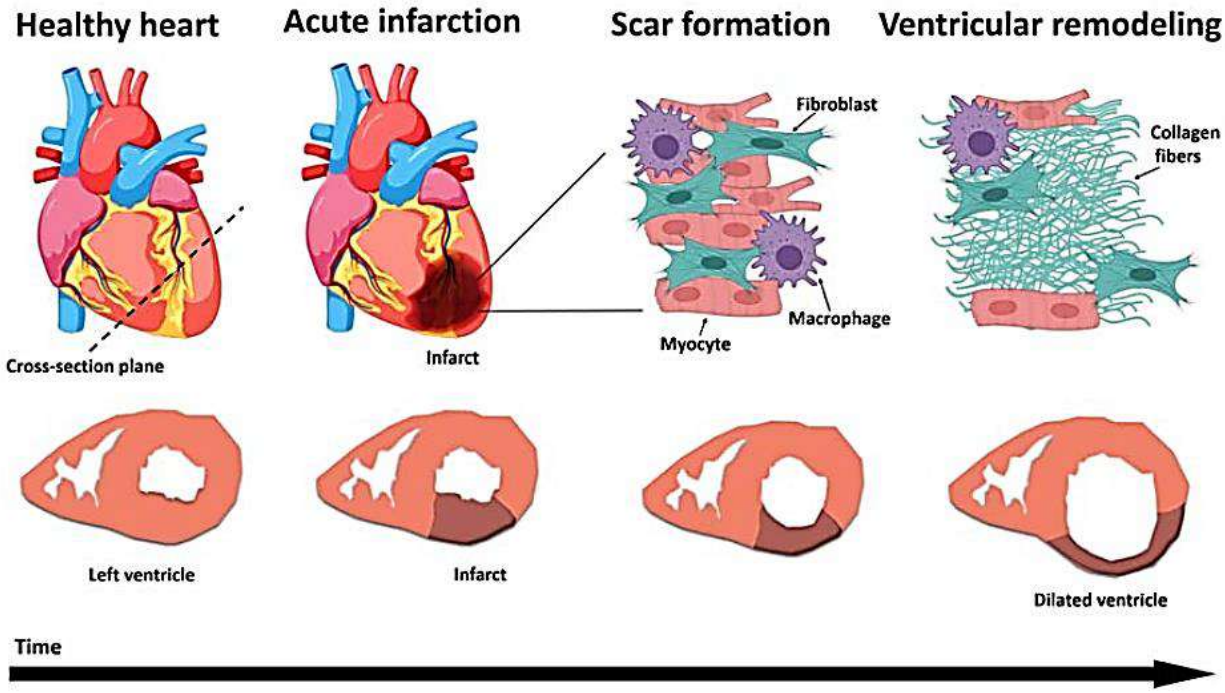


Figure (16): Heart failure: from acute crisis to chronic disease.

Cells represent a critical component in the tissue engineering paradigm. While preclinical research utilizes many readily available cell sources (cell lines, animal-derived isolated cells, etc.), translation of the developed strategies into the clinic requires human and preferably autologous cell sources of functional cardiovascular cells.

The major cell types used for cardiovascular tissue engineering and regeneration are as follows:

➢**Cardiomyocytes (CMs):** Cardiac muscle cells, responsible for contractile function of the

➢ **Fibroblasts**: Production of fibrillar collagen and differentiate to myofibroblasts after injury.

➢ **Macrophages**: Phagocytosis, inflammatory response.

➢ **Other cells Endothelial cells, smooth muscle cells, pericytes, neurons**.

Myocardial Regeneration Strategies

Five major processes associated with MI are targeted at present by various experimental regeneration strategies (Figure 17):

1- Cardio protection: the prevention of progressive (CMs) loss.

2- Inflammation: time-adjusted modulation of the post-myocardial infarction (MI) pro/anti-inflammatory or cellular responses (e.g., granulation tissue formation and macrophage infiltration) to induce effective tissue healing and repair and to avoid negative inflammatory effects (e.g., cell death, fibrosis, etc.).

3- ECM remodeling and cardiac fibrosis time-adjusted positive modulation of the fibrotic response (i.e., ECM remodeling and scar formation).

4- Angiogenesis: increasing the blood supply to ischemic regions is an extensively used approach for effective tissue healing. A variety of proteins, genes, or cells have been tested, aimed at inducing the formation of new vasculature at the infarct site.

5- Cardiomyogenesis: myocyte regeneration by activation and/or migration of distinct cell populations with stem- or progenitor-like properties in the adult myocardium which can contribute to de novo myocardium formation after MI.

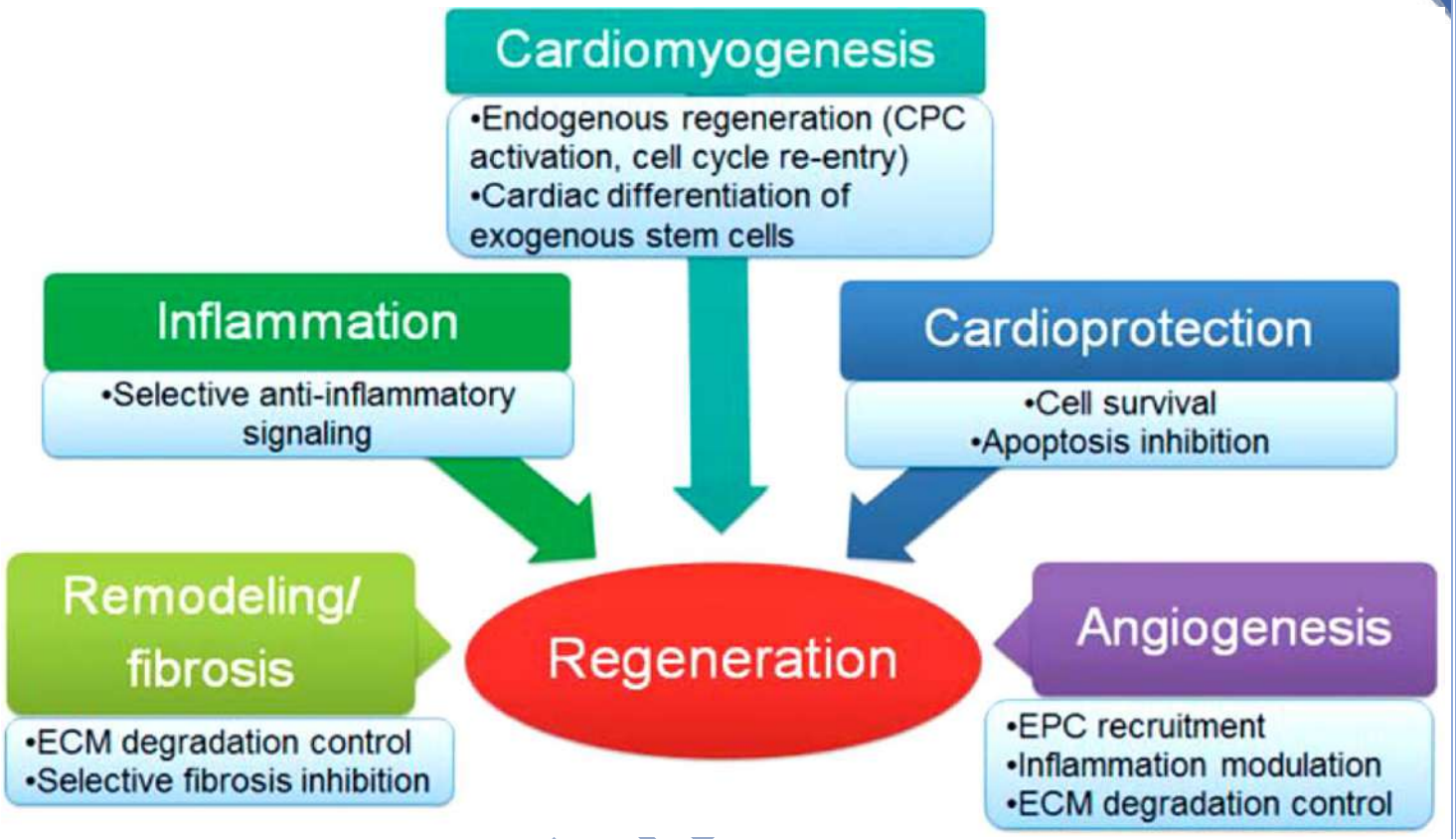


Figure (17): Therapeutic myocardial regeneration strategies.

In general, the biomaterials used in cardiovascular tissue engineering and regeneration strategies should comply with the following basic criteria:

✓ **Biocompatibility**: The ability of a scaffold to perform as a substrate supporting the appropriate cellular activity, including the facilitation of molecular and mechanical signaling, in order to optimize tissue regeneration, without eliciting any undesirable effects in these cells, or inducing any undesirable local or systemic responses in the host.

✓ **Mechanical strength**: Scaffolds that are used as ECM replacement of damaged myocardium should have the mechanical properties to contain and protect the seeded

or recruited cells and maintain their structure under mechanical perturbations existing during cultivation and at implant site. At the same time, the scaffold mechanical properties should be compatible with the host tissue to allow its integration without interfering with the normal function of the organ.

✓ **Biodegradation/bio resorption:** Ideally, the scaffold should disappear from the host when tissue regeneration has been accomplished and normal function is restored.

✓ **Scaffold fabrication:** Ideally, this process should use safe reagents which do not affect material properties, such as its cell recognition motifs.

✓ **Scaffold internal morphology:** When used as scaffolding for cells, the matrix should be porous with interconnecting pore structure and pore size larger than 50 mm, to enable cell-cell interactions and support vascularization after implantation.

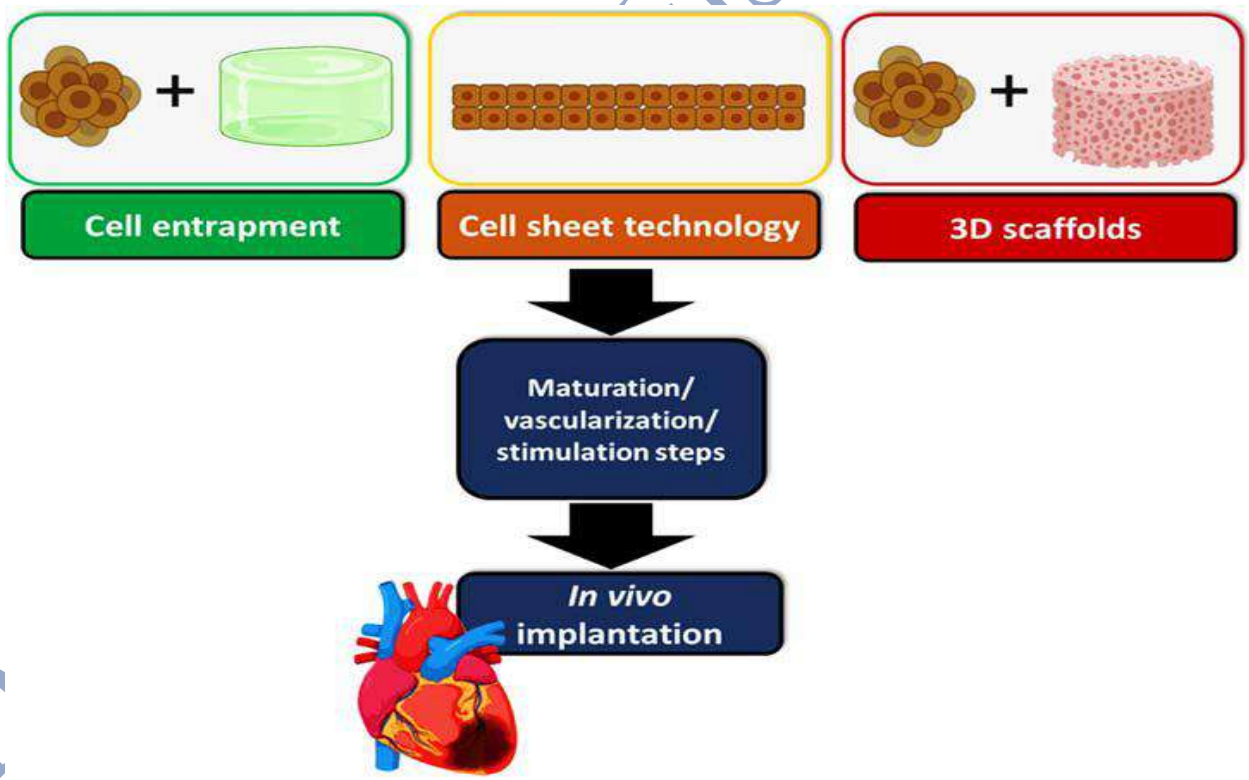


Figure (18): Strategies for reconstructing the cardiac patch.

Polymers commonly used for scaffold fabrication can be categorized by their source origin (natural or synthetic) and by their chemical structure (peptides/proteins, polyesters, and others).

Scaffolds can be fabricated in different shapes, sizes, and internal structure (porosity, pore size, and architecture of the pore structure (isotropic or anisotropic)). In tissue engineering of patches, either with seeded cells or without cells, the two main scaffolds in use are hydrogels and macroporous solid scaffolds.

Gastrointestinal Tissue Engineering

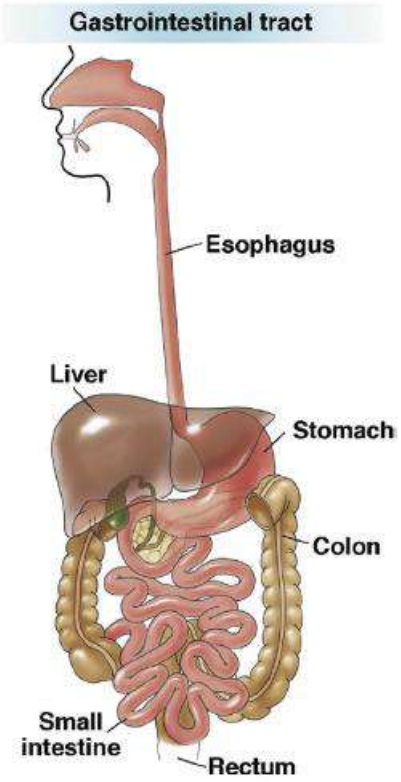


Figure (19): The organs of the gastrointestinal tract.

The gastrointestinal (GI) tract is a series of connected hollow organs, beginning at the mouth and ending at the anus (Figure 19). Swallowed food travels

through the esophagus to the stomach, where it is exposed to digestive enzymes and gastric acid. The resulting chyme—or partially digested food—passes through the pyloric sphincter to the duodenum, the upper portion of the small intestine. Digestion continues and nutrients are absorbed as the chyme is propelled through the small intestine to the colon, where water is reabsorbed and indigestible material is excreted as feces. Proper GI tract function relies upon complex coordination between multiple systems and an array of cell types.

As soft tissue-engineering techniques continue to develop, it may become possible to heal or replace GI tissues that have been: **removed**, **damaged**, or have otherwise lost normal functions, thus improving patient quality of life. **Cancers** of the esophagus, stomach, and colon. In addition to **leaks**, **infections**. Similar complications can result from surgeries used to treat inflammatory bowel diseases like **Crohn's disease and ulcerative colitis**. In these cases, replacement of removed tissues or even entire organs could help to restore structure and function.

Design Criteria for Engineered Gastrointestinal Tissues

Generally, any successful strategy would result in GI tissue satisfying the following minimum requirements:

(1) Regeneration of the epithelium and organ-specific epithelial function;

(2) Regeneration of the longitudinal and circular smooth muscle in proper alignment;

(3) Innervation of the smooth muscle and restoration of peristalsis; and

(4) Reintegration with the vascular and lymphatic systems with concurrent interactions with the immune system.

GI Tissue-Engineering Strategies

1- Select a proper scaffold:

The most successful preclinical GI tissue-engineering strategies to date have relied upon use of ECM-based scaffolds, often decellularized porcine small intestinal submucosa; in such cases, the scaffold is typically sutured to the wall of the GI tract, providing the necessary porous structure to facilitate tissue regeneration.

2- ***In vivo degradation*** of these biologically derived scaffolds also gradually releases growth factors, structural molecules, and other components important to the proliferation, differentiation, and phenotype of the varied and complex GI tissues.

3- Select a biomaterial:

Positive preclinical outcomes have also been demonstrated using synthetic biomaterials (e.g., lactic acid/caprolactone copolymer) for stomach regeneration and naturally derived scaffolds (e.g., chitosan) for colorectal tissue engineering.

4- In some cases, ***the use of healthy tissues from similar locations*** within the GI tract to generate ECM scaffolds has resulted in improved tissue-specific regeneration.

5- ***Seeding scaffolds with cells isolated from nearby locations prior to implantation*** can similarly improve outcomes. For example, collagen sponges seeded with autologous gastric smooth muscle cells enhanced the regeneration of the small intestinal epithelium and underlying musculature in canines relative to unseeded controls.

Stem cells have been used *in vivo* to successfully regenerate enteric neurons, smooth muscle, and functional small intestine complete with differentiated epithelium and neuromusculature.

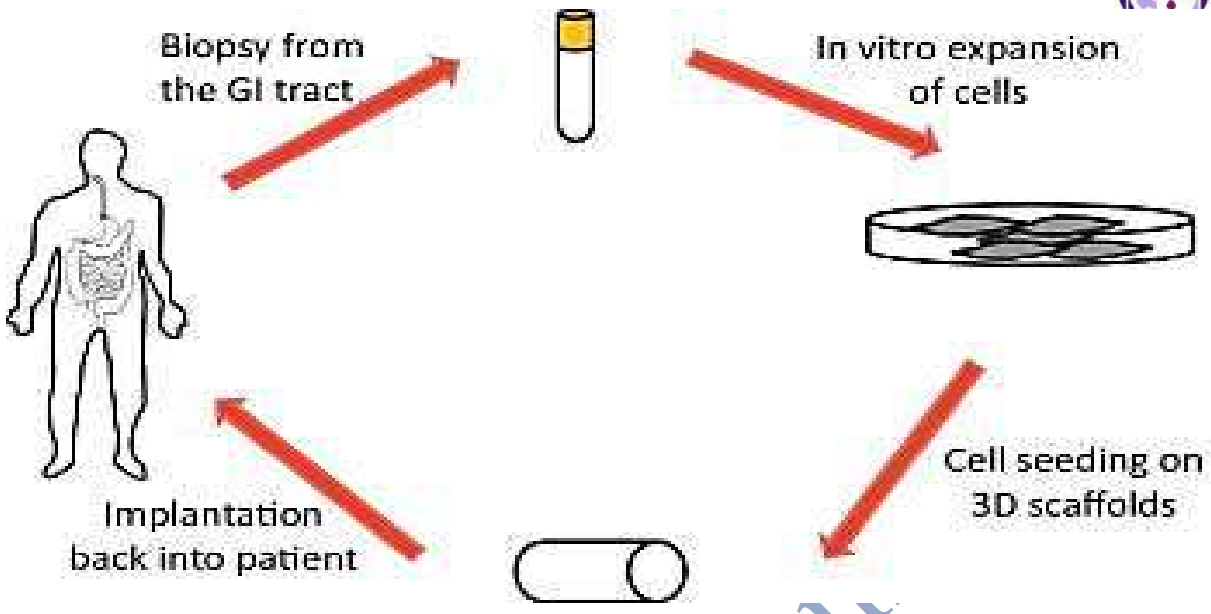


Figure (20): GI tissue-engineering strategies.

Wound Healing and Skin Tissue-Engineering

- The skin is the largest organ in the body and was one of the first organs to be tissue engineered.
- In general, skin substitutes are composed of a biomaterial matrix (typically collagen) with either fibroblasts (dermal component) and/or keratinocytes (epidermal component).
- Skin wound healing is a highly organized and complex series of processes comprising distinct but overlapping phases, which results in the restoration of tissue structure integrity and tissue functionality.
- Wounds are clinically divided into acute wounds such as burn injuries and chronic wounds such as diabetic or venous ulcers. Acute wound healing progresses in a timely and orderly pattern, whereas chronic wound healing is delayed.

- In general, skin substitutes are composed of a biomaterial matrix (typically collagen) with either fibroblasts (dermal component) and/or keratinocytes (epidermal component).
- The skin is a complex organ with multiple layers and functional components. The outermost layer of the skin, ***called the epidermis***, is mainly composed of differentiated, ***striated keratinocytes*** and ***melanocytes*** that organize into the following layers***: cornified layer (outermost), granular layer, spinous layer, and basal cell layer***, followed by the ***basement membrane***. Beneath the epidermis is the ***dermis***, which contains numerous cell types, including ***dermal fibroblasts, endothelial cells, neurons, and immune cells (including Merkel cells, Langerhans cells, macrophages, monocytes, and B cells, among others).***

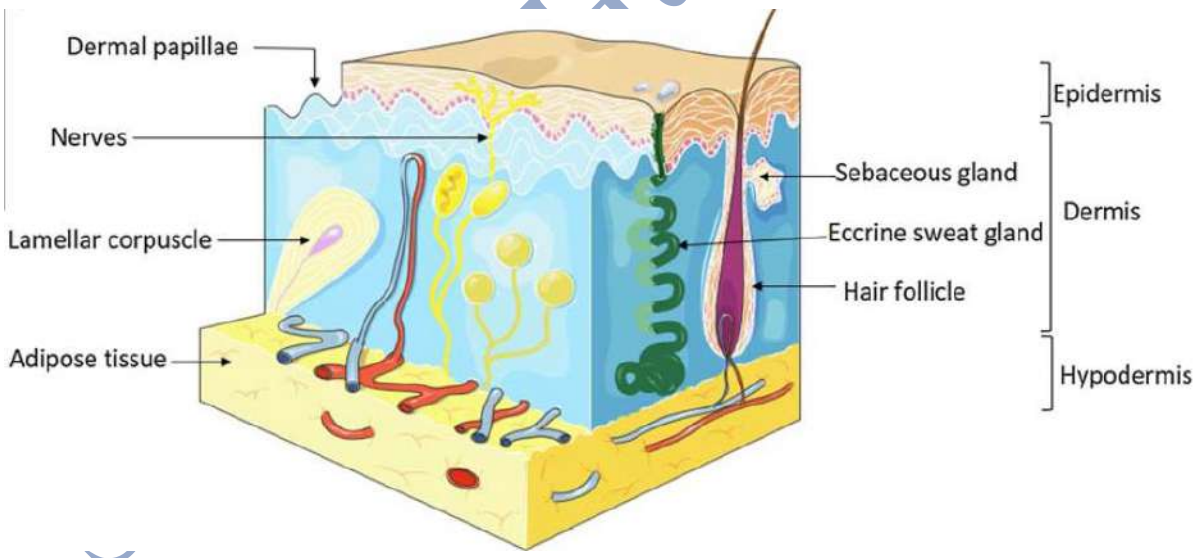


Figure (21): Schematic of normal skin structure, components, and layers.

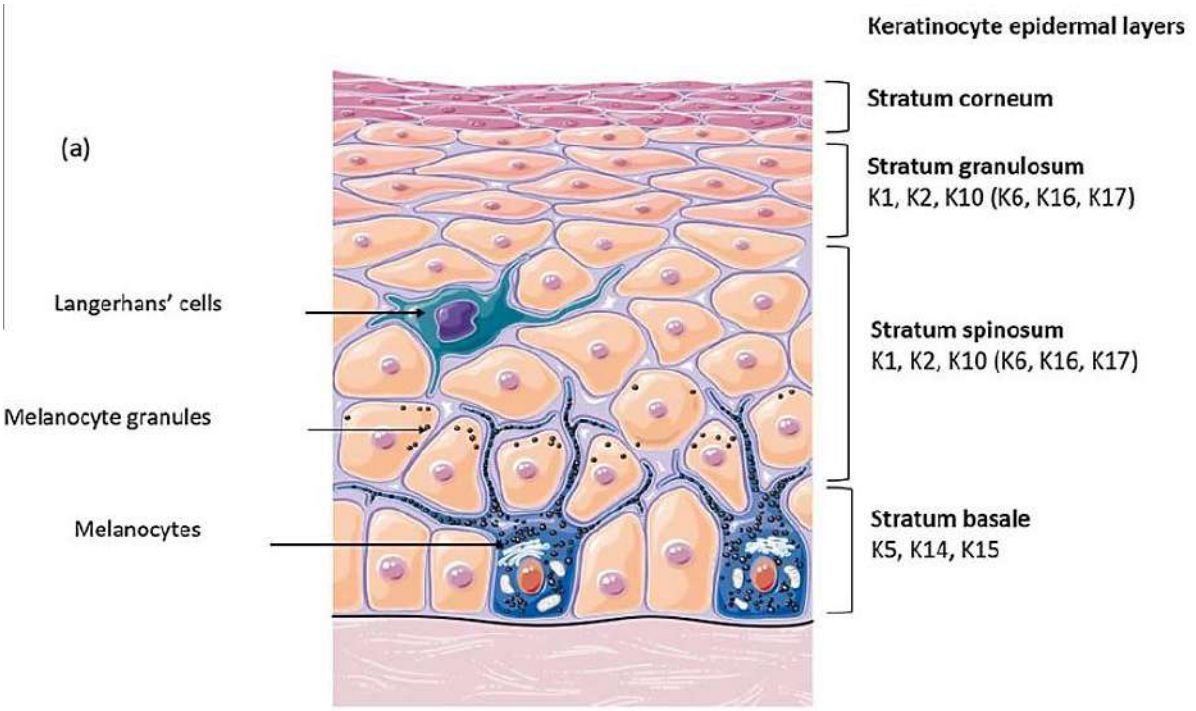


Figure (22): Schematic arrangement of keratins.

Design Criteria of Skin Tissue Engineering

The main goal of developing skin substitutes, either for skin replacements or for the development of *in vitro* tissue models, is to:

1- Create a system that is physiologically similar to that of native skin. Beyond mimicking the biochemical and structural components of skin, there are other

2- Cost effective

3- Has a long shelf-life

4- Low storage requirements,

5- Involves standardized processing (including sterility) and scaleup.

6- Skin replacements in the clinic need to adhere to the wound bed and protect this damaged tissue site from infection, be biocompatible and noninflammatory,

7- Match the physical and mechanical properties of skin,

8- Provide controlled degradation, allow for water vapor transmission,

9- Facilitate proper wound healing and angiogenesis.

10- Complexity (cell types, accurate barrier layers), especially in the development of *in vitro* tissue models to study mechanisms of skin disease damage and regeneration and for testing therapeutics.

Skin Substitute Technology

1- Biological skin substitutes can either be from:

i. Autologous,
ii. Allogenic, or
iii. Xenogenic sources and have been developed from human skin or from biomaterial-based strategies, including:

a) ECM components such as collagen or chondroitin sulfate, for example. In addition, some skin substitutes consist of cultured cell sheets or cell suspensions.

b) Synthetic materials used as substrates for these systems include:

- ✓ Silicone,
- ✓ Nylon,
- ✓ Poly(lactide-co-glycolide) PLGA,
- ✓ Poly (ethylene glycol) PEG, and
- ✓ Poly(caprolactone) PCL.

These scaffolds are often combined with either keratinocytes and/or fibroblasts to facilitate proper wound healing.

2- Novel technologies—3D printed skin equivalent constructs:

Three-dimensional (3D) printing of skin can provide:

1- High control over size and thickness of layers,
2- Spatial resolution in seeding cell types,
3- Selection of cell types,
4- The ability to seed cell-specific layers, and

5- Utility in patient-derived replacement applications.

One advantage of 3D printed systems versus standard engineered tissues involves improved reproducibility. The main approaches for 3D printing skin include:

1- Inkjet,

2- Laser, and

3- Bio extrusion processes or combination processes

The main considerations for 3D printed skin tissue constructs include:

1- The biomaterial or ink composition,

2- Cell type(s),

3- Cell sourcing,

4- Printing parameters, and

5- The general requirement of including an air/liquid interface to encourage differentiation and barrier function of the resultant skin tissue when compared to more traditional scaffold fabrication approaches.

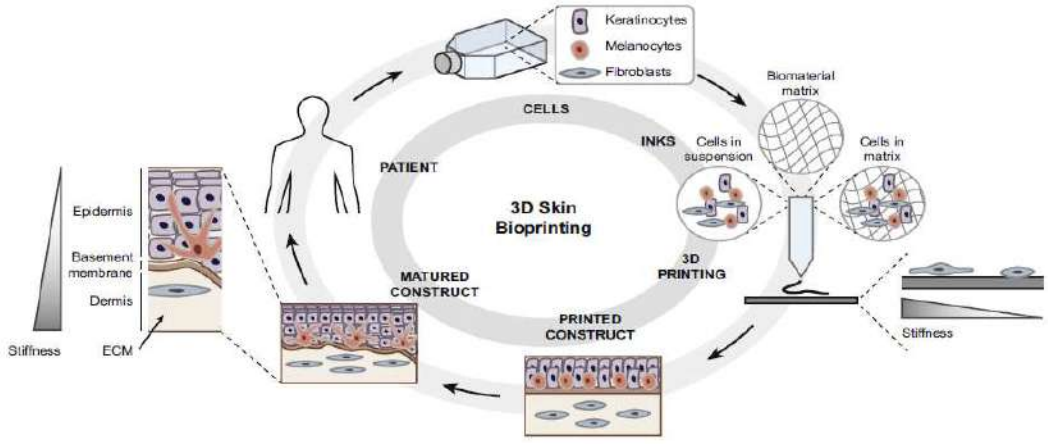


Figure (23): Schematic of patient-derived bioprinting of human skin and generalized approaches with design considerations—matrix stiffness is important to control both bioink and tissue structure. ECM, Extracellular matrix.

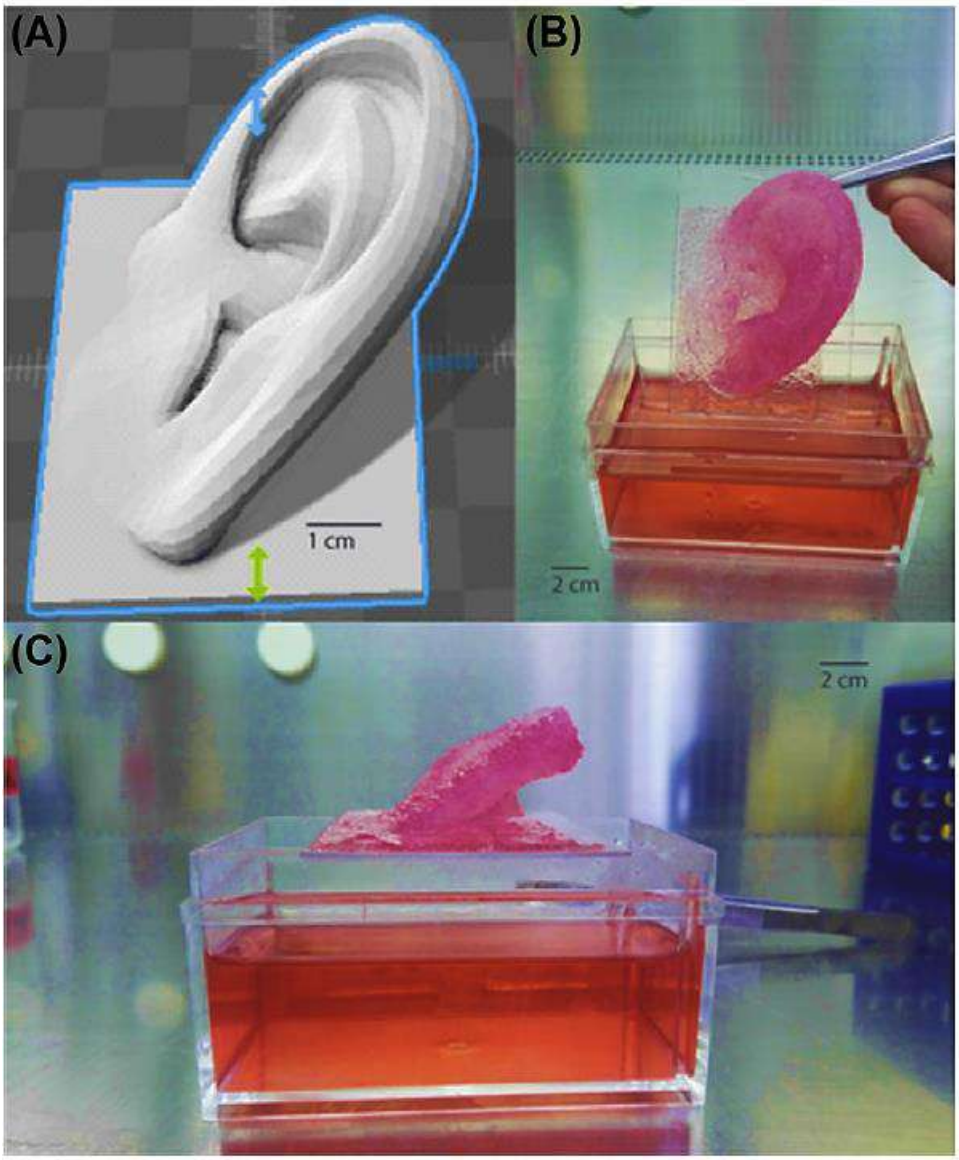


Figure (24): "Scaffold-free" bioprinting of human skin in complex 3D architectures allow for proof-of-concept printing of a human ear.

Hard Tissue Engineering

- Hard tissue regeneration that used to repair and replace damaged tissues such as the bones, and the teeth, advances significantly with advances in multiple tissue engineering strategies. The hard tissues, also called calcified tissues contain unique cell types and composed of both inorganic and organic matrices.
- The bone tissues consist of osteoblasts, bone lining cells, osteocytes, and osteoclasts.
- Bone is a dynamic tissue which could keep the remodeling and/or regeneration cycle constant. However, in critical conditions, the ability to re-generate is restricted, requiring special treatment for regeneration. The organic phase of bone contains 90% of the collagenous proteins (type 1 collagen) and 10% of non-collagenous proteins (e.g., osteocalcin, osteonectin, osteopontin, fibronectin, etc). Whereas, the inorganic phase consists of phosphate and calcium ions in the form of hydroxyapatite crystals ($Ca_{10}(PO_4)_6$ $(OH)_2$)). The collagenous and the non-collagenous matrix proteins organize to form the scaffold for hydroxyapatite deposition and impart typical stiffness and resistance to bone tissue.
- On the other hand, the tooth is a highly complicated organ composed of both hard tissues and soft tissues with unique characteristics and functions.
- Hard tissues in tooth include the enamel, cementum, dentin, and alveolar bone.
- Major cells types involved in dental tissue formation is ameloblasts (form enamel), odontoblasts (form dentin), cementoblast (form cementum), osteoblast and osteoclast (form alveolar bone). Enamel is the hardest tissue in the human body that contains the highest percentage of minerals (96%). In contrast to enamel, dentin is soft and flexible and able to absorb energy and resist fracture. Cementum helps to cover the tooth root and provides proper attachment to the periodontal ligament. The structure and cell composition of hard tissues (e.g. bone and tooth) are depicted in Fig. 10.1.

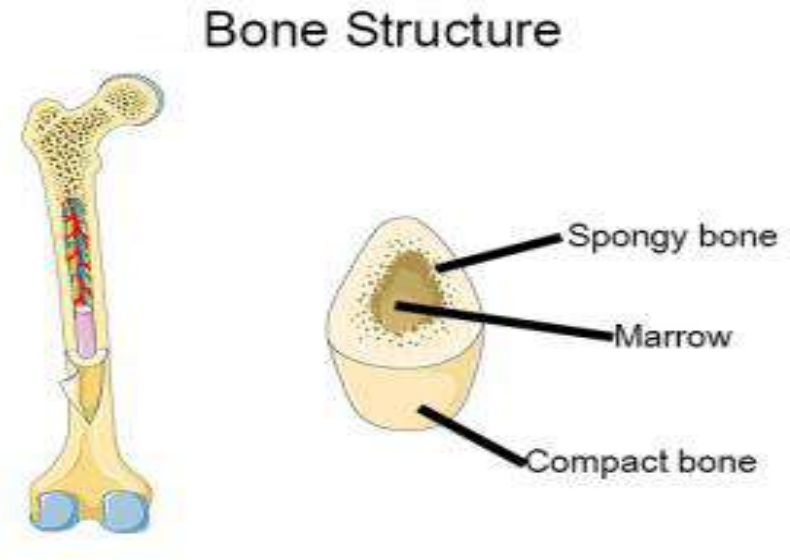


Figure (25): Bone structure.

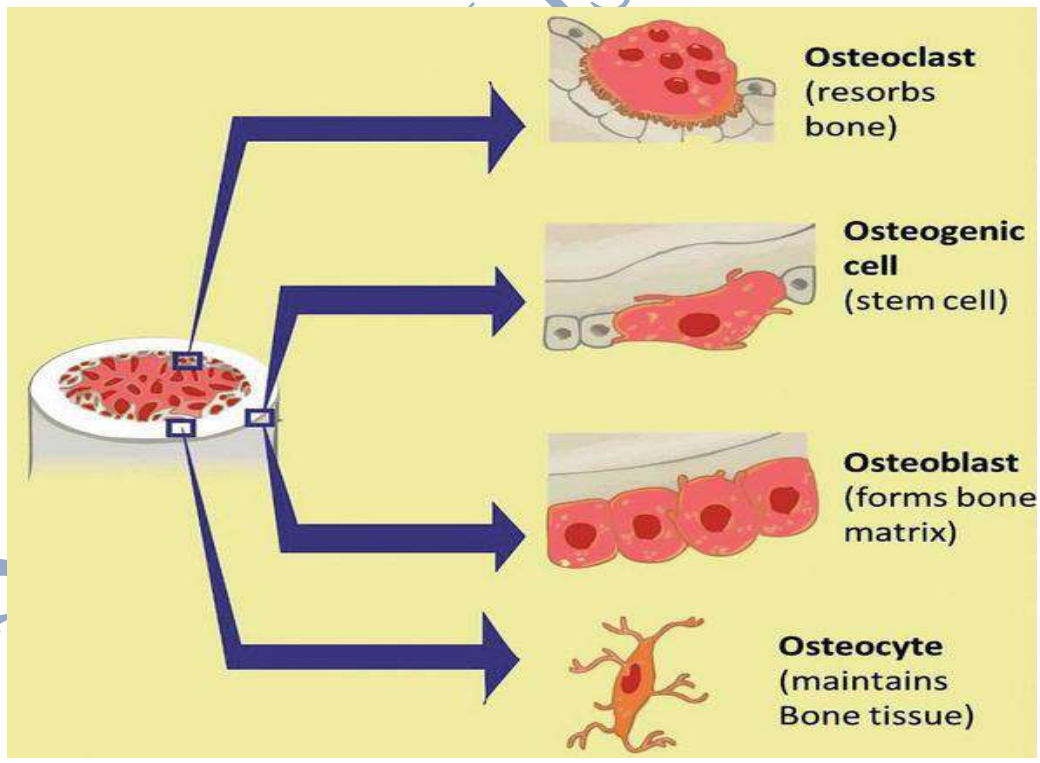


Figure (26): Bone cells.

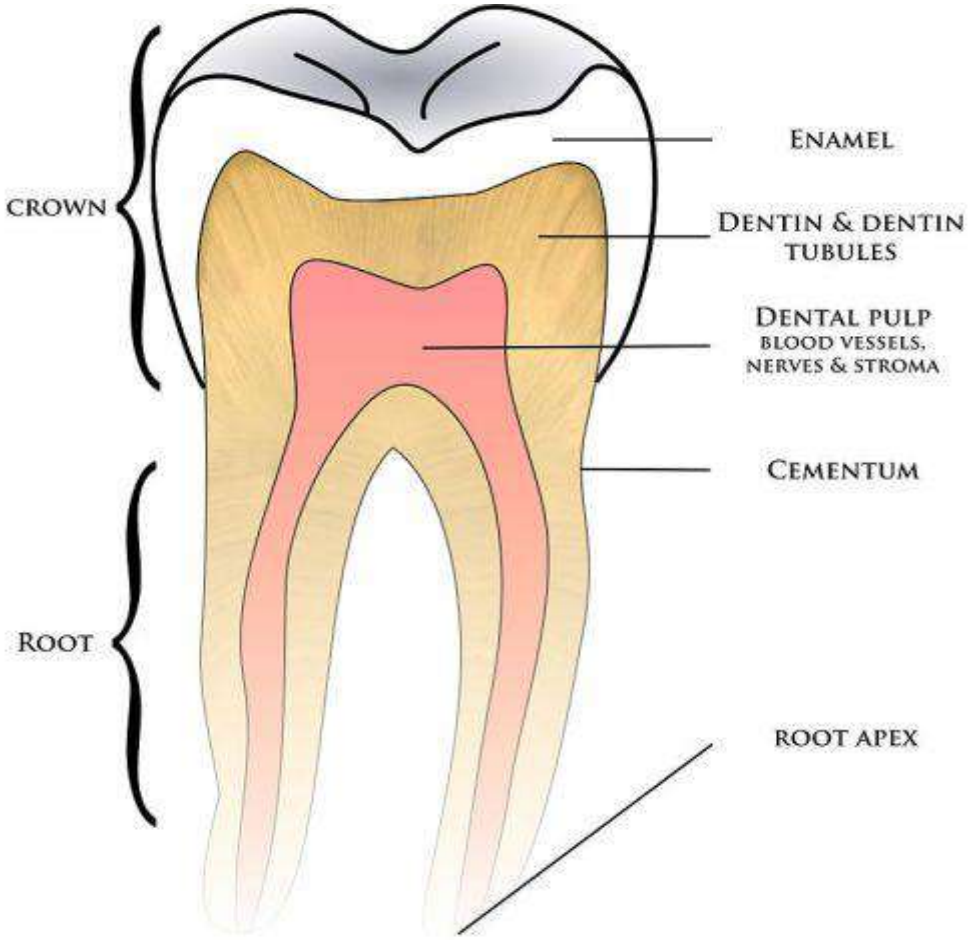


Figure (27): Tooth structure.

- The bone, which is a principal human tissue, consists of various components, like osteoclasts, osteoblasts, and osteocytes. It takes a big role in moving the body, controlling the body's physiological function, and protecting and supporting the organs.
- •Osteocytes and osteoblast take important roles in the formation of bone, while osteoclasts are associated with the re-absorption of the present bone tissue and re-generation. Also, osteoclasts help to stabilize bone formation ions.
- Synthetic materials for bone grafting should differ in shape, size, composition, mechanical strength, and porosity, since the structure and shape of the human

skeleton depends entirely on location and function. The subsequent three principal properties for an adequate bone re-generation must be presented according to a perfect scaffold for the tissue engineering of bone (BTE):

1- Osteoconduction

This describes the scaffold's ability to allow the growth of new tissue on the surfaces of the implant's external and internal (pores). The material should enable the bone cells to adhere on its surface and pores, proliferate, and make an extracellular matrix (ECM). The physical and chemical composition of the material governs these characteristics. In addition, the biocompatibility, porosity, and material mechanical

competence impact these properties. Perfectly, the scaffold should possess an optimum size of pore (at least 100 μm in diameter) to encourage the neovascularisation and the nutrients and gases diffusion needed to form the new bone.

2- Osteoinduction

This characterizes the scaffold's ability to attract and stimulate immature cells to a healing site to develop into bone-forming cells. The scaffold should also be able to fit in any shape of defects, so it must be malleable with shape and size varieties.

3- Osteointegration (Osseointegration)

It's a time-related healing process, via which a balanced implant anchorage is attained through the direct contact of bone-to-implant on the implant surfaces. There are three biological stages necessary to achieve the final bone regeneration. These are:

1. Incorporation through the woven bone formation,

2. Adaptation of bone mass to load (laminated and fibrous bone deposition) and

3. Adaptation of bone structure to load (bone remodeling).

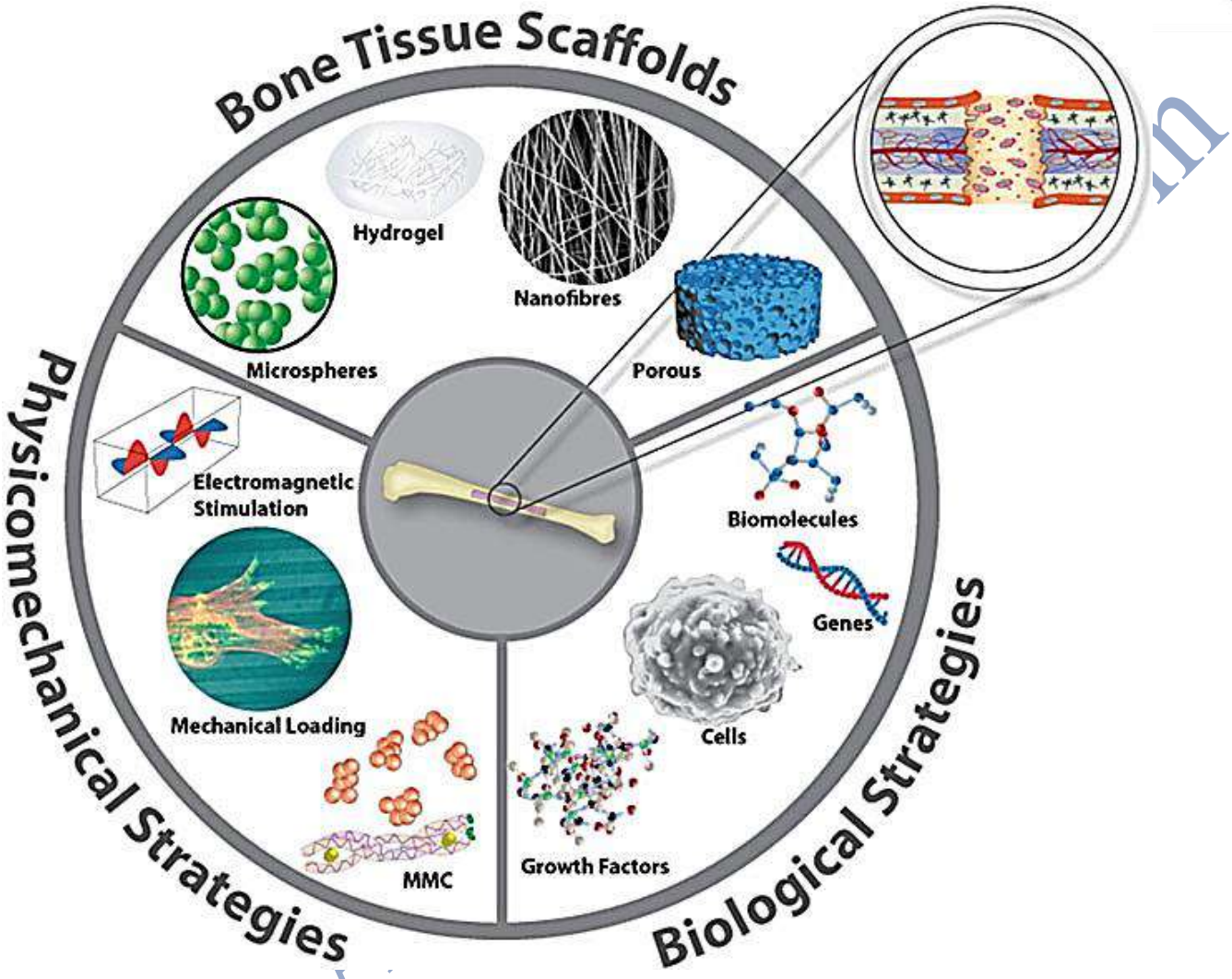


Figure (28): Different types of scaffolds (porous matrix, nano-fiber mesh, hydrogels, and microspheres) used to deliver bioactive molecules. This can be combined with a number of physic mechanical strategies to enhance treatment of various bone tissue defects and diseases.

Scaffold requirement of effective bone tissue regeneration:

Properties	*Description*
Cytocompatibility	✓ The scaffold or its released products should not elicit inflammation or toxicity *in vivo*.
Biodegradability	✓ The degradation rate of the scaffold should match the rate of tissue regeneration by external-enzymatic/biological process. ✓ Scaffold should resorb after fulfilling the purpose.
Bioactivity	✓ Scaffold should interact with the tissue according to osteoinductive and osteoconductive principles
High porosity	✓ Interconnected pores induce cell adhesion, cell distribution, migration, and thereby enhance bone tissue ingrowth. ✓ In addition, increased surface area of porous scaffold provides site for the formation of chemical bond between the bio ceramics and host bone. ✓ On the other hand, the porosity should not affect the mechanical stability
Mechanical features	✓ Scaffold should reproduce elastic and fatigue strength of the bones tissue site.
Tunable properties	✓ Scaffold should have customizable properties. ✓ Easy manufacturing
Processability	✓ Scaffold should be easy to be fabricated and sterilized. ✓ Easy clinical manipulation is a key factor

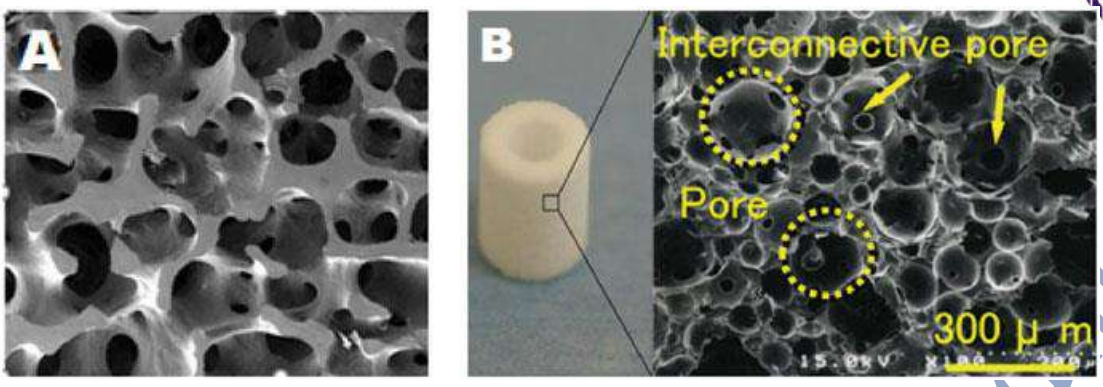


Figure (29): (a) SEM images showing interconnected porous structure of human trabecular bone (b) SEM image of hydroxyapatite scaffold. Interconnected pores are clearly visible.

Advanced materials used in Tissue Engineering: Shape Memory Polymers

Shape-memory polymers (SMPs) are polymeric smart materials that have the ability to return from a deformed state (temporary shape) to their original (permanent) shape when induced by an external stimulus (trigger), such as temperature change.

Properties of Shape-Memory Polymers

SMPs can retain two or sometimes three shapes, and the transition between those is often induced by:

1- Temperature change

2- The shape change of SMPs can also be triggered by an electric or magnetic field, light or solution.

3- Chemically Induced SMPs: Shape memory polymers induced by chemical triggers such as PH, water, solvents or biological agents are classified as chemically induced SMPs.

4- PH-Induced SMPs: Physiological human body PH varies depending on human body area and on pathological conditions. Therefore, PH-sensitive SMPs find an interesting application in the biomedical field.

These types of SMPs are obtained by addition of reversible crosslinks or sensitive PH groups (amino, carboxyl and sulfonic groups) that, when exposed to different PH environments, cause inclusion or complexes' dissociation.

Like polymers in general, SMPs cover a wide range of properties from stable to biodegradable, from soft to hard, and from elastic to rigid, depending on the structural units that constitute the SMP.

SMPs include thermoplastic and thermoset (covalently cross-linked) polymeric materials. SMPs are known to be able to store up to three different shapes in memory. SMPs have demonstrated recoverable strains of above 800%.

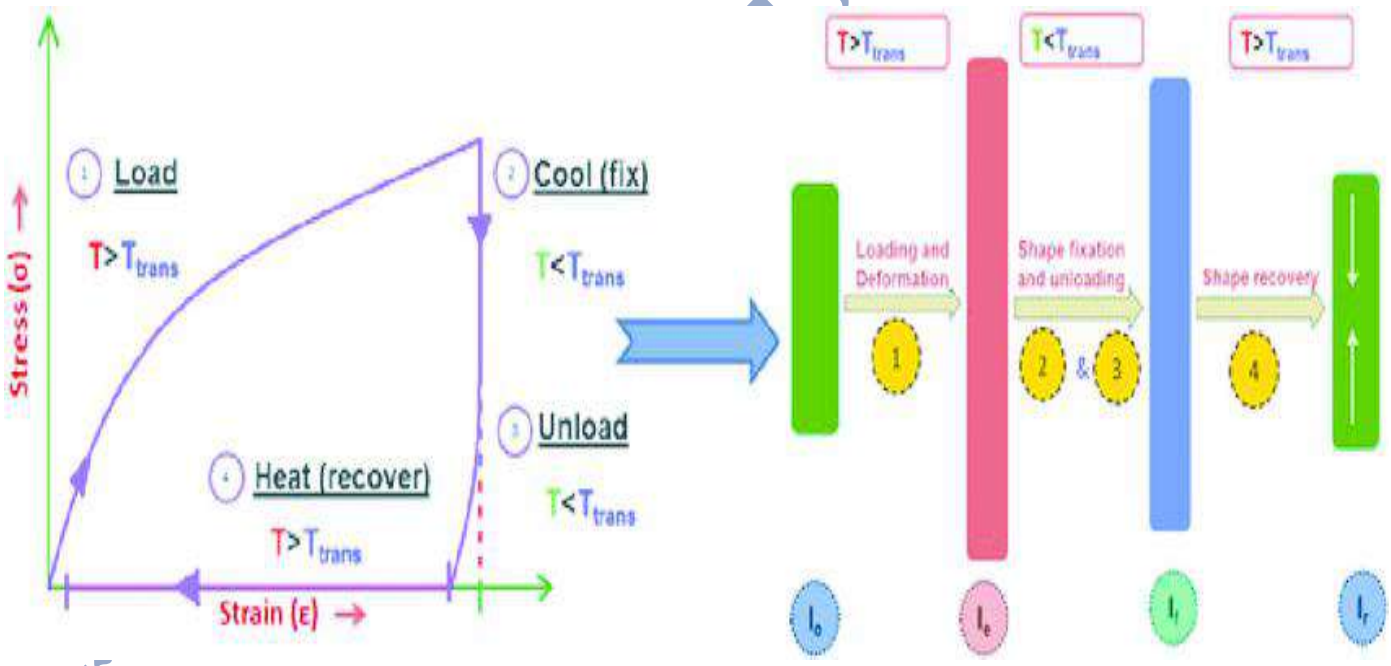


Figure (30): Polymer that, after heating and being subjected to a plastic deformation, resumes its original shape when heated above its glass-transition or melting temperature.

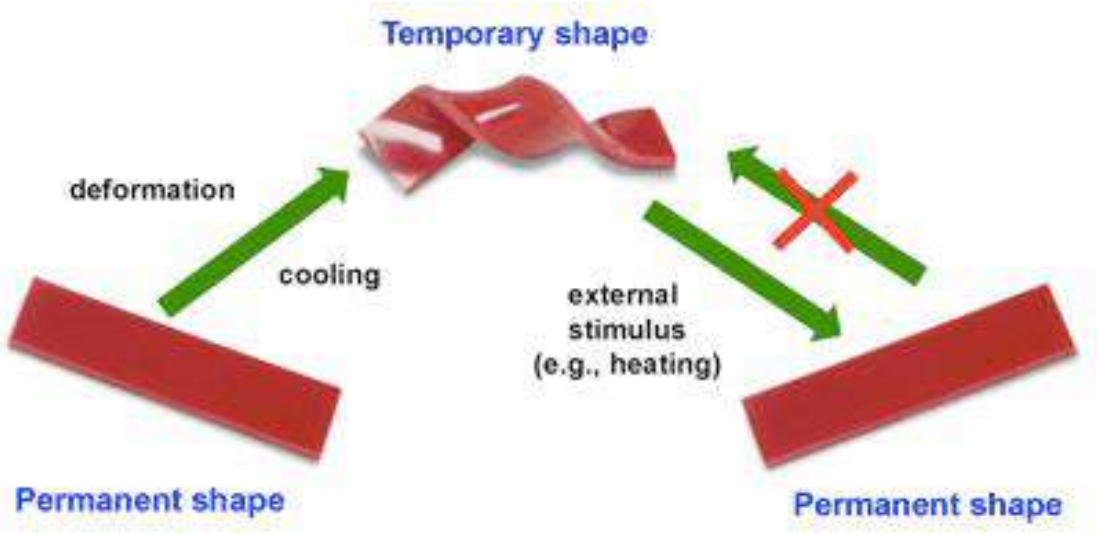


Figure (31): Shape memory polymers.

Biodegradable Shape-Memory Polymers (Bsmps) and Their Application in Biomedical Fields:

PCL	Tracheal stent Drug release Sutures
PU	Embolization Contraception 3D scaffolds Hemostatic devices
PLA	Stent Bone tissue engineering
PLGA	Embolization 3D scaffolds

SMPs can be also classified according to their shape-memory effect (SME), as one-way shape-memory effect (OWSME), two-way reversible shape-memory effect (TWSME) and multiple-SME (Figure 32), whose definitions are reported here below.

OWSMEs: materials lose shape reversibility meaning that when SMP recovers its original shape, another step is necessary to induce temporary shape.

TWSMEs: are materials able to switch between original and temporary shape several times without applying a further reshaping. TWSMEs can also be called reversible shape-memory effect (reversible SME), and the polymers with reversible SME are called reversible SMPs.

Multiple-SMEs: materials that show two or more than two temporary shapes in addition to original shape. Transition from first to second temporary shape is allowed by external stimulus and further stimulation permits them to return to the polymer original shape.

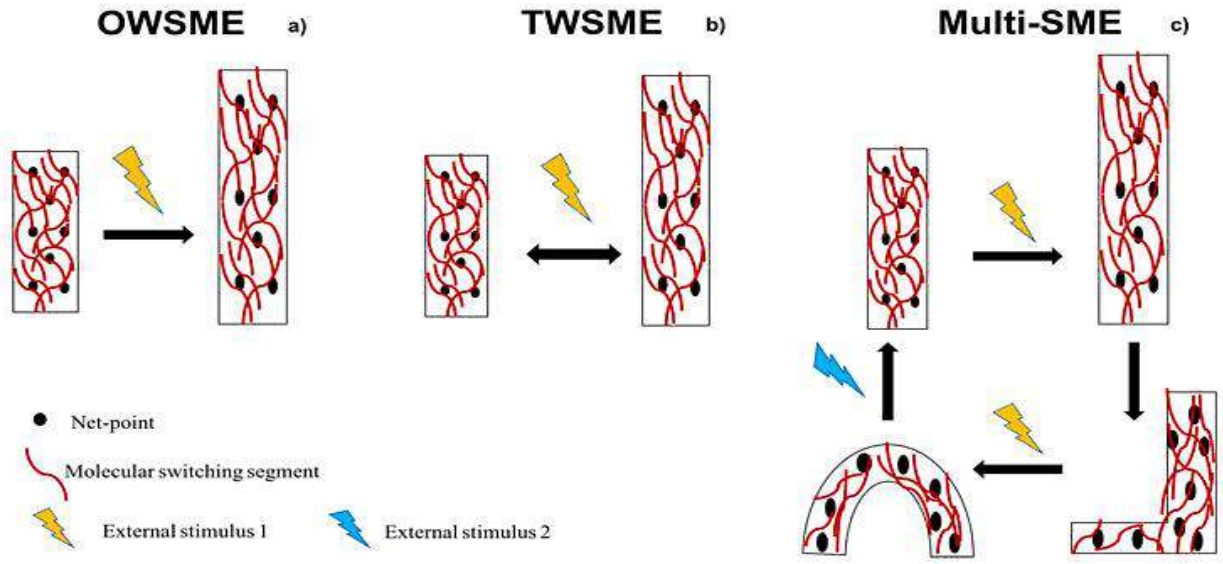


Figure (32): SMPs with different shape-memory effect (SME): (a) One-way (OWSME), (b) Two-way reversible (TWSME) and (c) Multiple-SME.