生醫材料導論

Hydrogel for Biomedical Application

水膠生物醫學應用

Ching-Li Tseng

Graduate Institute of Biomedical Materials & Tissue Engineering

Taipei Medical University

Hydrogels (水膠)

Wichterle and Lim, 1960

2-hydroxyethyl methacrylate
+
glycol dimethacrylate

Letters to Nature

Nature **185**, 117-118 (9 January 1960) | doi:10.1038/185117a0

Hydrophilic Gels for Biological Use

O. WICHTERLE & D. LÍM

1. Institute for Macromolecular Chemistry, Czechoslovak Academy of Sciences, Prague.

PLASTICS to-day enjoy wide use in many fields, and it is natural that the possibility of their employment in permanent contact with living tissues has been seriously considered. A study of the literature shows that almost all known plastics have been proposed for this purpose at various times. The question of structural similarity with the tissue has not, however, been emphasized although physiologically unfavourable effects were observed in most cases of application of normal type plastics.

▲ Top

- Gel are defined as a substantially dilute cross-linked system, and are categorized principally as weak or strong depending on their flow behavior in steady-state. (swelling behavior and three -dimensional structure)
- The excellent tolerance of the body towards hydrogels
- The **non-degradable hydrogels** got encapsulated in a fibrous capsule without eliciting any irritation to the surrounding tissue upon subcutaneous implantation.

* Advanced Drug Delivery Reviews 64 (2012) 18–23

Hydrogels

- Three-dimensional cross-linked hydrophilic polymer networks that have the ability to swell but **do not dissolve in water**.
- May absorb from 10～20% up to thousands of times their dry weight in water.

- Retain a large quantity of **water** within the matrix, enabling them to behave quite similar to natural living tissues.
- Provide a semi-wet, three-dimensional environment for molecular–level biological interactions.
- Provide a non-adhesive surface towards cells and allow for programmable cell adhesion upon attaching biological molecules to the hydrogel.

Water in hydrogels

Step 1

Begins to **absorb water**, the first water molecules entering the matrix will hydrate the most polar, hydrophilic groups, leading to 'primary bound water'.

Step 2

As the polar groups are hydrated, the network swells, and exposes hydrophobic groups, which also interact with water molecules, leading to hydrophobically-bound water, or 'secondary bound water'.

Step 3

Primary and secondary bound water are often combined and simply called the 'total bound water'.

Step 4

- The **additional swelling water** that is imbibed after the ionic, polar and hydrophobic groups become saturated with bound water is called 'free water' or 'bulk water'.
- **Fill the space between the network chains**, and/or the center of larger pores, macropores or voids.

Total water : Bound water + free water

Classification of hydrogel

Origin

Natural

- Most closely resemble the tissues they are meant to replace
- Almost always biocompatible
- Biodegradable
- Difficult to isolate from biological tissues
- Restricted versatility

Synthetic

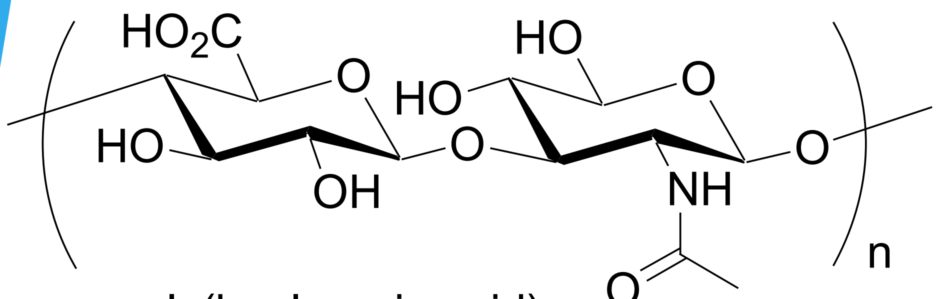
- Can be reliably produced
- Greater control over polymer structure
- May not be biocompatible
- Not always biodegradable
- Use of toxic reagents a problem

Lee, K. Y.; Mooney, D. J. *Chem. Rev.*, **2001**, *101*, 1869

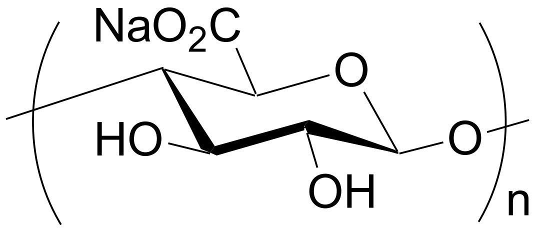
Classification of hydrogel

Natural

Some Hydrogel Forming Polymers

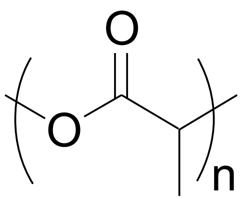


poly(hyaluronic acid)

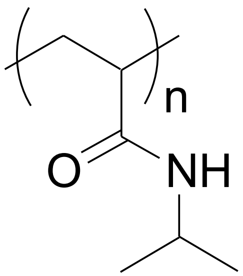


poly(sodium alginate)

Synthetic

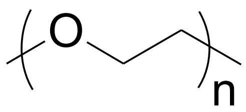


poly(lactic acid)



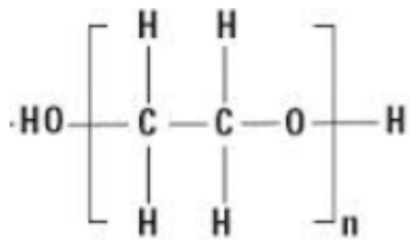
poly(N-isopropyl acrylamide)

PEG



poly(ethylene glycol)

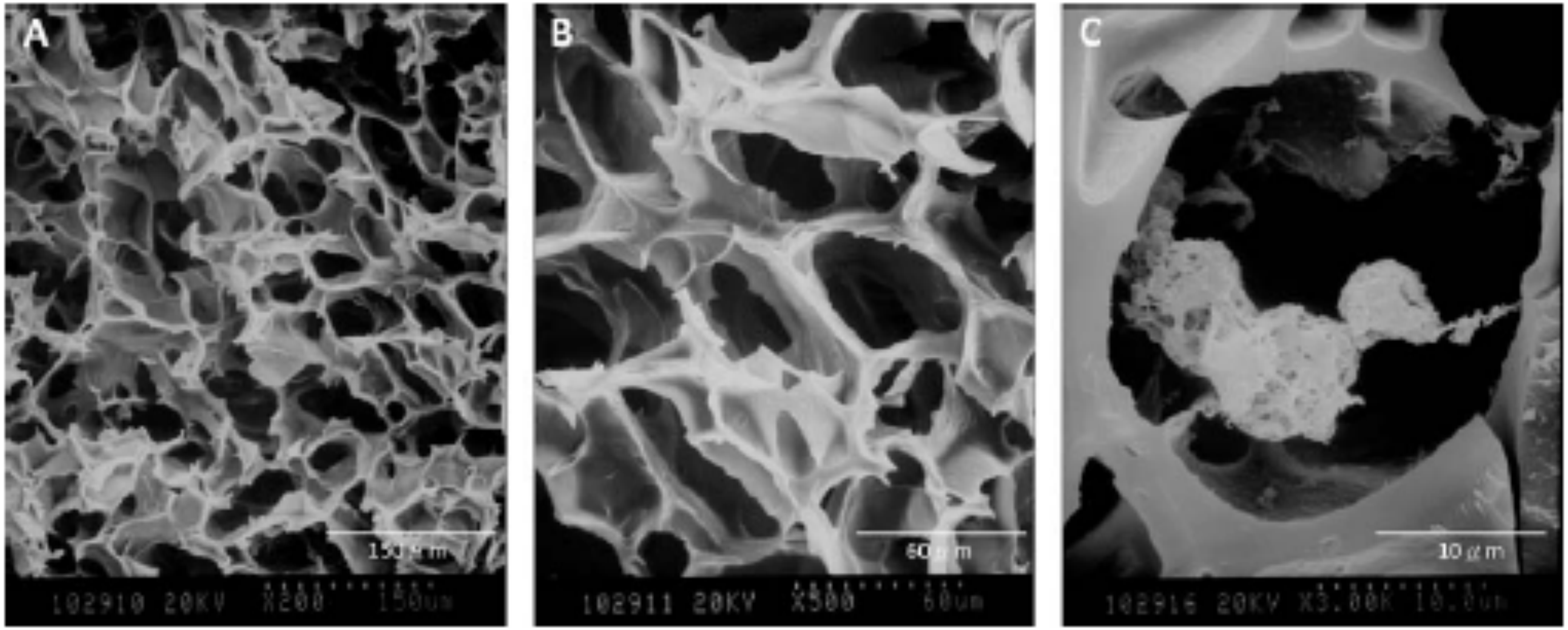
Poly(vinyl alcohol)



Classification of hydrogel

- various criteria for the classification of hydrogels

Water content or degree of swelling	Low swelling
	Medium swelling

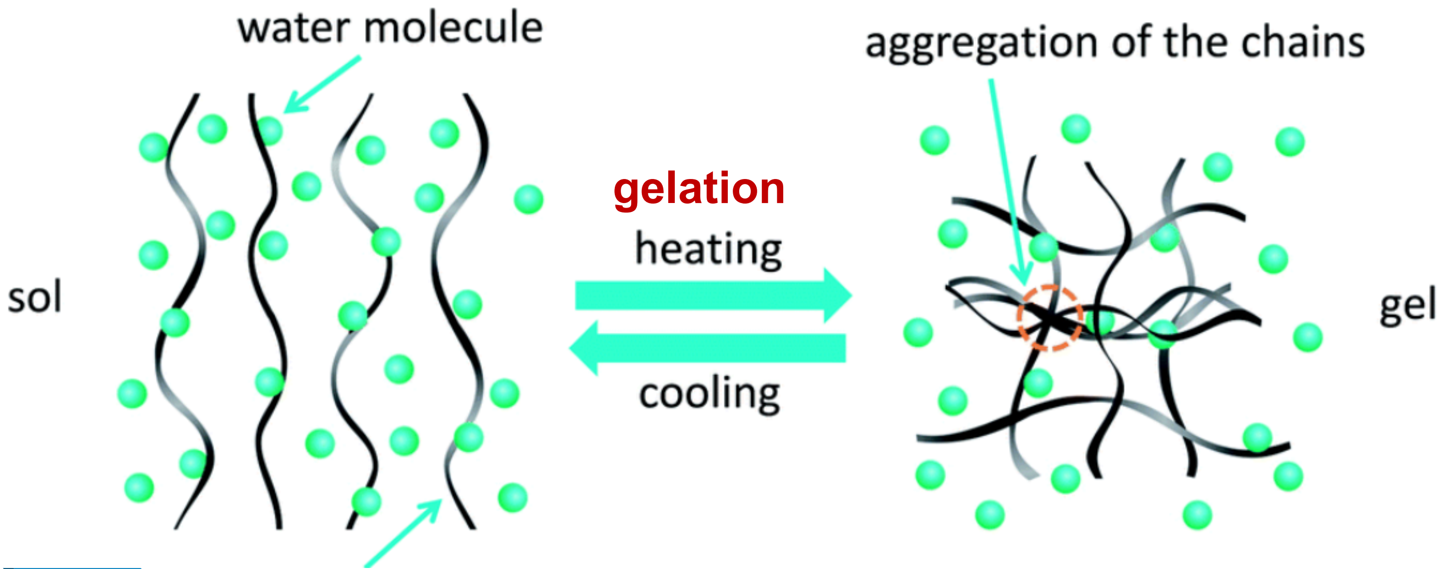


Biodegradability	Biodegradable
	Nondegradable

Methods to produce hydrogel

Gelation refers to the **linking of macromolecular chains together (gel)** which initially leads to progressively larger branched yet **soluble polymers (sol)** depending on the structure and conformation of the starting material.

➡ **sol-gel transition**

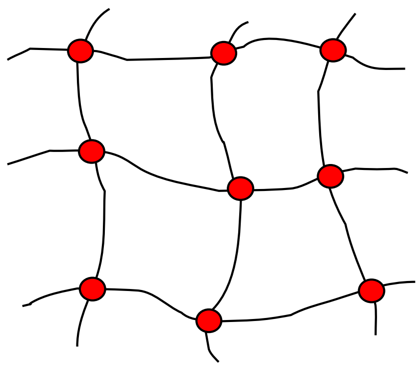


http://www.cailsilorin.com/solgel-transition-of/

Hydrogel Fabrication

Chemical hydrogels

Covalently cross-linked



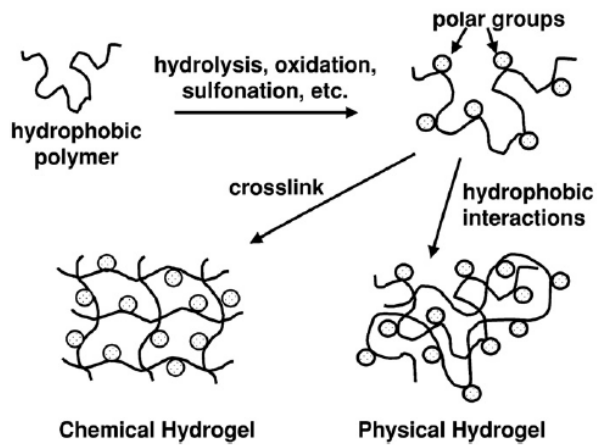
- **Thermoset hydrogels**
- **Volume phase transition**
- **Reliable shape stability and memory**

Physical hydrogels

Noncovalently cross-linked

- **Hydrogen bonding**
- **Hydrophobic interaction**
- **Stereocomplex formation**
- **Ionic complexation**

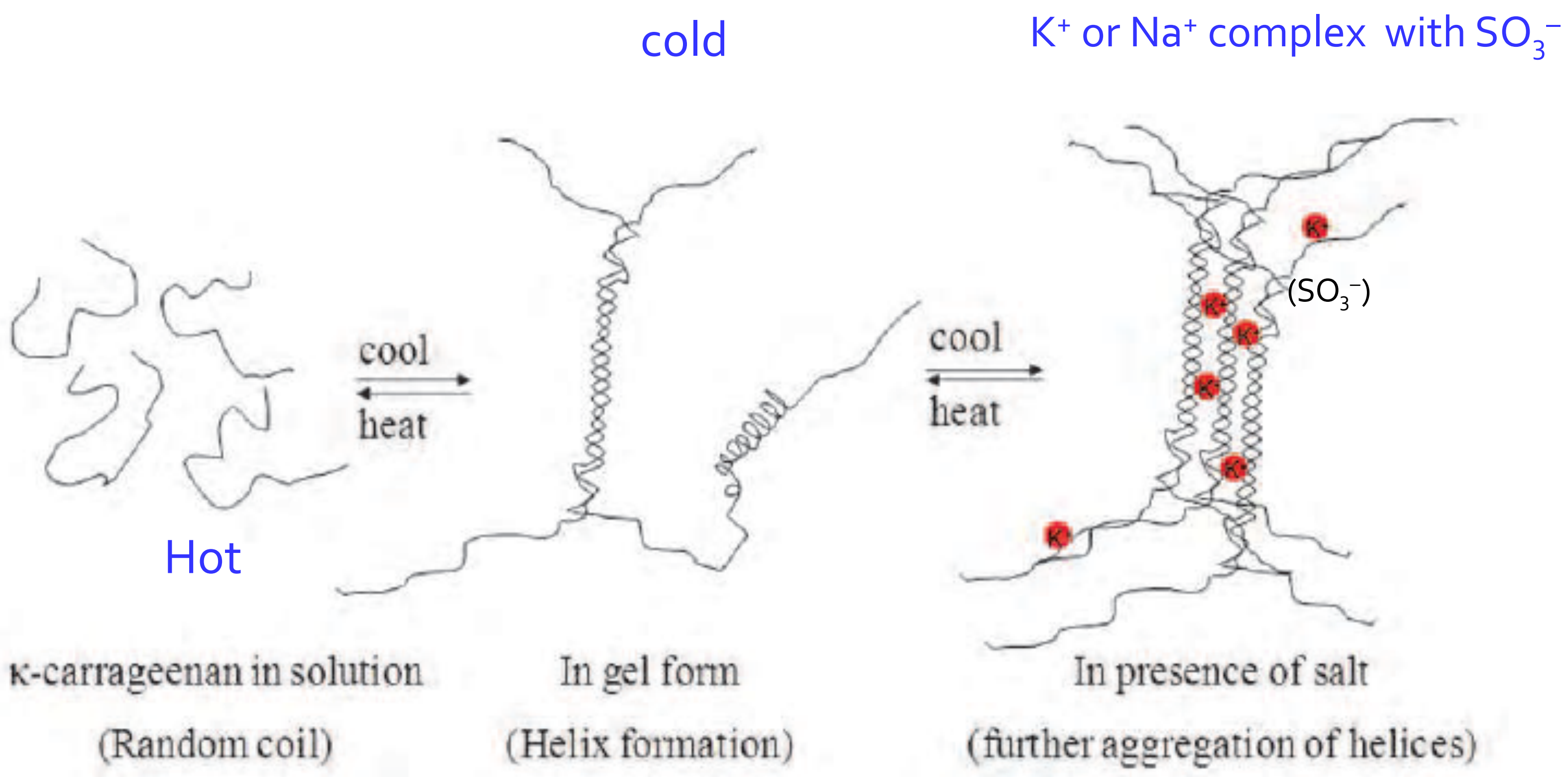
- **Thermoplastic hydrogels**
- **Sol-gel phase transition**
- **Limited shape stability and memory**



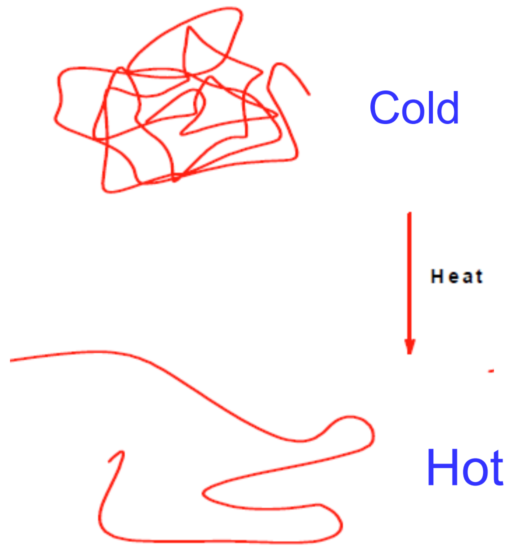
Physical cross-linking

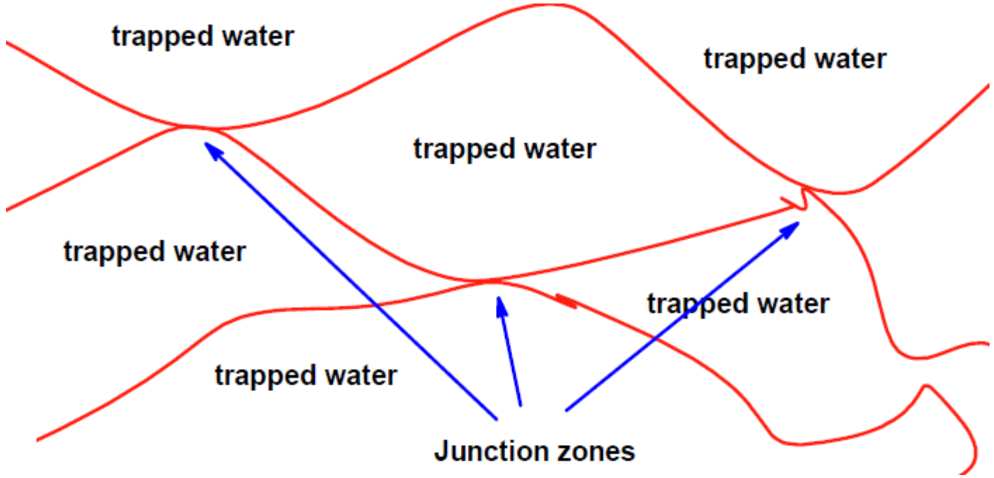
Heating/cooling a polymer solution

The gel formation is due to temperature change to form helix-formation, association of the helices, and forming junction zones.



Geltin gelation

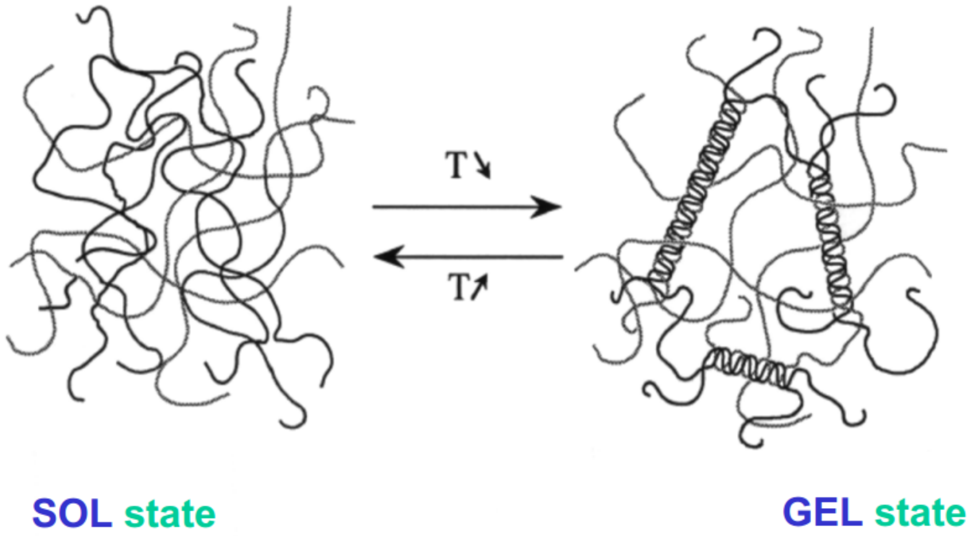




Mechanism of gelation

Gelatin gelation is related to the coil-triple helix transition.

Gelation and gel strengthening are kinetic processes



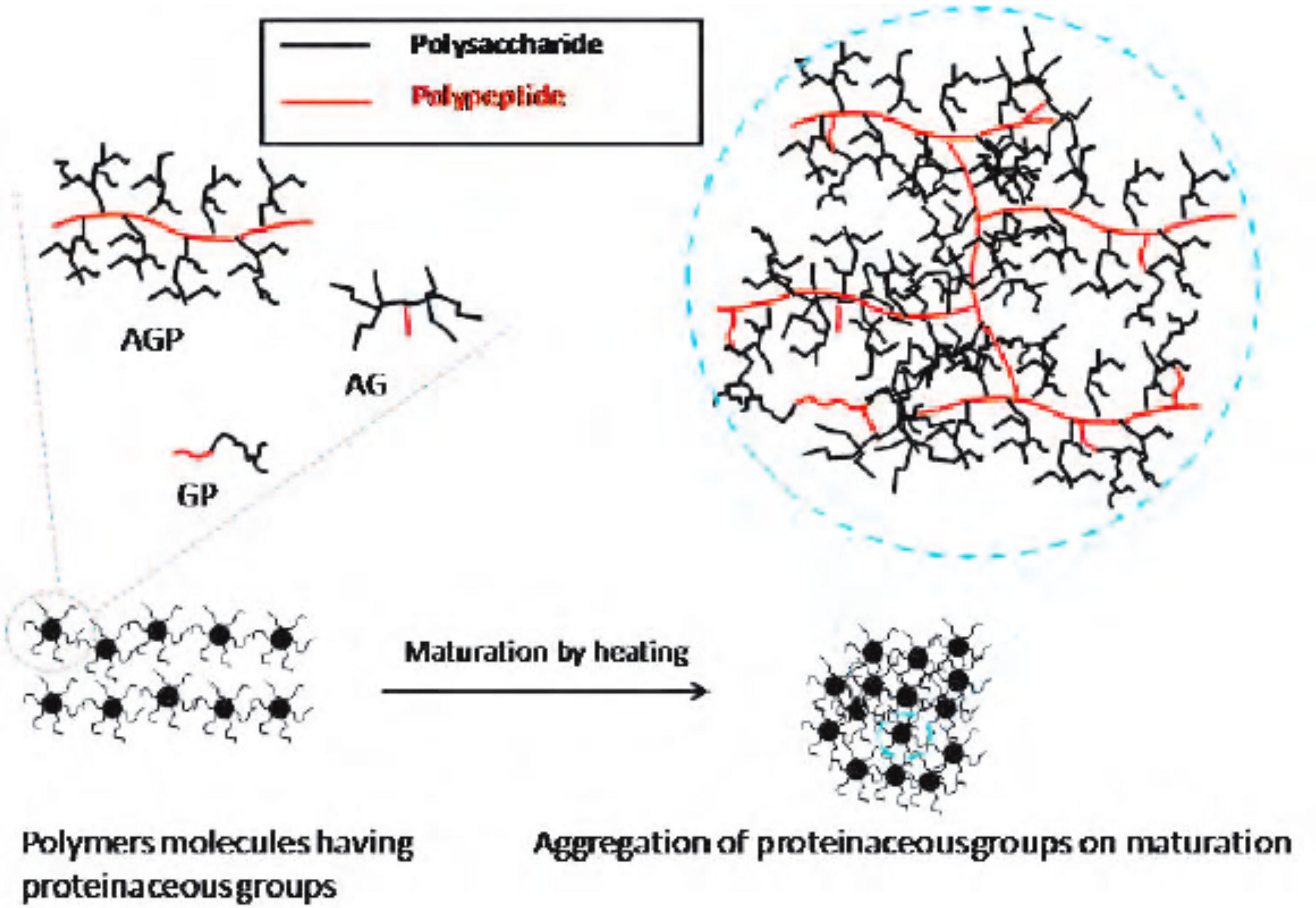
Coil-triple helix structure

Maturation
(heat induced aggregation)

Physical cross-linking

Gum arabic

- Arabinogalactan (AG)
- Arabinogalactan protein (AGP)
- Glycoprotein (GP)



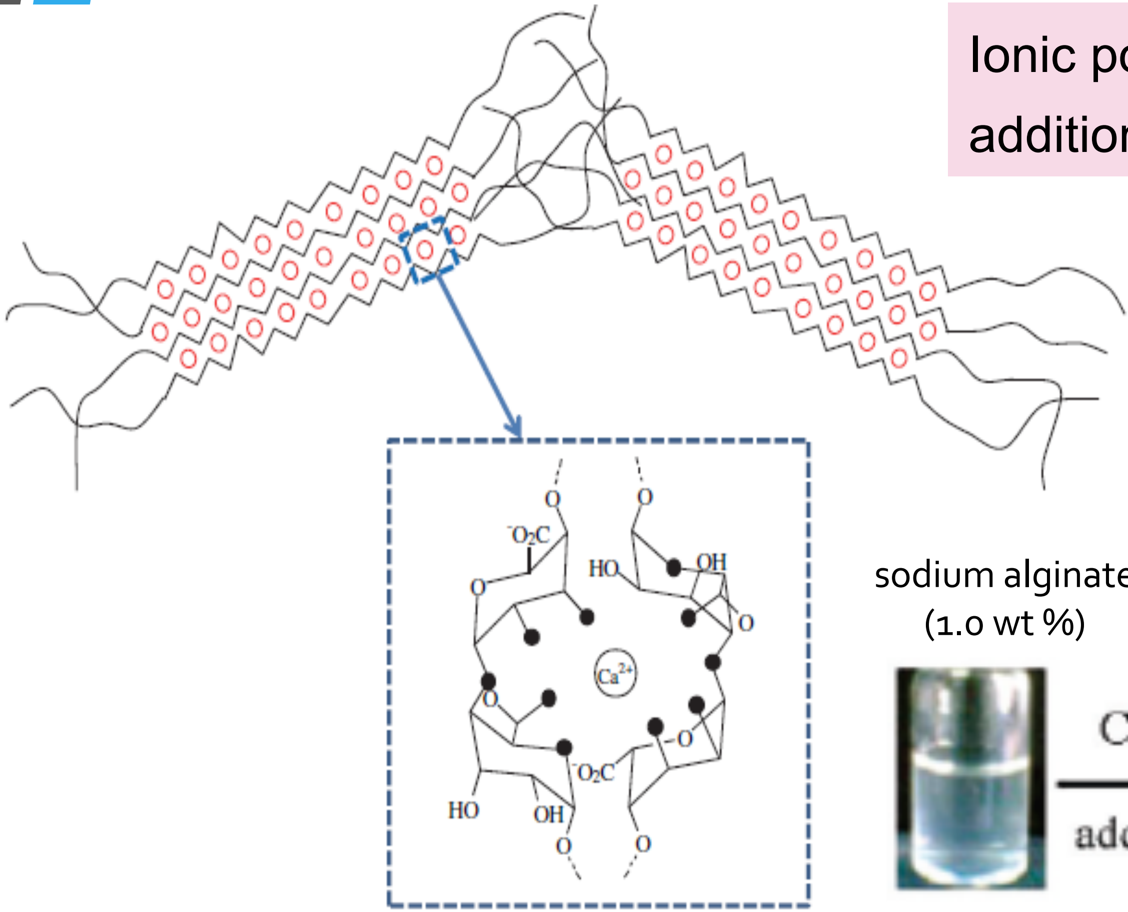
Maturation of gum arabic causing the aggregation of proteinaceous part of molecules leading to cross-linked hydrogel network.

Ionic hydrogel

Physical cross-linking

Calcium Alginate Hydrogels

Ionic polymers can be cross-linked by the addition of **di- or tri-vialent counterions.**

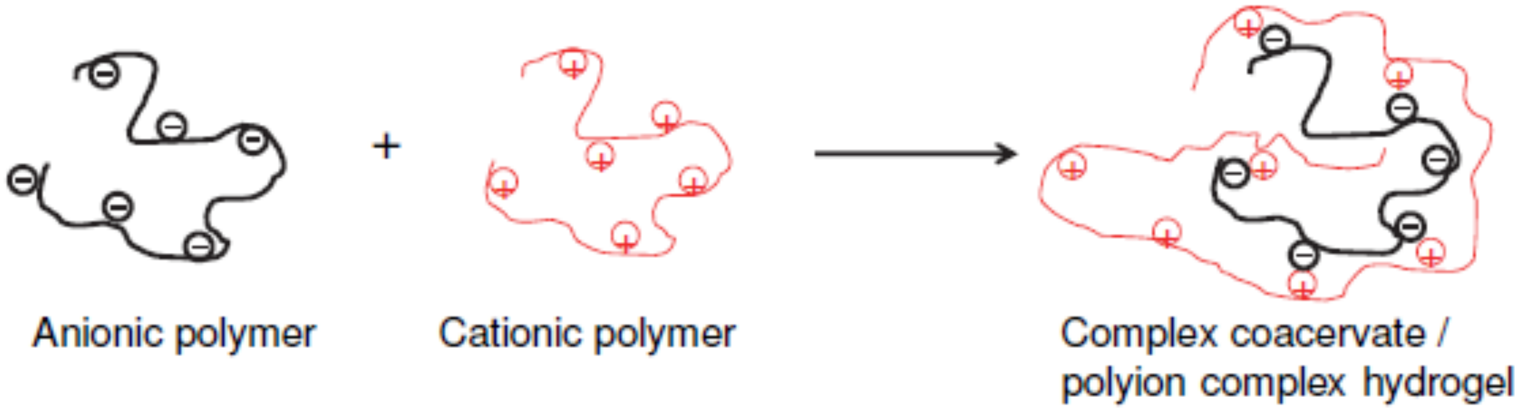


Crystal Growth & Design, Vol. 9, No. 8, 2009, 3470-3476

Complex coacervation

Physical cross-linking

Can be formed by mixing of a **polyanion** with a **polycation.** (polymers with opposite charges stick together and form soluble and insoluble complexes depending on the concentration and pH of the respective solutions)



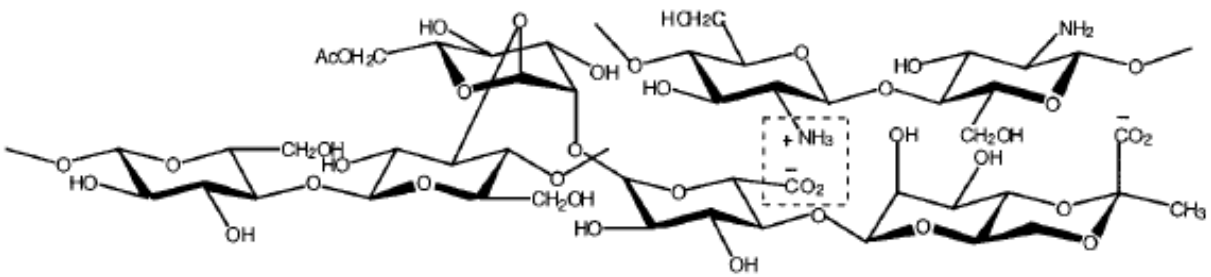
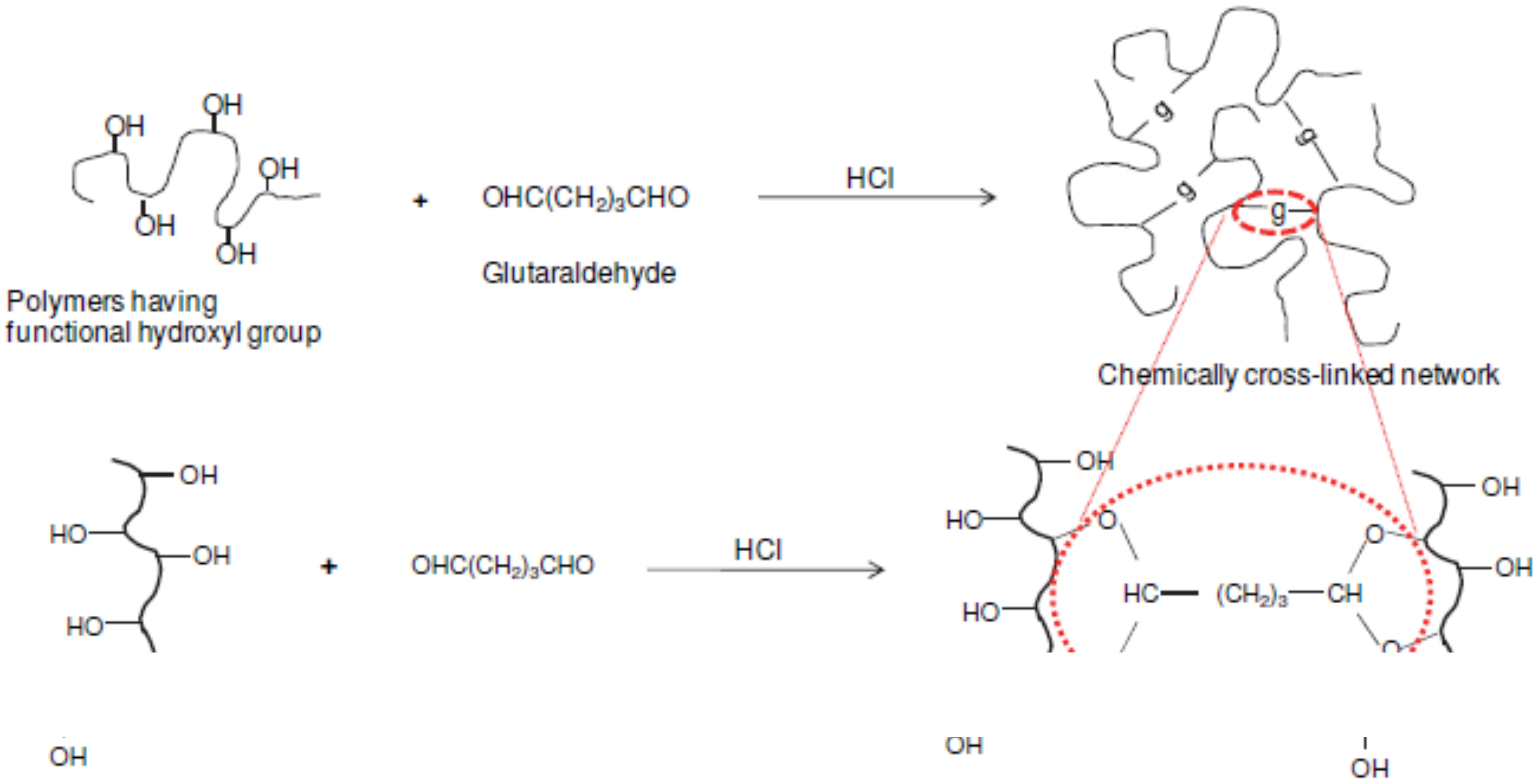


Figure 1. Schematic representation of Chitosan and Xanthan interaction to form the CH-X hydrogel.

Chemical cross-linkers

- Involves the introduction of new molecules between the polymeric chains to produce cross-linked chains.
- Functional groups (such as OH, COOH, and NH_2)

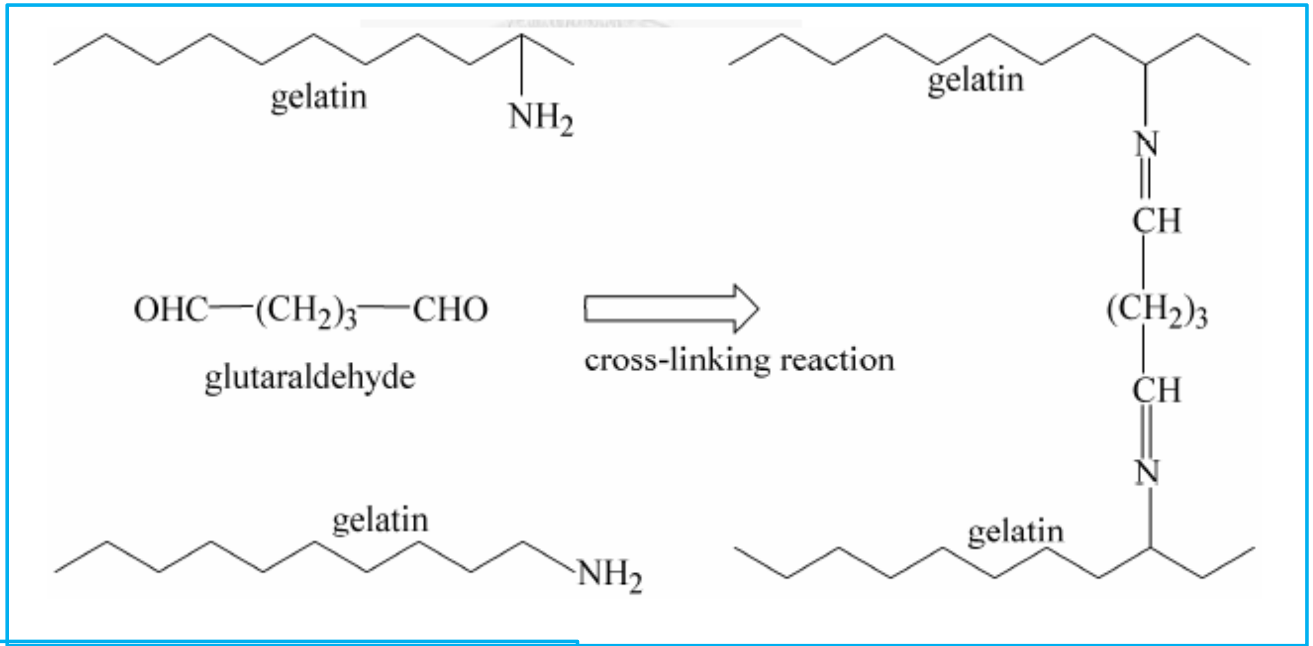


Schematic illustration of using chemical cross-linker to obtain cross-linked hydrogel network.

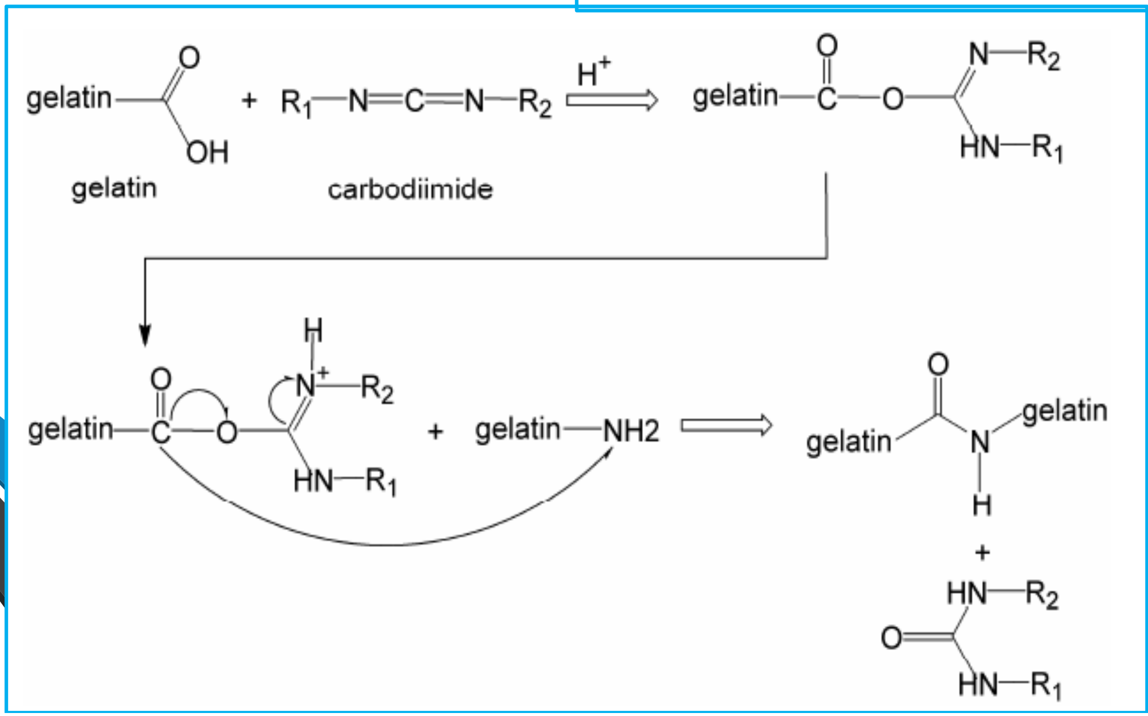
Chemical cross-linkers

Gelatin

Glutaraldehyde



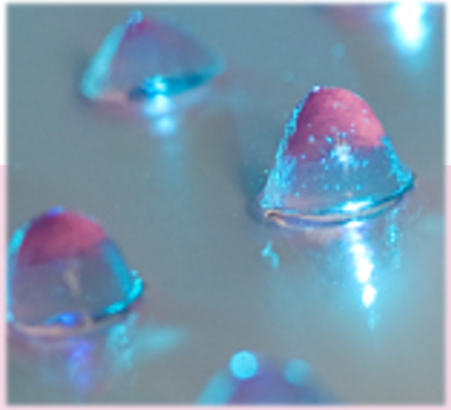
Carbodiimide



Monomers used in the synthesis hydrogels for pharmaceutical applications

Monomer abbreviation	Monomer
HEMA	Hydroxyethyl methacrylate
HEEMA	Hydroxyethoxyethyl methacrylate
HDEEMA	Hydroxydiethoxyethyl methacrylate
MEMA	Methoxyethyl methacrylate
MEEMA	Methoxyethoxyethyl methacrylate
MDEEMA	Methoxydiethoxyethyl methacrylate
EGDMA	Ethylene glycol dimethacrylate
NVP	N-vinyl-2-pyrrolidone
NIPAAm	N-isopropyl A A m
AA	Acrylic acid
HPMA	N-(2-hydroxypropyl) methacrylamide
EG	Ethylene glycol
PEGMA	PEG methacrylate

Different physical forms



(a) Solid molded forms

soft contact lenses

(b)Pressed powder matrices

pills or capsules for oral ingestion

(c) Microparticles

bioadhesive carriers or wound treatments

(d) Coatings

on implants or catheters; on pills or capsules
on the inside capillary wall in capillary electrophoresis

(e) Membranes or sheets

a reservoir in a transdermal drug delivery patch
2D electrophoresis gels

(g) Liquids

form gels on heating or cooling

Characteristic of hydrogel

http://www.hannahkeller.com/ME2/dirmod.asp?sid=&nm=&type=Publishing&mod=Publications%3A%3AArticle&mid=8F3A7027421841978F18BE895F87F791&tier=4&id=B86C8DCD26724945B6CC7624176F1F33

Solubility

Method A

- The hydrogel content of a given material is estimated by measuring its insoluble part in dried sample after immersion in deionized water for 16 h or 48 h at room temperature.
- The sample should be prepared at a dilute concentration to ensure that hydrogel material is fully dispersed in water.

$$Gel\ Fraction\ \ (hydrogel\%) = \left(\frac{W_d}{W_i}\right) * 100$$

Wi: initial weight of dried sample

Wd: weight of the dried insoluble part of sample after extraction with water

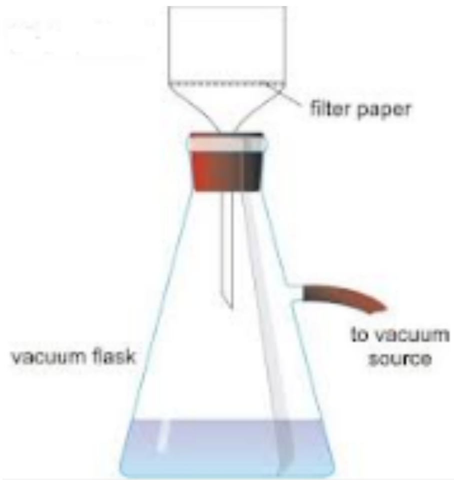
Solubility

Method B

Pore size: 1.2 μm

- The weight (W_1) of a 70 mm glass fiber paper is determined
- **1-2 wt% (S) sample** dispersion can be prepared in distilled water followed by **overnight hydration** at room temperature.
- The hydrated dispersion is then centrifuged prior to filtration.
- Drying of the filter paper is carried out in a oven followed by cooling to a constant weight (W_2).

$$\%Hydrogel = \left(\frac{W_2 - W_1}{S}\right) * 100$$



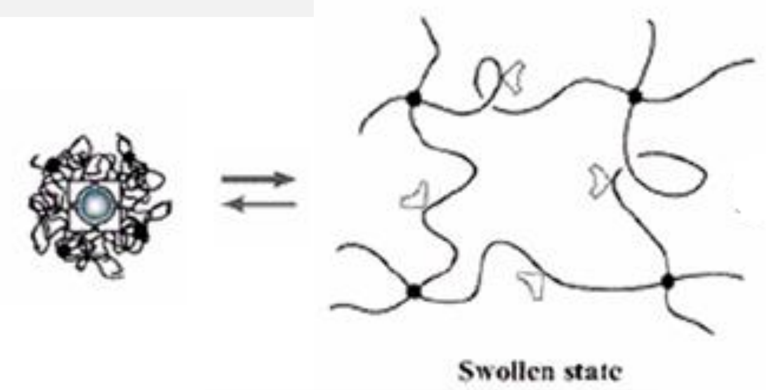
Swelling measurement

according to the Japanese Industrial Standard (JIS) K8150

- Determined by swelling the hydrogels in PBS for 72 h to reach equilibrium followed by overnight lyophilization to obtain their dry weight.
- Changes in hydrogel weight between the dried and swollen states were used to determine the volumetric swelling ratio and equilibrium water content as follows

$$\text{Swollen Ratio, } Q = \left(\frac{W_{swollen} - W_{dry}}{W_{dry}}\right)$$

$$\text{Equilibrium Water Content} = \left(\frac{W_{swollen} - W_{dry}}{W_{swollen}}\right)$$



http://www.pharmainfo.net/reviews/recent-trends-novel-drug-delivery-system

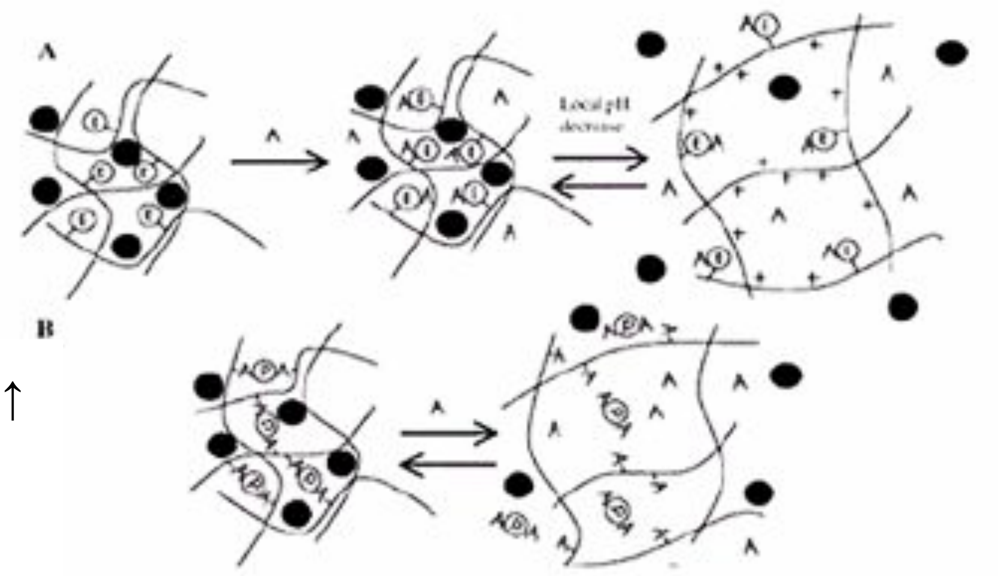
Swelling measurement

Swelling property is influenced by:

- **Type and composition of monomers**
- **Other environmental factors such as :**

  Temperature, pH, ionic strength
- **Cross-linking**

Mechanical strength and permeability



- **hydrophobic comonomers density ↑**
- **mechanical strength ↑**
- **swelling property ↓**

Rheology

流變學

- Describe the elastic/viscous behavior of materials **(Viscoelastic).** 黏彈體
- **Liquid-like as viscous; Solid-like as Elastic**
- Corresponds to Maxwell-type behavior with a single relaxation time that may be determined from the crossover point and, this relaxation time increases with concentration.

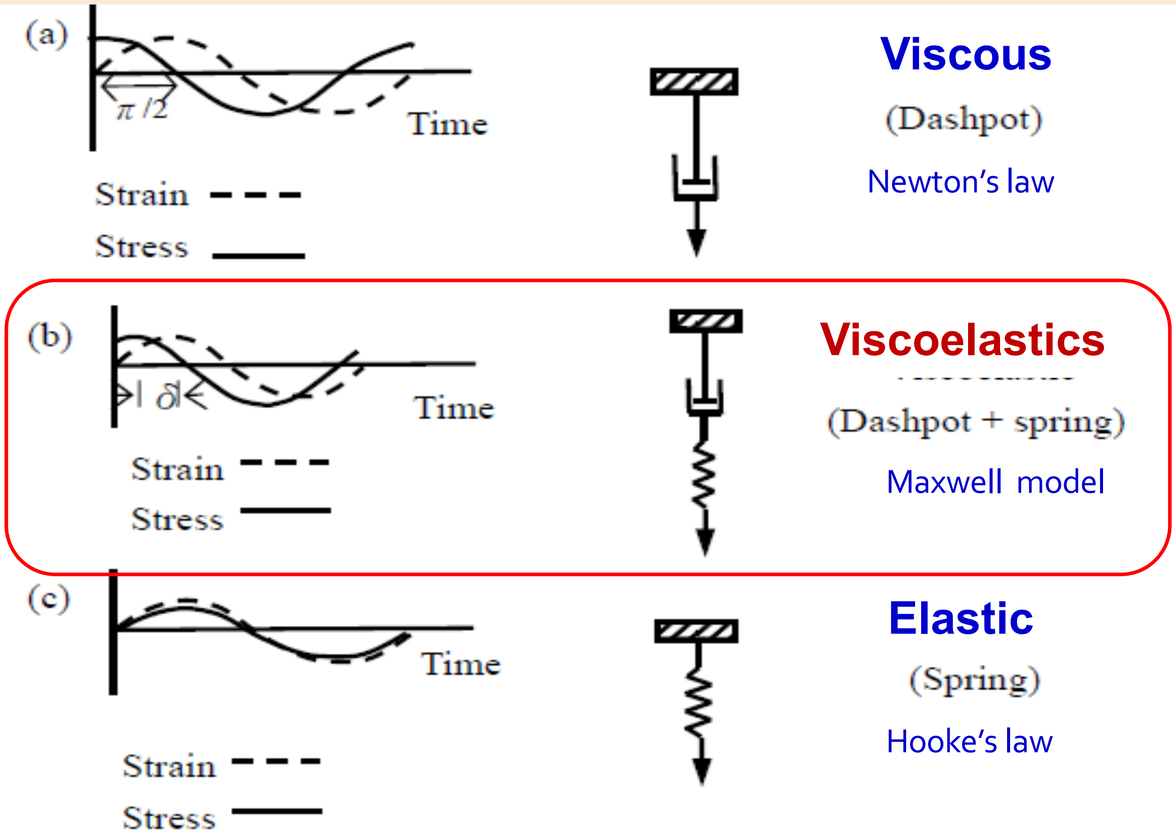
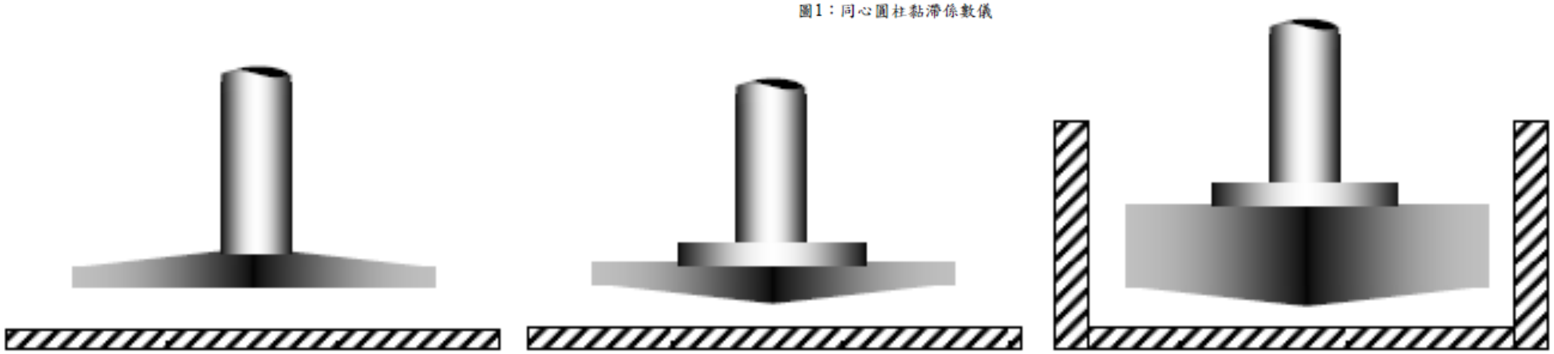


Figure 6 Maxwell model element.

Rheology

圖1：同心圓柱黏滯係數儀



•Gelatinization Time	G’, G”, η*	Parameters: τ=1 Pa, f=1Hz, CS mode, t =150/300s, T =25/37˚C

流變儀（Rheometer）

G′ : Storage modulus 儲存模數
G″ : Loss modulus 損失模數

- The **storage modulus G′** provides information regarding the **elasticity** or **energy stored** in the material during
- The **loss modulus G″** describes the **viscous character** or energy dissipated as heat.

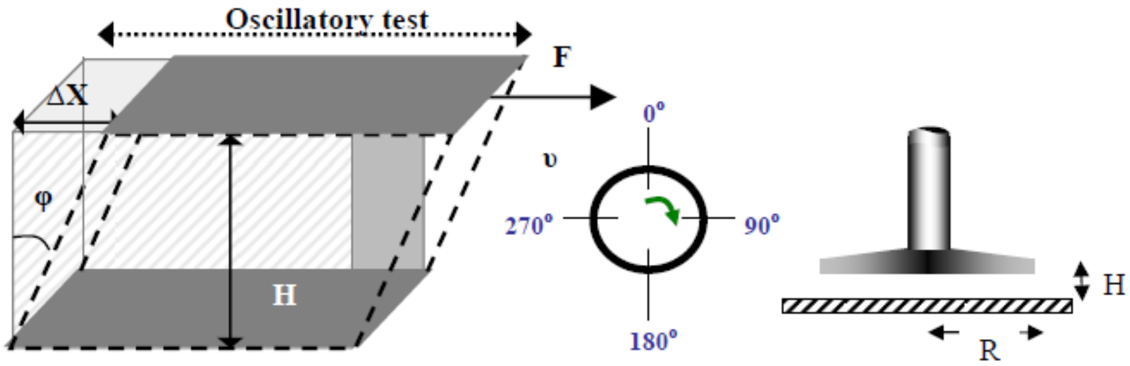
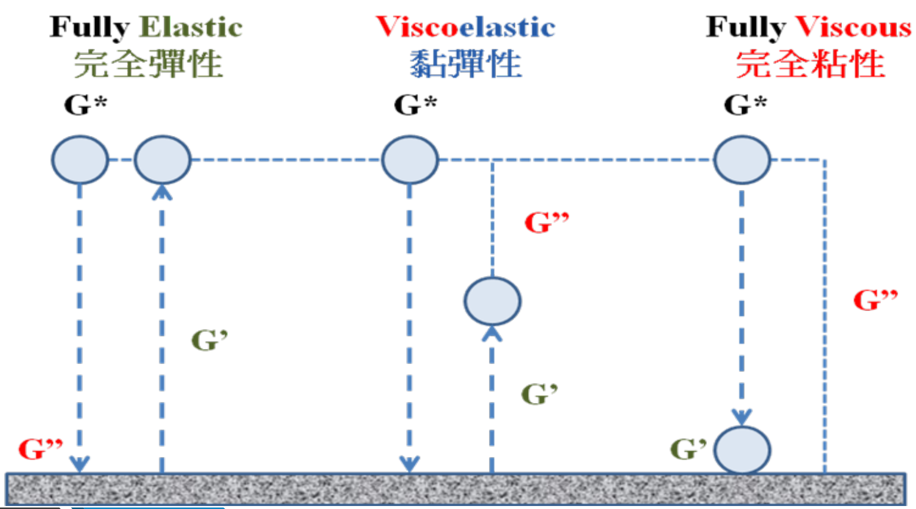


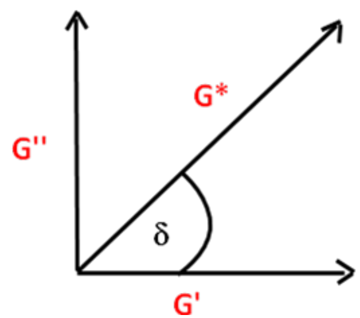
Figure 7 Dynamic oscillatory test model of rheometer.



G*: complex modulus

$$G^{*}(\omega) = G^{'}(\omega) + iG^{''}(\omega)$$

$$G^{'} = \frac{\tau_0}{\gamma_0}\cos\delta \quad , \quad G^{''} = \frac{\tau_0}{\gamma_0}\sin\delta$$



$$\tan\delta = \frac{G^{''}}{G^{'}}$$

tan **δ** **> 1**, in a **solution** form

< 1, in a **gel** form.

Rheology

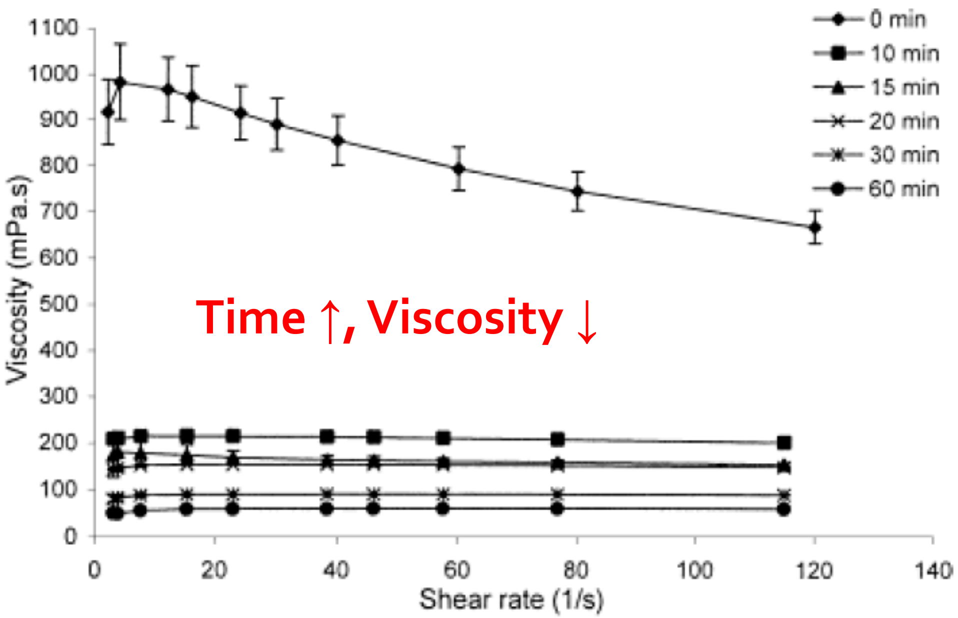


Figure 2. Dynamic viscosity as a function of shear rate for chitosan solutions at 20°C following steam sterilization (0−60 min). Mean ± sd (n = 3).

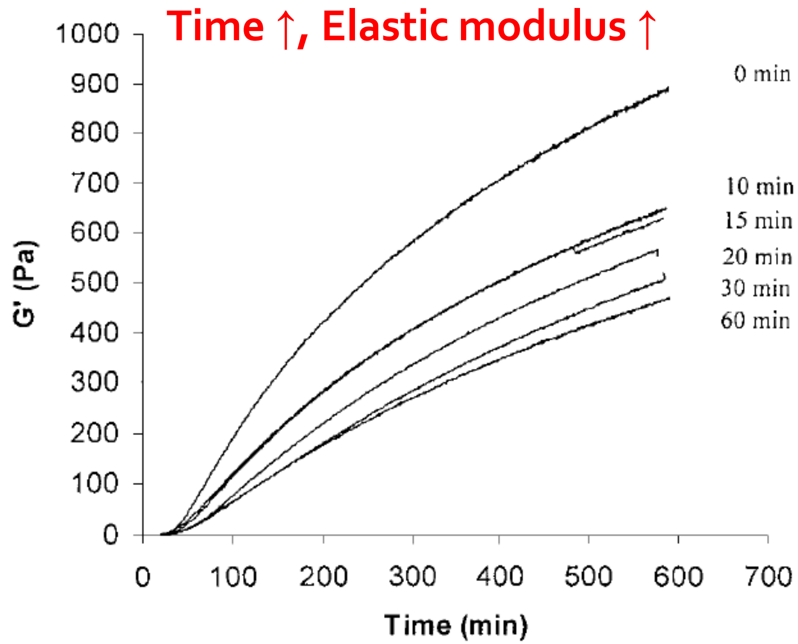


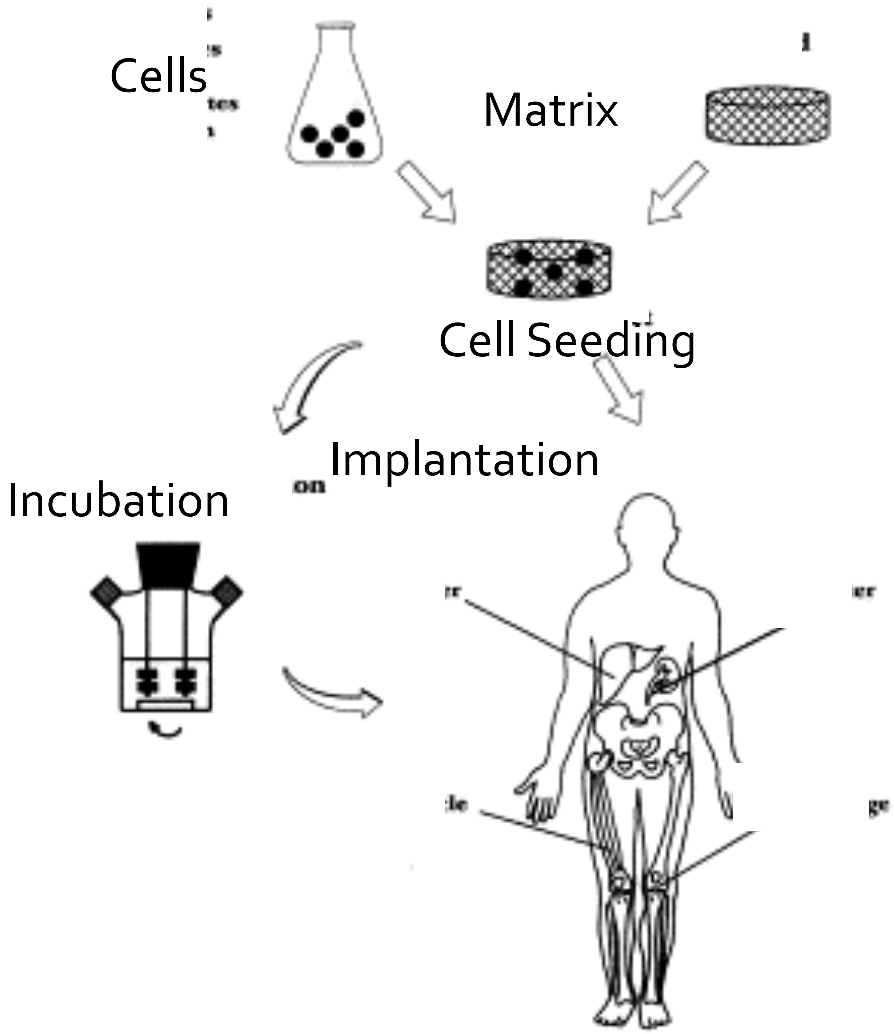
Figure 3. Elastic Modulus (G') of chitosan/GP systems at 37°C following steam sterilization of chitosan solutions (0−60 min). Mean (n = 3–6).

The difference between initial gelling and gelling temperature is a critical factor

Smart /intelligent stimuli-responsive hydrogels and application

Matrix Based Cell Transplantation

- Matrix purposes
  - Maintain structural integrity of the implant
  - Guide the growth of new tissue
  - Allow for the invasion of blood vessels
  - Provide necessary mechanical forces to cells



Marler, J. L.; Upton, J.; Langer, R.; Vacanti, J. P. *Adv. Drug Deliv. Rev.*, **1998**, *33*, 165

Hydrogels as Tissue Engineering Matrices

Advantages

- Aqueous environment can protect cells and fragile drugs
- Good transport of nutrient to cells and products from cells
- Easily modified with cell adhesion ligands
- Can be injected in vivo as a liquid that gels at body temperature
- Usually biocompatible

Disadvantages

- Can be hard to handle
- Usually mechanically weak
- Difficult to load drugs and cells and then crosslink
- In vitro as a prefabricated matrix
- Difficult to sterilize

Hoffman, A. S. *Adv. Drug Deliv. Rev.*, **2012**, *64*, 18-23

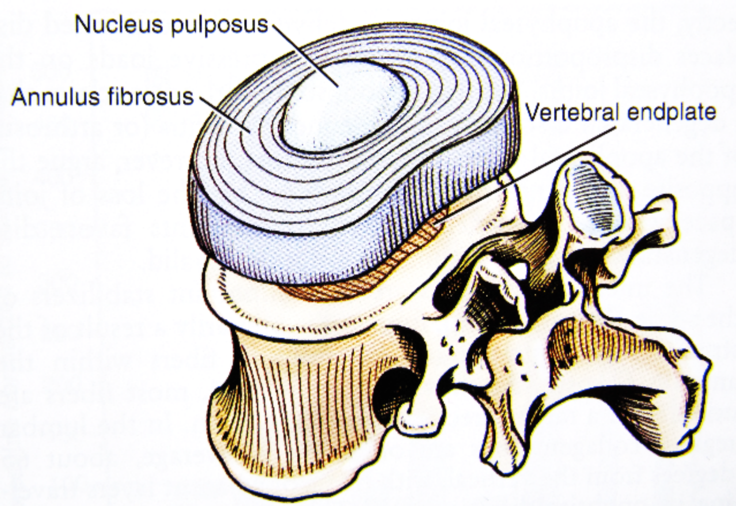
Hydrogel for cartilage repair

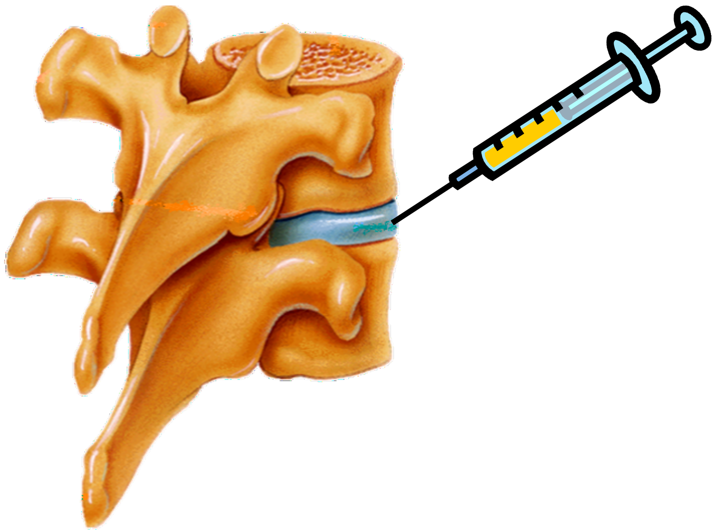
Hydrogels as Tissue Engineering

Table 2
Complex shear modulus of native tissues and polymers.

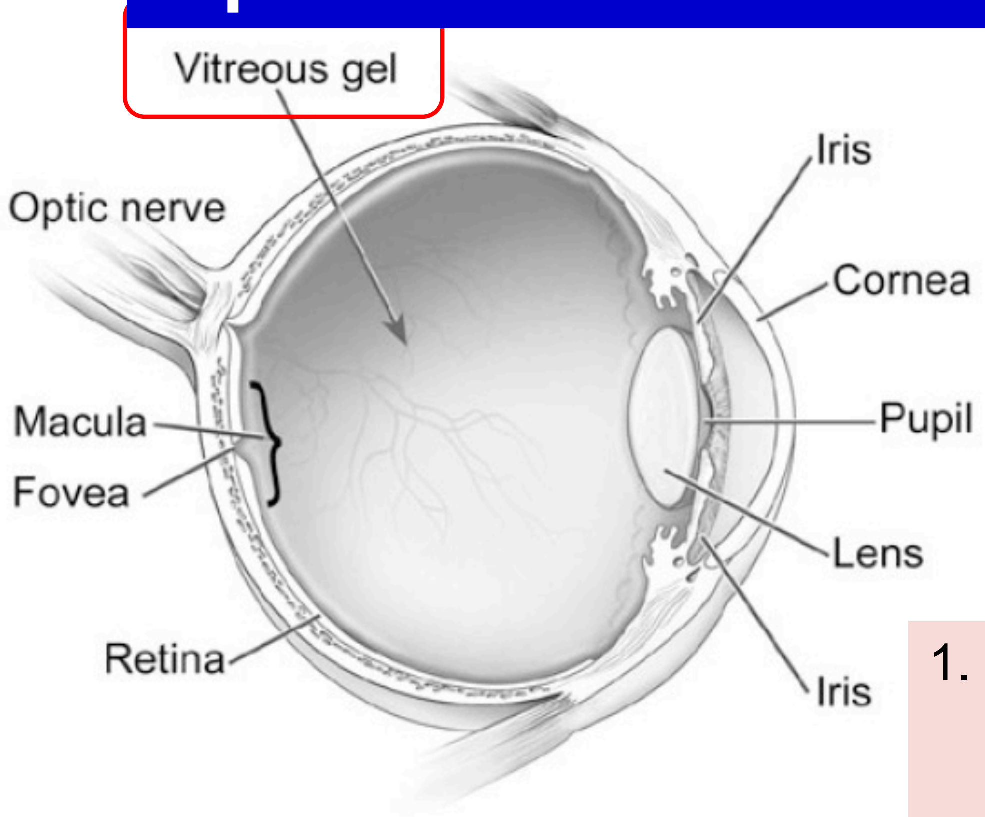
	Component	$\|G^*\|$ (kPa)[a]	δ (°)
Nucleus pulposus	NA	11.3	24
Anulus fibrosus[b]	NA	540	NA
Articular cartilage	NA	440	13
Hyal50%	10 mg ml^{-1}	0.019	20.56
Collagen–proteoglycan mixture	Coll:PG = 28:9	0.04	60
Elastin-like polypeptide (ELP)	324 mg ml^{-1}	0.08	NA
Hyaluronan	20 mg ml^{-1} in PBS	0.09	NA
GMHA	1% w/v	0.109–0.154	NA
HA-MA	1.5% w/v	0.3	1.1
Alginate	2% in 0.15 M NaCl and 1.8 mM $CaCl_2$	2.31	3
Cross-linked ELP	50 mg ml^{-1} (ELP[KV_6–112]), 37 °C	3	6.5
Amidic alginate hydrogel	1% in distilled water	16	19.7
Oxi-HA/ADH8 hydrogel	6% (w/v) HA with 8% (w/v) ADH	30	17.3

[a] Values are determined at 10 rad s^{-1}.
[b] Tissue was tested at a frequency of 628 rad s^{-1}.





Hydrogel as Artificial vitreous replacements



Vitreous body:

1. Two-third of the eye with a weight of approximately 4 g and a volume of about 4 ml
2. The fluid that fills the space inside the sclera. It has an index of refraction of 1.337
3. Density: 1.0053~ 1.0089 g/cc
4. pH 7.0~7.4

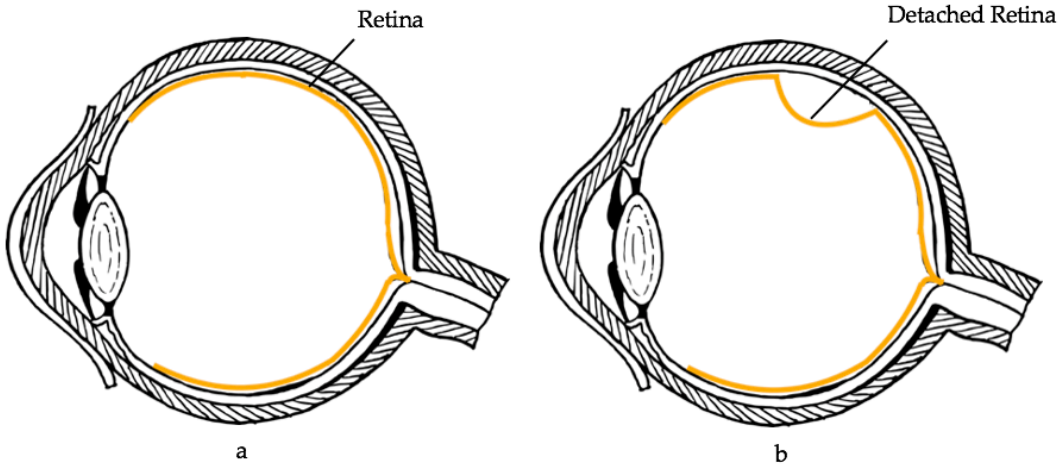
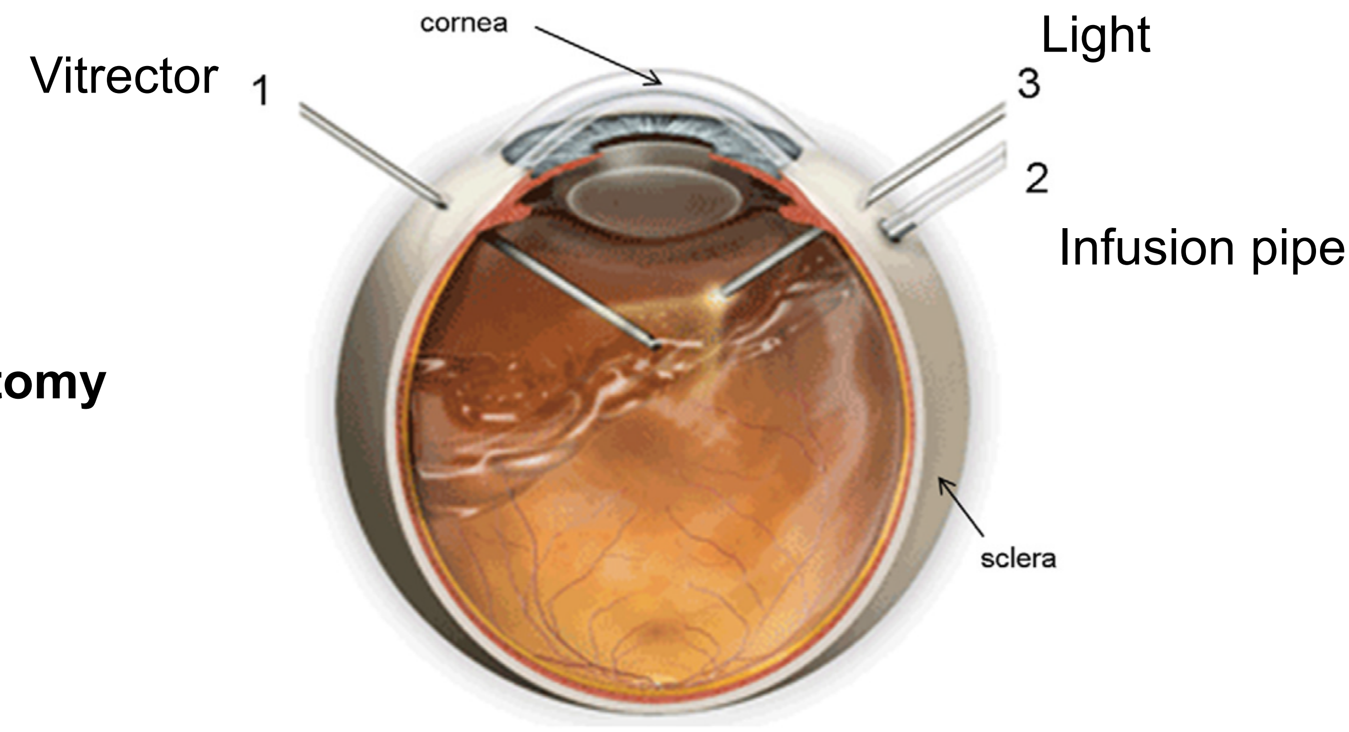


Figure 2. (a) Healthy eye (b) Retinal detachment.

Vitrectomy



Hydrogel as Artificial vitreous replacements

Classification of vitreous substitutes

- **Gases**
  - Air
  - Perfluorocarbon gases
- **Liquid**
  - Aqueous solutions: water, PBS
  - Silicone oil and its derivatives
  - Perfluorocarbon liquids (PFCLs)
- **Gel**
  - Semisynthetic polymers
  - Synthetic polymers

Table 3

Comparison of the properties of biomaterials used as vitreous substitutes (compiled from [6,10,17,30])

Material	Refractive index	Specific gravity	Viscosity (cSt)	Surface tension (dyn/cm)
Natural vitreous	1.33	1.086	2813 (M)* 385 (K)	high
Silicone oil	1.404	0.97	1000–12,000	20
Fluorosilicone oil	1.382	1.29	1000–10,000	23
Perfluoro-n-octane	1.27	1.76	0.8	14
Perfluorophenanthrene	1.28	2.03	8.03	16

*M – Maxwell viscosity and K – Kelvin viscosity. The value given is the average of the three regions of the vitreous.

Hydrogel as Artificial vitreous replacements

Criteria for an ideal vitreous substitute

- Clear, transparent and colorless
- Density and refractive index
- Storable and serializable, chemically and biologically inert
- Biocompatible with the vitreous humor itself and with the adjacent tissues
- Not be absorbable or biodegradable
- Viscoelastic properties
- High surface tension
- Allow the transfer of necessary metabolites and proteins inside the vitreous
- Injectable through a small-gauge needle
- Hydrophilic material with high equilibrium water content

Hydrogel as Vitreous substitute

poly[(R)-3-hydroxybutyrate-(R)-3-hydroxyhexanoate] (PHBHx)-based polyurethane thermogel named PHxEP (poly(PHBHx/PEG/PPG urethane)

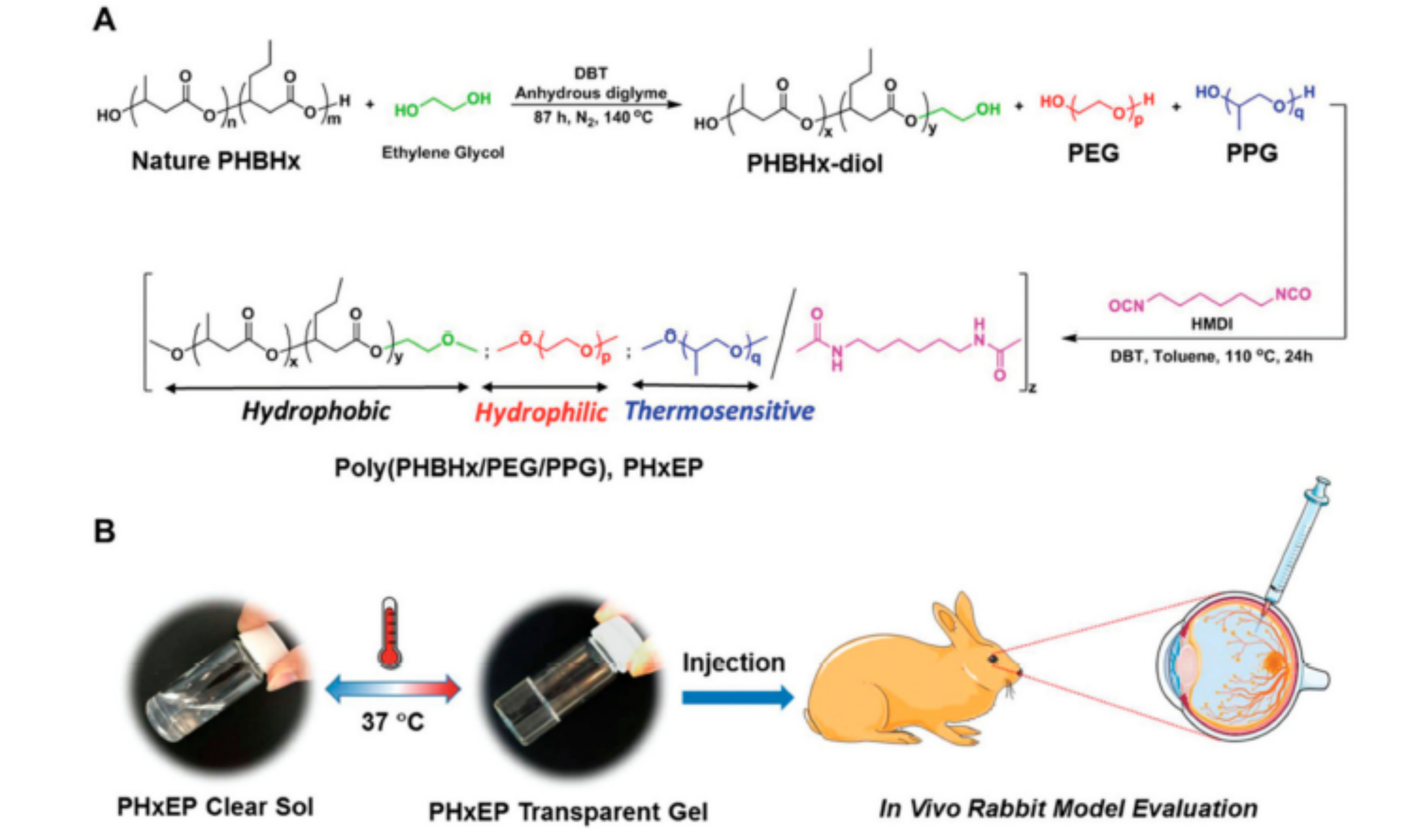


Figure 4. (A). Schematic illustration of the synthetic route of poly(PHBHx/PEG/PPG urethane); (B). hydrophobic PHBHx content is varied within the hydrogel, and that can change the gel properties from cloudy to transparent at 37 °C. The gels are then tested for in vivo eye application. Image adapted from Loh et al., (2019) and reprinted with permision from Royal society of chemistry.

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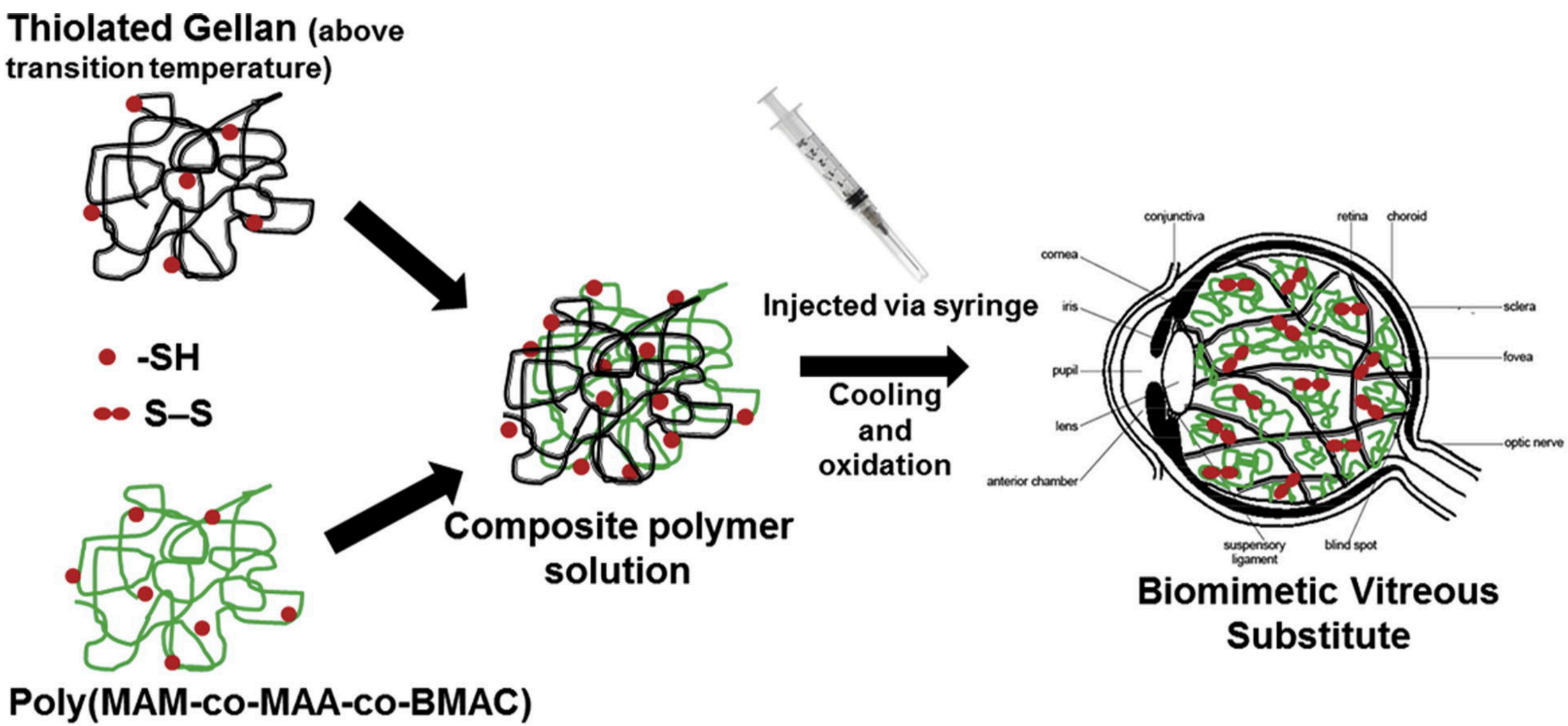

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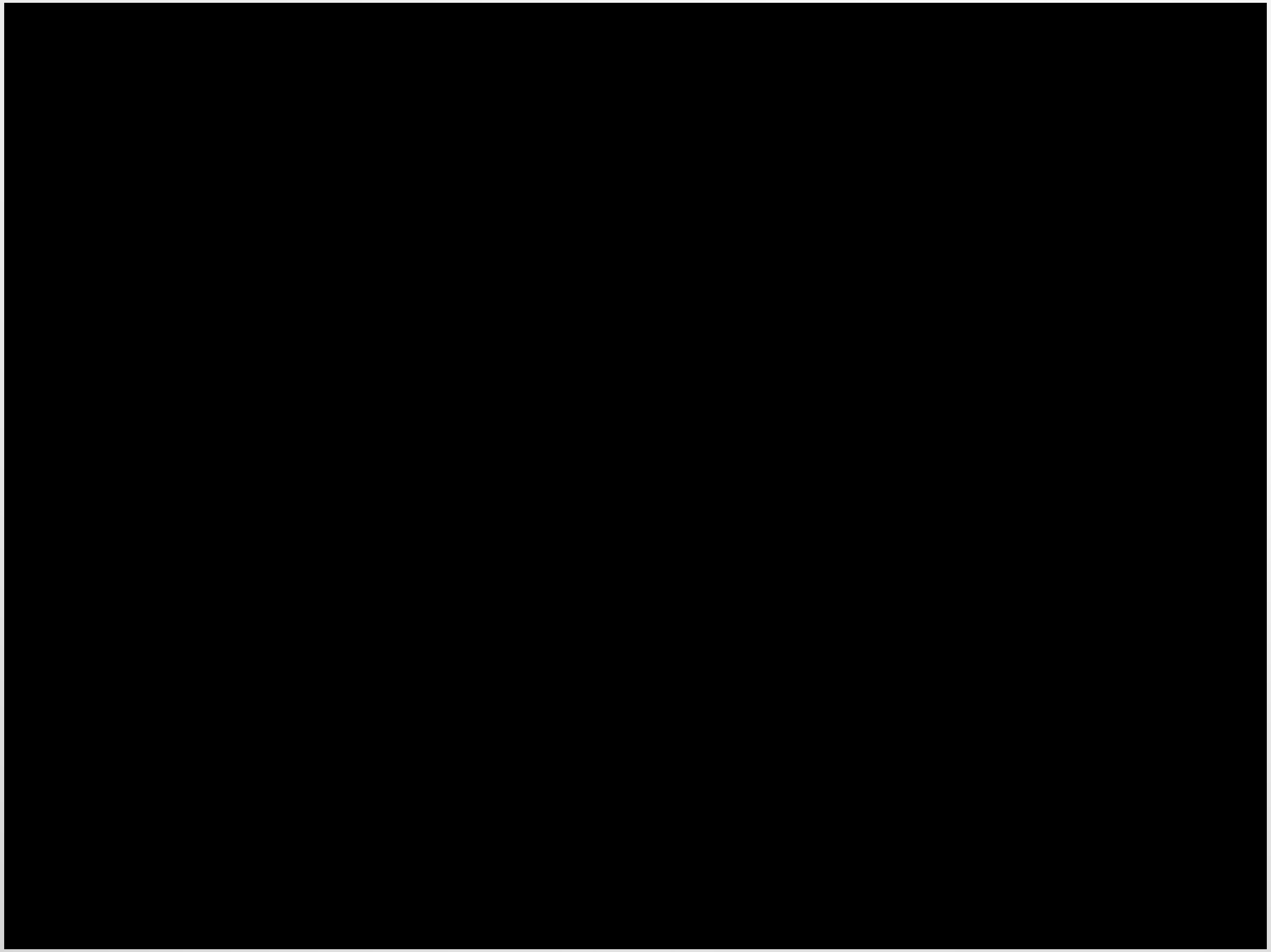
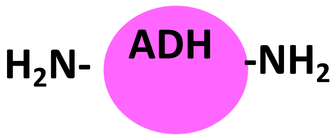
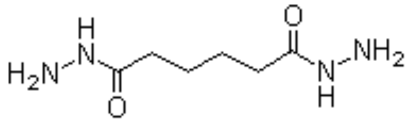
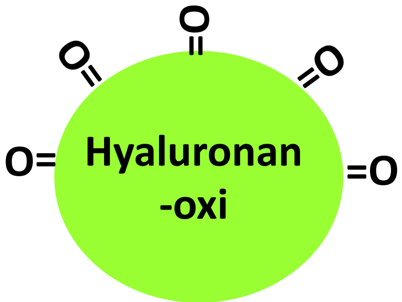


Hydrogel as Artificial vitreous replacements

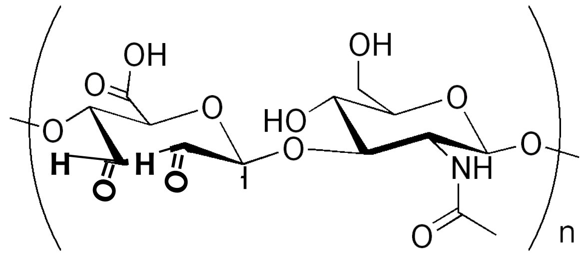


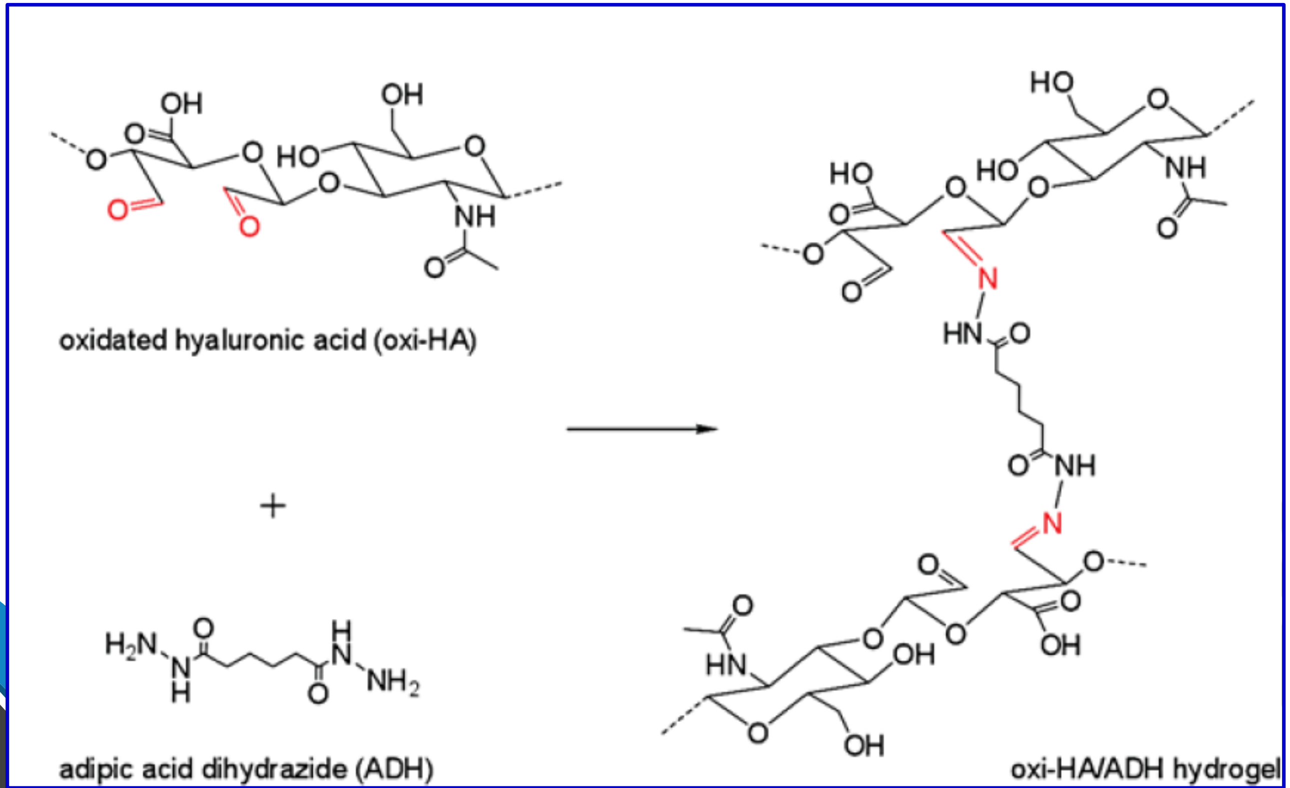
Low toxicity





Physicochemical properties
Biocompatibility
Biodegradability





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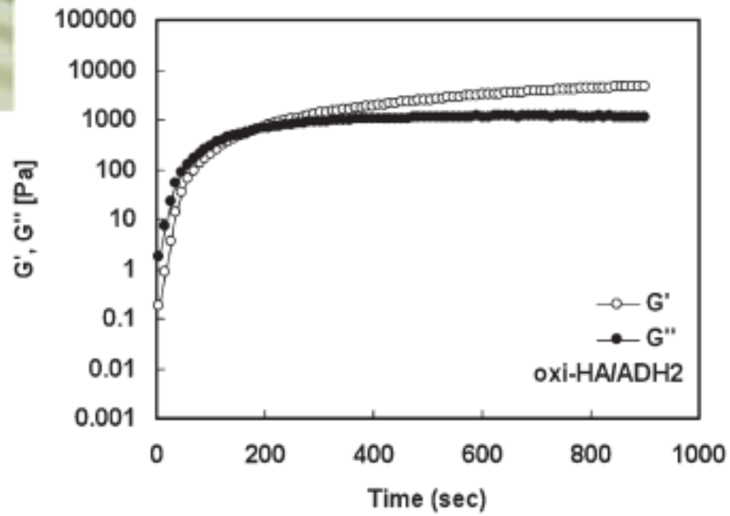
Hydrogel as Artificial vitreous replacements

Table 2.
Characteristics of oxi-HA/ADH hydrogels prepared with 6% (w/v) oxi-HA cross-linked with different concentrations of ADH (2, 4 and 8%, w/v)

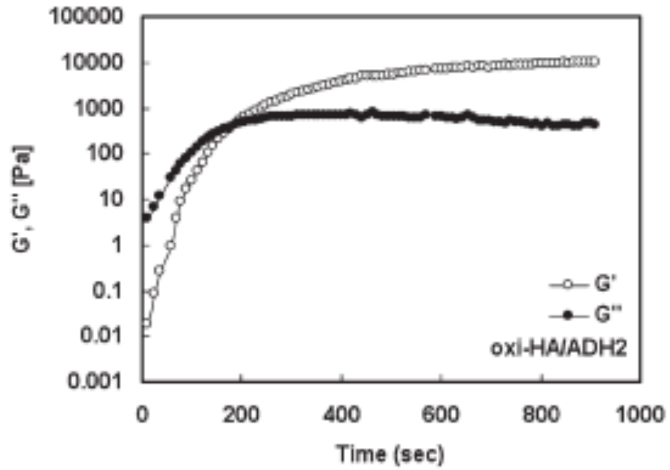
Hydrogel	RI	Gel point (s)		*In vitro* degradation time (days)
		4°C	37°C	
Oxi-HA/ADH2	1.3420 ± 0.0000	180.3	175.4	2
Oxi-HA/ADH4	1.3427 ± 0.0001	202.2	185.7	14
Oxi-HA/ADH8	1.3442 ± 0.0001	491.7	143.4	>35

The gel point was defined at the cross-over point of G' and G'' determined by rheological measurement ($G' = G''$); the time required for the gel point to occur is referred to as the gelation time.

4°C



37°C



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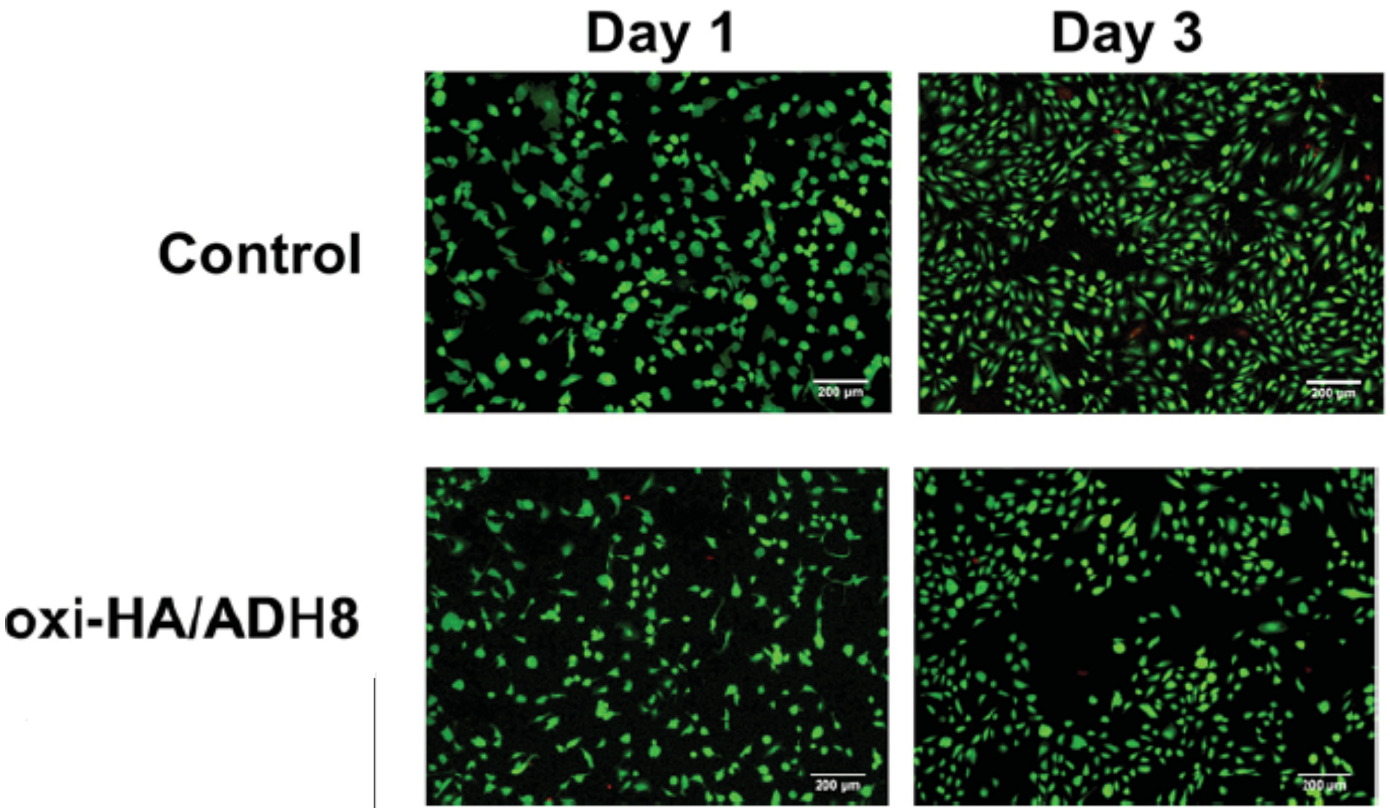
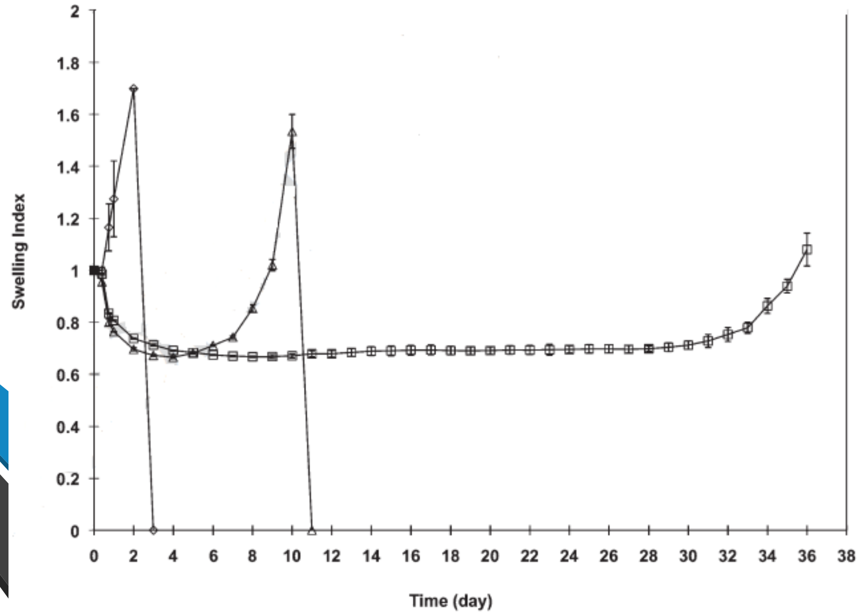
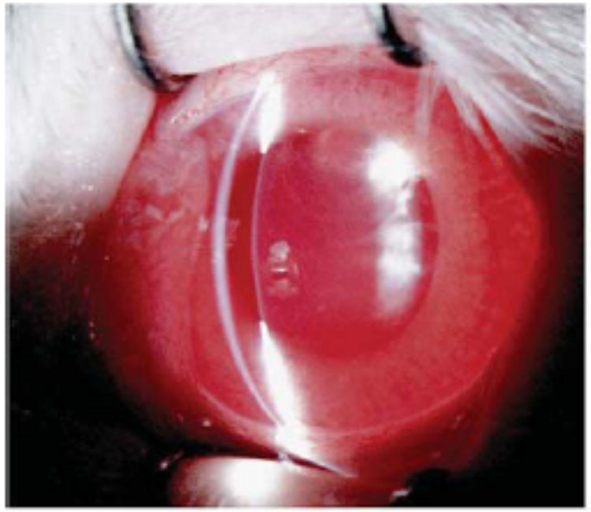
Hydrogel as Artificial vitreous replacements

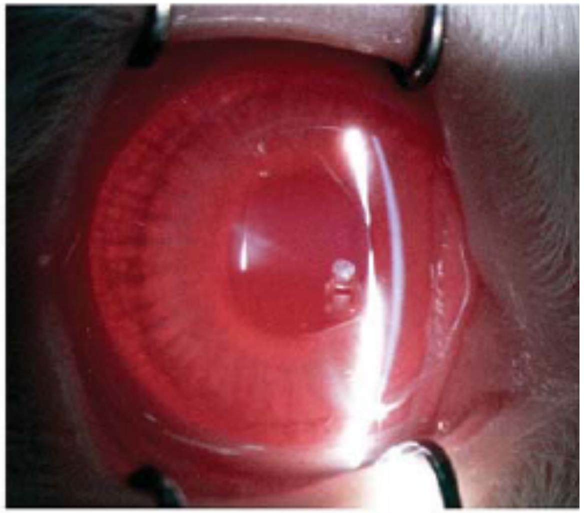




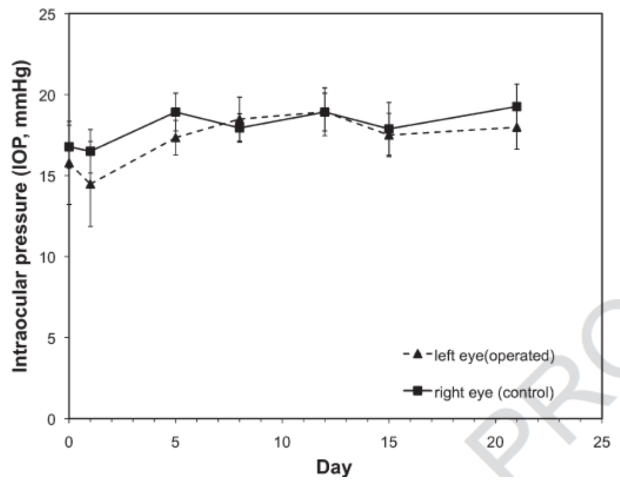
Hydrogel as Artificial vitreous replacements

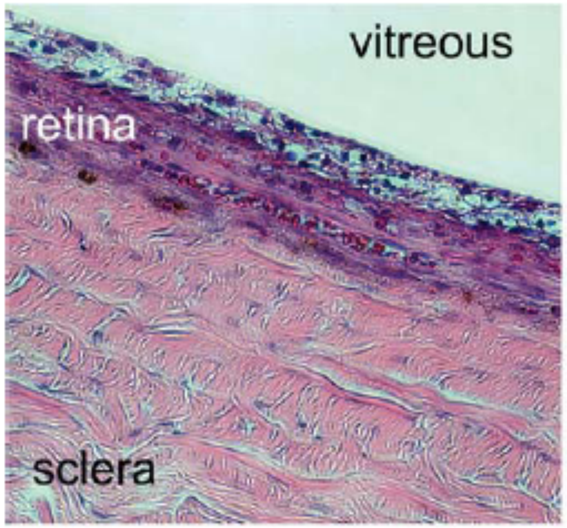


left eye (operated)

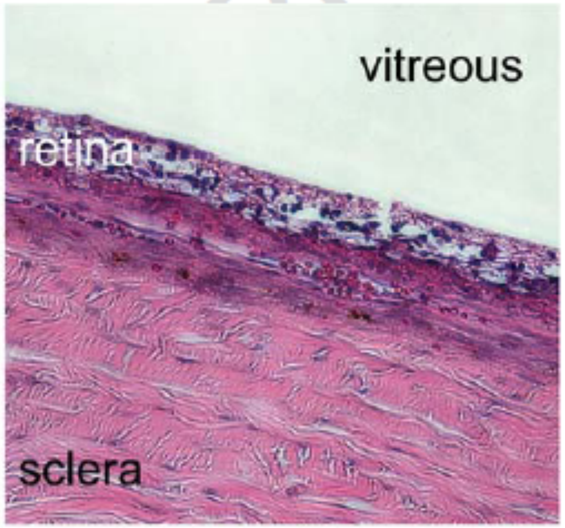


Right eye (control)





left eye (operated)



Right eye (control)

oxi-HA/ADH8

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Summaries

- Unique biocompatible injectable hydrogels for biomedical applications due to the several advantages of *in situ* forming gels compared to implantable systems.
- Injectable systems responding to various stimuli such as light, chemical/physical reactions, temperature.
- The feasibility of tuning the degradation and gelation time as well as the water content of the gels, coupled with the mild gelation process and ability to deliver them in a minimally invasive manner, make them potential protein and cell carrier vehicles.
- Determine the suitability of the injectable gel for tissue engineering and drug delivery applications include rate of gelation, degradability, rate of degradation, extent of water content, non - toxicity of the process, as well as physical and mechanical properties of the gel.
- Properties of various tissues will be a crucial parameter that will determine hydrogel applicability as tissue engineering scaffolds.

Reference

1. Syed K. H. Gulrez, Saphwan Al-Assaf and Glyn O Phillips (2011). Hydrogels: Methods of Preparation, Characterization and Applications, Progress in Molecular and Environmental Bioengineering - From Analysis and Modeling to Technology Applications.

2. Allan S. Hoffman. Hydrogels for biomedical applications. Advanced Drug Delivery Reviews 64 (2012) 18–23.