Hydrogel Stiffening

Corso Materiali intelligenti e Biomimetici
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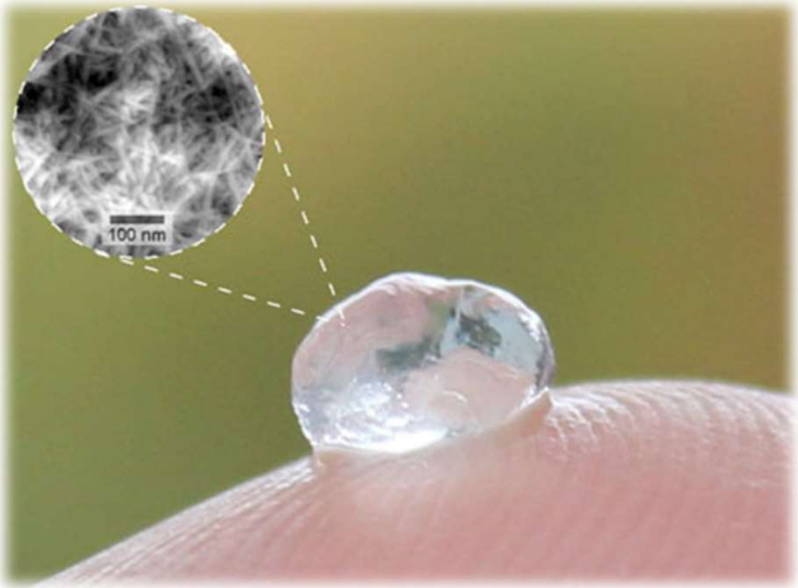
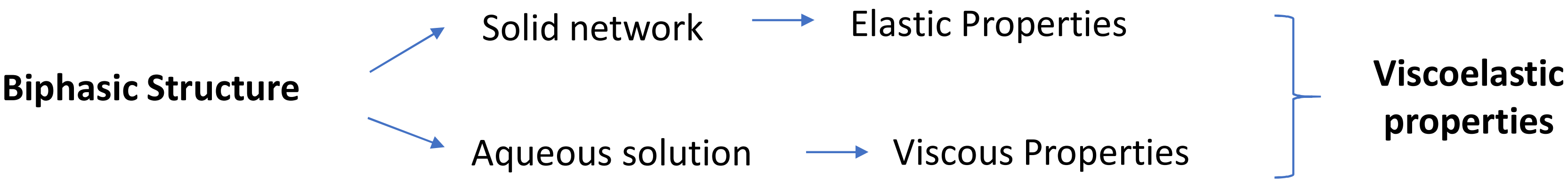
Hydrogels



Hydrogels **are hydrophilic polymer networks** which may absorb from 10–20% up to thousands of times their dry weight in water.

As the term ‘network’ implies, **crosslinks** have to be present to avoid dissolution of the hydrophilic polymer chains/segments into the aqueous phase.



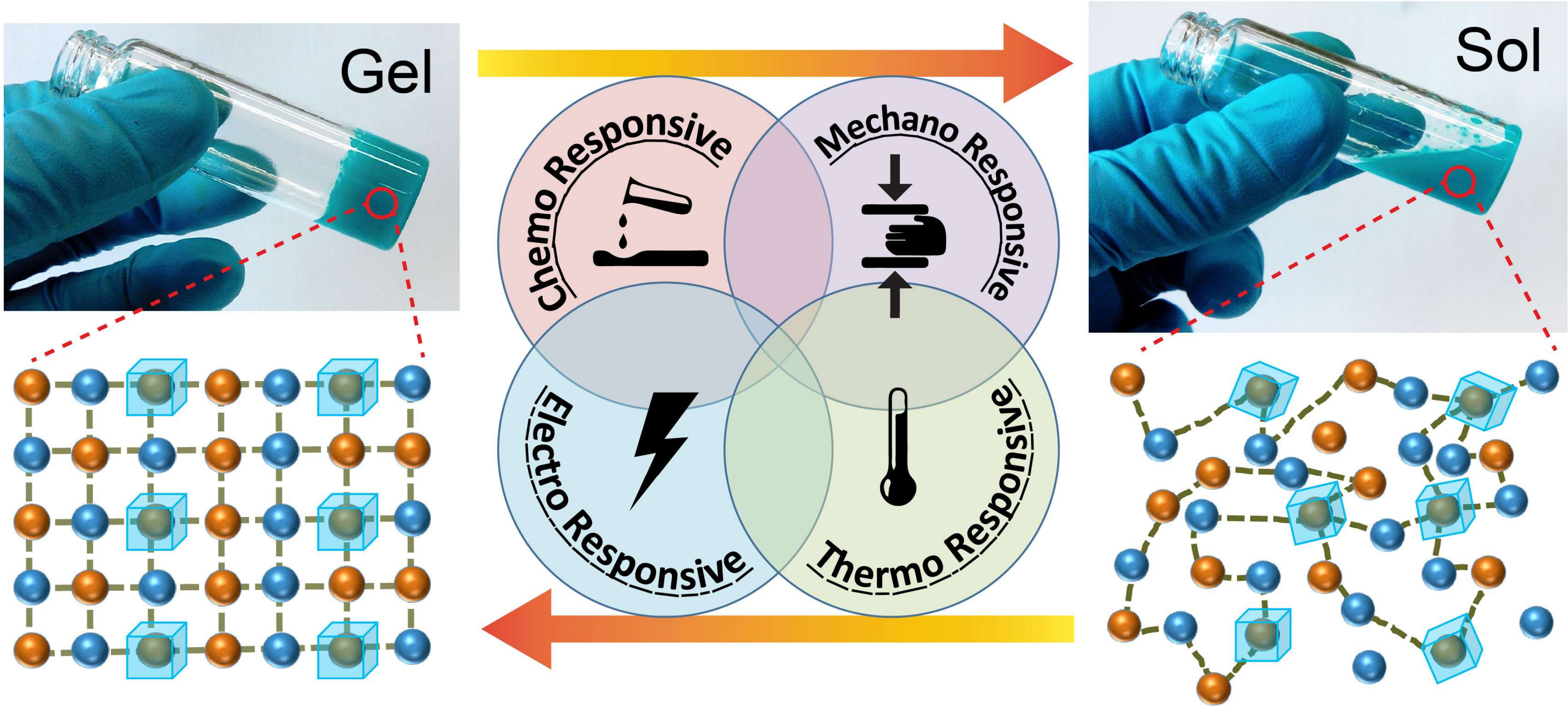
Biphasic Structure

Solid network → Elastic Properties

Aqueous solution → Viscous Properties

Viscoelastic properties

Polymeric solution-gel transition

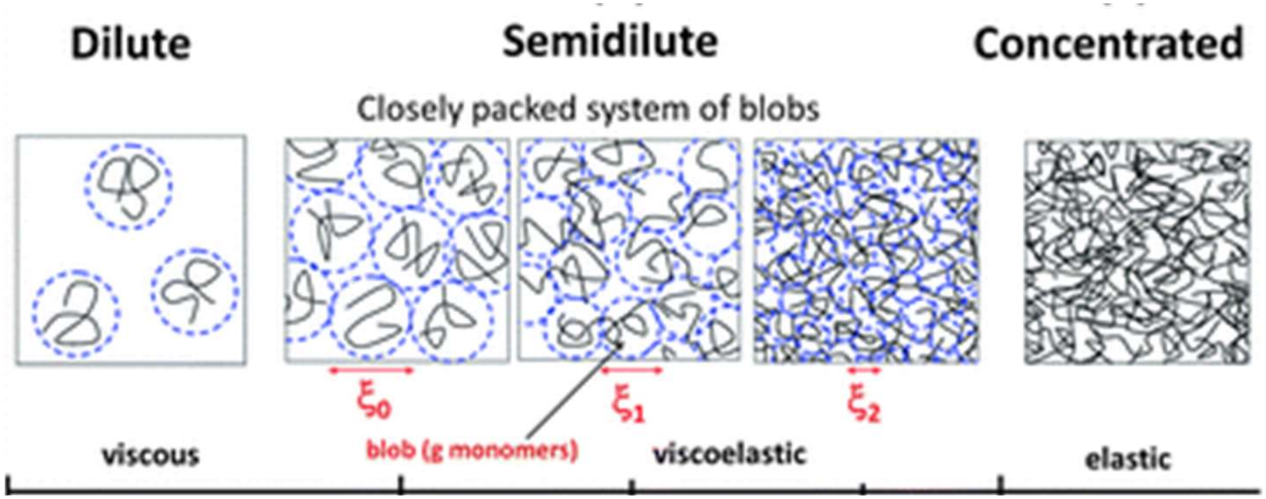


Hydrogels (2)

Hydrogels can also be described in a **rheological way:**

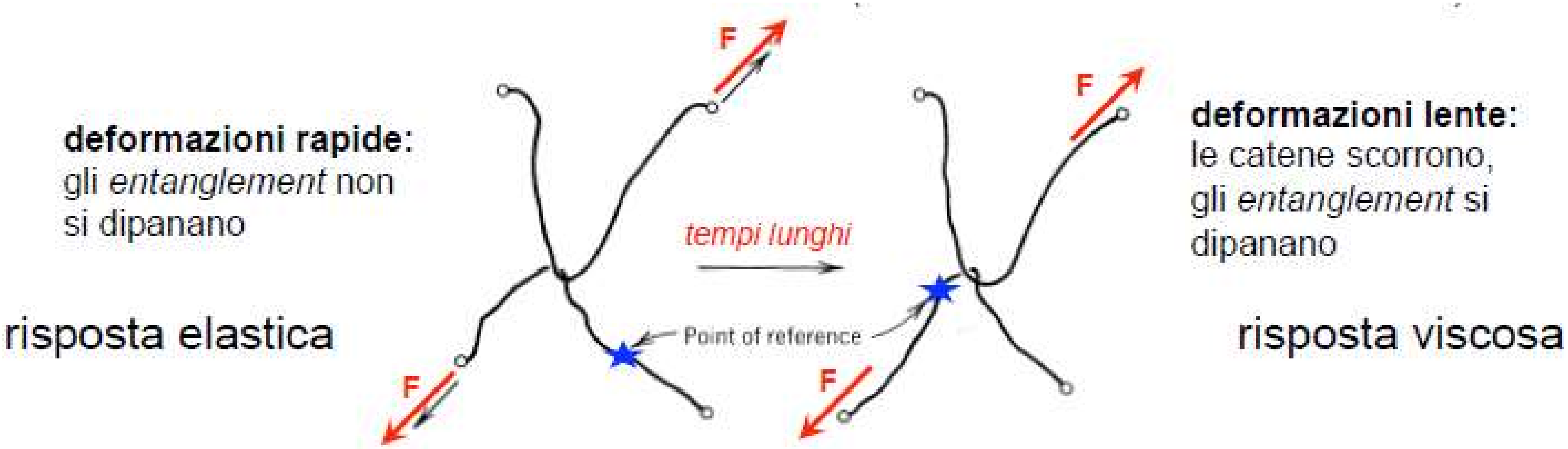
Aqueous solutions of hydrophilic polymers at low or moderate concentrations, where **no substantial entanglement of chains occurs,** normally show **Newtonian behavior**.

On the other hand, once **crosslinks** between the different polymer chains are introduced, the so obtained networks show **viscoelastic and sometimes pure elastic behaviour.**



Hydrogel viscoelasticity

- Biphasic structure (solid/aqueous phase)
- Entanglements behaviours
- Inter molecular and intramolecular forces

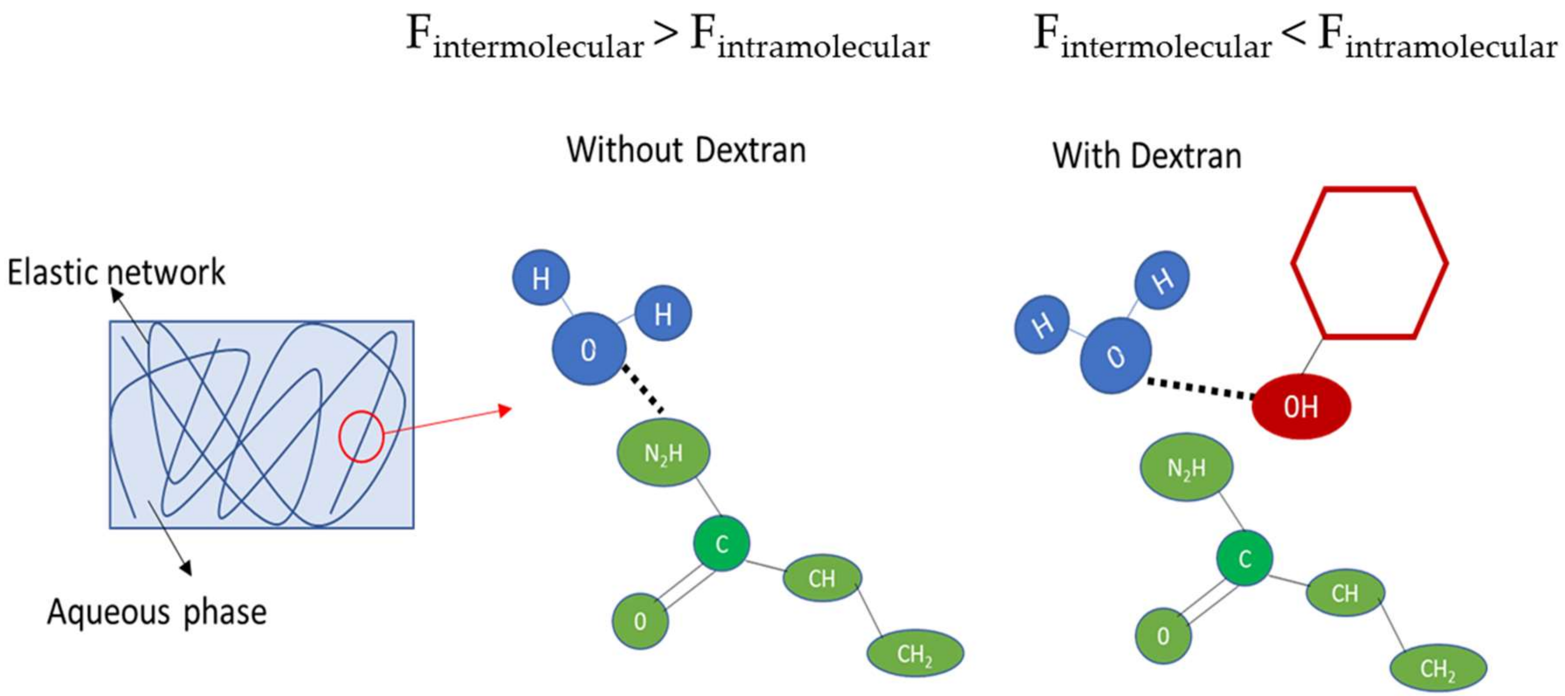


deformazioni rapide: gli *entanglement* non si dipanano

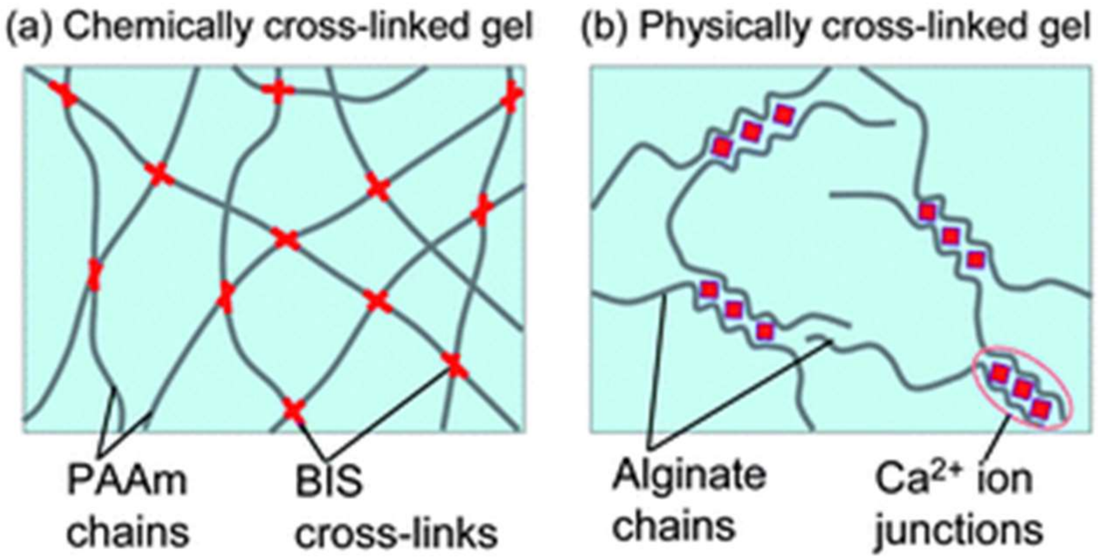
risposta elastica

deformazioni lente: le catene scorrono, gli *entanglement* si dipanano

risposta viscosa


$F_{intermolecular} > F_{intramolecular}$
$F_{intermolecular} < F_{intramolecular}$
Without Dextran
With Dextran
Elastic network
Aqueous phase
H
H
O
N_2H
C
CH
O
CH_2
H
H
O
OH
N_2H
C
CH
O
CH_2

Crosslinking types



(a) Chemically cross-linked gel

(b) Physically cross-linked gel

- **covalently-crosslinked** networks

- molecular **entanglements**
- **secondary forces** (ionic, H-bonding or hydrophobic forces)

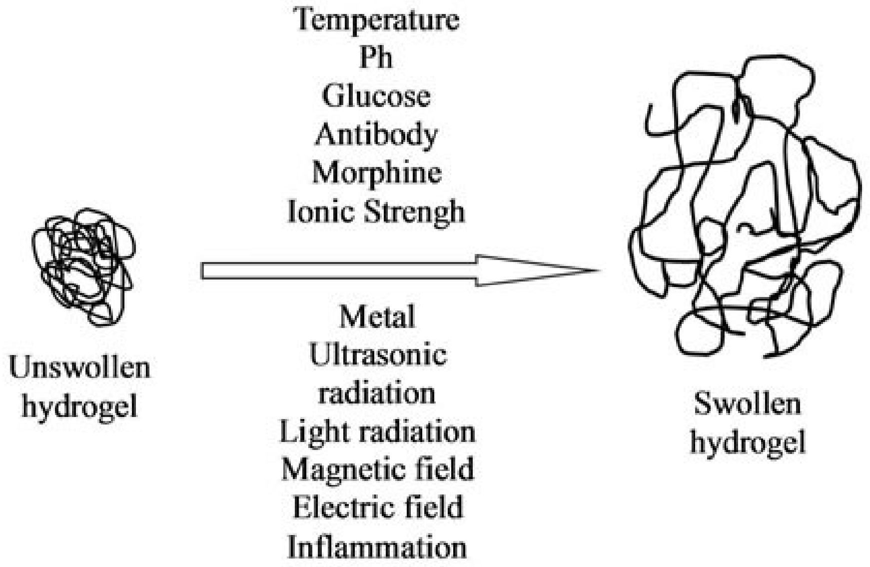
- All of these interactions are **reversible**, and can be disrupted by changes in physical conditions such as ionic strength, pH, temperature, application of stress, or addition of specific solutes.

Swelling

In the crosslinked state, **crosslinked hydrogels reach an equilibrium swelling level** in aqueous solutions which depends mainly on the crosslink density, but also from environment conditions (pH, T, etc)

Equilibrium Water Content:

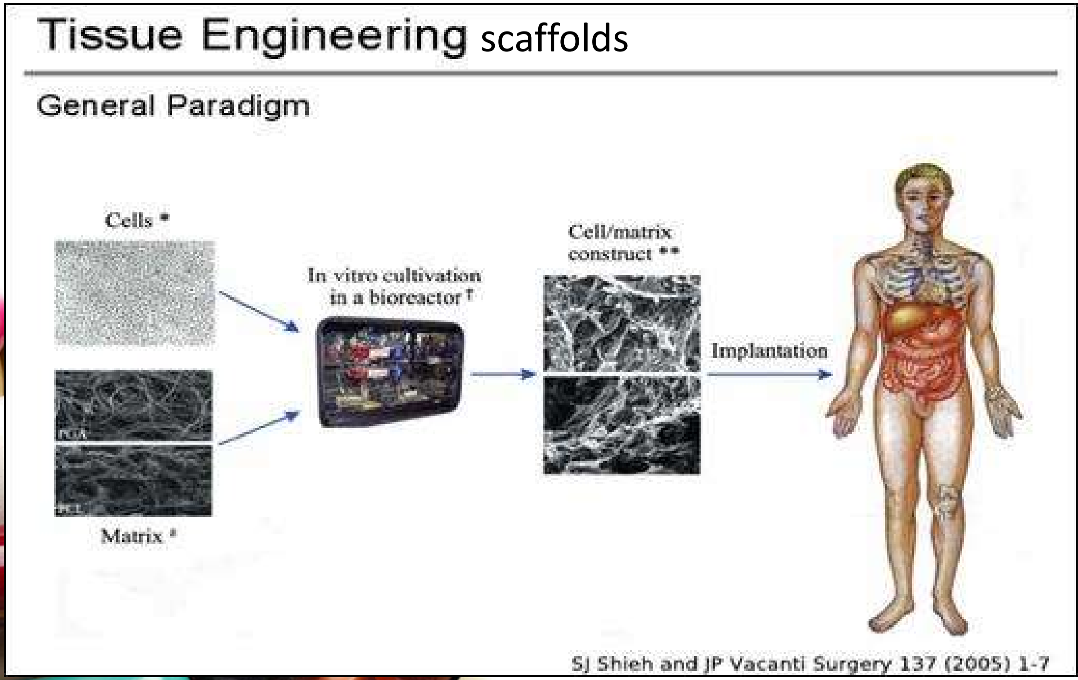
$$EWC = \frac{W_w}{W_t} \cdot 100\%$$



Applications

soft contact lenses

pills or capsules for oral ingestion



SJ Shieh and JP Vacanti Surgery 137 (2005) 1-7

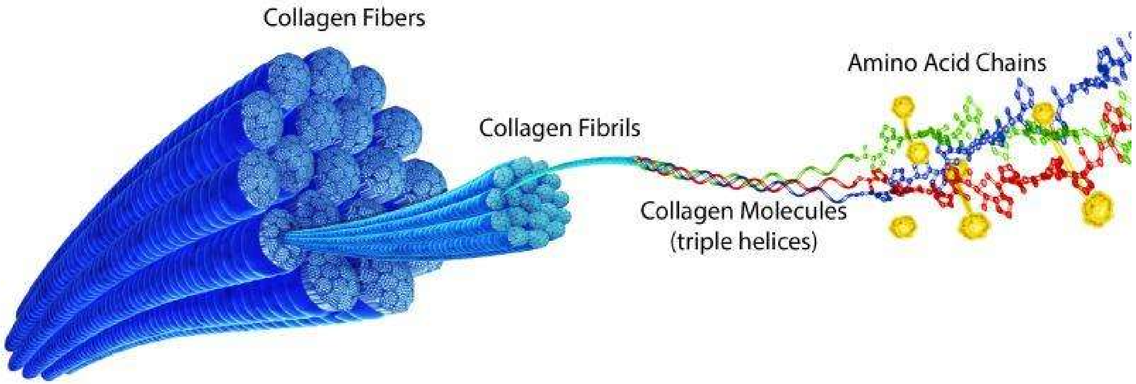
Adequate design and material selection for each specific application depend on several variables, including physical properties (e.g. **mechanics, degradation, gel formation**), mass transport properties (e.g. **diffusion)**, and biological properties (e.g. **cell adhesion** and signaling).

Examples

Hydrophilic Polymers used to synthesize hydrogels				
Natural Polymers	**Anionic polymers:** HA, alginic acid, pectin	**Cationic polymers**: chitosan, poly-lysine	**Amphipathic polymers**: collagen (and gelatin), fibrin	**Neutral polymers**: dextran, agarose
Synthetic Polymers	**PEG** (polyethylene glycol), **PVA** (Polyvinyl alcohol), **PCL** (Polycaprolactone), **PolyHEMA** (Poly-hydroxyethyl methacrylate), **PU** (polyurethane) , **PA** (Polyacrylate), PVP (Polyvinylpyrrolidone)			

Example 1 - Collagen

Collagen is an attractive material for biomedical applications as it is the most abundant protein in mammalian tissues and is the **main component of natural ECM** (extra-cellular matrix).



There are at least 19 different types of collagen, but the basic structure of all collagen is composed of three polypeptide chains, which wrap around one another to form a **three-stranded rope structure** . The strands are held together by both hydrogen and covalent bonds. Collagen strands can self aggregate to form **stable fibers**.

Collagen solutions form **physical gels passing from 4°C to 37°C.**

Mechanical properties of collagen hydrogel can be enhanced by introducing various *chemical crosslinkers* (i.e. glutaraldehyde, formaldehyde, carbodiimide), by *crosslinking with physical treatments* (i.e. UV irradiation, freeze-drying, heating), and by *blending it with other polymers* (i.e. HA, PLA, PGA, PLGA, chitosan, PEO).

Example 2 - Gelatin

Gelatin derives from **collagen denaturation**, resulting in a biodegradable, biocompatible and nonimmunogenic product, suitable for medical applications.

Gelatin aqueous solutions **(50°C)** form physical **thermo-reversible gels on cooling**. During gelling, the chains undergo a conformational disorder-order transition and tend to recover the collagen triple-helix structure.

With respect to collagen, which is also known to have wide biomedical applications, gelatin *does not express antigenicity in physiological conditions*, and it is much *cheaper and easier to obtain* in concentrate solutions.

On the other hand, gelatin exhibits **poor mechanical properties**. *In order to create stable gelatin hydrogels at 37°C, chemical crosslinking agents such as glutaraldehyde are typically used*.

Example 3 - Agarose

Agarose is a typical naturally-occurring **polysaccharide** that is known to form **thermo-reversible gels** when a homogeneous solution is **cooled from 99°C** to a temperature below 35°C. The melting and gelling temperatures may be dependent on the concentration of the gel.

The major drawbacks of agarose are that it shows significantly *low cell adhesiveness* and cell proliferation, as it does not contain any adhesive proteins.

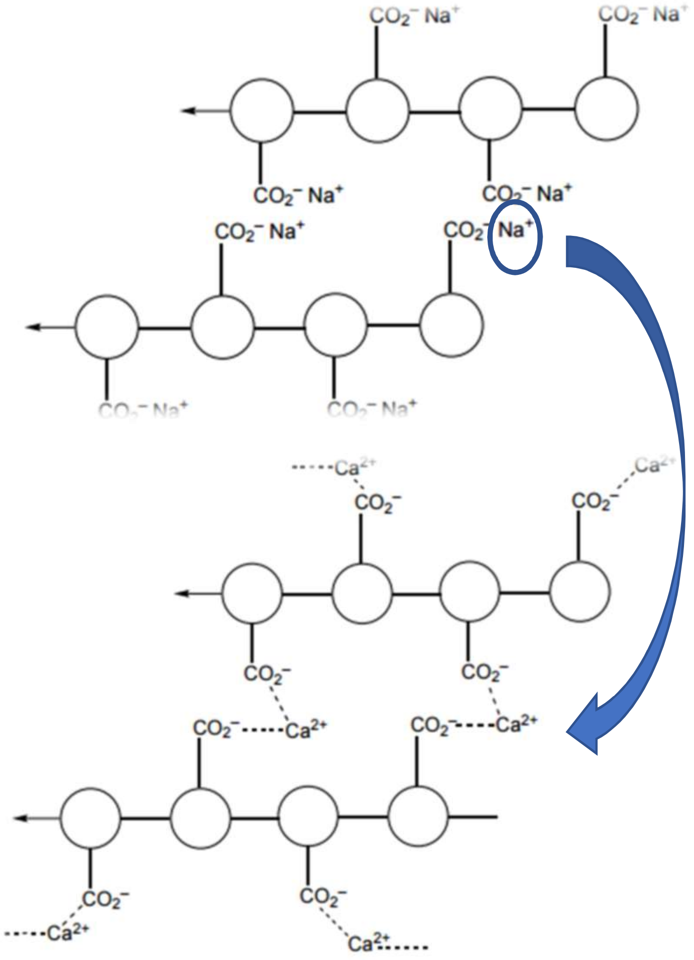
Modification of polymers with peptides containing the cell recognition motif RGD (R, arginine; G, glycine; D, aspartic acid) has recently attracted much attention for enhancing the cell adhesiveness of substrates in tissue engineering

Example 4 - Alginate

Alginate is a linear **polysaccharide extracted from brown algae** has been used in a variety of medical applications including cell encapsulation and drug stabilization and delivery, because it gels under gentle conditions, has low toxicity, and is readily available.

Gels are formed when **divalent cations** such as Ca2+, Ba2+, or Sr2+ cooperatively interact with monomers to **form ionic bridges between different polymer chains**.

Ionically crosslinked alginate hydrogels do not specifically degrade but undergo **slow, uncontrolled dissolution**. Mass is lost through ion exchange of calcium followed by dissociation of individual chains, which results in loss of mechanical stiffness over time.



Calcium ions replace the sodium ions in the polymer. Each calcium ion can attach to two of the polymer strands.

Example 5 – Liver ECM

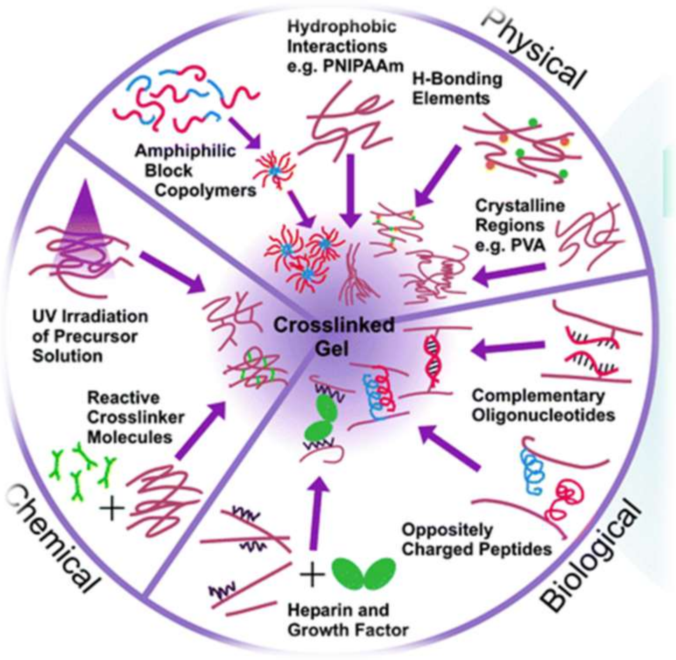
Decellularization maintains **microstructures of native extracellular matrices and its biochemical compositions**, providing tissue-specific microenvironments for efficient tissue regeneration.

***Digestion*,** its necessary to solubilize decellularized ECM (i.e. breaks down proteins into smaller peptides).

The digested ECM solution is brought from 4°C to 37°C to form hydrogels.

Methods for synthesizing physical and chemical hydrogels

Physical gels
- Warm a polymer solution to form a gel (e.g. collagen)
- Cool a polymer solution to form a gel (e.g., agarose or gelatin)
- ‘Crosslink’ a polymer in aqueous solution, using freeze–thaw cycles to form polymer microcrystals
- Lower pH to form an H-bonded gel between two different polymers in the same aqueous solution
- Adding ions in solution (e.g. alginate)
- Mix solutions of a polyanion and a polycation to form a complex coacervate gel (e.g., sodium alginate plus polylysine)
- Gel a polyelectrolyte solution with a multivalent ion of opposite charge

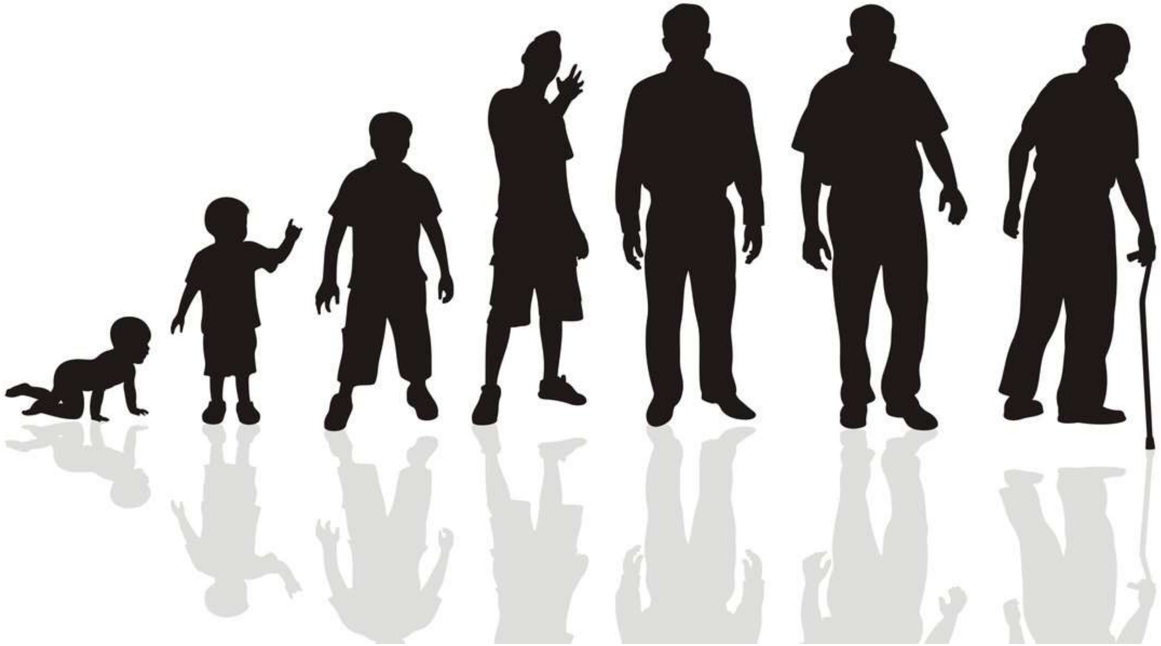


Chemical gels

Crosslink polymers in the solid state or in solution with:
- Radiation
- Chemical crosslinkers (e.g., treat collagen with glutaraldehyde)
- Copolymerize a monomer+crosslinker in solution/multifunctional macromer
- Chemically convert a hydrophobic polymer to a hydrogel

Why Stiffening?

- Stabilize hydrogels
- Enhance mechanical properties
- Modulate Mechanical Properties



Pathophysiological models of foetal growth, ageing, fibrosis

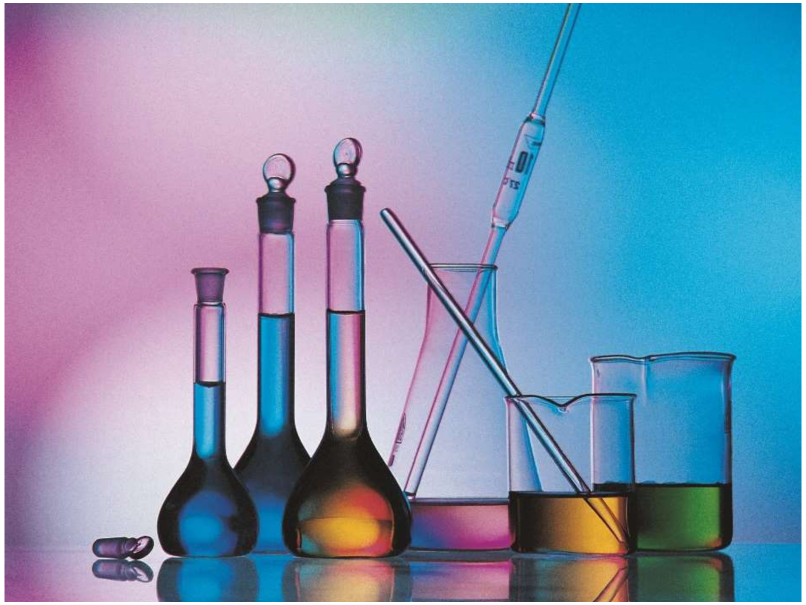
Crosslinker types

- Chemical/UV → Toxics effects
- Biological
- Enzimatic

Biological, Enzimatic: In general, non toxic

Toxics effects, In general, non toxic: Stiffening depends on concentration/dose

Enzimatic → Stiffening depends on concentration and time



More stable hydrogels can be created by using either UV-light or chemical crosslinkers (e.g. glutaraldehyde). Despite the improved mechanical strength and proteolytic stability of synthetically crosslinked hydrogels, the crosslinkers often elicit **either cytotoxic side-effects** or immunological responses from the host. Photocrosslinked hydrogels may also encounter a limitation in applications of deep tissue implants, where light is unable to penetrate the host tissue.

Chemical stiffening: Glutaraldehyde (GTA)

Crosslinking of amine containing polymers (i.e. collagen, gelatin, ecm) with GTA (glutaraldehyde) involves the *reaction of free amino groups of lysine* or hydroxy-lysine amino acid residues of the polypeptide chains *with the aldehyde groups of GTA*

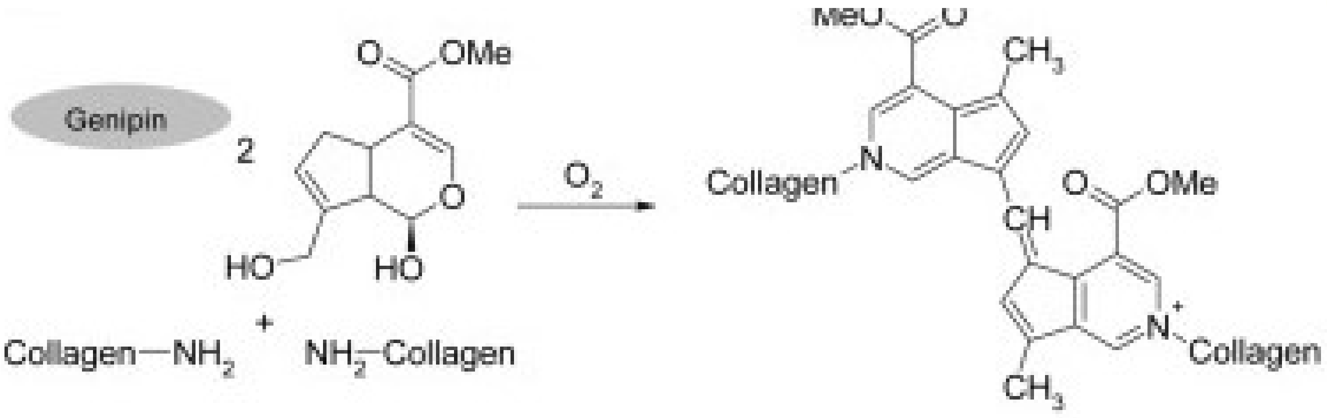
Since glutaraldehyde is a **toxic** compound that even at low concentration shows cell-growth inhibition, hydrogels need to be careful washed before use.

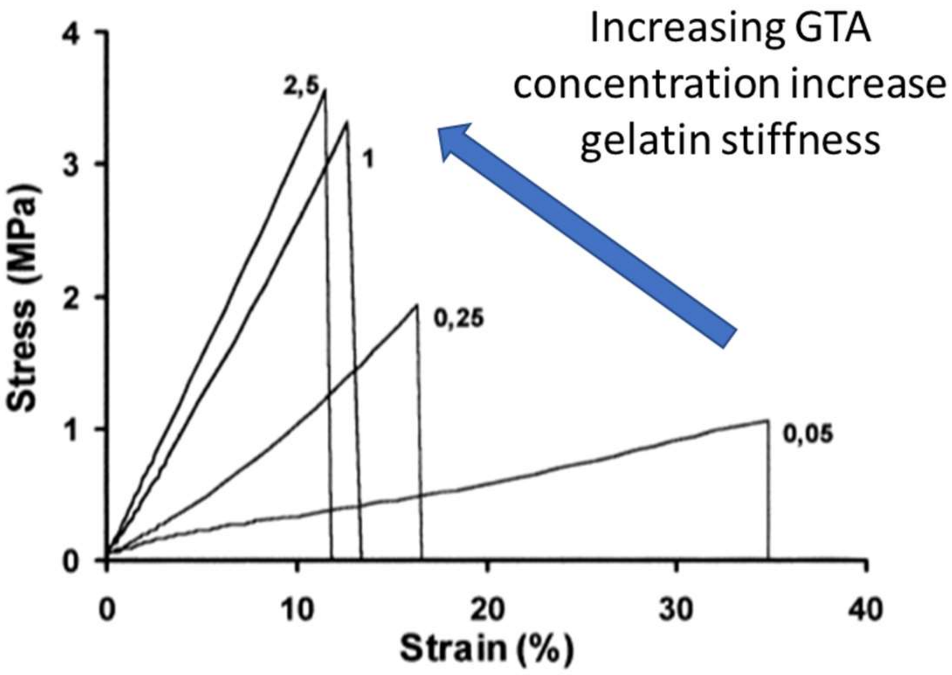
Biological crosslinkers: Genepin

Toxicity of chemical reagents such as GTA is the reason of the increasing demand for a crosslinking agent able to form stable and biocompatible crosslinked products.

Genipin is a **naturally occurring crosslinking agent**, which seems to display promising characteristics.

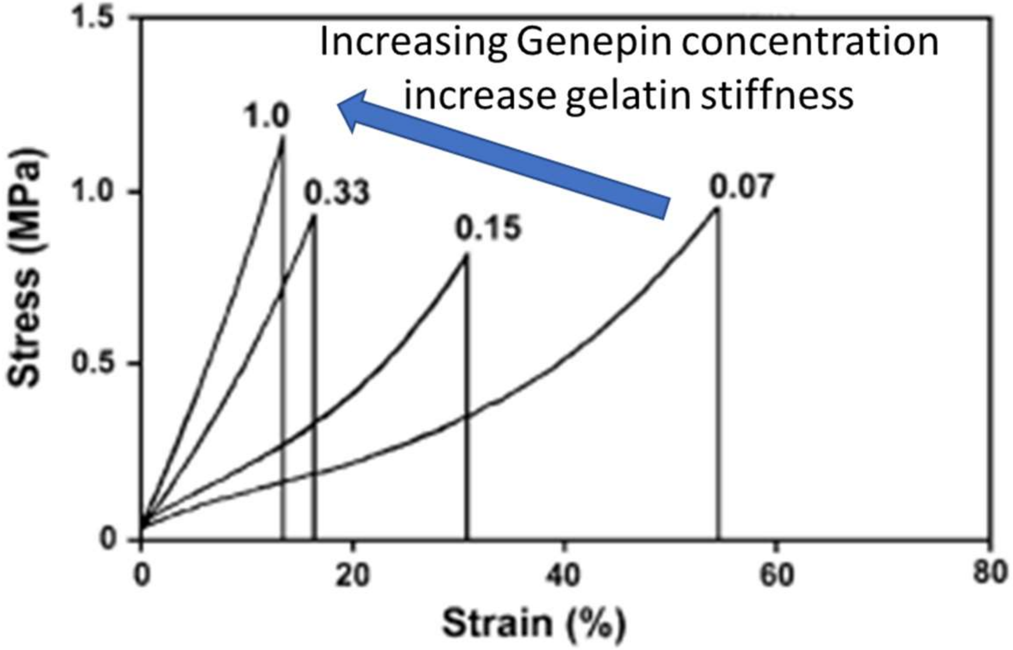
Genipin can be obtained from an iridoid glucoside, geniposide, abundantly present in **gardenia fruits**. Genipin has been widely used in herbal medicine, and the *dark blue pigments obtained by its spontaneous reaction with amino acids* or proteins have been used in the fabrication of food dye.





Increasing GTA concentration increase gelatin stiffness

Fig. 1. Typical stress–strain curves recorded from gelatin films cross-linked with GTA. The numbers near the curves indicate the concentration of GTA, expressed as wt%.



Increasing Genepin concentration increase gelatin stiffness

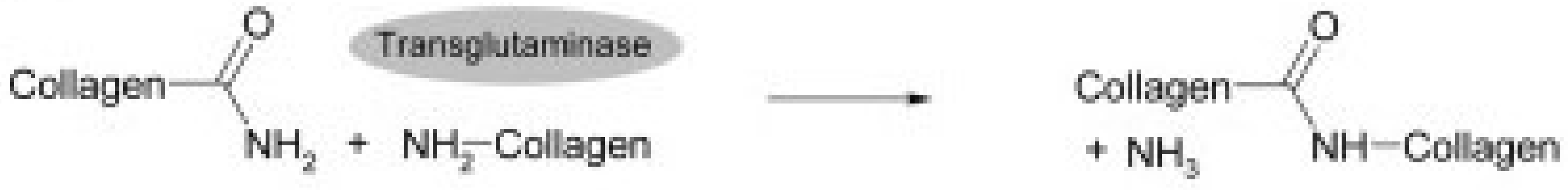
Fig. 1. Typical stress–strain curves recorded from gelatin films cross-linked with genipin. The numbers near the curves indicate the concentration of genipin, expressed as wt%.

Enzymatic stiffening: mTG

A naturally occurring protein crosslinking enzyme, **microbial transglutaminase**, was used to form a thermally stable hydrogel from gelatin. This enzyme is ubiquitous in nature, being found in many species of the plant and animal kingdoms (e.g. peas, oysters, shrimp, tuna, chickens, cows, and humans).

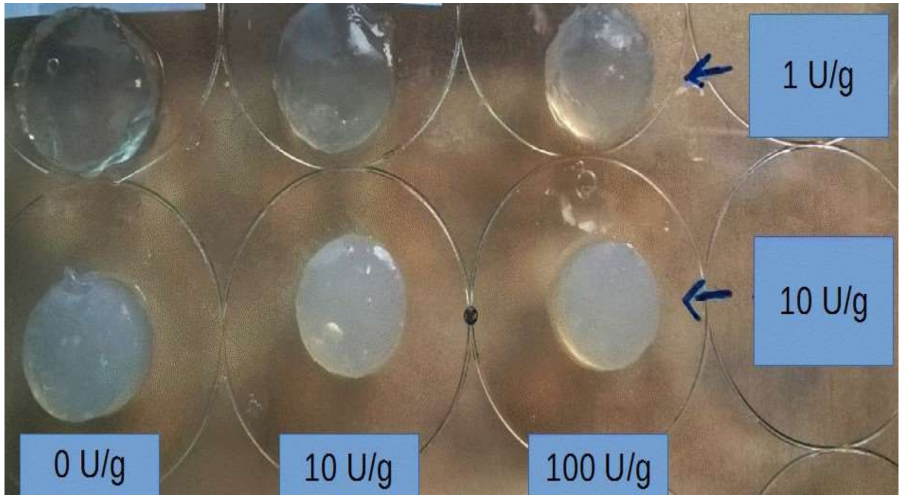
Microbial transglutaminase (mTG) is a native protein that is innocuous and **commonly used in food manufacturing processes** approved for human consumption by the U.S. Food and Drug Administration.

Transglutaminase functions by **catalysing the formation of covalent N e-(g-glutamyl) lysine amide bonds** between individual gelatin strands to form a permanent network of polypeptides.



Example: mTG-Gelatin Hydrogels

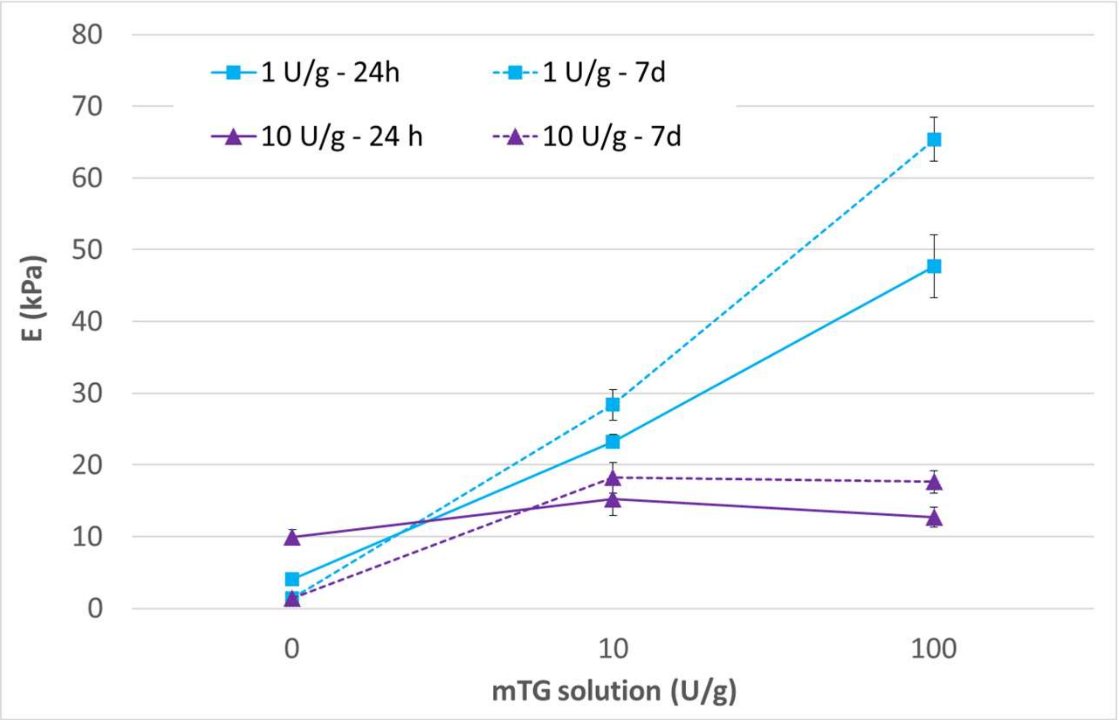
+ swelling



Inside the gel

+ shrinking

Solution (outside the gel)



*One **U (unit)** is defined as the **amount of the enzyme that produces a certain amount of enzymatic activity** (i.e. the amount that catalyzes the conversion of 1 micro mole of substrate per minute).*

E increase both with mtg concentration on incubation time

Domande articoli

- Descrivere le differenze tra stiffening con GTA e con mTG
- Qual è la differenza tra lo stiffening con mTG prima e dopo la gelazione fisica della gelatina
- Descrivere possibili applicazioni