EPFL

Biomaterials for MX

MSE – 471 (2022)

Prof. Maartje M.C. Bastings

Course 2: Naturally derived biomaterials

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Definition of Biomaterials

'Any nonviable material (synthetic or natural) that comes in contact with a biological environment (cell, tissue, organ), and is intended for use in biomedical applications (prosthetic, diagnostic, therapeutic or storage) without adversely affecting the living organism and its components'

Biomaterials are defined by their **application**, NOT by the chemical composition or physical property

Biomaterials Science: An Introduction to Materials in Medicine
By Allan S. Hoffman, Frederick J. Schoen, Jack E. Lemons

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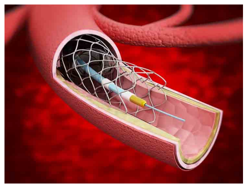
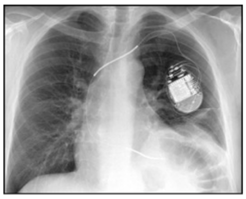
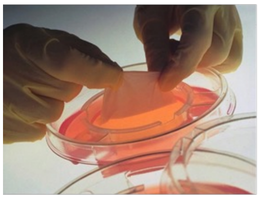
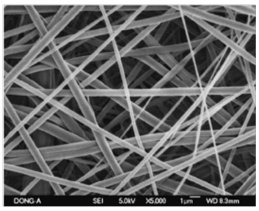
Everyday examples





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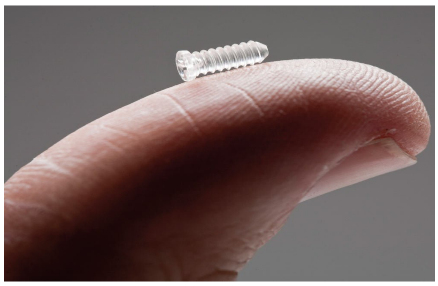
Biopolymers

Polymers are substances composed of **repeating structural units** that are linked together to form **long chains**. Humans have relied extensively on biological materials like wool, leather, silk, and cellulose, e.g. **natural polymers**.

1) polymers that are **produced by biological systems** such as micro-organisms, plants and animals, in short natural products;

2) polymers that are **synthesized chemically** but are **derived from biological starting materials** such as amino-acids, sugars, and natural fats or oils.



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Biopolymer classes

Polyesters	*Polysaccharides (fungal)*	Lipids/surfactants
Polyhydroxyalkanoates	Pullulan	Acetoglycerides, waxes
Polylactic acid	Elsinan	Surfactants, emulsan
	Yeast glucans	
Proteins		*Polysaccharides (bacterial)*
Silks	*Polysaccharides (plant/algal)*	Xanthan
Collagen/gelatine	Starch (amylose/amylopectin)	Dextran
Elastin	Cellulose	Gellan
Resilin	Agar	Levan
Adhesives	Alginate	Curd lan
Polyaminoacids	Carrageenam	Polygalactosamine
Soy, zein, wheat glutein,	Pectin	Cellulose (bacterial)
casein, and serum albumin	Konjac	
	Various gums (guar)	*Specialty Polymers*
Polyphenols		Shellac
Lignin	*Polysaccharides (animal)*	Poly-γ-glutamic acid
Tannin	Chitin/chitosan	Natural rubber
Humic acid	Hyaluronic acid	Synthetic polymers from natural fats and oils (nylon from castor oils)

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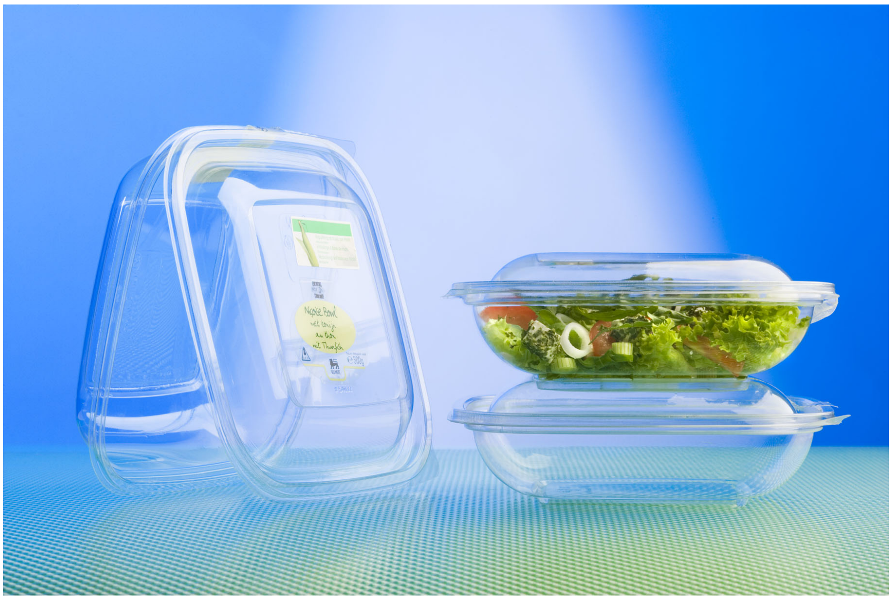

https://www.ecomena.org/poly-lactic-acid/

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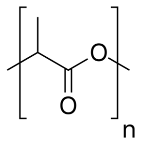
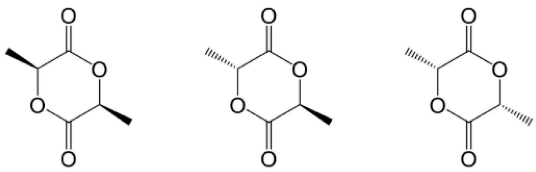
PLA (Poly Lactic Acid) materials

gold standard for many orthopaedic regenerative engineering applications (and esthetic medicine)

- versatility in fabrication,
- biodegradability,
- Biocompatibility,
- Processability
- Optical clarity
- Low cost



Mechanical Parameter	PLA	PLLA	PDLLA
Melting temperature, (°C)	150–162	170–200	Amorphous
Glass transition temperature (°C)	45–60	55–65	50–60
Ultimate tensile strength, (MPa)	21–60	15.5–150	27.6–50
Ultimate tensile strength, (MPa)	21–60	15.5–150	27.6–50
Tensile modulus (GPa)	0.35–0.5	2.7–4.14	1–3.45
Ultimate tensile strain (%)	2.5–6	3.0–10.0	2.0–10.0
Specific tensile modulus (kNm/g)	0.28–2.8	0.80	2.23–3.85



L-lactide meso-lactide D-lactide

Polylactic acid (PLA) controlled delivery carriers for biomedical applications
https://doi.org/10.1016/j.addr.2016.06.018

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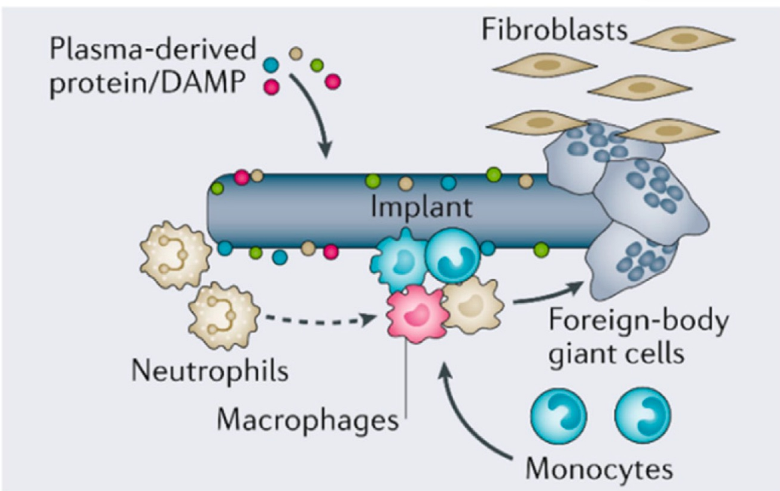
PLA Degradation



- **Degradation by cells, enzymes, and environment**
- **Degradation product is non toxic and metabolized!!**

Ref: Giant, 18, 2024, 100261 (on moodle)

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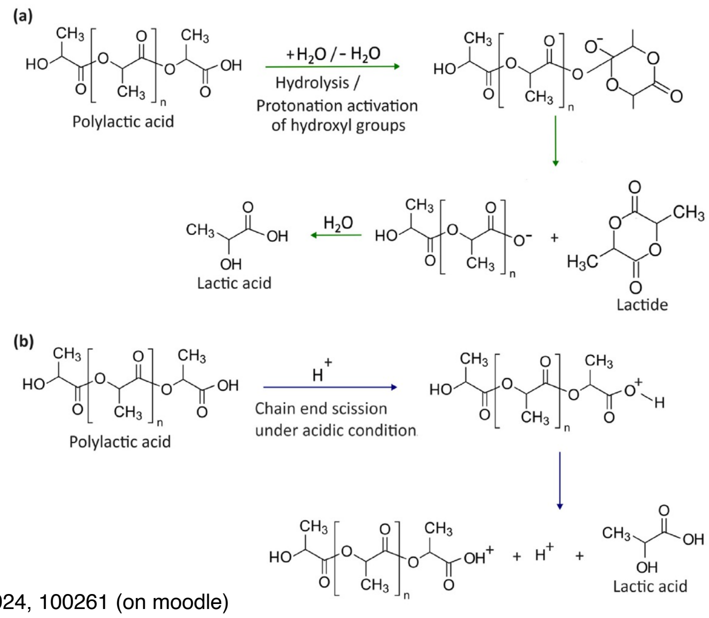
PLA Degradation



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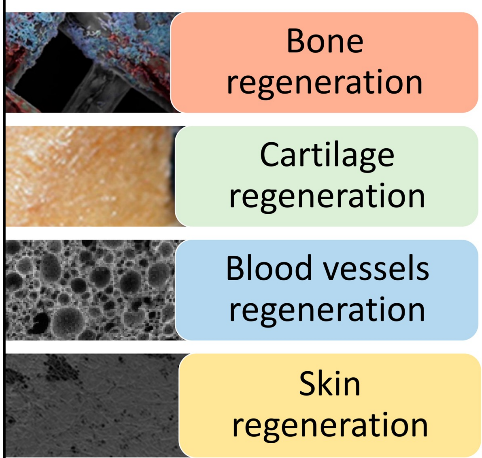
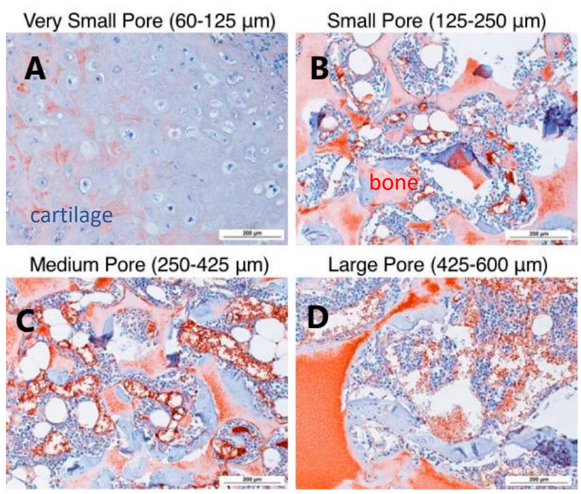
PLA (Poly Lactic Acid) materials



relationship between pore size and chondrogenesis



histological analysis after 8 weeks of subcutaneous implantation

Polymers **2022**, *14*(6), 1153; https://doi.org/10.3390/polym14061153

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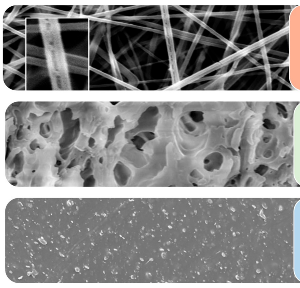
PLA (Poly Lactic Acid) hybrid materials

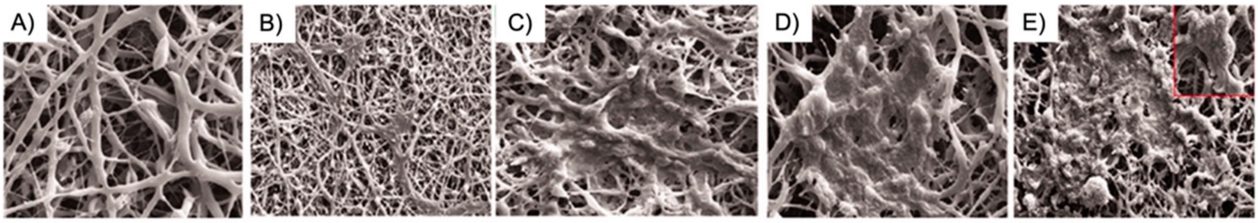


PLLA/natural polymers

PLLA/synthetic polymers

PLLA/inorganic biomaterials

Most often PLLA, why?



No cells | Seeded with cells | Start of growth | Islet like clusters

Polymers **2022**, *14*(6), 1153; https://doi.org/10.3390/polym14061153

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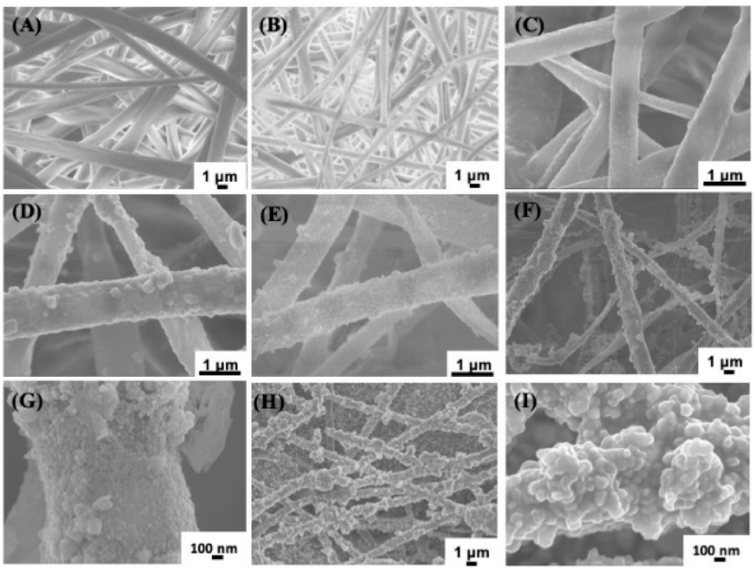
PLA (Poly Lactic Acid) hybrid materials



Coating with silver nanoparticles

Why?

Polymers **2022**, *14*(6), 1153; https://doi.org/10.3390/polym14061153

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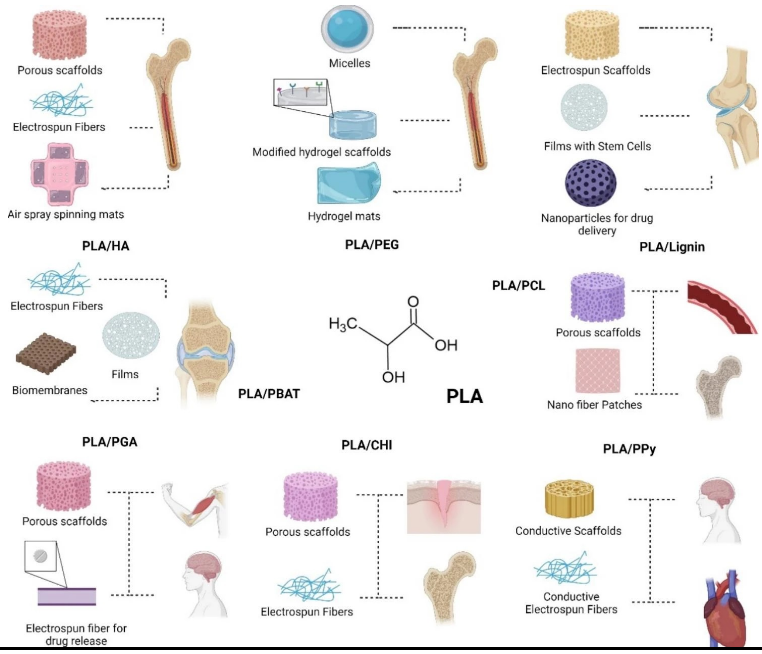
PLA (Poly Lactic Acid) hybrid materials



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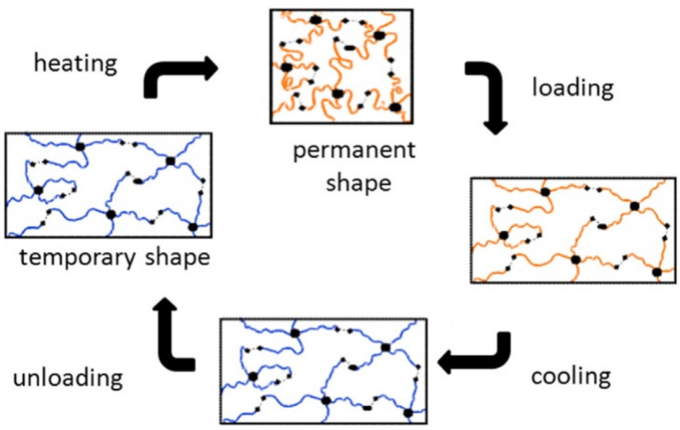
Shape memory and bio-resorbable behavior

Change between permanent and temporal cross-linked network

Co-polymer present with physical cross-links

T < Tg = temporal locked in shape

T > Tg = chemical crosslinks only



The shape memory effect (SME).

Shape memory materials (SMMs) are featured by the ability to recover their original shape from a significant and seemingly plastic deformation when a particular stimulus is applied.

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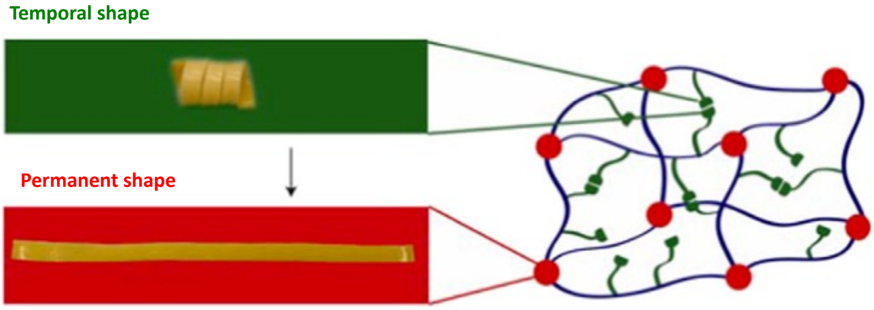
Shape memory and bio-resorbable behavior

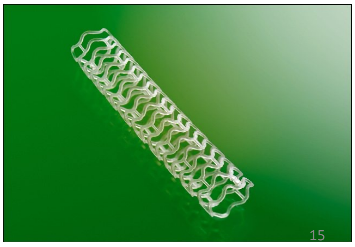


The shape memory effect (SME).

Shape memory materials (SMMs) are featured by the ability to recover their original shape from a significant and seemingly plastic deformation when a particular stimulus is applied.

Useful for:

Injection
Drug delivery
Low-invasive surgery



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PLA degradation

Why is biodegradation useful?

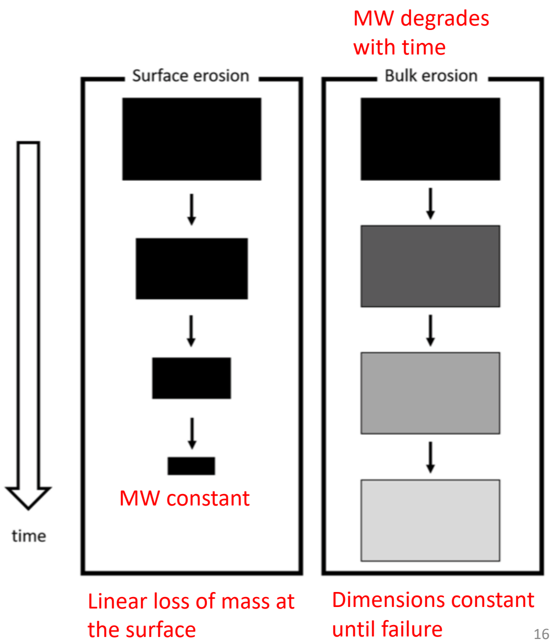
1) Temporary needs → only 1 surgery needed
2) Avoid chronic inflammation

PLGA → lactic acid / glycolic acid
→ CO2 and H2O

Degradation influenced by:
1) Bond stability
2) Hydrophobicity
3) Steric effects
4) Production of autocatalytic products
5) Microstructure (pores, crystallinity,…)
6) Environment

Helps degradation

Resists degradation



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Protein-based natural materials: Silk

Silk is **natural fibrous protein** which is spun by *Lepidoptera larvae* such as silkworms, spiders, scorpions, mites, and flies

Silk has been used as a biomaterial in various forms such as **films, membranes, gels, sponges, powders, and scaffolds.**

Can be prepared as:
gel, powder, film, matrix, or fibre

Applications:
scaffolds, vascular prostheses, structural implants, nets, and sutures

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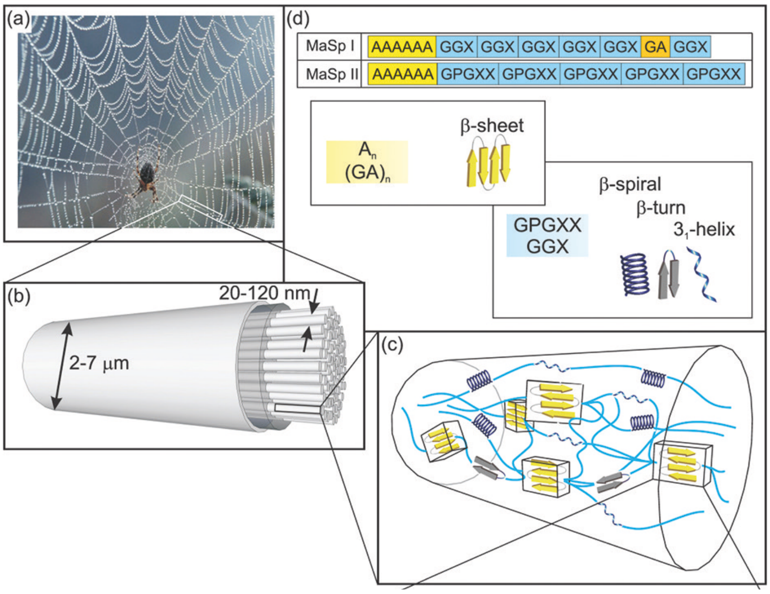
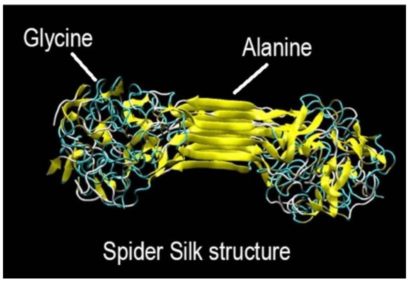
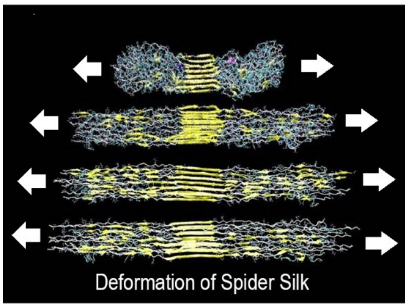
Hierarchical structure







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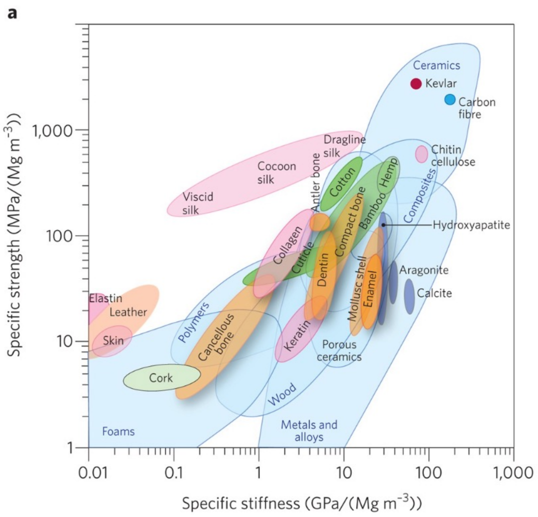
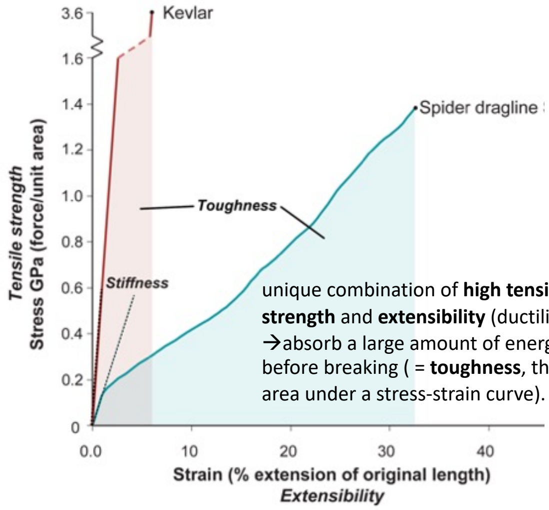
Exceptional properties

Hierarchical mix of structured semi-crystalline domains and random coil polymer domains





unique combination of **high tensile strength** and **extensibility** (ductility).
→absorb a large amount of energy before breaking (= **toughness**, the area under a stress-strain curve).

Bioinspired structural materials. Nat Mater

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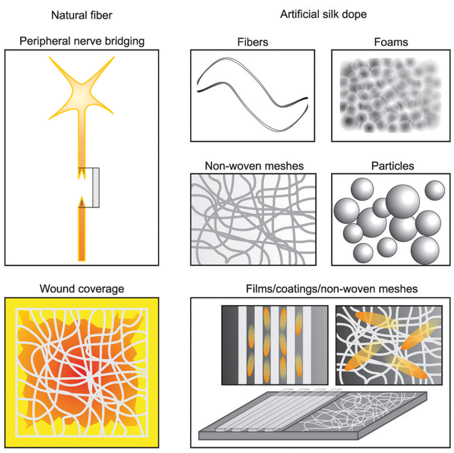
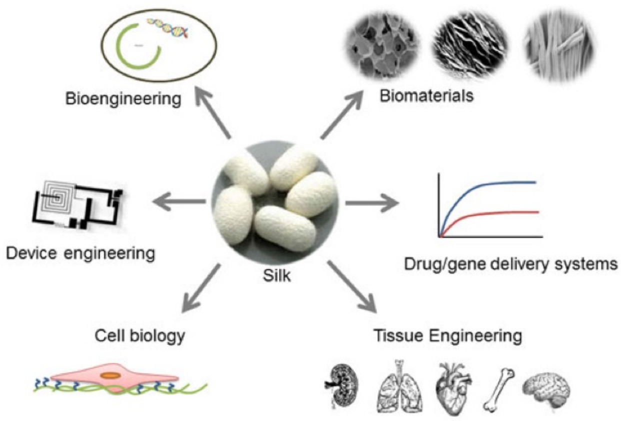
Silk as Biomaterial





Typical fabrication strategies for silk proteins and architectures produced.

Technique	Typical architectures	Length scales
Self-assembly	Nanofibers, films, micro and nanospheres [80]	nm–μm
Electrospinning	Fibers, mats [51]	μm–mm
Casting, molding	Membranes, mats, micro needles [68,75]	nm–mm
Spray drying	Micro and nanospheres [61]	nm–μm
In situ polymerization	Fibers, hydrogels [53,87]	μm–mm
Freeze drying	Porous sponges [19,94]	μm–mm
Soft lithography, nano-imprinting, inkjet printing	Nanostructures, wires, photonic elements [122,194]	nm–μm

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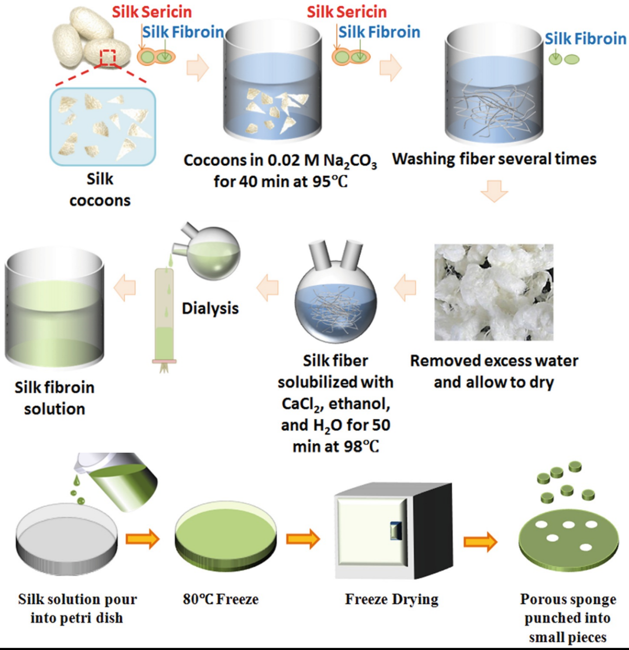
Example: Silk Wound Dressings

The unique properties of SF, as its **biocompatibility**, **biodegradability**, **high water and oxygen uptake**, **low immunogenicity**, and **robust mechanical properties**, make it an exceptional choice for wound healing.

Scaffolds containing different growth factors and signaling molecules are preferable for wound regeneration because they can **interact with cells and consequently enhance cell behavior during the healing process.**

Despite the successful use of SF in wound dressings, it is **not yet approved as an artificial skin**.



Park C.H. (2018) Silk Fibroin in Wound Healing Process.
In: Chun H.,. Et al Novel Biomaterials for Regenerative Medicine.
Advances in Experimental Medicine and Biology, vol 1077. Springer
https://doi.org/10.1007/978-981-13-0947-2_7

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Silk pavilion

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Protein-based natural materials: Collagen

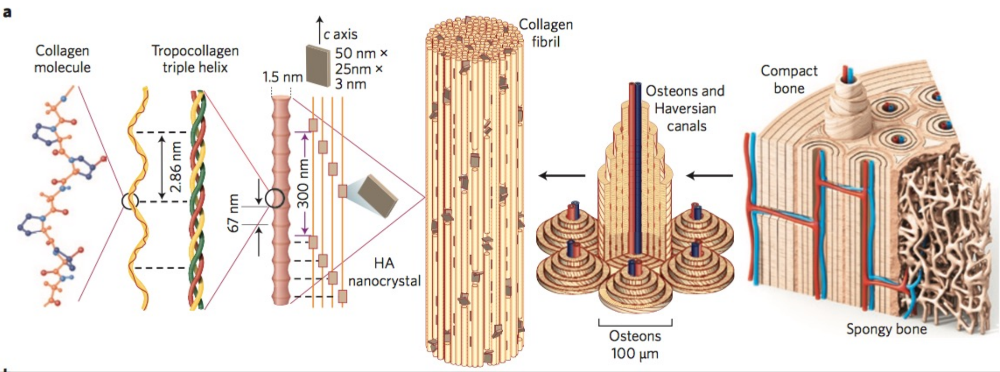
Collagen is a protein that acts as a **structural support** in a wide range of tissues including skin, bone, tendons, ligaments, cartilage, blood vessels, and nerves.

It has a characteristic **triple-helix structure** that forms **fibrils** and is the most abundant protein in mammals, typically accounting for **more than 30% of body protein**.

10 different types of collagen in the body: **fibril forming and non-fibril forming**

The **arrangement** of collagen fibers is parallel to one another resulting in a tensile strength of a steel wire.

In skin, where strength and flexibility are required, collagen fibers are **randomly oriented** and woven together like felt.



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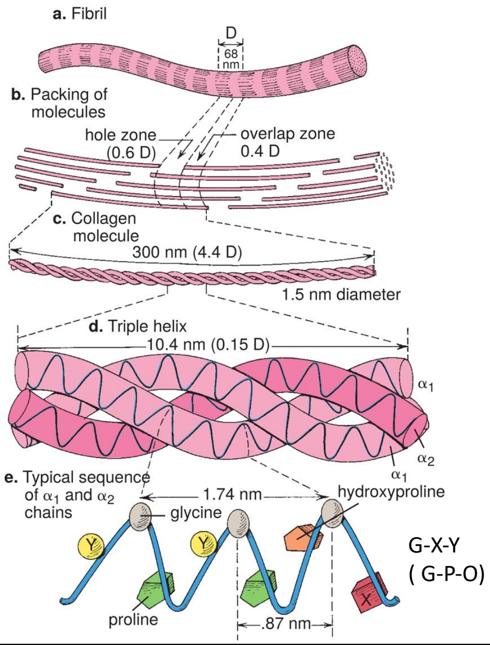
Collagen Types



	Type	Molecular formula	Polymerized form	Tissue distribution
Fibril-Forming (fibrillar)	I	$[\alpha1(I)]_2\alpha2(I)$	fibril	bone, skin, tendons, ligaments, cornea (represent 90% of total collagen of the human body)
	II	$[\alpha1(II)]_3$	fibril	cartilage, intervertebrate disc, notochord, vitreous humor in the eye
	III	$[\alpha1(III)]_3$	fibril	skin, blood vessels
	V	$[\alpha1(V)]_2\alpha2(V)$ and $\alpha1(V)\alpha2(V)\alpha3(V)$	fibril (assemble with type I)	*idem* as type I
	XI	$\alpha1(XI)\alpha2(XI)\alpha3(XI)$	fibril (assemble with type II)	*idem* as type II
Fibril-associated	IX	$\alpha1(IX)\alpha2(IX)\alpha3(IX)$	lateral association with type II fibril	cartilage
	XII	$[\alpha1(XII)]_3$	lateral association with type I fibril	tendons, ligaments
Network-forming	IV	$[\alpha1(IV)]_2\alpha2(IV)$	Sheet-like network	basal lamina
	VII	$[\alpha1(VII)]_3$	anchoring fibrils	beneath stratified squamous epithelia

Alberts B., Johnson A., Lewis J., Raff M., Roberts K. Molecular Biology of The Cell. Garland Science; 2002

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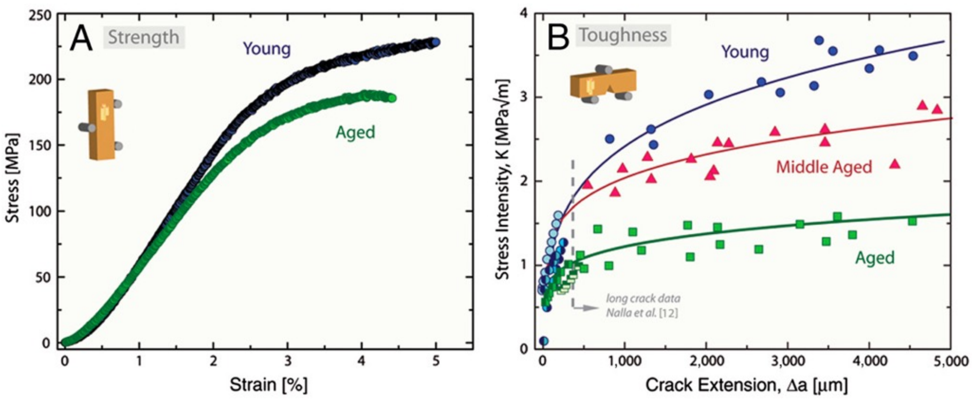
Collagen: molecular changes in aging

Aging increases cross-linking between the collagen molecules, which causes **plasticity at the nanoscale**.

Collagen fibrils can no longer slide with respect to one another as a way to **absorb energy from an impact**



Role of microstructure in the aging-related deterioration of the toughness of human cortical bone

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Collagen Biomaterials

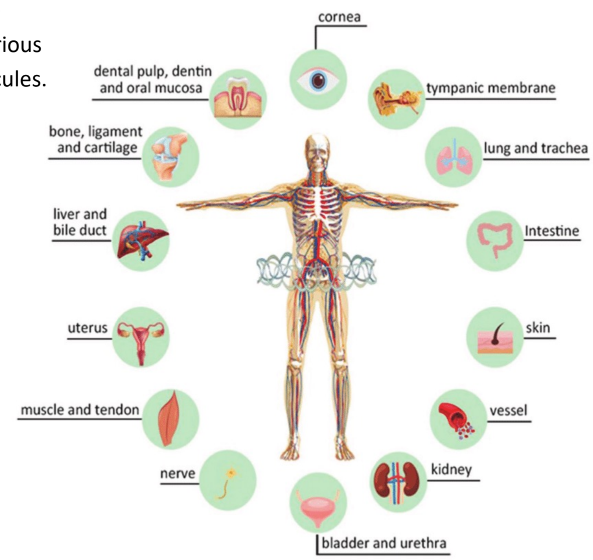
Collagen-based biomaterials are of great importance for tissue engineering and regenerative medicine. It is extracted from various tissue sources and assembled in combination with other molecules.

- Superior biocompatibility
- Low immunogenicity

- Used as decellularized ECM in fundamental studies
- Tissue replacement material in medical applications

Current challenges:

optimization of collagen-based biomaterials for medical applications by enhancing mechanical strength, biodegradability or delivery characteristics.



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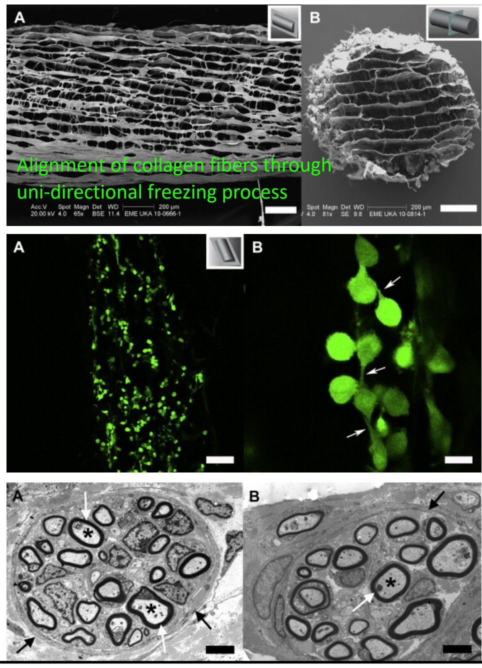
Example: Collagen for Neural Migration

Collagen-based biomaterials have been extensively studied as a promising nerve guide in regenerative medicine.

most collagen nerve guides are engineered from crosslinked collagen solution molded into tubular shape.

Engineering parameters:
Pore orientation
addition of neurotrophic factors
cell delivery
Mechanical properties

Tissue-engineered nerve grafts are always constructed by the combination of **supporting scaffolds** and **cells** or **growth factors**. Collagen scaffolds with cells can also release the various growth factors from cells that **promote axonal growth**.



https://doi.org/10.1016/j.biomaterials.2011.10.069

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Besides Collagen: Elastin

Responsible for the **elasticity** of the aorta and skin, and **connective tissue** such as ligaments.

Rubber-like material r**esponds to changes in temperature** and is able to **convert chemical energy into mechanical energy**.

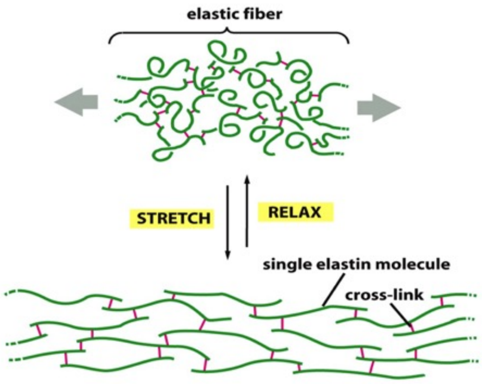
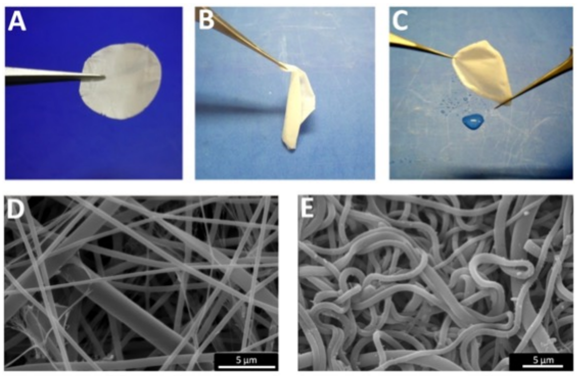


Figure 19-71 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Material could be used as a **replacement** for ligament tissue, blood vessels, or any other tissues requiring the elastic properties of elastin.



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Elastin-like synthetic materials

Elastin-mimetic protein polymers [(Val-Pro-Gly-Xaa-Gly)n] display reversible, temperature-dependent phase behavior in aqueous solution

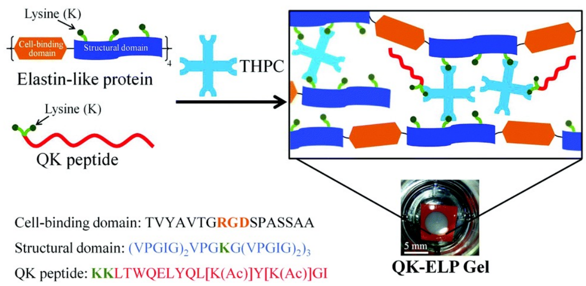
[(Val-Pro-Gly-Val-Gly)4(Val-Pro-Gly-Xaa-Gly)]
(Xaa = Lys, 1; Ile, 2)

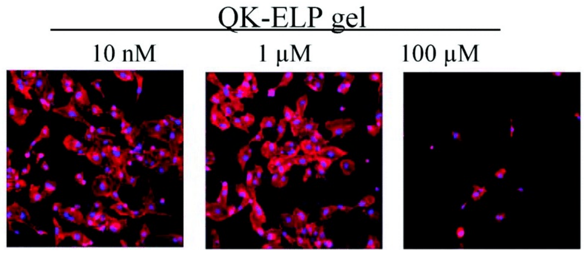
two structural criteria for the formation of a hydrogel:

(1) a flexible, hydrophilic (polyamide) backbone

(2) chemically reactive, selectively cross-linkable functional groups, i.e., the ε-amino of the lysine side chain

QK= small angiogenic peptide





Reiersen, H.; Clarke, A. R.; Rees, A. R. J. Mol. Biol. 1998, 283, 255−264.

Heilshorn, **Biomater. Sci.**, 2014,**2**, 757-765

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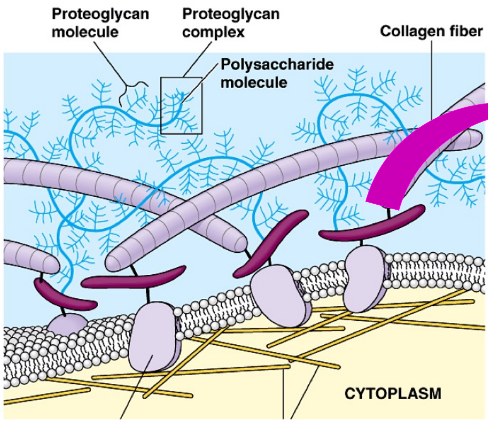
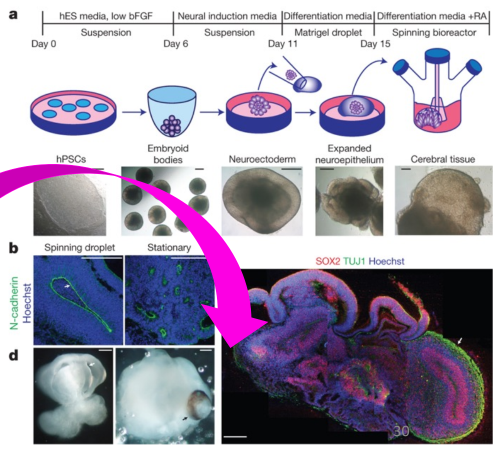
Matrigel



The current **"Magic" hydrogel** for tissue engineering
= decellularized ECM from mouse tumors
Composed of hundreds off ill-defined components
→ Cannot be used for clinical translation (why?)



* **Extracellular matrix** gives strength, adhesion and proliferative cues to tissues

* Composed of mix of biopolymers (proteins, sugars, soluble factors)

* Specific "niche" for each organ

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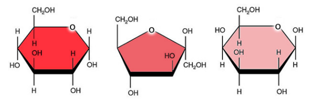
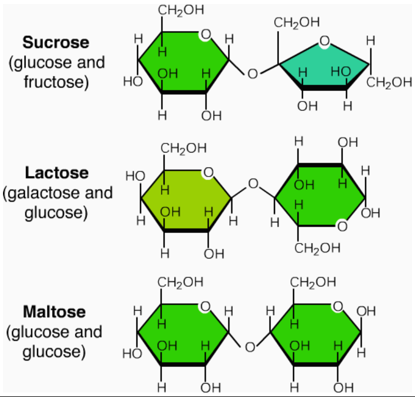
Polymers: Saccharides (Sugars)

Monosaccharides



Disaccharides



Polysaccharides are inexpensive, natural biopolymers which are widely used as raw materials and are easily biodegradable.

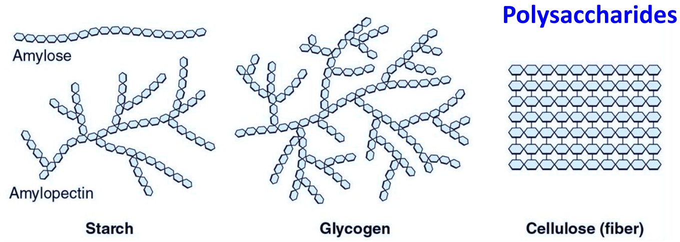
75% of all organic material on earth is present in the form of polysaccharides !!!

two principal functions:
structural support & energy storage

Polysaccharides as biomaterials:

- wound management
- tissue engineering
- drug delivery systems

Polysaccharides



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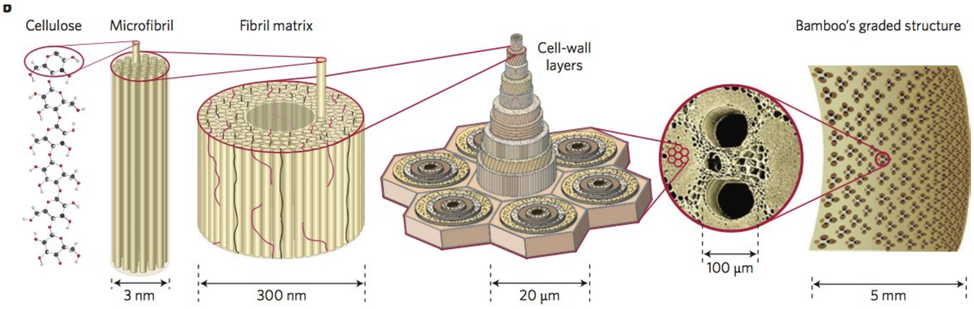
Saccharides as Biomaterial: Cellulose

Cellulose is a molecule, consisting of hundreds – and sometimes even thousands – of carbon, hydrogen and oxygen atoms.

Cellulose is the main substance in the walls of plant cells, helping plants to remain stiff and upright.

Humans cannot digest **cellulose**, but it is important in the diet as fiber.

	Cellulose
Source	Plant
Subunit	β-glucose
Bonds	1-4
Branches	No
Diagram	
Shape	



Note: Hierarchical structure!

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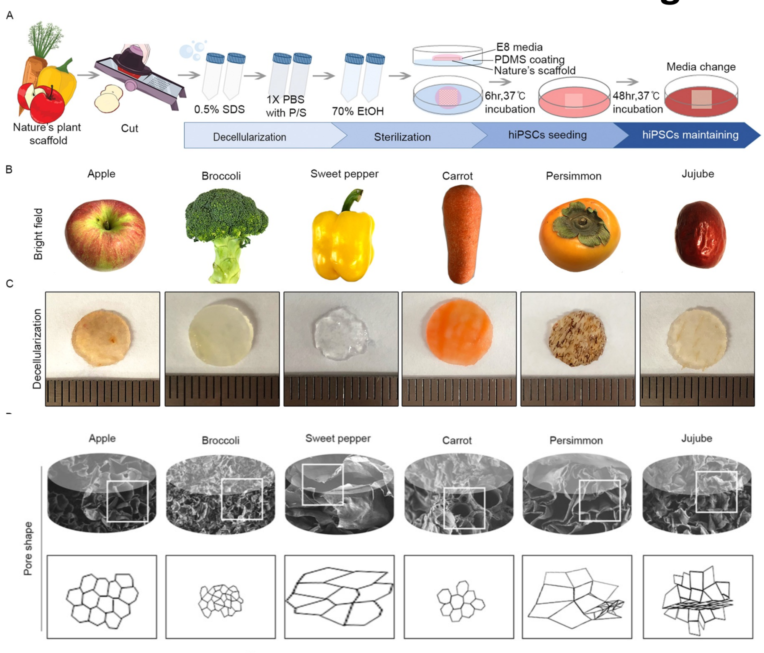
Cellulose as tissue engineering scaffold



Protocol:

cut into pieces (1 × 1 cm) for sequential immersion in:

(1) 0.5% sodium dodecyl sulphate (SDS) solution (to decellularise)
(2) 70% ethanol (to sterilise).

The remaining cellulose constructs show pores of various shapes and sizes

Lee, J., Jung, H., Park, N. *et al.* Induced Osteogenesis in Plants Decellularized Scaffolds. *Sci Rep* **9,** 20194 (2019)

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Induced Osteogenesis in Cellulose

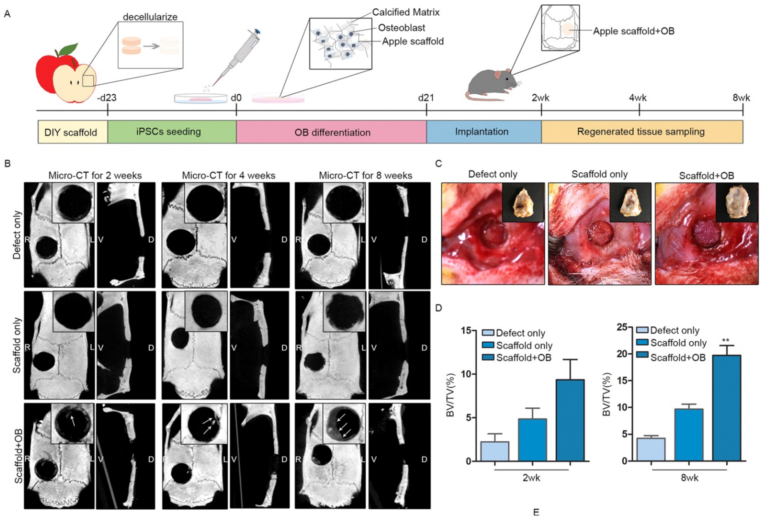
3D culture system that closely replicates the microenvironment of calcifying osteoid is essential for **cultivation of bone-like material**.

3D cellulose constructs of plants may serve as scaffolds to promote growth and differentiation of osteoblasts.

Study to **generate bone-like tissue** by seeding pluripotent stem cells (hiPSCs), stimulated to differentiate as osteoblasts in culture, onto the decellularised scaffolds of various plants.

Apple scaffolding bearing regular **pores of 300 µm** helped generate **calcified tissue** in rats.

Facilitates production of mineralized bone.



Lee, J., Jung, H., Park, N. *et al.* Induced Osteogenesis in Plants Decellularized Scaffolds. *Sci Rep* **9,** 20194 (2019)

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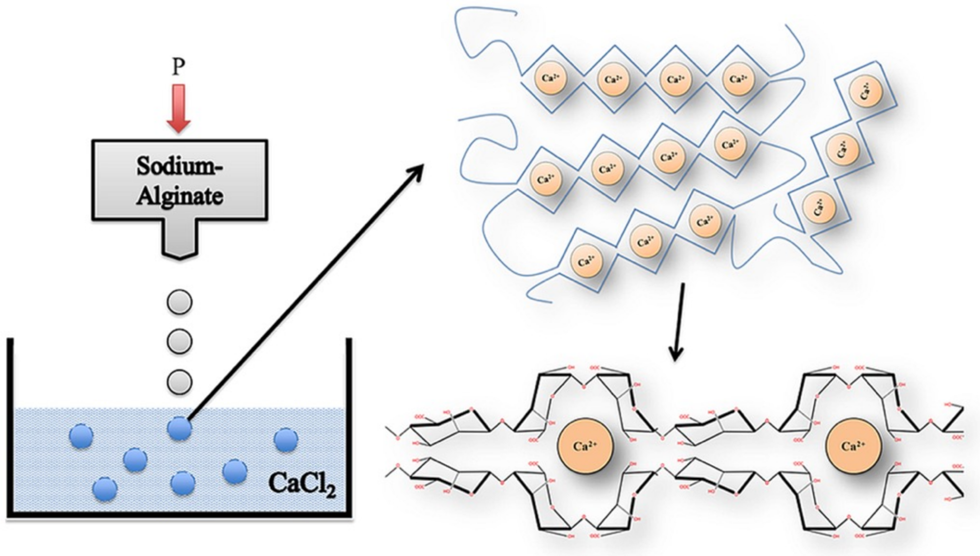
Alginate



Mannuronate + guluronate units

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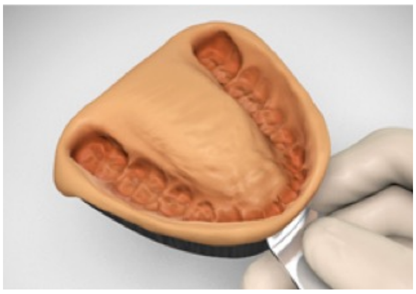
Alginate

Applications in:
Drug delivery
Wound dressing
Dental impression
Tissue engineering



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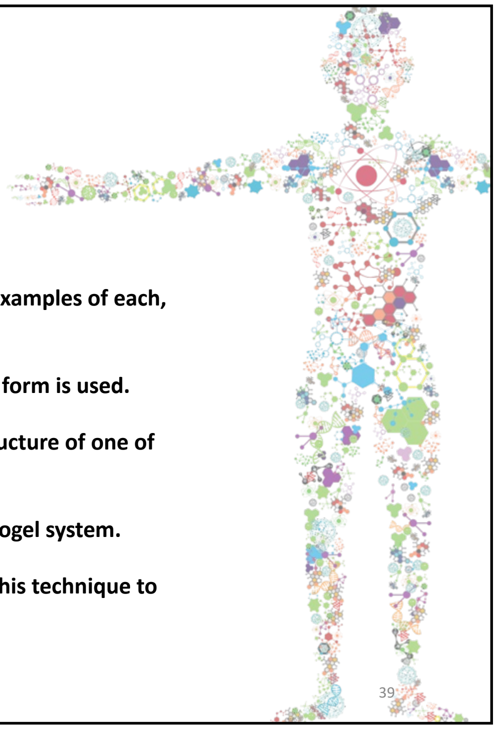
Test Questions



1) What are the 2 categories of natural biomaterials and give 2 examples of each, including medical applications.

2) Name 5 applications of PLA and explain why most often the L form is used.

3) What do collagen and silk have in common and sketch the structure of one of the two.

4) Why is elastin elastic? Design a elastin inspired synthetic hydrogel system.

5) What is decellularization and give an example of how to use this technique to make a biomaterial.

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