Polymers & Hydrogels in bioengineering

Corso Materiali intelligenti e Biomimetici
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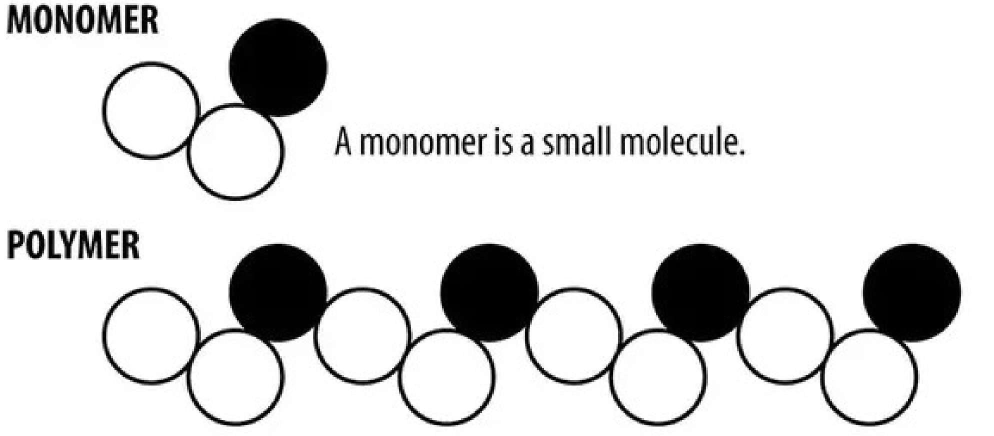
Polymers

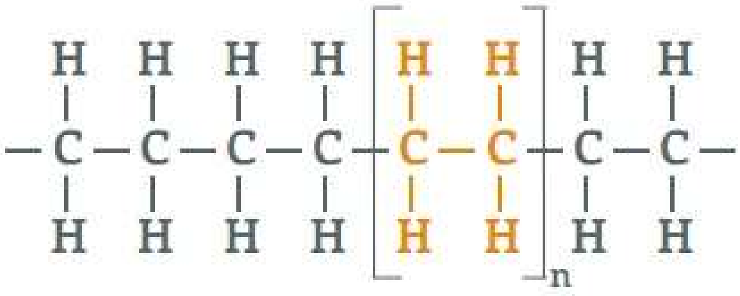
A **polymer** molecule consists of a chemical entity (repeat unit) which is covalently bonded to its identical neighbors forming a polymeric chain. The repeat unit is usually a called **monomer**.

How individual polymer molecules move when supplied with thermal or mechanical energy and how groups of molecules interact and entangle can explain the *observed behavior*.



A polymer is a long-chain molecule made up of a repeated pattern of monomers.

Example: PE - $(C_2H_4)_n$



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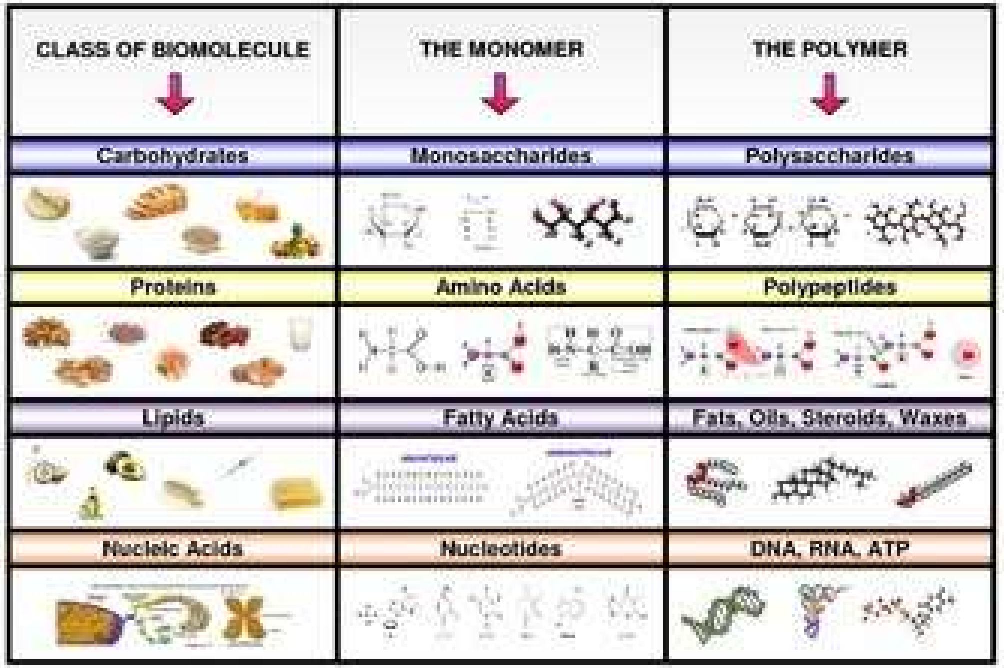
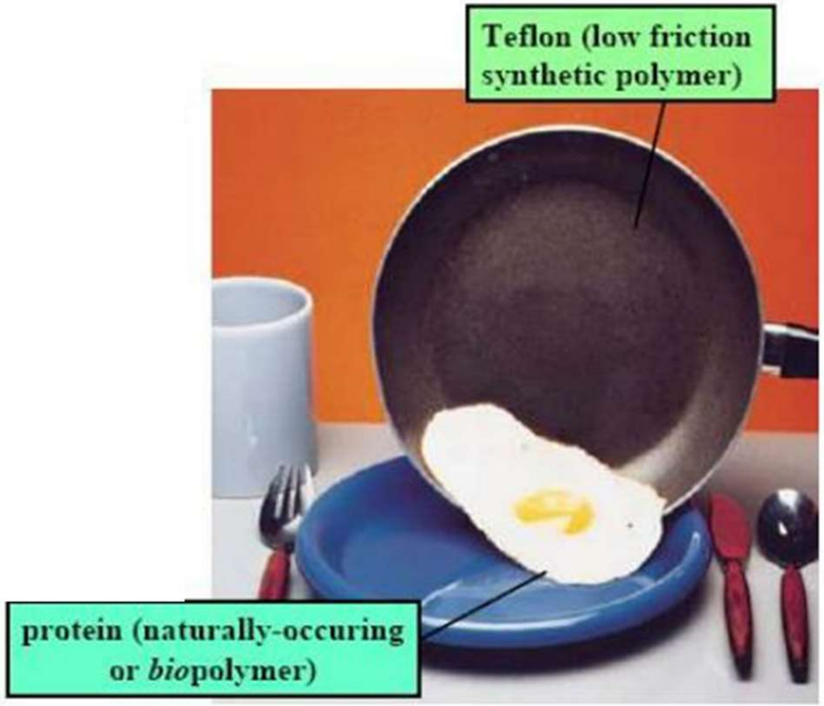
Natural and Synthetic Polymers

Polymers are abundant in nature, found in all living systems and **organic materials** such as wood, paper, leather, natural fibers have found extensive use. Today **synthetic materials** are mostly used.



CLASS OF BIOMOLECULE	THE MONOMER	THE POLYMER
Carbohydrates	Monosaccharides	Polysaccharides
Proteins	Amino Acids	Polypeptides
Lipids	Fatty Acids	Fats, Oils, Steroids, Waxes
Nucleic Acids	Nucleotides	DNA, RNA, ATP



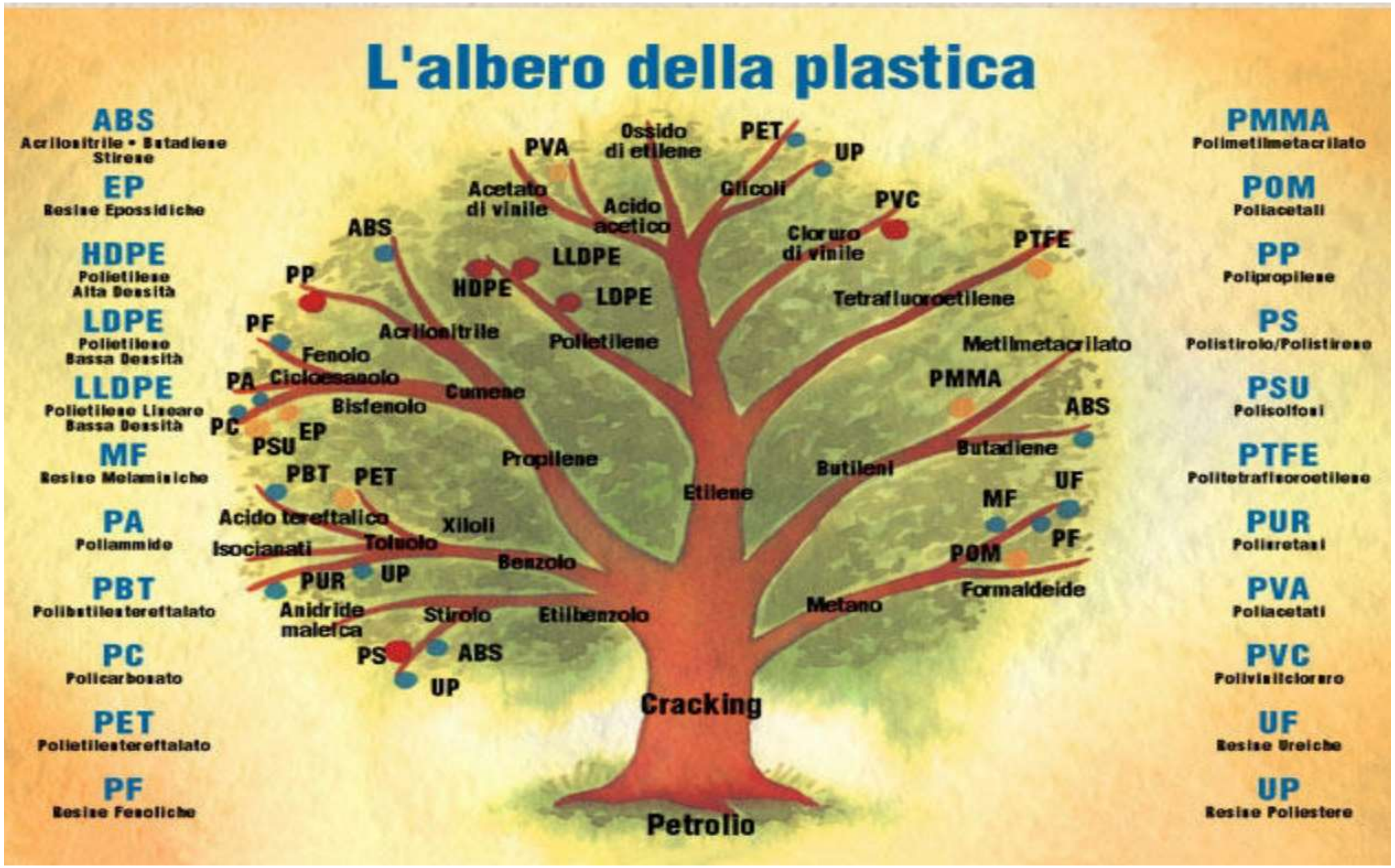
Teflon (low friction synthetic polymer)

protein (naturally-occuring or *bio*polymer)

Synthetic Polymers

The first man-made polymers, formed by **chemical modification of natural materials,** were produced in the second half of the nineteenth century.
E.g.: *Celluloid (*from nitrocellulose and camphor*)*

Fully **synthetic polymers** were developed in the twentieth century, most in the period 1950–1970s driven by chemical industry expansion.
Eg: *Bakelite* (formed from a condensation reaction of phenol with formaldehyde)



Fully synthetic polymers are the so-called plastics of modern society. The feedstock for polymerization processes is *petrochemical*, and environmental concerns have led to more recent developments of polymers from renewable resources.

TIPI DI POLIMERI TERMOPLASTICI

- **POLIETILENE (PE)** ***1935***

usato per flaconi per liquidi, **tubi, giocattoli, pellicole, sacchi per la spazzatura, cassette**.

Dal PE riciclato si producono nuovamente sacchi per la spazzatura, flaconi e nastri adesivi, tappi.
È una delle materie plastiche più diffuse per il basso costo e la versatilità.

- **POLIPROPILENE(PP)**

utilizzato per la produzione di **contenitori per alimenti, oggetti di arredo, flaconi per detersivi e per l'igiene personale, giocattoli, moquette, mobili da giardino, tubi .**

- **POLIETILENTEREFTALATO (PET)**

usato per produrre bottiglie per bevande. Dal suo riciclo si ottengono alcuni tessuti come il pile, interni ed accessori per auto, contenitori, fibre per imbottiture

- **ACRILONITRILE-BUTADIENE-STIRENE (ABS)**

usato per produrre telefonini, tower pc molto resistente agli Urti.

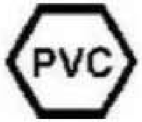
- **POLICARBONATO(PC)**

utilizzato per produrre caschi protettivi e parti di automobili presenta una buona resilienza e durezza.

- **POLIVINILCLORURO (PVC)**

usato per vaschette e contenitori di alimenti, per finestre e porte, per piastrelle, per i tesserini magnetici (BANCOMAT), per i nastri magnetici delle videocassette e pellicole cinematografiche. Come per il PE, il basso costo e la versatilità rendono il PVC uno dei polimeri più diffusi nel panorama delle plastiche.

Dal PVC riciclato si producono tubi usati in edilizia e negli impianti fognari.



- **POLISTIROLO o polistirene (PS)**

usato per produrre vaschette per alimenti, posate, piatti, tappi, pannelli isolanti per l'edilizia, imballaggi. **NON RICICLABILE.**

- **POLIAMMIDE (PA) (NYLON)**

E' stata la prima fibra tessile sintetica (1940). Calze da donna, paracaduti, pellicola trasparente........

Durante guerra le donne si disegnavano 1 riga nera sulla gamba per fingere di indossare le calze di nylon

TIPI DI POLIMERI TERMOINDURENTI

NON RICICLABILI

- **BACHELITE (resine Fenoliche)**

La prima materia prima sintetica (1909 belga Leo Baekeland). Usata per spine, prese di corrente, telefoni, articoli casalinghi.

- **FÒRMICA (resine Melaminiche)**

Resistono bene all'umidità e alle alte temperature, sono altamente colorabili

EP – Resine epossidiche.

Utilizzate nella produzione di vernici, adesivi, laminati, isolatori elettronici. Si usano anche rinforzate con fibre di vetro, di carbonio e altre resine

PU – Poliuretani.

Utilizzati nella fabbricazione di finte pelli, suole e tacchi da scarpe, film per isolamento elettrico, articoli per lo sport, per uso tecnico e nel settore medicale

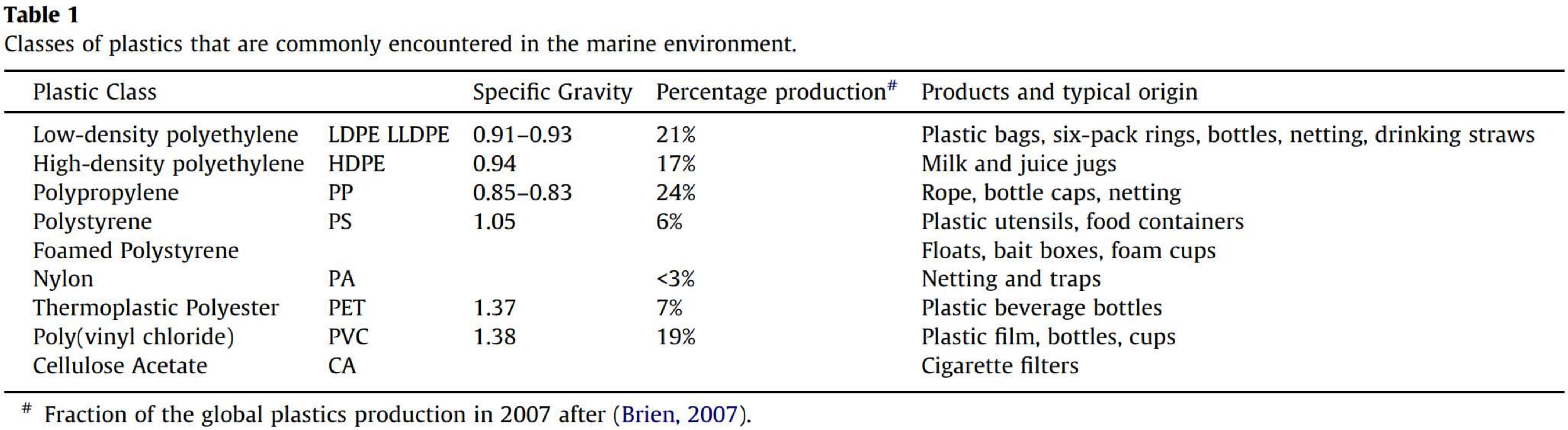


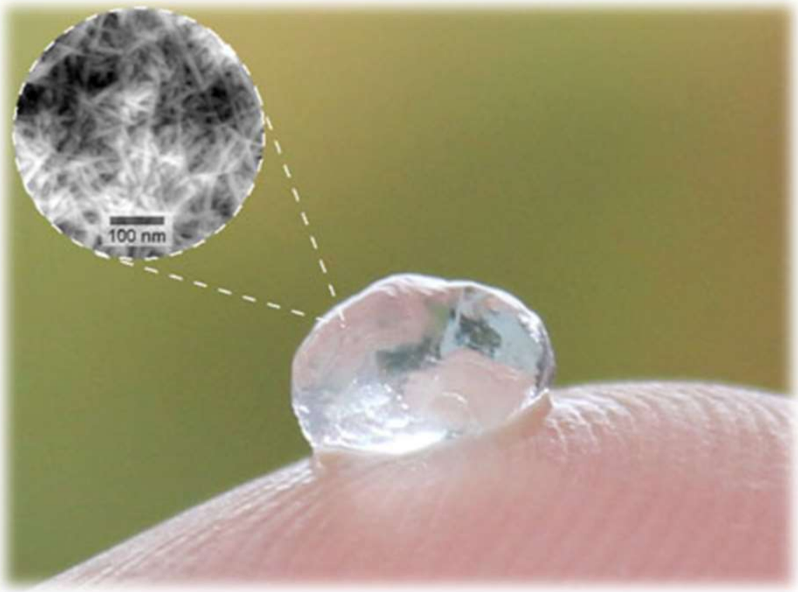
Table 1
Classes of plastics that are commonly encountered in the marine environment.

Plastic Class		Specific Gravity	Percentage production#	Products and typical origin
Low-density polyethylene	LDPE LLDPE	0.91–0.93	21%	Plastic bags, six-pack rings, bottles, netting, drinking straws
High-density polyethylene	HDPE	0.94	17%	Milk and juice jugs
Polypropylene	PP	0.85–0.83	24%	Rope, bottle caps, netting
Polystyrene	PS	1.05	6%	Plastic utensils, food containers
Foamed Polystyrene				Floats, bait boxes, foam cups
Nylon	PA		<3%	Netting and traps
Thermoplastic Polyester	PET	1.37	7%	Plastic beverage bottles
Poly(vinyl chloride)	PVC	1.38	19%	Plastic film, bottles, cups
Cellulose Acetate	CA			Cigarette filters

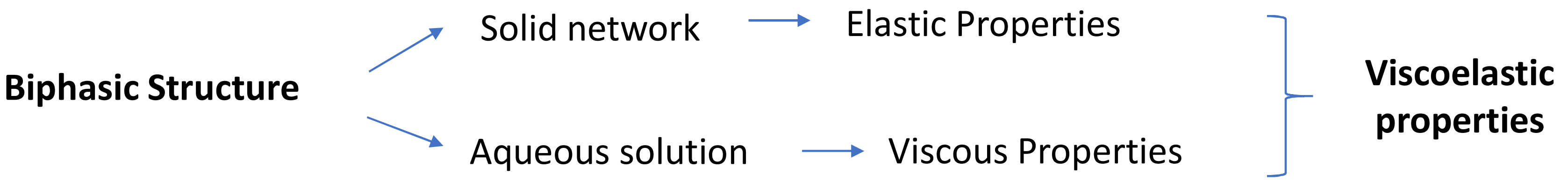
Fraction of the global plastics production in 2007 after (Brien, 2007).

la **regola delle tre “R”**: Reduce, Re-use, Recycle (Riutilizzare, ridurre, riciclare)

Hydrogels



Hydrogels constitute a group of **polymeric materials**, characterized by **the hydrophilic structure** of which renders them capable of holding large amounts of water in their three-dimensional networks (10–20% up to thousands of times their dry weight in water).
Crosslinks have to be present to avoid dissolution of the hydrophilic polymer chains/segments into the aqueous phase.



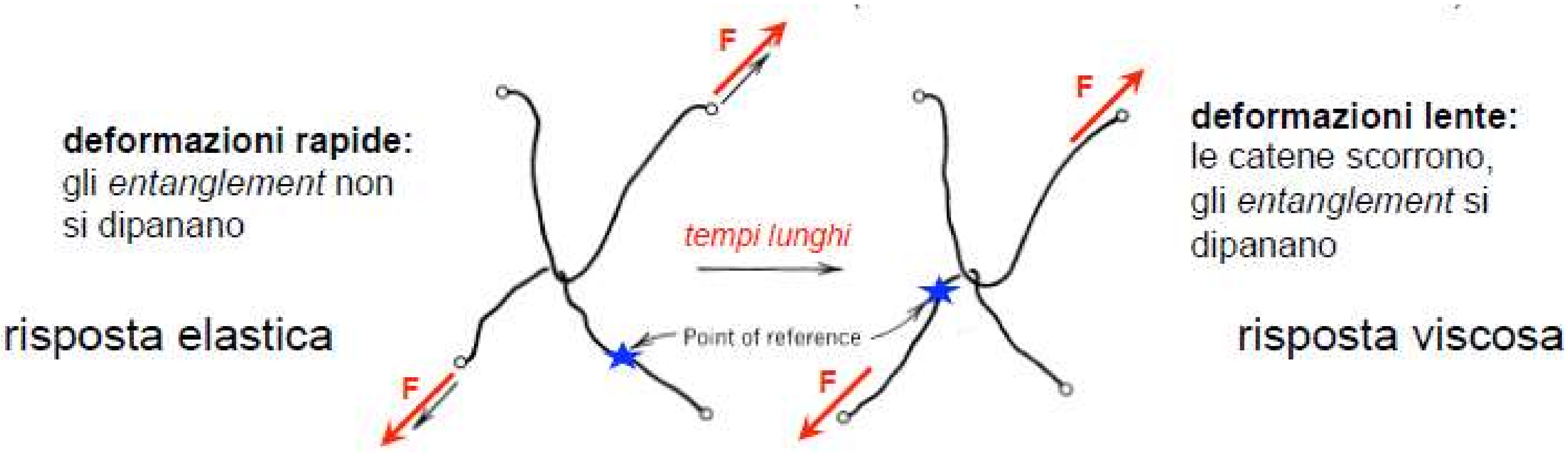
Biphasic Structure → Solid network → Elastic Properties

Biphasic Structure → Aqueous solution → Viscous Properties

Elastic Properties + Viscous Properties → **Viscoelastic properties**

Hydrogel viscoelasticity

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



deformazioni rapide:
gli *entanglement* non
si dipanano

risposta elastica

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

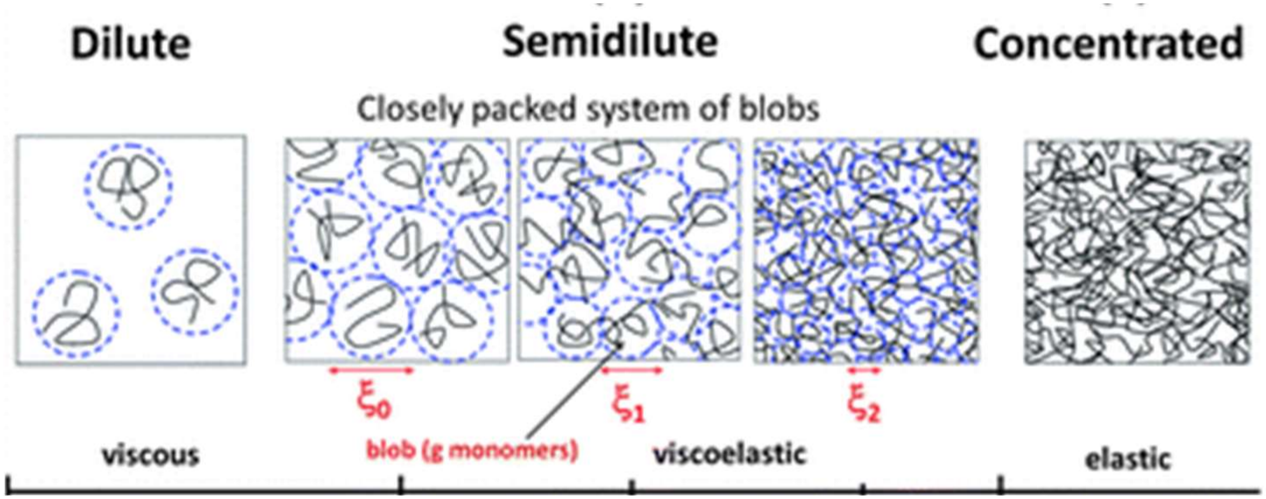
risposta viscosa

Hydrogels (2)

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

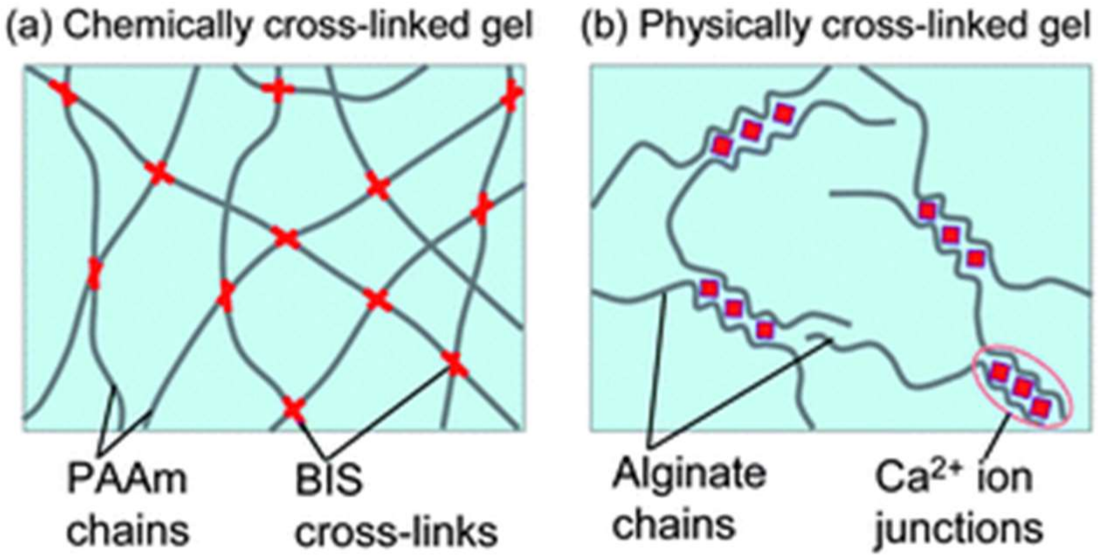
Aqueous solutions of hydrophilic polymers at low or moderate concentrations, where **no substantial entanglement of chains occurs,** normally show **Newtonian behavior**.

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



Crosslinking types

Considering inter-molecular chains:



- **covalently-crosslinked** networks

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

⬇

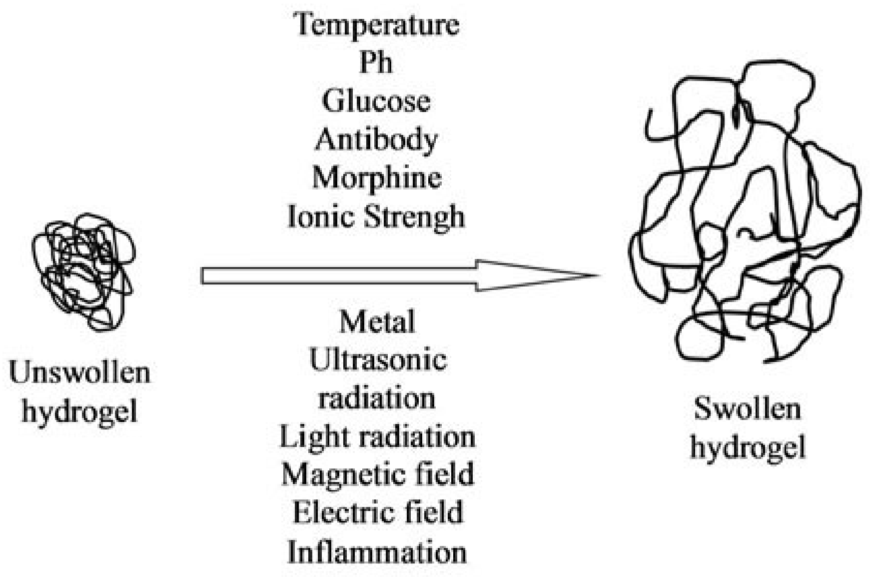
All of these interactions are **reversible**, and can be disrupted by changes in physical conditions such as ionic strength, pH, temperature, application of stress, or addition of specific solutes.

Hydrogel Swelling

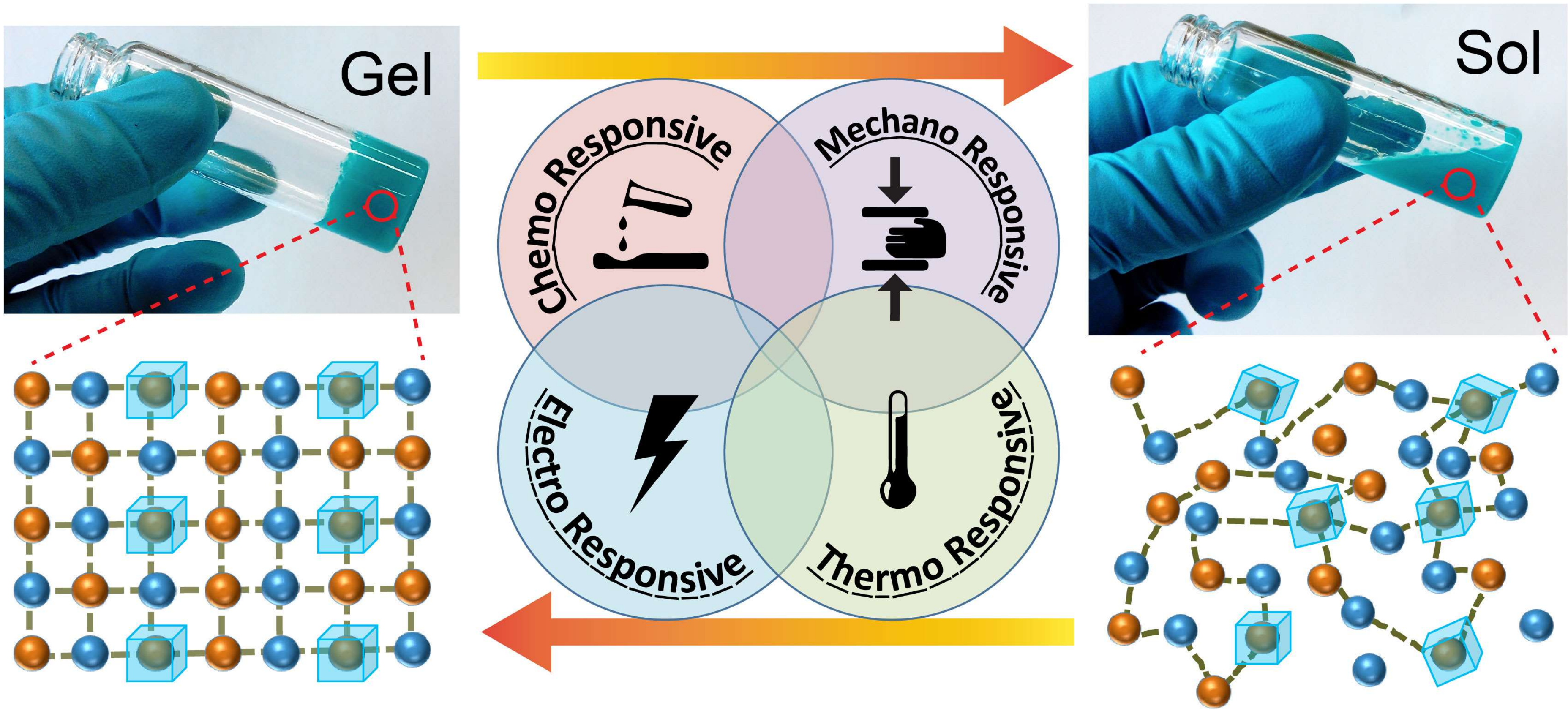
In the crosslinked state, **crosslinked hydrogels reach an equilibrium swelling level** in aqueous solutions which depends mainly on the crosslink density, but also from environment conditions (pH, T, etc)

Equilibrium Water Content:

$$EWC = \frac{W_w}{W_t}$$



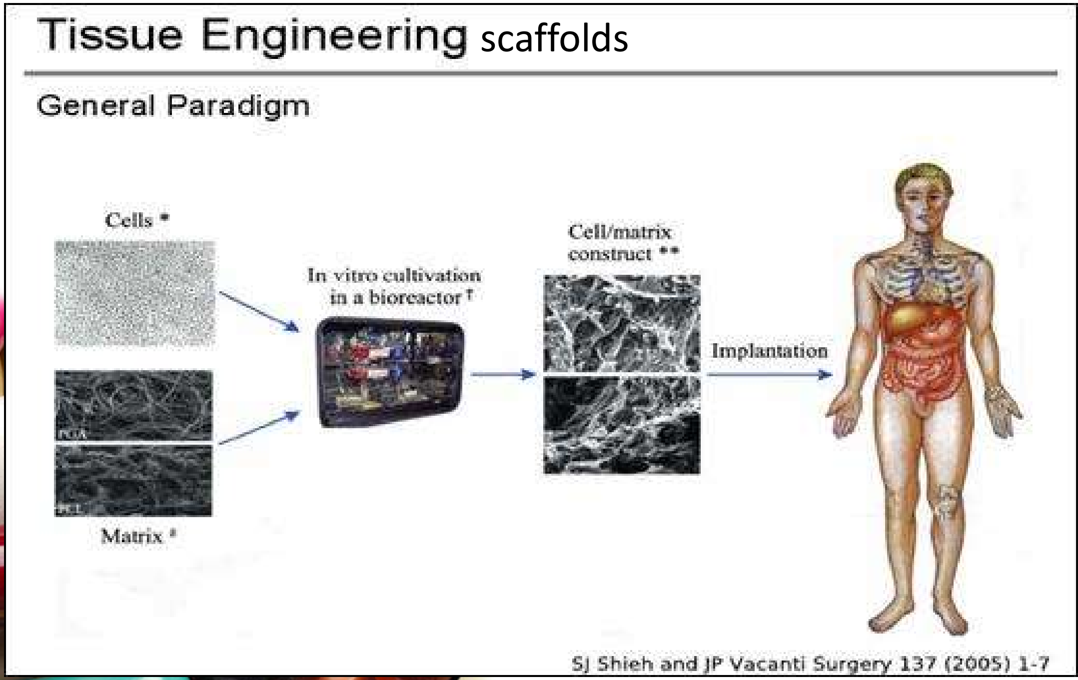
Polymeric solution-gel transition



Applications

soft contact lenses

pills or capsules for oral ingestion



Tissue Engineering scaffolds

General Paradigm

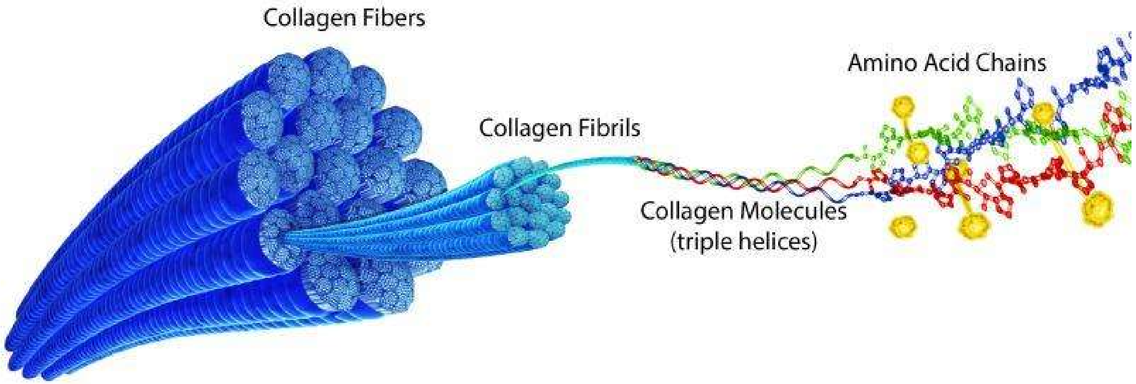
SJ Shieh and JP Vacanti Surgery 137 (2005) 1-7

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).

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**.
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 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,** generally *extracted from red seaweed*, which 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

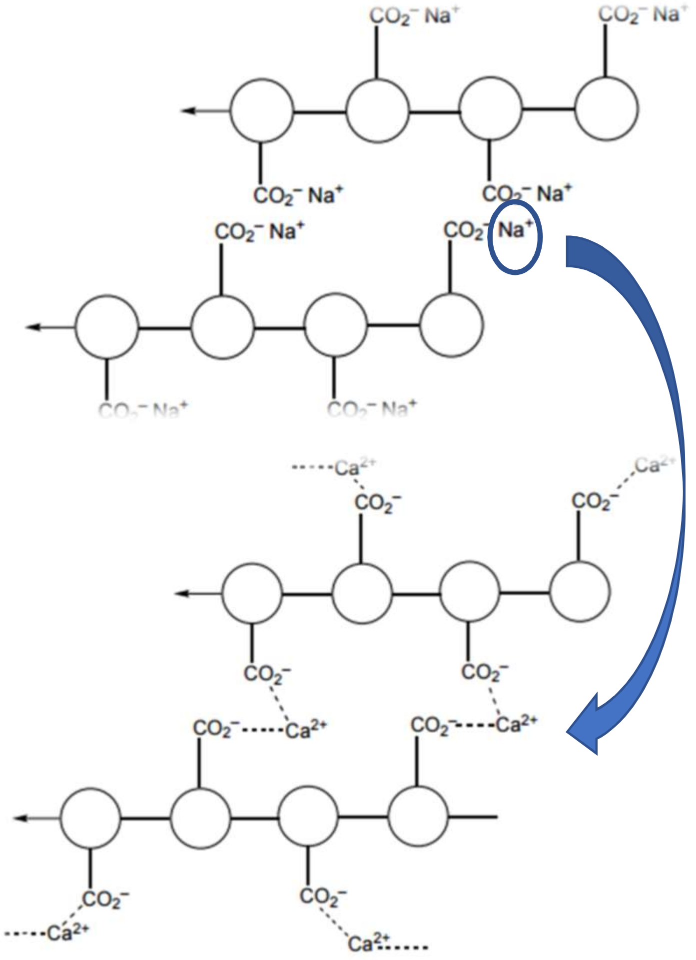
Example 4 - Alginate

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.

$$\{\text{Chelator-Ca}\}_{\text{complex}} \Longleftrightarrow \text{Chelator} + \text{Ca}^{2+}$$



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

Example 5 – decellularised ECM

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

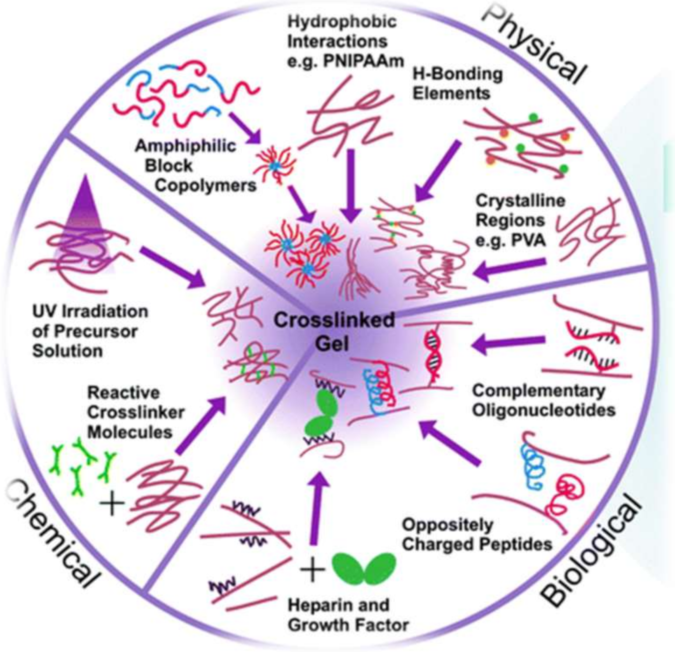
Digestion, its necessary to solubilize decellularized ECM (i.e. breaks down proteins into smaller peptides).

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



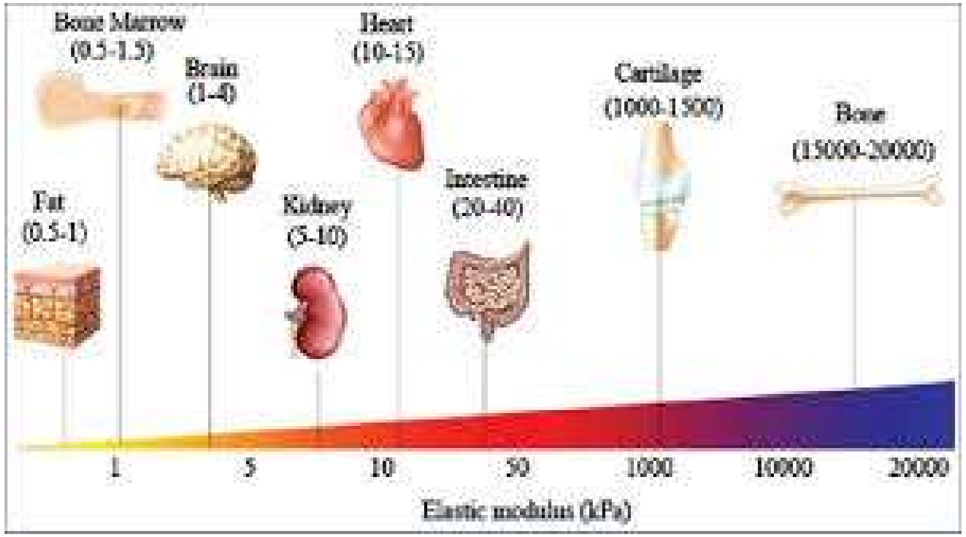
Chemical gels

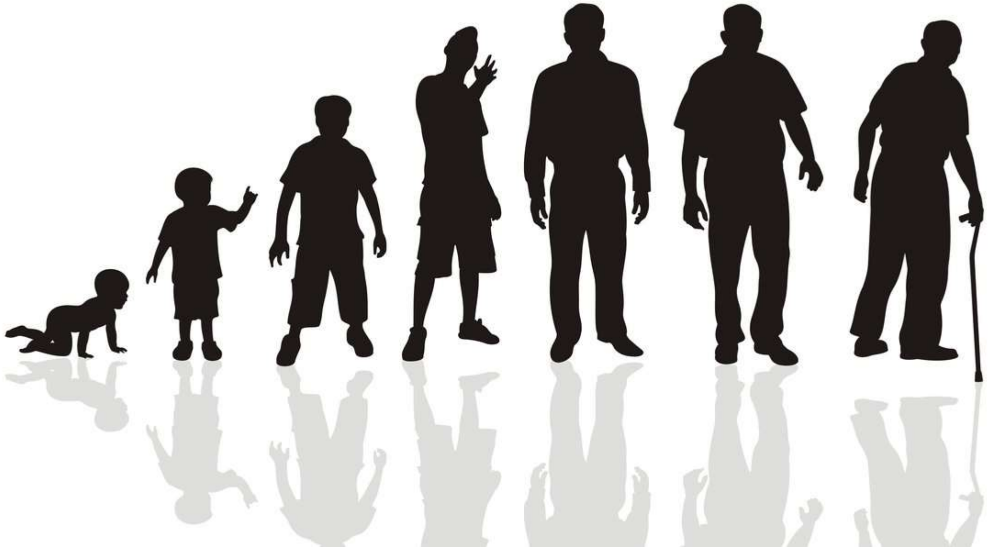
Crosslink polymers in the solid state or in solution with:

- 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?

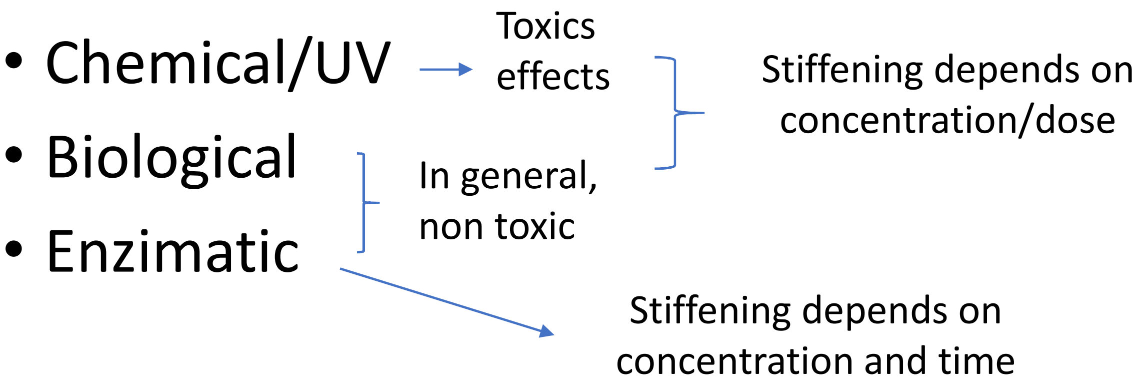
- Stabilize hydrogels
- Enhance mechanical properties
- Modulate Mechanical Properties





Pathophysiological models of foetal growth, ageing, fibrosis

Crosslinker types



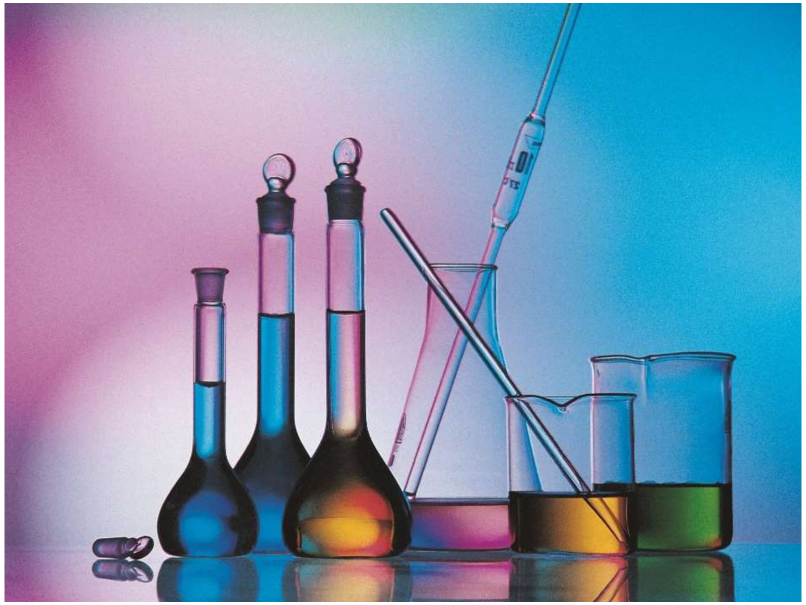
- Chemical/UV
- Biological
- Enzimatic

Toxics effects

In general, non toxic

Stiffening depends on concentration/dose

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)

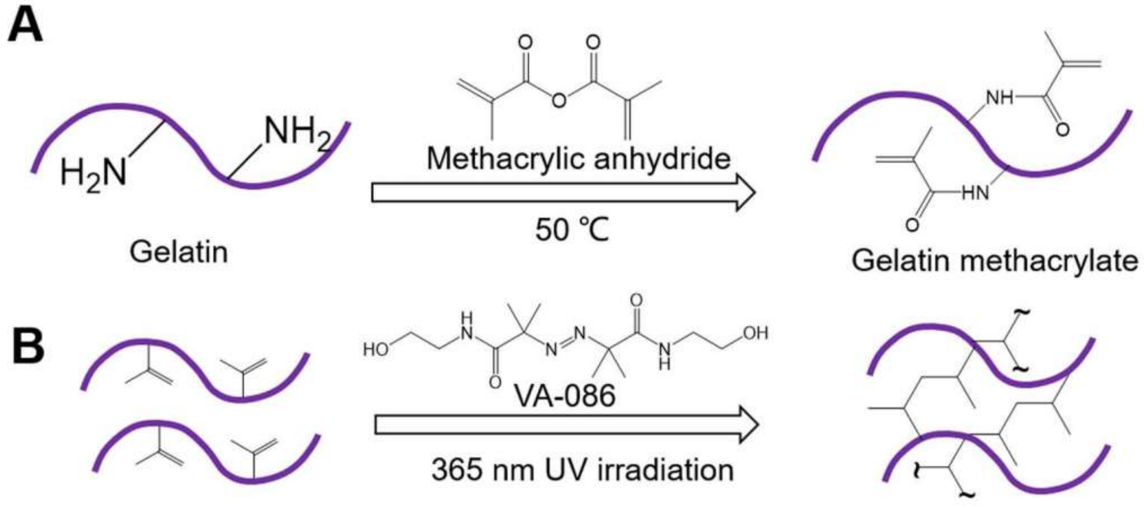
Crosslinking of amine containing polymers (i.e. collagen, gelatin, ecm) with GTA (glutaraldehyde) involves the *reaction of free amino groups of lysine* or hydroxy-lysine amino acid residues of the polypeptide chains *with the aldehyde groups of GTA*

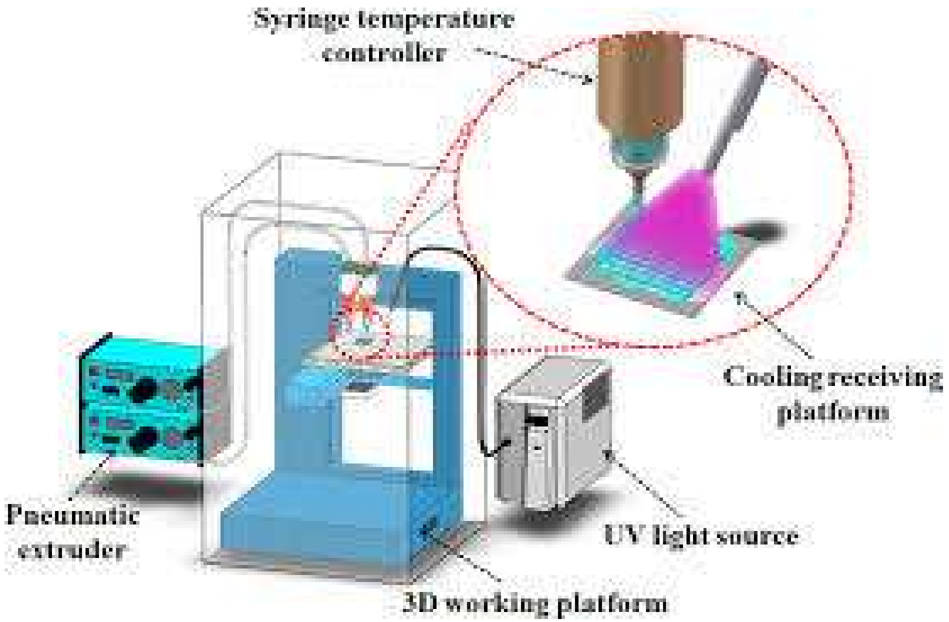
Since glutaraldehyde is a **toxic** compound that even at low concentration shows cell-growth inhibition, hydrogels need to be careful washed before use.

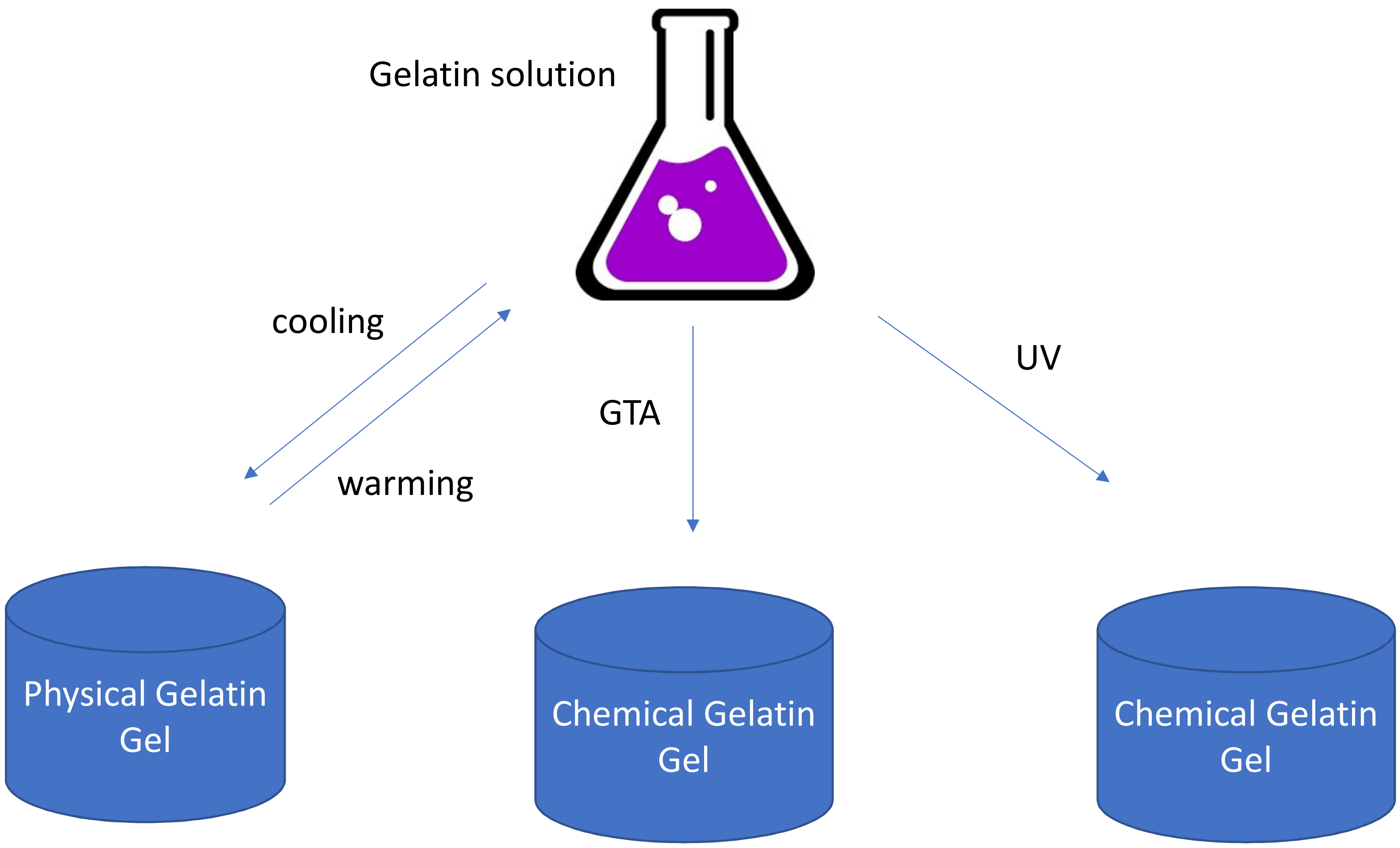
UV crosslinking: GelMA

Gelatin methacrylate (GelMA) is a widely used natural hydrogel for biofabrication because of cost-effectiveness, the ease of synthesis and photocrosslinking, as well as the great biocompatibility to allow cell adhesion and proliferation.

GelMA has been employed as the bioinks for stereolithography bioprinting or laser-based bioprinting. Building blocks for bioassembly, such as microdroplets and microfibers [18], can be also fabricated using the GelMA.





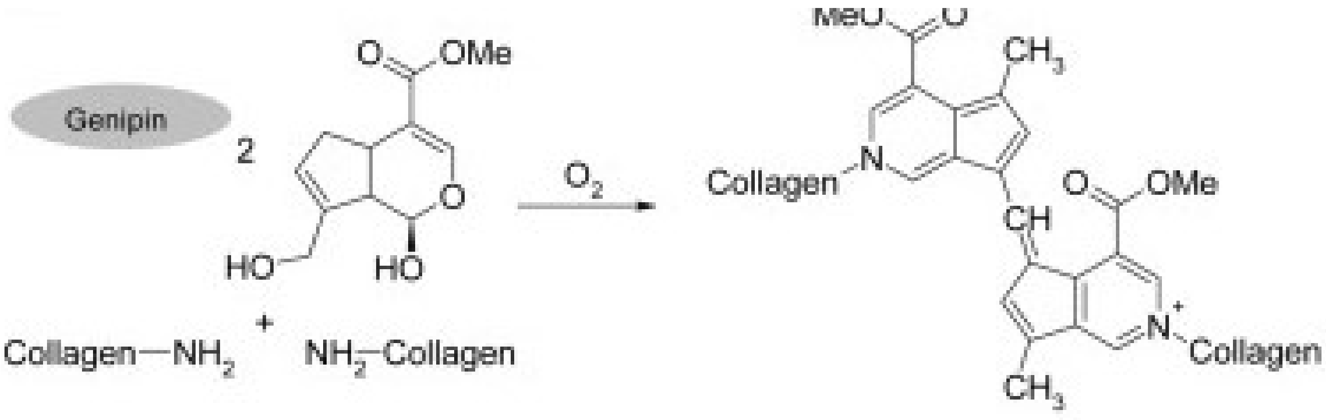

Gelatin solution
cooling
warming
GTA
UV
Physical Gelatin Gel
Chemical Gelatin Gel
Chemical Gelatin Gel

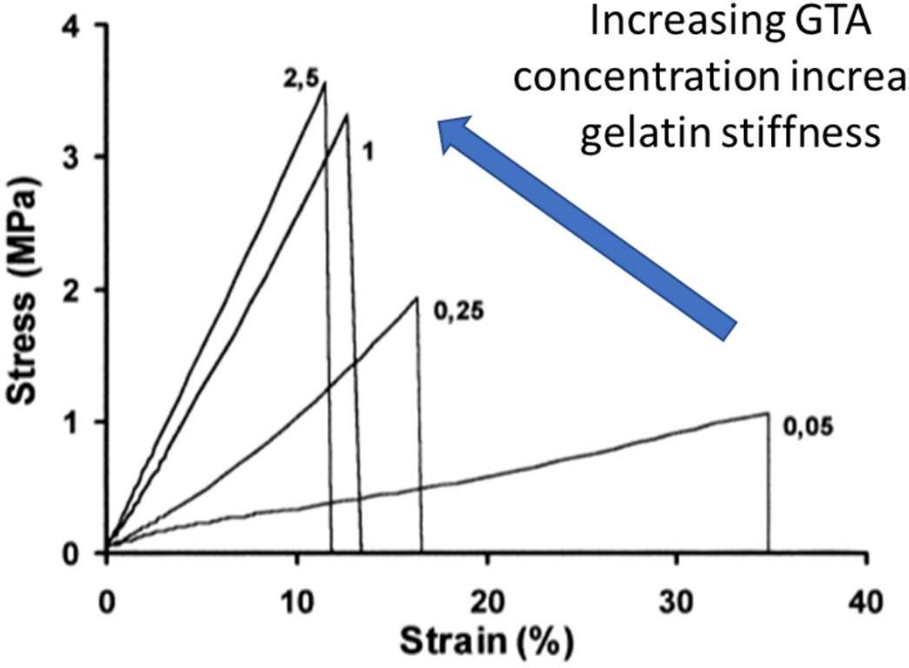
Biological crosslinkers: Genepin

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.

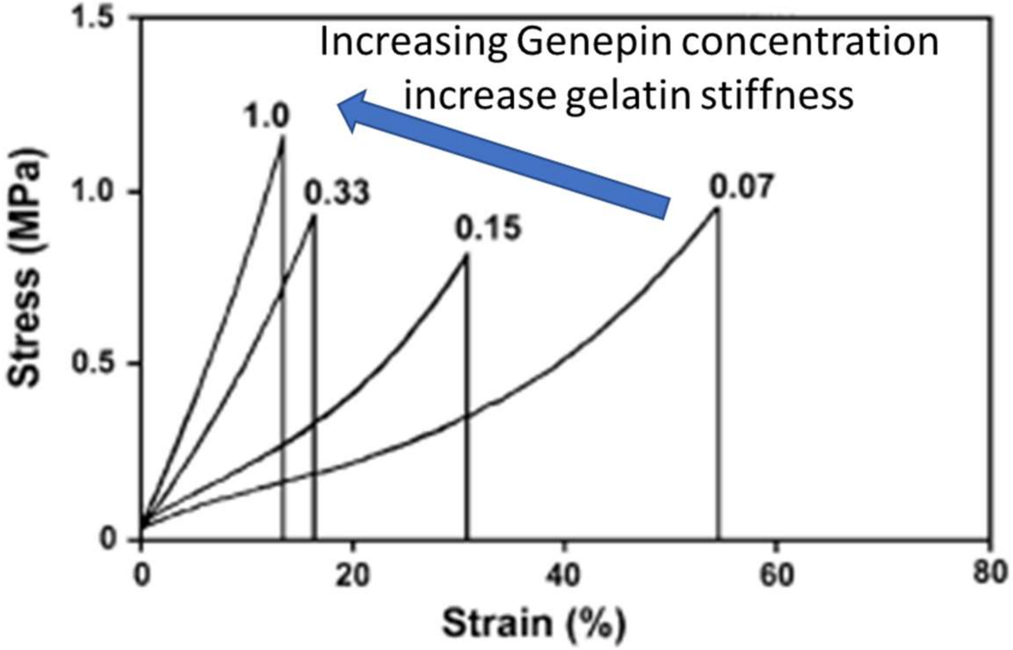
Genipin can be obtained from an iridoid glucoside, geniposide, abundantly present in **gardenia fruits**. Genipin has been widely used in herbal medicine, and the *dark blue pigments obtained by its spontaneous reaction with amino acids* or proteins have been used in the fabrication of food dye.





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

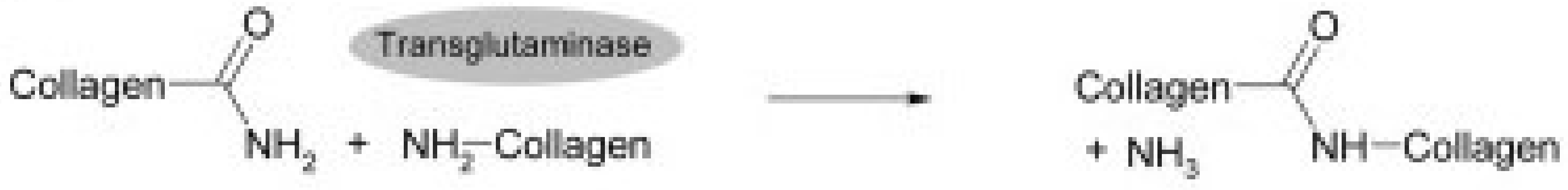
Fig. 1. Typical stress–strain curves recorded from gelatin films cross-linked with genipin. The numbers near the curves indicate the concentration of genipin, expressed as wt%.

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).

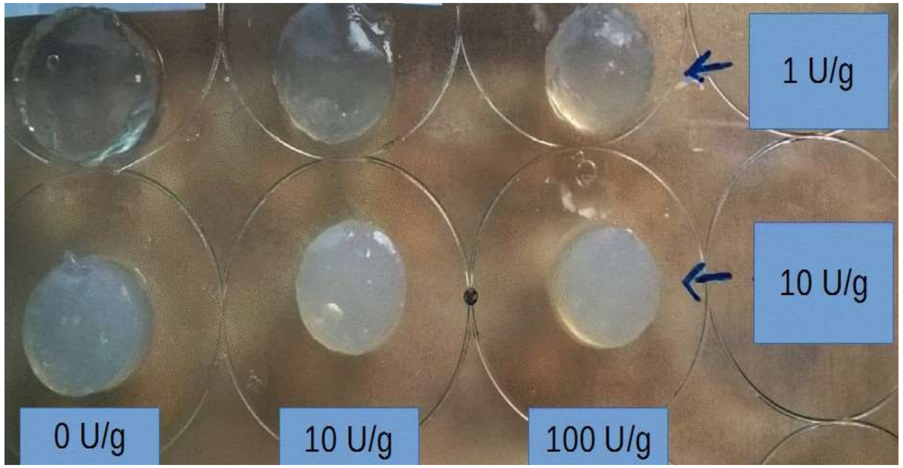
Microbial transglutaminase (mTG) is a native protein that is innocuous and **commonly used in food manufacturing processes** approved for human consumption by the U.S. Food and Drug Administration.

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 & TIME-VARIANT stiffness

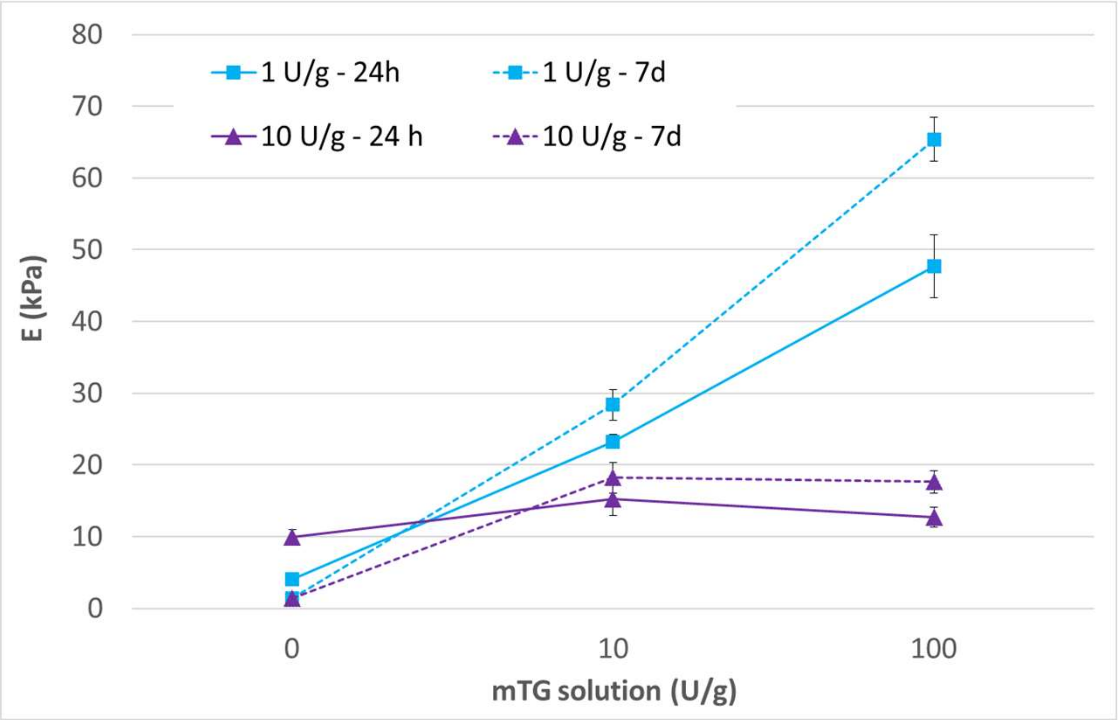
+ swelling



Inside the gel

+ shrinking

Solution (outside the gel)

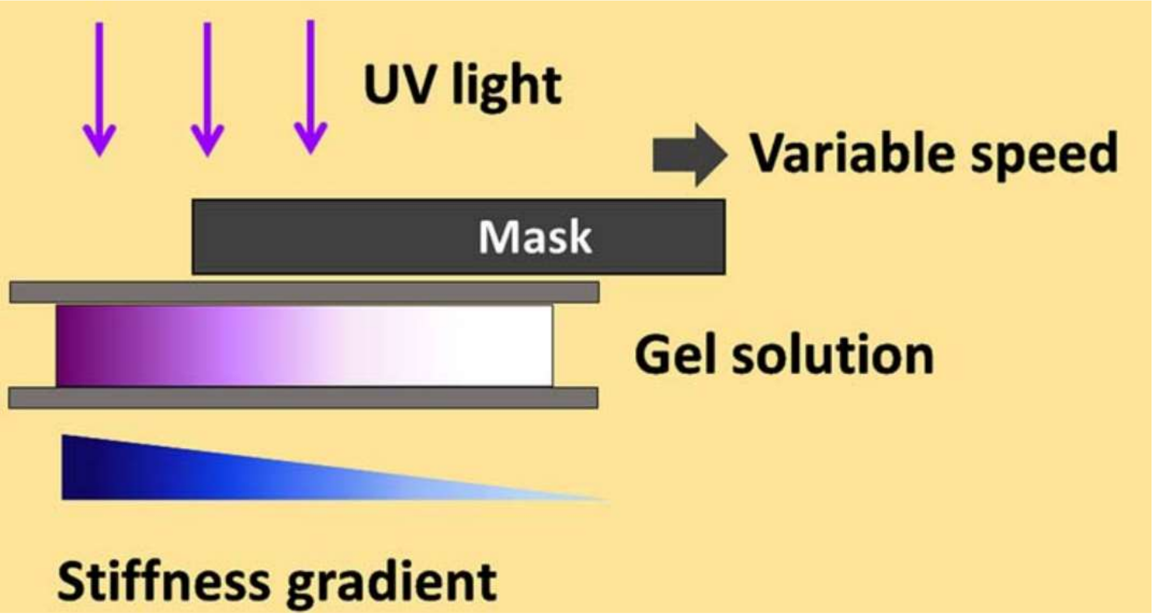


One ***U (unit)*** *is defined as the* ***amount of the enzyme that produces a certain amount of enzymatic activity*** *(i.e. the amount that catalyzes the conversion of 1 micro mole of substrate per minute).*

E increase both with mtg concentration on incubation time

SPACE-variant mechanical properties

Within the body, tissues do not present an uniform stiffness but complex **stiffness gradients,** which can be found at the interface within the tissue, between different tissues or in pathologic conditions.



Methods to generate mechanical gradients:

- Sliding mask
- Mask with an opacity gradient
- Crosslinker diffusion

Domande articoli

1) Indentificare le differenze tra gli hydrogel ottenuti con le due diverse modalità di stiffening descritte nel paper

2) Cosa succede al variare di GTA in termini viscoelastici?

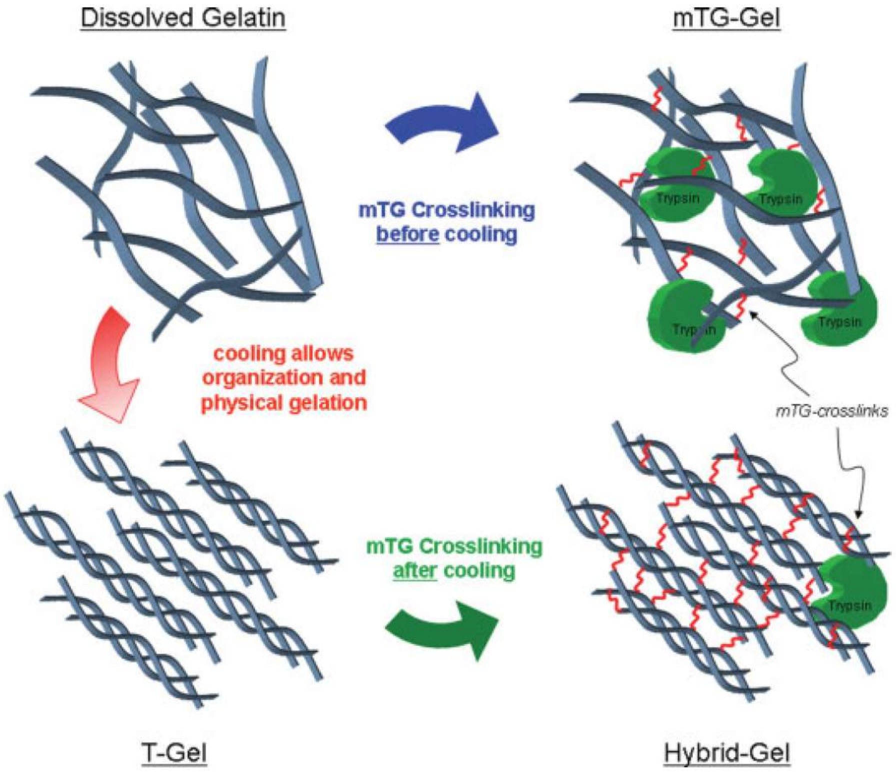
3) Spiegare cosa succede variando la viscosità della fase acquosa di un gel

https://padlet.com/lu_cacopardo/6cqcl59eo9jv

	Gruppo 1	Gruppo 2	Gruppo 3	Gruppo 4	Gruppo 5	Gruppo 7	Gruppo 8	Gruppo 9
Proprietà Materiali	ok	ok	ok	ok	ok	ok	ok	ok
Polymers&Hydrogels								

Legenda: ok+ ok ok-

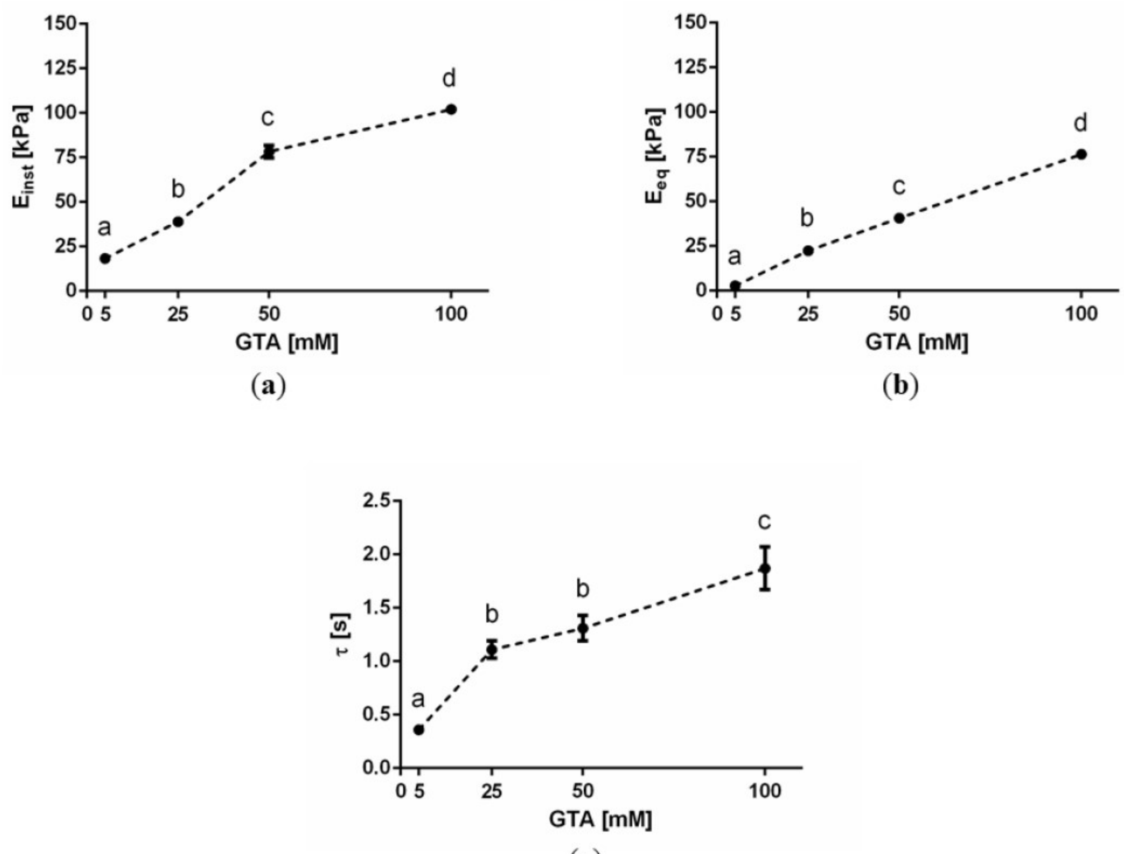
Articolo 1



Le differenza è che nel caso degli mTG gel i crosslink catalizzati dall’enzima si formano tra catene polimeriche orientate casualmente, mentre nel Hybrid-gel (sottoposto prima a gelazione termica e poi a trattamento enzimatico) i legami si formano tra catene che hanno assunto una struttura organizzata.

‘Hybrid-Gels were found to be **more resistant** to proteolysis compared mTG-Gels. An explanation may be that thermal cooling allows gelatin to first self-organize into a tight network of polypeptides through hydrogen bonding, while mTG-gels have a looser network more susceptible to proteolytic attack (from trypsin) despite being thermally stable’

Articolo 2

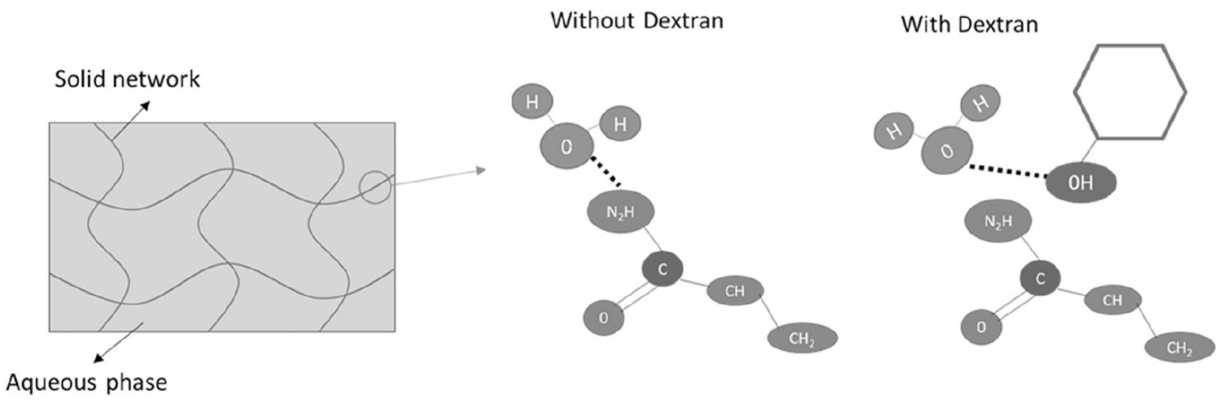


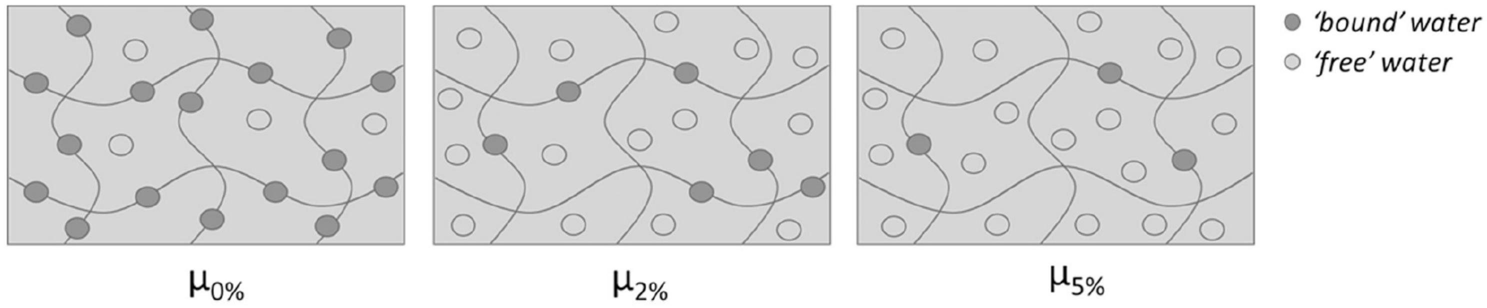
(a) (b)

(c)

il crosslinking con GTA non solo rende I gel più rigidi (Eeq aumenta) ma anche più elastici (tau aumenta)

Articolo 3





Aumentando la viscosità della fase acquosa, la componente viscosa del gel aumenta lasciando inalterata quella elastica (non vario il network 'elastico'). Tau aumenta nelle spugne, quindi tende ad avere un comportamento più elastico 'solid-like' e diminuisce nei gel, quindi tende ad avere un comportamento più viscoso o 'fluid-like' (questo dipende dal fatto che nei gel la presenza di destrano fa si che l'acqua sia meno legata alle catene e quindi più libera di scorrere).