UNIVERSITÁ DEGLI STUDI DI TERAMO

Corso di Laurea Magistrale in Biotecnologie Avanzate
AA 2021-2022

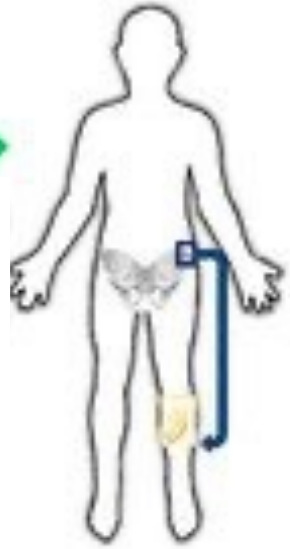
INTRODUCTION TO TISSUE ENGINEERING

HISTORY OF TISSUE ENGINEERING

Figure 1.1 Some random images showing the development of regenerative medicine throughout different eras in history. (Nerlich 2000 [13]. Reproduced with permission of Elsevier.) (a) 2500 BC: false big toe developed in ancient Egypt. (b) 278 AD: Saints Cosmas and Damian performing a leg transplant from a deceased donor onto a patient with an amputated leg. (Zimbler 2001 [15]. Wikipedia, public domain, https://commons.wikimedia.org/wiki/File: Fra_Angelico_064.jpg.) (c) In 2013, Chinese doctors saved a man's severed hand by grafting it to his ankle before later reattaching it to the patient's arm. (Gordon 2006 [21]. Reproduced with permission of John Wiley and Sons.)

CURRENT TREATMENTS

AUTOGRAFT



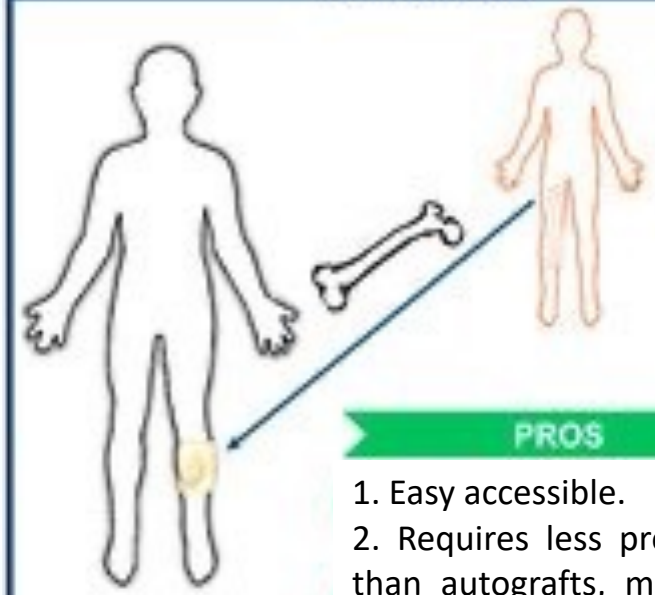
PROS

1. Provides matrix with osteogenic cells and osteoinductive factors to support new bone growth.
2. Better osseointegration and lack of immunological rejection

CONS

1. Short availability and more than one surgery is required.
2. Risk of site morbidity, pain, infection and scarring.
3. Weakening of the donor bone.

ALLOGRAFT



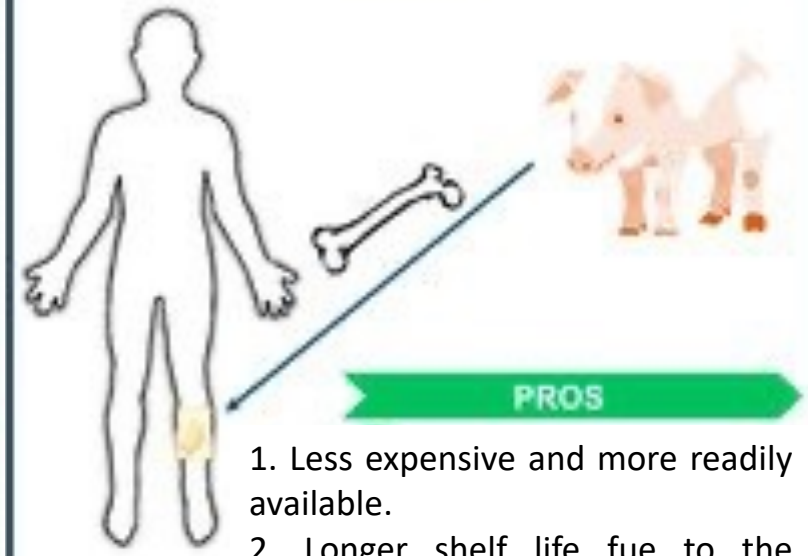
PROS

1. Easy accessible.
2. Requires less procedures than autografts, minimizing the surgical time.

CONS

1. Higher risk of immunological rejection.
2. Risk of infection
3. Reduced osteoinductive factors.

XENOGRAFT



PROS

1. Less expensive and more readily available.
2. Longer shelf life fue to the extensive sterilization processes.

CONS

1. Higher risk of immunological rejection.
2. Risk of infection and slower osseointegration.
3. Reduced osteoinductive factors.

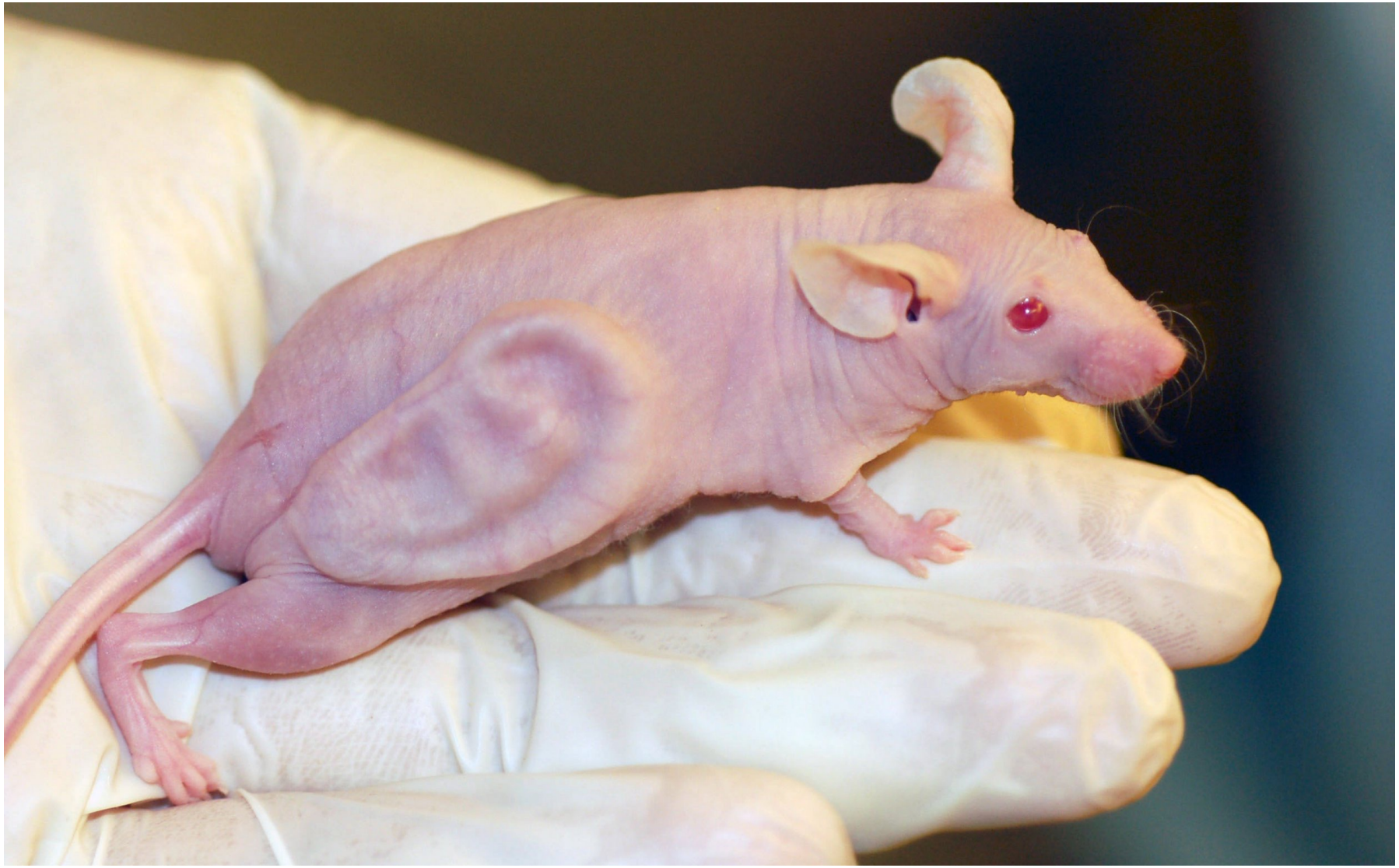
TISSUE ENGINEERING (TE)

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 3D artificial tissue and organs which can be used to augment, repair and/or replace damaged and/or diseased tissue.

"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

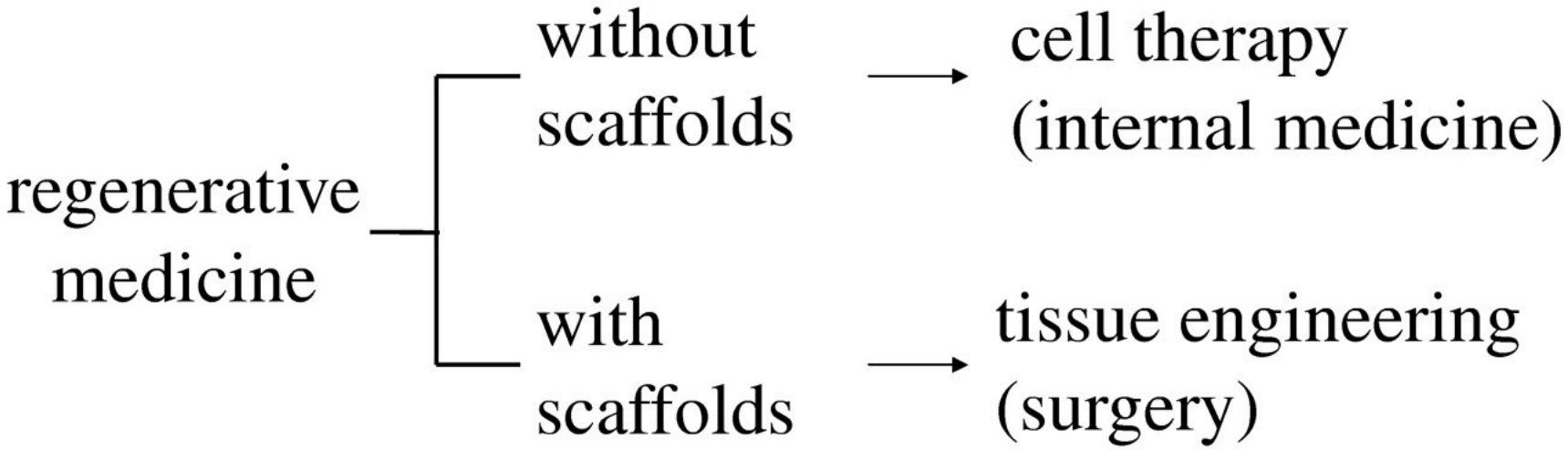
VACANTI MOUSE MODEL: LIMITATIONS?



TE AND REGERNATIVE MEDICINE

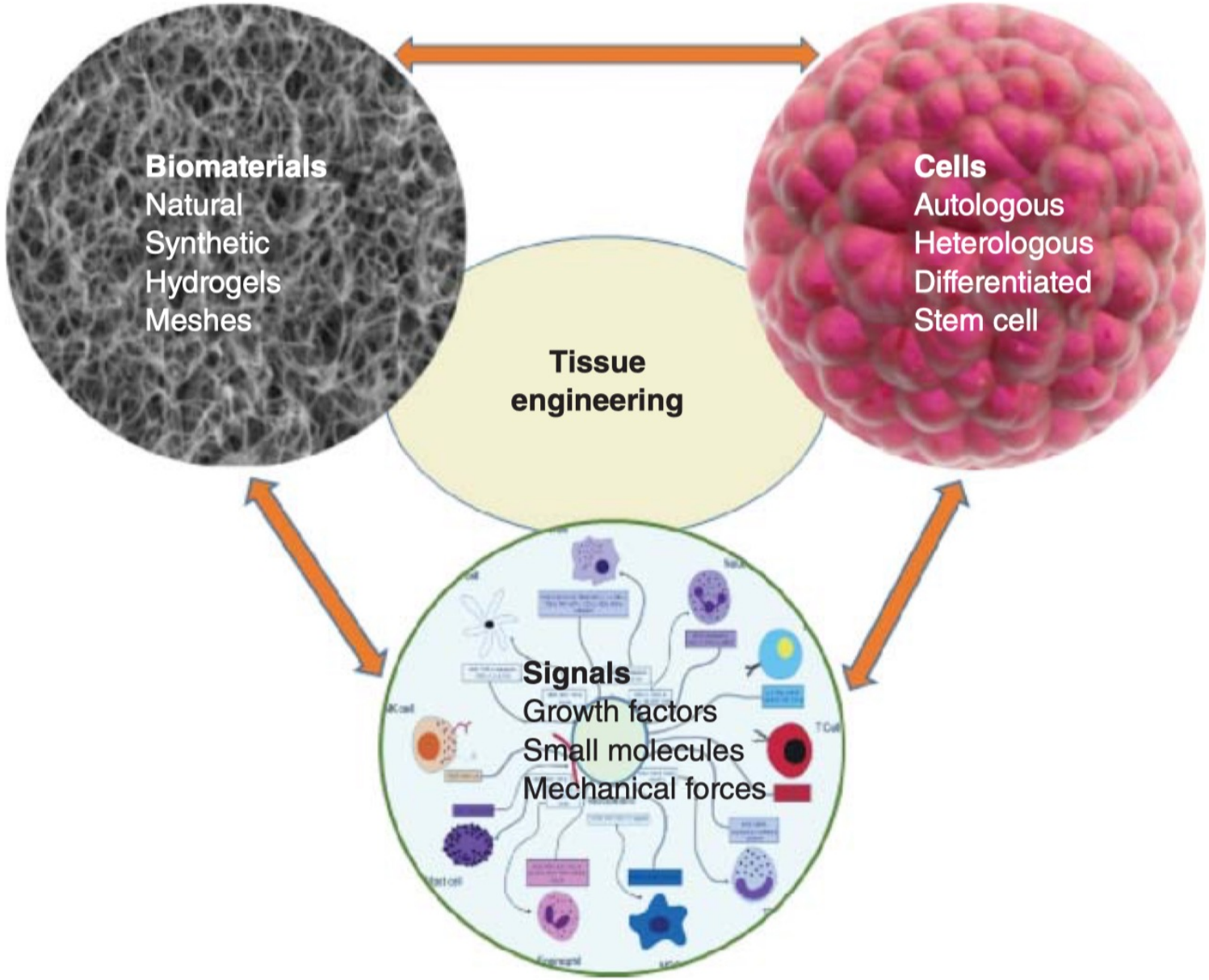
Tissue engineering and regenerative medicine are often used interchangeably.

- TE involves the construction of a tissue in vitro.
- Regenerative Medicine refers to tools for helping the body regrow a damaged tissue in vivo in the patient. The need for cell sources in tissue engineering was a major limiting factor in the advancement of the field. This shortage of cell sources ignited the use of renewable cells such as stem cells and progenitors, leading to the term “regenerative medicine.”
- **Regenerative medicine** is mostly based on understanding morphogenesis and natural, inherent self-repair mechanisms, and, as such, regenerative medicine typically involves the use of stem cells and progenitors.



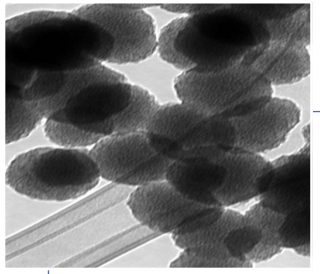
Tissue engineering and regenerative medicine, often abbreviated as “TERM,” are today complementary.

The TRIAD Of TE

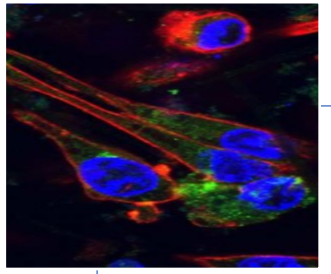


LONG-TERM OBJECRTIVE Of TE RESEARCH

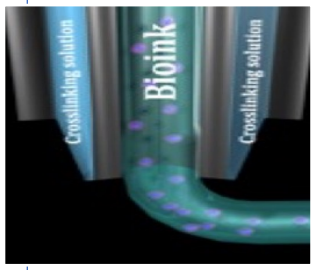
Multidisciplinary unit composed of chemists, engineers and biologists.



Synthesis of novel biomaterials

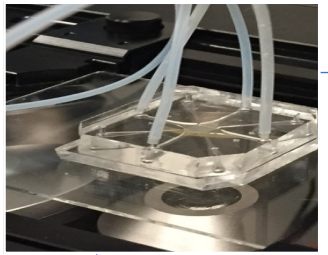


Chemical & biological characterization of biomaterials



Advanced manufacturing processes

- electrospinning
- additive manufacturing



Cell/organ-on-a-chip models

TISSUE ENGINEERING AND RELATED FIELDS

The field of tissue engineering is closely related to the field of controlled release and can be viewed as an extension of gene/protein/cell therapy.

- **Gene Therapy** is defined as the process by which genes, small DNA, or RNA molecules are delivered to human cells, tissues, or organs to correct a genetic defect, or to provide new therapeutic functions for the ultimate purpose of preventing or treating diseases.
- **Protein therapy** is defined as the delivery of proteins to cells, tissues or organs to provide a therapeutic function. Therapeutic angiogenesis, a specialized case of protein therapy, involves delivering angiogenic growth factors to support the formation of new blood vessels.
- **Cell therapy** involves use of isolated cells that are delivered to damaged or diseased tissue for therapeutic purposes.

Comparison Between Gene Therapy, Protein Therapy, Cell Therapy and Tissue Engineering—
The field of tissue engineering can be viewed as an extension to work in protein, gene and cell therapy. While the goal of these latter fields is to deliver specifically targeted cellular components at the site of damaged tissue, the goal of tissue engineering is to deliver 3D tissue at the site of injury.

TISSUE ENGINEERING AND RELATED FIELDS

❖ **Controlled Release**—The field of controlled release is very closely related to the field of tissue engineering, partly due to research in both fields being started by the same person, Dr. Robert Langer.

Controlled release strategies involve the release of a specific drug over time. Rather than providing a single dose at the time of administration, the goal is to provide sustained release over time, preferably with zero order kinetics, which means that the release rate is consistent with time.

The main advantage of controlled release strategies involves the ability to maintain therapeutic plasma drug levels without reaching extremely high or dangerously low concentrations.

❖ **Controlled Release and Tissue Engineering**—There are clear differences and similarities between the two fields.

The most important difference between the two fields is that controlled release is focused on delivery of a therapeutic agent to the injury site and does not involve cells. Tissue engineering, as we have seen, revolves around cells and the development of strategies to support tissue fabrication.

The commonality between the two fields is the use of scaffolds.

- In the case of controlled release technology, properties of the scaffold regulate release kinetics of drugs, which in turn dictate effectiveness of the therapy.
- In the case of tissue engineering, properties of the biomaterial are important to support tissue fabrication.

TISSUE ENGINEERING AND RELATED FIELDS

- **Cell encapsulation** is focused on the culturing of cells within a scaffold, which regulates the release of a therapeutic agent produced by cells into the culture environment.
  Cells are encapsulated within a 3D scaffold to protect from the host immune system; immune cells like neutrophils and macrophages cannot penetrate the barrier created by the scaffold. The scaffold acts as a semi-permeable membrane and blocks host immune cells; however, nutrients like oxygen and glucose can pass through the scaffold and reach cells.
  In the case of controlled release, polymer degradation is used to regulate the release kinetics of the drug while in the case of cell encapsulation, material degradation is not a prerequisite for success of the therapy; rather, the scaffold acts as a semipermeable membrane to support the release of therapeutic agents by encapsulated cells.

Cell Encapsulation and Tissue Engineering—The relationship between the two fields is the utilization of cells for therapeutic purposes. In the case of cell encapsulation, cells function to release specific proteins in the host environment, which serves a therapeutic purpose. In the case of tissue engineering, cells are used to support artificial tissue fabrication, which then acts to replace or restore function in damaged or diseased tissue.

BIOMATERIALS

According to the National Institutes of Health (NIH), a biomaterial is defined as

"any substance (other than a drug) or combination of substances **synthetic or natural in origin**, which can be used for any period of time, as a whole or part of a system which **treats, augments, or replaces tissue, organ, or function of the body."**

annual biomaterials symposia at Clemson University, the Sixth Annual International Biomaterials Symposium in April 20–24 th 1974, Clemson's Advisory Board for Biomaterials provided the following definition:

"a biomaterial is a systematically**, pharmacological inert substance** designed for implantation within or incorporation with a living system."

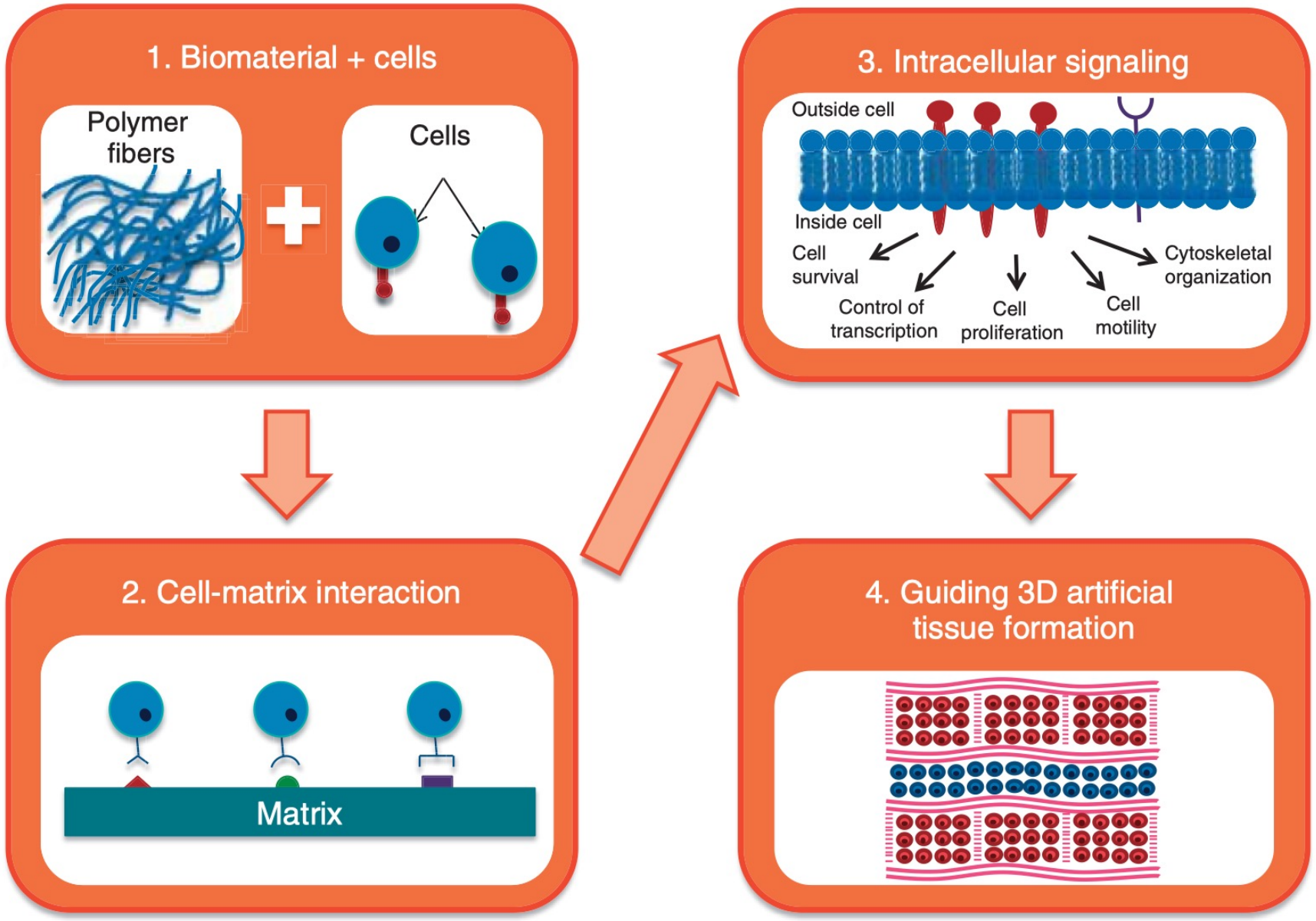
In Dr. Black publication in 1982:

"a biomaterial is any **pharmacological inert material**, **viable or non-viable**, natural product or man-made, that is a part of or **is capable of interacting in a beneficial way within a living organism**."

Sharma:

"biomaterials are materials designed **for interfacing and/or interacting with a living system**, **inducing no adverse reaction at the site of implantation in vivo or ex vivo and systematically**."

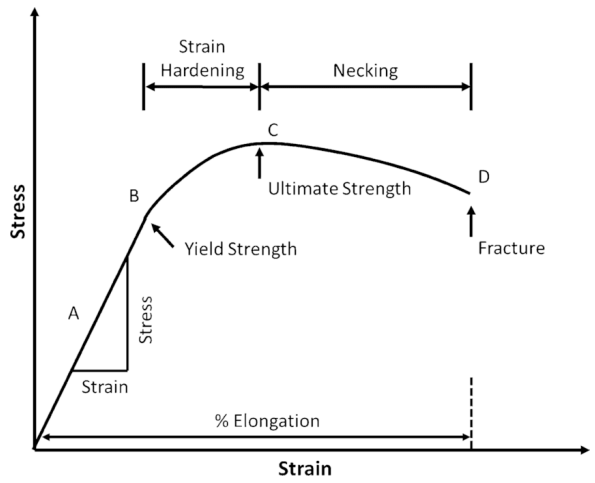
BIOMATERIALS



cell surface integrins and specific binding sites on the surface of biomaterials.

BIOMATERIALS DEVELOPMENT

Biomaterial degradation refers to the gradual breakdown of a biomaterial mediated in a controlled manner to support the fabrication of 3D artificial tissue



(e) Material degradation

(b) Biomimetic properties

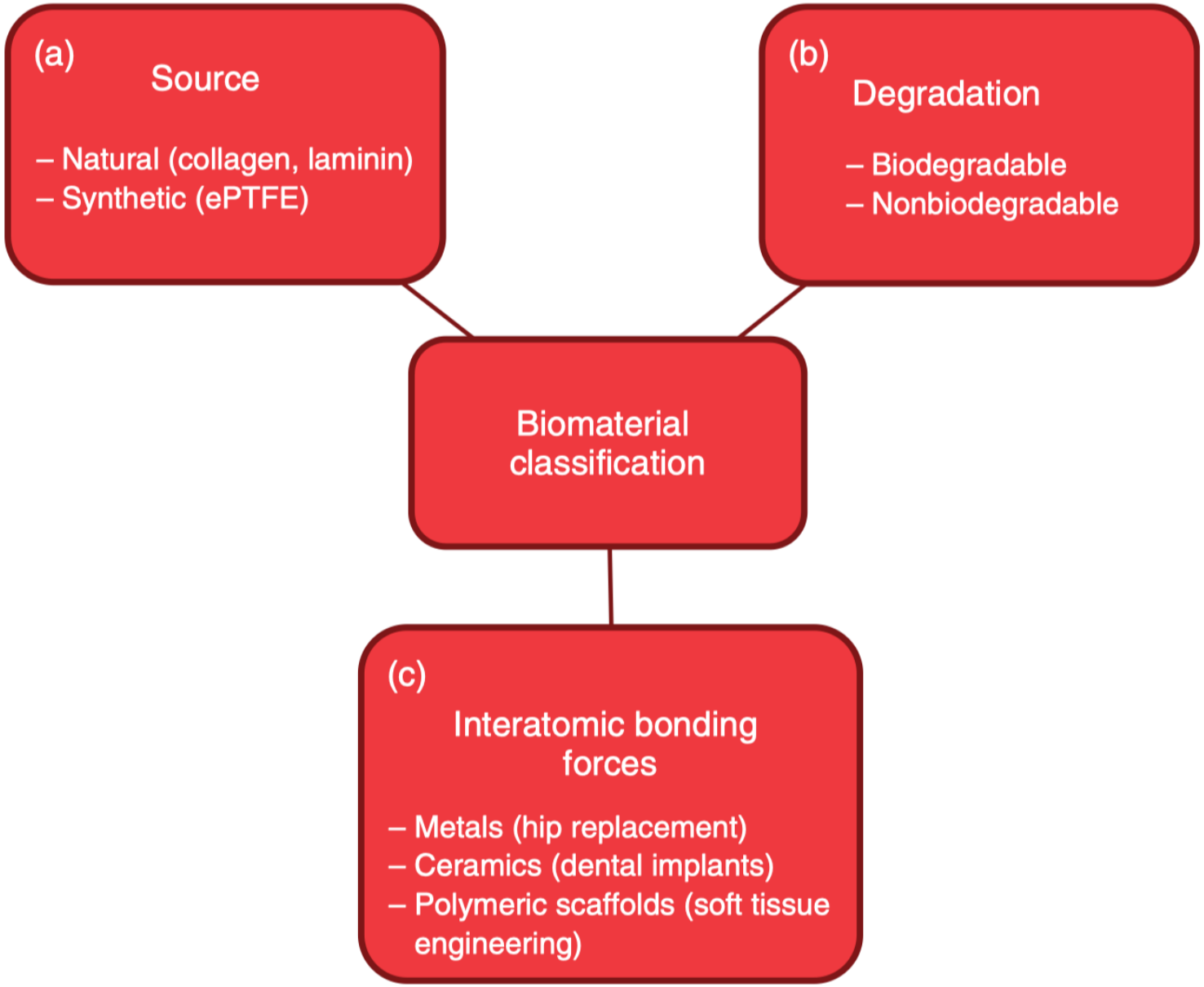
(a) Biomaterial platforms
- Biodegradable hydrogels
- Polymeric scaffolds
- Acellular scaffolds

(d) Mechanical properties

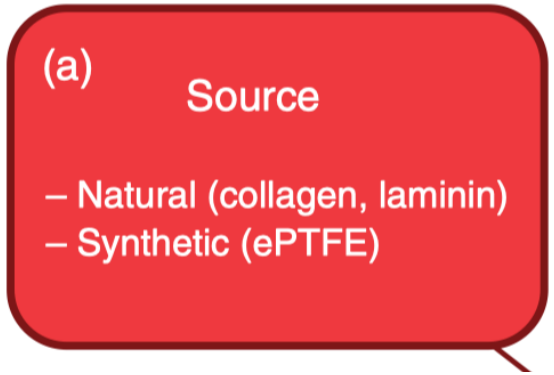
(c) Biocompatibility

The ability of 3D artificial tissue to be accepted by host defense mechanisms upon implantation, while maintaining functional capacity, is known as biocompatibility.

BIOMATERIALS CLASSIFICATION



BIOMATERIALS CLASSIFICATION



Natural materials have anatomically matched 3-dimensional architecture and are biologically active, thereby supporting functional interaction with isolated cells. However, the main disadvantage of natural materials is batch variability inherently due to differences in isolation efficiency.

Synthetic materials have the advantage of reproducibility, as synthesis is tightly regulated, and tenability, as materials of different properties and functionality can be fabricated. However, the main disadvantage of synthetic materials is the lack of biological functionality, as many synthetic materials do not have functional binding sites for isolated cells.

BIOMATERIALS CLASSIFICATION

(b)

Degradation

– Biodegradable
– Nonbiodegradable

The degradation kinetics of a material define the rate at which the material disintegrates or loses structural stability as a function of time.

On the surface, material degradation may appear to be a nondesirable material property, as loss of structural integrity can lead to catastrophic effects.

This is indeed the case for numerous medical applications, as in the case of knee and hip replacements, which are often fabricated using metallic components like stainless steel, and in which longevity of the implant is a critical functional determinant. Metallic materials fall into the category of nondegradable materials where long-term structural stability is essential for function.

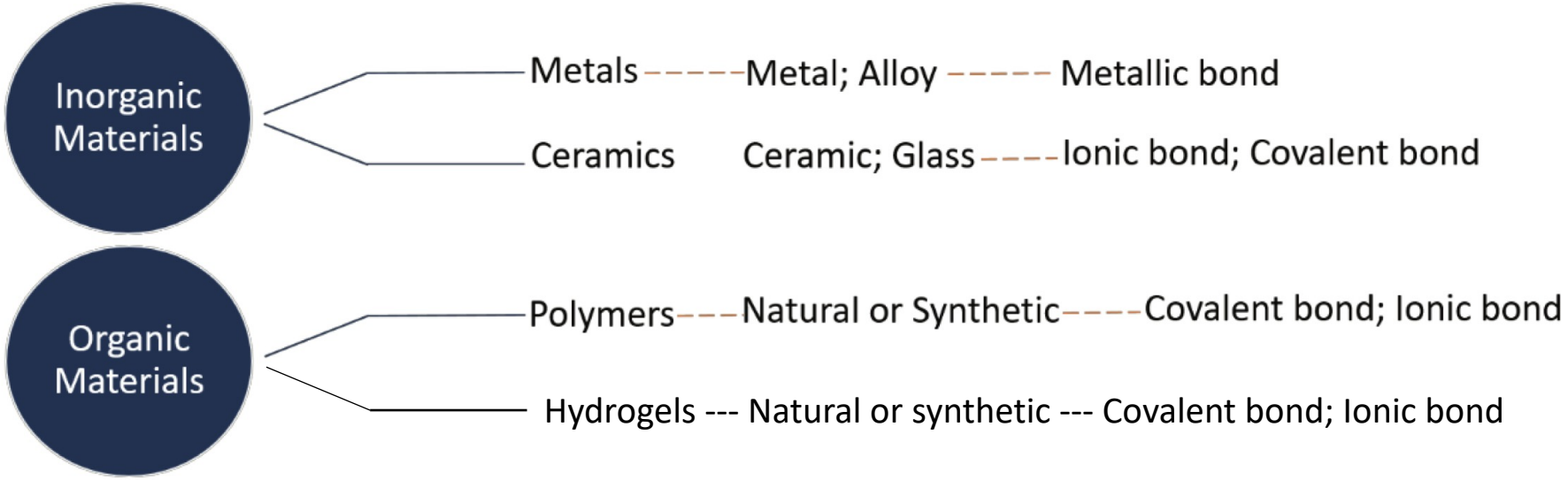
In the case of tissue engineering, a biodegradable material is molded into a scaffold and then populated with isolated cells. The cells use the scaffold as a temporary support matrix, and during the culture period, extracellular matrix components are fabricated by cells. During the tissue fabrication process, the material degrades and is replaced by extracellular matrix fabricated by the cells. In this case, material degradation is an important property required to support 3D tissue formation. The degradation kinetics of scaffolds is an important material property and is the focus of many tissue engineering studies.

BIOMATERIALS CLASSIFICATION

(c)

Interatomic bonding forces

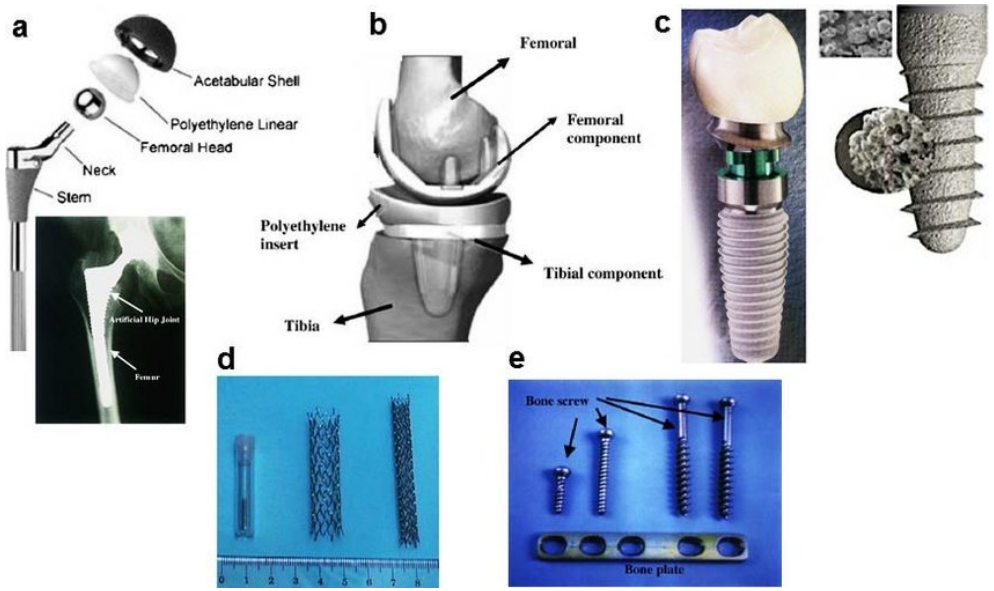
- Metals (hip replacement)
- Ceramics (dental implants)
- Polymeric scaffolds (soft tissue engineering)



BIOMATERIALS CLASSIFICATION

1. Metals

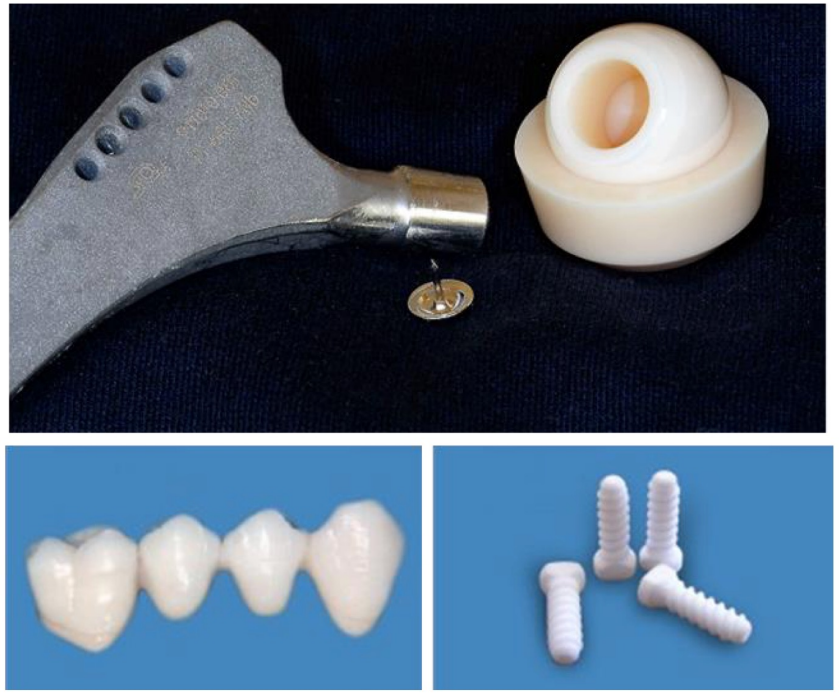
Metals are the most widely known biomedical materials and are indispensable in the medical field. Nearly all of the metal biomaterials are crystalline in nature, i.e., with regular atomic arrangements. Metals have great strength, resistance to fracture toughness, better elasticity, and rigidity compared with ceramics and polymers. Their outstanding mechanical reliability properties are controlled by dislocation and crystallization. For this reason, metals are extensively employed for loadbearing implant applications such as orthopaedic, dental, and maxillofacial surgery. Apart from these, metals are also used in making stents and stent-grafts for cardiovascular surgeries. The most common metals and alloys used for biomedical applications are stainless steel, titanium, titanium-based alloys, cobalt-based alloys, magnesium-based alloys, and tantalum-based alloys.

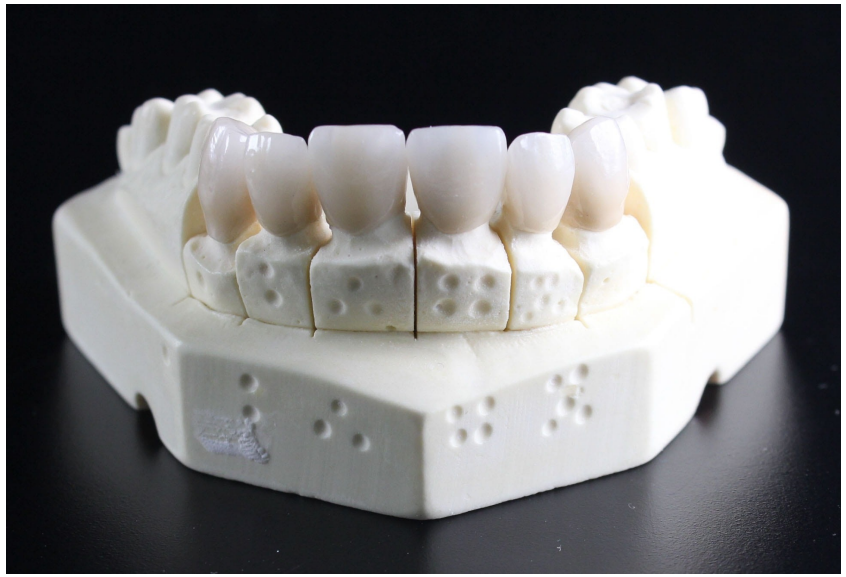


BIOMATERIALS CLASSIFICATION

2. Ceramics

Ceramics are inorganic solid materials consisting of metallic and nonmetallic elements that are predominantly bound together by ionic bonds. They exist as both crystalline and non-crystalline (amorphous) compounds. Ceramics are typically characterized by excellent biocompatibility, high corrosion resistance, high wear, high strength, extremely high stiffness, and hardness. The advancement of ceramic material applications in the biomedical industry has focused mostly on orthopaedics and dentistry. Bioinert ceramics such as alumina (Al_2O_3), zirconia (Zr_2O_3) and pyrolytic carbon, and bioresorbable ceramics such as calcium phosphates are some of the widely employed bioceramics.



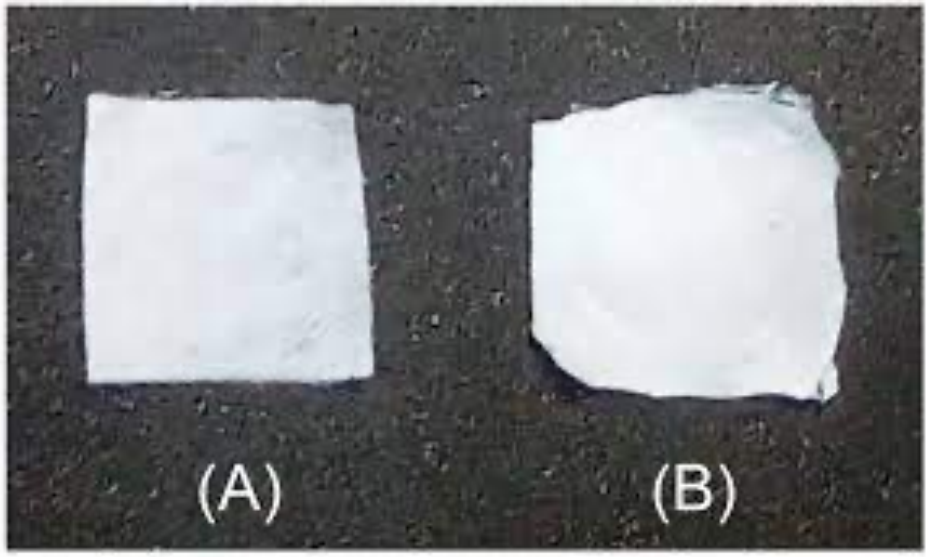


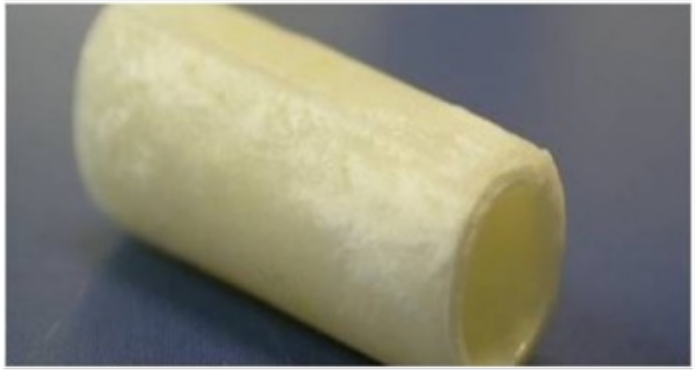
BIOMATERIALS CLASSIFICATION

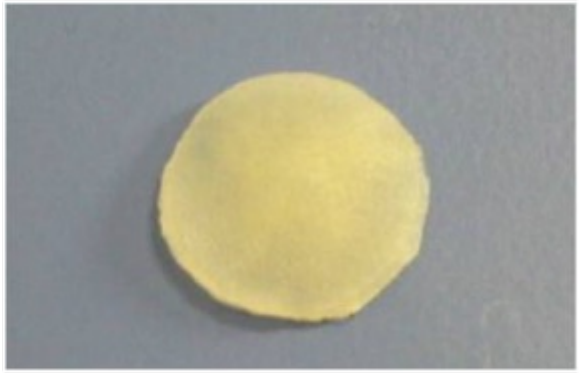
3. Polymers

Polymers are macromolecules and they represent a major and versatile class of biomaterials being widely applied in biomedical applications due to their low toxicity in biological fluids, easy pre/post-processing, sterilization, better shelf life, lightweight nature, and remarkable physical and chemical properties.

The main advantages of the polymeric biomaterials compared to metal or ceramic materials are ease of manufacturability to produce various shapes (latex, film, sheet, fibers, etc.), ease of secondary processability, reasonable cost, and availability with desired mechanical and physical properties. The required properties of polymeric biomaterials are similar to other biomaterials, that is, biocompatibility, sterilizability, adequate mechanical and physical properties, and manufacturability.







BIOMATERIALS CLASSIFICATION

3. Hydrogels

Hydrogels are three-dimensional network of hydrophilic cross-linked polymer that do not dissolve but can swell in water or can respond to the fluctuations of the environmental stimuli.

Hydrogels are highly absorbent (they can contain over 90% water) natural or synthetic polymeric networks.

Hydrogels also possess a degree of flexibility very similar to natural tissue, due to their significant water content.

Table 4.2: Classification of biomaterials and typical properties associated with them (Wagner, 2020).

Attributes	Polymers	Metals & Alloys	Ceramics
Type of bonds present	Covalent & van der Waals forces	Metallic	Ionic/Covalent
Melting point	Low	Intermediate	High
Chemical stability	Poor	Good	Very high
Electrical conductivity	Very low	High	Very low, but varies
Thermal conductivity	Very low to intermediate	High	Low
Properties and advantages	Degradable, inert, similar density to soft tissues and ease of processing	High strength and hardness	Non-conductive and inert; closely mimic biological properties of bone
Mechanical deformation	Very high, plastic (can be easily shaped and processed)	High (ductile)	Low (brittle)
Major issues	Thermally unstable; low strength	Wear and corrosion	High density and brittle
Biomedical applications	Soft tissue implants; drug delivery systems; tissue engineering	Hard tissue applications (Orthopedic and dental implants)	Tissue engineering