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Inorganic Polymerization: An Attractive Route to Biocompatible Hybrid Hydrogels

Titouan Montheil,^a Cécile Echalié,^{a,b} Jean Martinez,^a Gilles Subra^a and Ahmad Mehdi²

As an intermediate state between liquid and solid materials, hydrogels display unique properties, opening a wide scope of applications, especially in the biomedical field.

Organic hydrogels are composed of an organic network cross-linked *via* chemical or physical reticulation nodes. In contrast, hybrid hydrogels are defined by the coexistence of organic and inorganic moieties in water. Inorganic polymerization, i.e. sol-gel process, is one of the main techniques leading to hybrid hydrogels. The chemoselectivity of this method proceeds through hydrolysis and condensation reactions of metal oxide moieties. In addition, the mild reaction conditions make this process very promising for the preparation of water-containing materials and their bio-applications.

1. Introduction: Hydrogels

Since the pioneering work of Wichterle and Lim in 1960 on cross-linked hydroxyethyl methacrylate (HEMA),¹ hydrogels have been of great interest to biomaterials scientists, especially for the last two decades² and mainly exploited in the biomedical field.^{3–6}

Hydrogels are water-swollen three-dimensional polymer networks,² cross-linked either through covalent bonds (chemical hydrogels) or held together *via* physical interactions (physical hydrogels).⁵ Many materials, both naturally occurring and synthetic, are included in the definition of hydrogels.

By comparison with organogels in which the three-dimensional network is swollen in an organic solvent, the main characteristic of hydrogels is the presence of a large amount of water trapped in its structure. Indeed, hydrogels may absorb up to thousands of times their dry weight in water.³ This ability is due to the presence of hydrophilic functional groups among the macromolecular backbone. A water-swollen network is ideal to trap hydrophilic molecules or living cells. Hydrogels are also more attractive than organogels precursors to prepare a wide range of materials (aerogels, xerogel, membranes, scaffolds, etc.).⁷ Indeed, the removal of water is safe and does not generate organic wastes; it can for example be performed by lyophilisation.

Hydrogels are swollen by water but not dissolved in it. This resistance to dissolution arises from cross-links between chains resulting in the network. The number of cross-links in a given volume is characterized by the cross-link's density, noted ρ_x and expressed in network chains per gram (mol/g). It affects the fundamental properties of the hydrogel (Figure 1), like the mesh

dried material, the shear modulus (G , in MPas) which measure the stiffness of materials, and the diffusion coefficient (D , in $\text{cm}^2 \cdot \text{s}^{-1}$) of entrapped molecules.^{8–12}

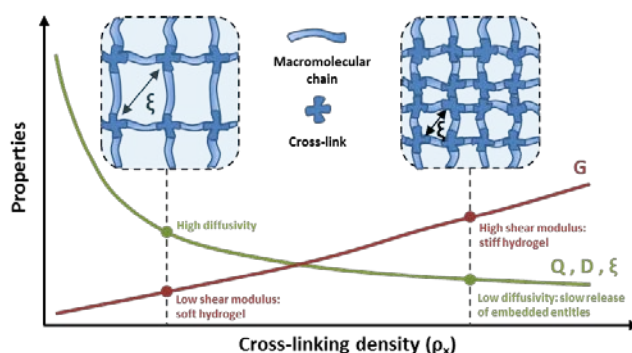


Figure 1: Schematic representation of the relationship between cross-linking density (ρ_x) and hydrogel properties (G : shear modulus; Q : swelling ratio; D : diffusion coefficient; ξ : mesh size). Adapted from Kirschner *et al.*⁹

Various classifications have been applied to hydrogels.^{5,13,14} Among them, two chemical characteristics can be used to sort the hydrogels: the origin of the macromolecular chain (i.e. natural and synthetic), and the nature of the cross-links (Figure 2).

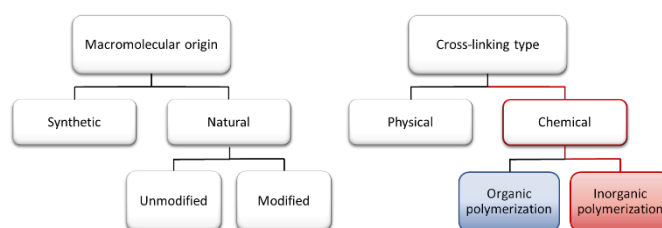


Figure 2: Hydrogel's classification highlighting the filiation of hybrid hydrogels (red line).

Natural hydrogels are made of naturally-occurring polymers like polysaccharides (alginate, chitosan, agar, etc.),^{15–19} and polypeptides (collagen,^{20–22} fibrin,^{23,24} etc.). They can be used as they are or post-modified (e.g. deacetylation for hyaluronic acid). Most of synthetic hydrogels are made of synthetic polymers (polyethylene glycol, polyacrylic acid, poly(N-isopropylacrylamide)

^a Institut des Biomolécules Max Mousseron (IBMM), Univ Montpellier, CNRS, ENSCM, France.

^b Institut Charles Gerhardt de Montpellier (ICGM), Univ Montpellier, CNRS, ENSCM, France.

Corresponding authors : ahmad.mehdi@umontpellier.fr ; gilles.subra@umontpellier.fr

size (ξ , in nm) which defines the average distance between cross-links, the swelling ratio (Q , expressed in percent) which is the ratio between weight increase of swelled material over the weight of

and polyvinyl alcohol, etc.)² but they can also be obtained from low-molecular weight precursors such as synthetic self-assembling peptides.^{25,26} The second classification distinguishes physical hydrogels from chemical hydrogels. Physical hydrogels result from the formation of a physical network involving weak and reversible interactions such as ionic and π - π stacking interactions, hydrogen bonds, Van der Waals forces, and hydrophobic interactions.^{13,27-29} The large number of these interactions counterbalances their weakness to stabilize the three-dimensional network. Because physical hydrogels do not require any chemical reaction to cross-link the network, they can be prepared more easily in a biocompatible way.

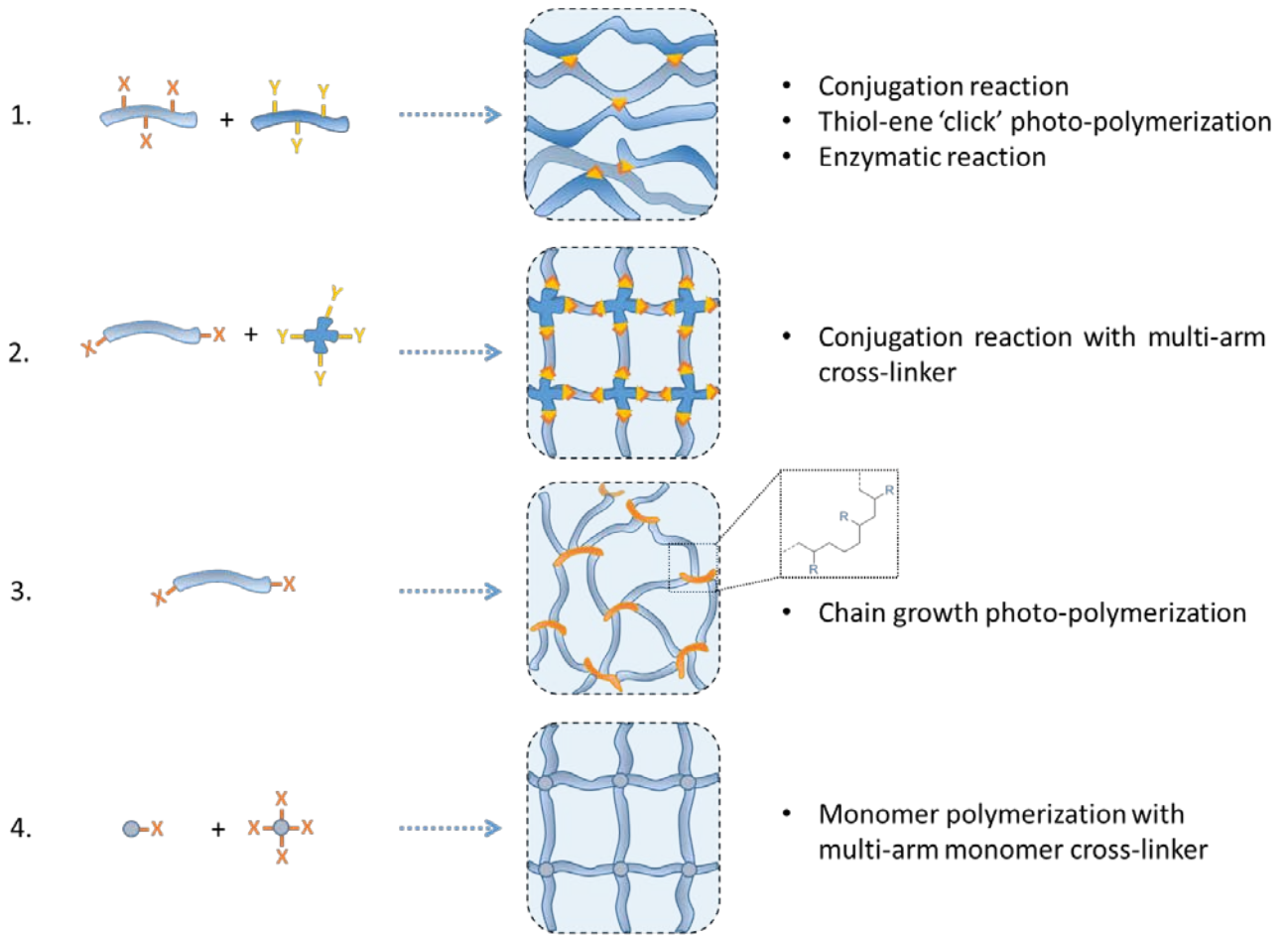
On the other hand, thanks to covalent bonds between elements of the network, chemical hydrogels display better mechanical properties and higher stability than physical hydrogels. Chemical hydrogels can be cross-linked by organic or inorganic reticulation, leading respectively to organic and inorganic networks. Organic chemical hydrogels have been widely studied for many years, mainly for biomedical applications.^{3-5,30,31} The covalent network can be obtained by different strategies. Most of the time, it involves the reticulation of macromolecular precursors. The covalent cross-linking may occur between mutually reactive moieties (e.g. X and Y in Figure 3) displayed by two polymer chains (Figure 3-a1)^{32,33} or by a linear polymer chain and a multi-arm cross-linker (Figure 3-a2).³⁴ Classical conjugation reactions involving for example aldehydes and hydrazides,³⁵ maleimides and thiols,³⁴ click azide alkyne chemistry³⁶ were described, but step growth thiol-ene photo-polymerization is also very popular, yielding thioether bonds from thiol- and vinyl-functionalized polymers.³⁷ At last, enzymatic reaction linking two peptide sequences carried by different polymer chains was also reported.³⁸

Besides, polymers bearing just vinyl groups can be engaged in chain growth photo-polymerization. Free radical species open homolytically the π -bond of the vinyl group, regenerating a radical and propagating the formation of covalent bonds (Figure 3-a3).³⁹ It results in the formation of a polymeric reticulation node involving several macromolecular chains. In another way, polymer chains can be cross-linked randomly by gamma or electron beam irradiation.⁴⁰ Organic hydrogels can also be obtained by free radical polymerization of water soluble monomers such as 2-hydroxyethyl methacrylate (HEMA) and multifunctional monomers as cross-linker.¹

Recent reviews summarizing advances in the cross-linking chemistries are available. They are focused on popular PEG-based hydrogels.^{41,42} Organic hydrogels are not always obtained in biocompatible conditions. Toxicity may arise from reagent, catalyst,

by-products or reaction conditions (elevated temperature, photo-irradiation, etc.). An interesting alternative to organic hydrogels lies in hybrid hydrogels synthesized by inorganic sol-gel polymerization (Figure 3-b). A hybrid network is defined by the coexistence of organic and inorganic moieties. The formation of the covalent network does not require two different mutually reactive functions as it is obtained by condensation of a single type of function: M-OH, with M being a metal or metalloid (see section 2.b.). Moreover, this reaction occurs in water, and can be performed at neutral pH and ambient temperature. Interestingly reaction by-products are most of the time water and low molecular weight alcohols (e.g. MeOH, EtOH, iPrOH). Avoiding organic solvents and chemical reagents, this procedure is greener than those used to obtain organic chemical hydrogels, and not using organic solvents can be considered as a biocompatible pathway. Surprisingly, such hybrid hydrogels obtained by inorganic polymerization have been scarcely described in the literature. They are the subject of less than 10% of the papers dealing with hydrogels. This review will focus on the different types of hybrid hydrogels, first deciphering the chemical processes for their fabrication, and then establishing relationships between their composition, properties and potential applications.

a. Organic hydrogel synthesis



b. Hybrid hydrogel from inorganic polymerization

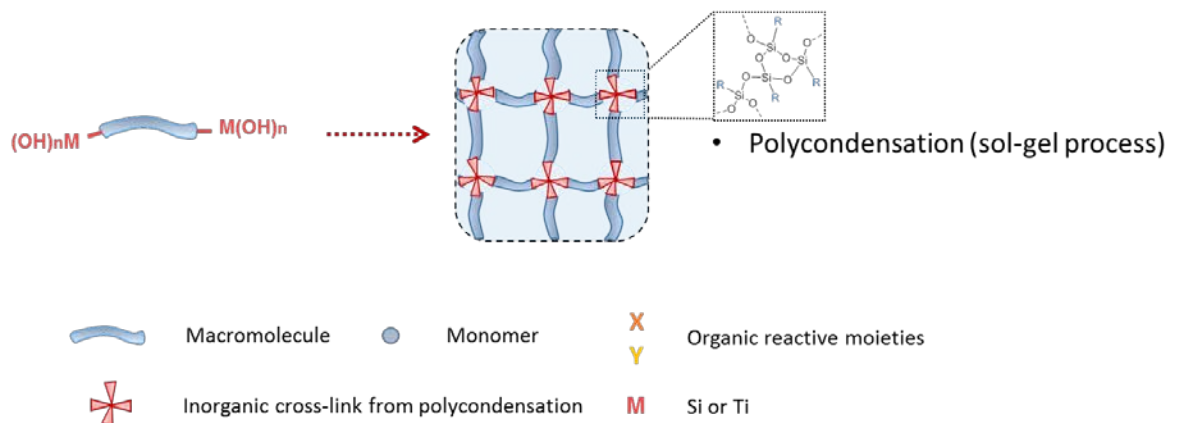


Figure 3: (a) Organic and (b) inorganic hydrogel syntheses.

2. Sol-gel inorganic polymerization

a. History and principle

In the 19th century, pioneering works in sol-gel chemistry by Ebelmen^{43,44} and Graham⁴⁵ described that under acidic conditions, $\text{Si}(\text{OEt})_4$ (tetraethyl orthosilicate, TEOS) underwent hydrolysis, yielding to silica in the form of a glass-like material. The sol-gel

process began to reach many academic and industrial domains, notably for manufacturing hybrid materials.^{7,46–51}

The sol-gel process is an inorganic polymerization⁴⁹ as it involves metal-containing molecular precursors, which react together to form a metal oxide as an inorganic network. This polymerization can be performed under mild experimental conditions, i.e. neutral pH, in water and at low temperature. This is the reason it was referred to '*Chimie Douce*'.⁵² Metal oxide of general formula $M(OR)_n$ ($M = Si, Al, Ti$, etc. and R an alkyl group such as Me, Et, ⁱPr, etc.), and metal halides (MX_n) can be used as precursors. The most investigated metal is silicon, and silicon alkoxides are the molecular precursors of choice for the sol-gel process.

Inorganic polymerization involves two distinct states: colloidal solution (*sol*) and the *gel*. The *sol* state is characterized by a colloidal suspension of solid particles. The *gel* state is constituted by solid particles forming an interconnected network in a secondary liquid phase.⁵³ The evolution from the molecular state to the network is driven by two main reactions: hydrolysis and condensation. In the case of silicon, the first step consists in the hydrolysis of alkoxyisilyl groups ($Si-OR$) to form silanols (hydroxysilyl, $Si-OH$) (Figure 4-a). From the formation of the first silanol entity, condensation starts, and the two reactions occur simultaneously. Two types of condensation take place. When two hydroxysilyl groups react together, the term oxolation is used (Figure 4-b). Alkoxolation occurs when a hydroxysilyl group reacts with an alkoxyisilyl group, (Figure 4-c). Successive condensations yield to siloxanes units ($Si-O-Si$ bonds) and linear growth of the polymeric chain. In addition, the tetravalence of the silicon atom allows the linkage of three to four different partners. In this case, a reticulation node is created. These covalent bonds lead to a stable three-dimensional network. Hence, sol-gel process allows formation of a network in a bottom-up way, combining relatively small molecular blocks to form a supramolecular matrix.

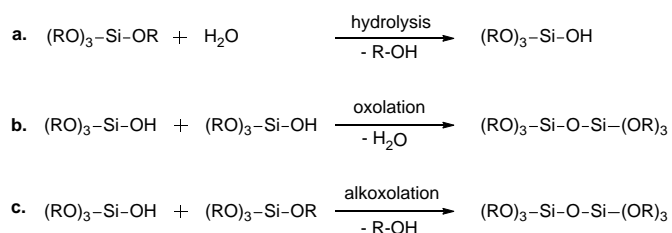


Figure 4: (a) Hydrolysis of a silicon alkoxide into silanol; (b) Condensation *via* oxolation; (c) Condensation *via* alkoxolation.

At neutral pH, hydrolysis and condensation reactions are very slow. Brinker *et al.* described the profile of hydrolysis and condensation rates as a function of pH (Figure 5).⁷

Hydrolysis is favoured at acidic and basic pHs, while condensation is fast at very low pH, decreases dramatically at pH 1.5-2 to finally increase gradually until PH 10-11. Under basic conditions (pH higher than 12), the hydrolysis rate is much higher than condensation. This difference lead to depolymerization and the network collapses.

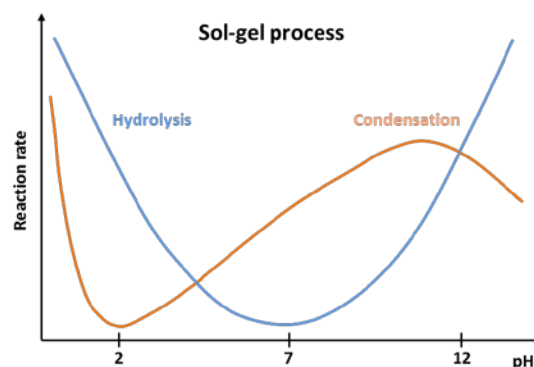


Figure 5: Reaction rates of tetraethyl orthosilicate hydrolysis (blue curve) and condensation (orange curve) as a function of pH.⁷

Acidic and basic catalysts can be used to increase reaction rates (Figure 6-a and 6-b). When a neutral or a physiological pH is needed, nucleophile catalysts can be used as an alternative to speed up the process (Figure 6-c).^{54,55} Owing to their high affinity for silicon, fluorides hold a prime position as nucleophile catalysts.

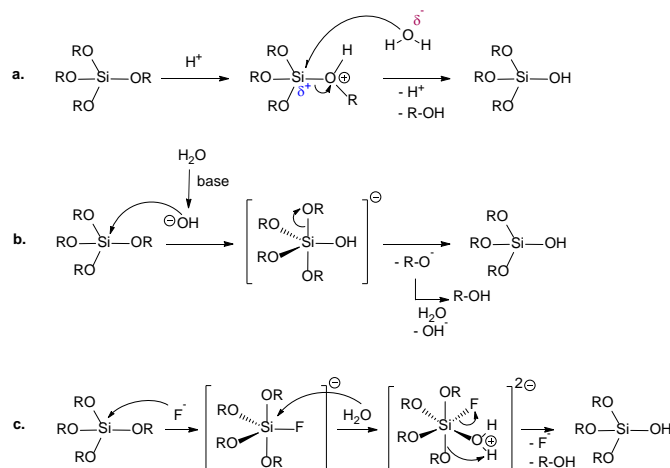


Figure 6: (a) Acidic, (b) basic, and (c) nucleophilic catalysis of the sol-gel process (example for the hydrolysis step).

Noteworthy, the choice of the catalyst is crucial regarding physico-chemical properties (porosity, surface area, transparency, etc.) of the final hydrogel. Indeed, under acidic conditions, hydrolysis is favoured over condensation which results in a loose network of polymeric chains highly swollen in water. In an opposite way, basic conditions favour condensation, and thus the nucleation of inorganic precursors takes place, yielding a colloidal hydrogel (Figure 7).^{7,56,57} Fluorides catalyse equally both hydrolysis and condensation at neutral pH, which leads preferentially to the formation of a colloidal gel. To a lesser extent, the nature of the metal alkoxide, concentration and temperature also influence the process.

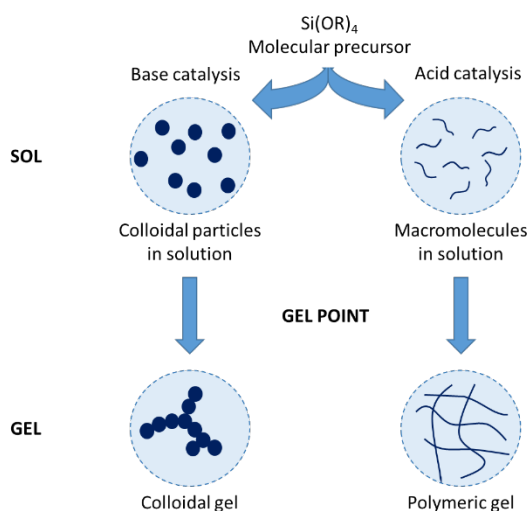


Figure 7: Influence of the catalyst on hydrogel textures.

Because its kinetics can be easily controlled, sol-gel is compatible with a lot of processing methods (Figure 8). During the 90's, Brinker *et al.* described many types of materials accessible by the sol-gel process, such as membranes, monoliths, fibres, porous gels, particles, etc.⁷

The mild conditions used make the sol-gel process compatible with almost any type of other components, including functionalized organic molecules and biomolecules, leading to a variety of hybrid materials.^{58,59} Even microorganisms and living cells can be encapsulated during the sol-gel polymerization. These characteristics pave the way to a large scope of applications.

b. Hybrid materials

The cohabitation between organic and inorganic fractions within the hydrogel implies that the properties of the resulting material (porosity, specific area, mechanical, physical and chemical

properties, etc.) are not the sum of the individual contribution of each component, but result from the synergy between each phase and their interface. Typically, the inorganic part contributes to both the mechanical strength and the thermal stability of the material. On the other hand, the organic part may contribute to a specific chemical and biological functionality of the hybrid material (thermo sensitivity, antibacterial property, etc.). These materials (powders, thin films, monoliths, nanoparticles, etc.) find applications in various fields such as human healthcare,^{53,60,61} housing, energy, environment, etc.^{62,63} Indeed, such materials are water swollen three-dimensional networks obtained in very soft conditions (temperature, pH, solvent). Surprisingly, literature is scarce on this type of hydrogels.

Hybrid hydrogels are classified into class I and class II, according to the nature of the interaction between organic and inorganic parts (Figure 9). Noteworthy, this classification is also applicable to hybrid materials in general.⁶⁴ In class I materials (also called nanocomposites), organic components are simply embedded in the inorganic matrix (or vice versa) through weak bonds (hydrogen, Van der Waals and ionic bonds) with no covalent interaction. Easy and straightforward to prepare, Class I hydrogels are the most common type of hybrid hydrogels. Class II materials are composed of a network formed by organic and inorganic domains linked through covalent, ionic-covalent or Lewis acid-base chemical bonds.⁶⁵ Organic and inorganic groups are usually linked through stable Si-C bonds. The term 'co-gel' refers to a hybrid hydrogel obtained by mixing inorganic precursors with organic mono-silylated hybrid molecules ($\text{R-Si}(\text{OR})_n$). When the network is obtained exclusively from bi (or multi) functional hybrid precursors (named bridged organosilica $[(\text{R}'\text{O})_n\text{Si-R-Si}(\text{OR})_n]$) the term 'nanostructured hybrid hydrogel' is used. In that case, there is a perfect distribution, of inorganic and organic moieties, at the molecular level, in the final material.

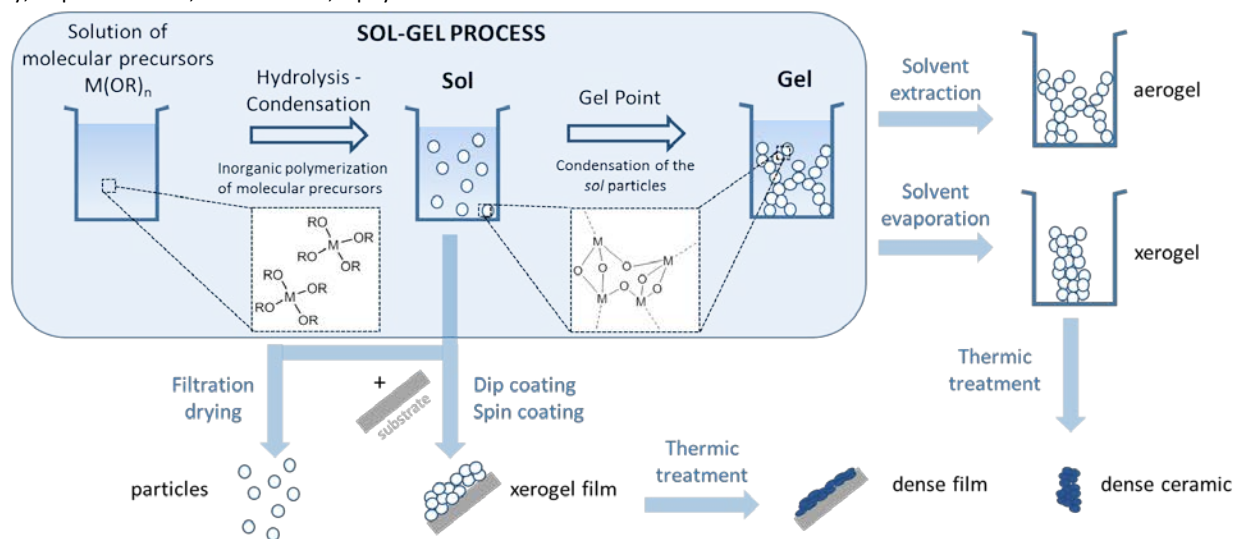


Figure 8: Principle of the sol-gel process and associated resulting materials. Adapted from Brinker *et al.*⁷ and Kołodziejczak-Radzimska *et al.*⁶⁶

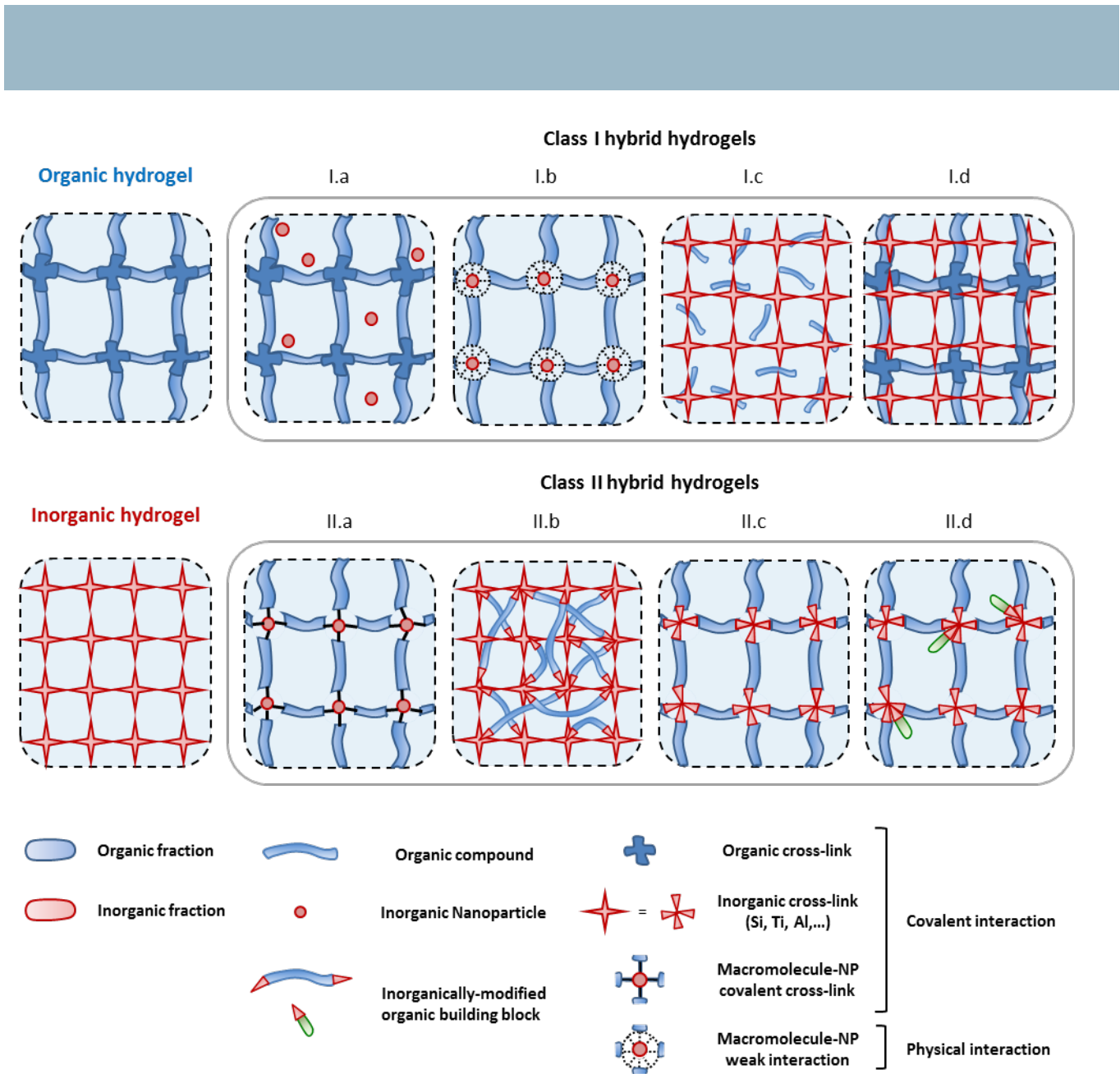


Figure 9: Classes and subclasses of hybrid hydrogels. (I.a) Nanoparticles (NPs) dispersed within the organic hydrogel. (I.b) NPs creating reticulation nodes through weak interaction with organic macromolecules. (I.c) Inorganic network containing organic compounds. (I.d) Inorganic and organic interpenetrating networks. (II.a) NPs form covalent reticulation nodes for organic chains. (II.b) Inorganic and organic interpenetrating networks linked together by inorganic covalent bonds (M-O-M). (II.c) Hybrid network formed by hybrid inorganic-organic macromolecules. (II.d.) Same as (II.c) with additional hybrid inorganic-organic molecules.

3. Class I hybrid hydrogels (nanocomposites)

Nanocomposites are materials constituted by organic and inorganic phases mixed in a non-covalent way. Organic and/or inorganic phases constitute the network through covalent bonds, and the other phase is dispersed at a nanometric scale in the hydrogel, with only weak interactions with the network.

a. Inorganic/organic nanocomposites

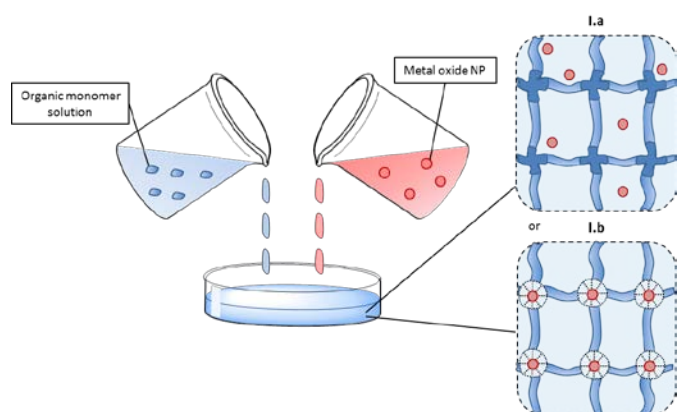


Figure 10: Synthesis of class I.a and I.b hydrogels.

Class I.a and I.b hybrid hydrogels are characterized by the incorporation of inorganic charges, i.e. nanoparticles (NPs), within an organic hydrogel (Figure 10). Added during the hydrogel synthesis, these NPs may have been prepared by the sol-gel process (e.g. silica⁶⁷ or titanium oxide⁶⁸ NPs). However, the hydrogel network is not obtained by inorganic polymerization but by organic polymerization. Inorganic components can either be simply entrapped (I.a),^{67,69–72} or adsorbed by weak interactions into the network.^{69,70,73} In the latter case, NPs may help in the establishment of the organic network, behaving as reticulation nodes *via* weak interactions with the organic part (I.b).^{68,74,75}

The main reason for adding inorganic charges in a hydrogel is to improve mechanical properties such as the compressive strength, the traction, the viscoelastic modulus, and the toughness.^{67,70,72} Physico-chemical properties can also be improved by addition of an inorganic charge. This charge can be used to control the swelling ratio^{72,73} and to improve the stability to pH, temperature variations, hydrolysis and enzymatic cleavage.⁷⁶ Inorganic components have also been used to enhance bioactive properties. For example, hydroxyapatite was incorporated into alginate^{77,78} or PEG derivative^{28,79} hydrogels for bone regeneration purposes. Besides, drug-loaded silica particles were embedded in collagen and chitosan hydrogels⁸⁰ as local reservoirs to reduce the toxicity of anticancer drugs (e.g. doxorubicin⁸¹), and deliver drugs at the site of the tumour. Silica particles also ensure a controlled release of the drug, avoiding a burst effect.

Silica nanoparticles are the most commonly used, but hydroxyapatite,^{77,78} titanium dioxide (TiO₂),^{68,75} zinc oxide (ZnO),^{71,82} aluminium oxide (Al₂O₃),⁷⁵ zirconium oxide (ZrO₂),⁸³ cerium oxide (CeO₂),⁸⁴ and graphene oxide,⁷³ have also been reported as doping agents.

b. Organic/inorganic nanocomposites

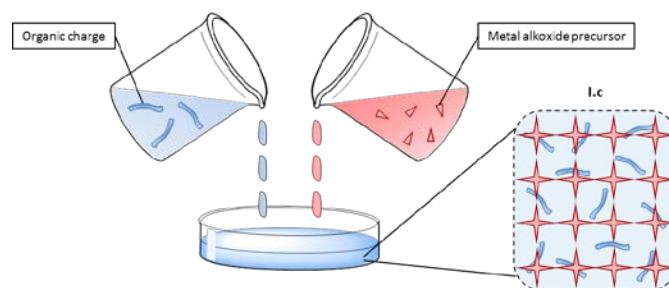


Figure 11: Synthesis of class I.c hydrogels.

i. Inorganic network synthesis

By contrast to inorganic/organic nanocomposites (I.a and I.b), class I.c hybrid hydrogels are prepared by entrapping organic and biological entities in an inorganic network obtained by the sol-gel process (Figure 11).

Most of the studies reported on this class of hydrogels involved silica. Indeed, silicon alkoxides are preferred because they are less reactive than other metal alkoxides including elements such as titanium or aluminium. This is due to the fact that silicon has a low electrophilicity and zero degree of unsaturation, while elements such as titanium or aluminium display higher unsaturation.^{56,85,86} As a consequence, non-silicon metal alkoxides are difficult to handle; they are very sensitive to moisture, and precipitation of the corresponding oxides occurs as soon as water is present.⁸⁶

Silica-based hydrogels are mainly obtained by hydrolysis and condensation of silicon tetraalkoxide precursors, and by condensation of silicic acid colloidal solutions.^{87,88} Typically, silicon tetraalkoxide precursors are diluted in an alcohol/water mixture,^{7,89} and a catalyst is added.⁹⁰ Under these conditions, gelation occurs at room temperature within a few hours. Gelation time depends strongly of the amount of silica precursor: the higher is the concentration, the faster the gelation proceeds.

However, one of the factors limiting the use of fully inorganic silica hydrogels is the formation of alcohol as by-product resulting from hydrolysis of silica precursors, which can have a detrimental effect on the activity of entrapped biomolecules or cells.⁸⁷ Moreover, this may hamper the design of polysaccharide-based hybrid hydrogels which are not fully soluble in alcohol-containing solution.⁹¹

Silicon tetraglycolate can be used to overcome this problem.⁹² In fact, it displays the advantage of being water-soluble (a paramount advantage compared to TEOS) and biocompatible. The resulting hydrogel contains water and glycerol, which results from the precursor hydrolysis. The released glycerol is biocompatible and non cytotoxic. In addition, it is a good solvent for organic compounds (e.g. polysaccharides), allowing their use for the design of hybrid hydrogels.⁹¹ Khonina *et al.* also reported the modification of tetraalkoxytitanium with glycerol to synthesize alcohol-free titanium-based hydrogels.⁵⁶

ii. Applications

Class I.c hybrid hydrogels were found to be an attractive way to study the behaviour of confined organic compounds or biological

entities within a water-swollen network. This is enabled by the transparency⁹⁰ and the chemical and biological inertness of silica hydrogels.⁸⁸ In addition, the pore radius of silica hydrogels (1 to 6 nm) is smaller than the average cell diameter. This feature allows nutrients and waste diffusion while entrapping and protecting the cells against the immune system for *in vivo* studies.⁹² Bhatia *et al.* reported the immobilization of enzymes within silica networks without interfering with their activity.⁸⁷ These materials found applications as biosensors and immobilized enzyme reactors. Recently, Mutlu *et al.* described a synthetic ecosystem in which silica hydrogel matrices allowed for the precise control of microbial populations and their microenvironment.⁹³ This system acts as a nano-reactor and allows co-encapsulation of a mixed culture of synergistically acting bacterial species, which can perform more varied and complex transformations of chemical substances than each species by itself.

Encapsulation of organics (molecules and polymers),^{94,95} and biological materials (enzymes, cells, etc.)^{88,93} also yielded innovative biomaterials for biomedical applications (tissue engineering, biosensors, etc.). Oh *et al.* synthesized hybrid hydrogels by inorganic polymerization of TMOS (tetramethyl orthosilicate) in the presence of alginate.⁹⁵ TMOS being the only source of building block, the alginate did not take part in the network construction, but conferred biocompatibility to the hydrogel. Hybrid silica-alginate hydrogels were found to display better mechanical properties, long-term stability, and cell culture behaviour than pure alginate hydrogels. Thus, these silica-alginate hydrogels were good hybrid scaffold candidates for tissue engineering. Silica-polysaccharide based hybrid hydrogels coming from silicon-glycerolate precursors were also described, and found applications as sensor materials (dye immobilization⁹⁶), and biomaterials for tissue engineering (enzymes,⁹⁴ cell entrapment⁸⁸).

Among the few examples of non silica-based hydrogels, Johnson *et al.* succeeded in encapsulating a protein (bacteriorhodopsin) within titania hydrogels.⁹⁷ This bio nanocomposite was used for visible light photocatalysis.

organic class I.a-c hydrogels, which are only reinforced by organic or inorganic inclusions, IPN hydrogels show a significant increase in thermal and mechanical stability,⁹⁹ thanks to a better repartition of the mechanical stress. Besides, Interpenetration can also prevent cracks from spreading.¹⁰⁰ TEOS is commonly used to get the inorganic network,¹⁰⁰⁻¹⁰² but TMOS¹⁰³ and sodium silicate¹⁰⁴ were also studied. By varying the nature and the concentration of these inorganic precursors, the morphology of IPN hydrogels can be tuned.¹⁰⁵ Various organic polymers were used to establish the organic network: poly(acrylic acid),^{100,104} poly(N-isopropylacrylamide) (PNiPAAm),^{102,103,106} and chitosan.¹⁰¹ However, even when the two types of networks are closely entangled, organic and inorganic parts of the IPN may form microdomains, thus resulting in a non-homogeneous material at the nanometric scale. An elegant alternative is to covalently combine the two networks in a single hybrid network, which will belong to the second class of hybrid hydrogels.

4. Class II hybrid hydrogels

The establishment of stronger interactions between inorganic and organic networks, ideally through covalent bonds, can prevent phase separation even at nanoscale, affording the resulting hybrid hydrogel a perfect homogeneity at micrometric scale.^{107,108} The effects of this homogeneity are witnessed by a good optical transparency and a significant improvement of the mechanical properties.¹⁰⁹

Hybrid hydrogels in which organic and inorganic domains are covalently bound are classified as class II hybrid hydrogels. In all the cases, stable Si-C bonds link the organic and inorganic parts. Most of the time, the organic precursor is chemically modified with inorganic moieties prior to the preparation of the hydrogels. Upon hydrolysis and condensation of the alkoxy silyl groups, siloxane bonds are formed between the different silicon-containing precursors, and ensure the hydrogel solidification. The chemical introduction of a silyl group on an organic molecule (e.g. a polymer) is referred to *silylation*, and can be performed following several approaches (Figure 13). Obviously, the choice of the silylating reagent depends on the available functions displayed on the organic molecules (e.g. the backbone or the extremities of a polymer, the N or C-termini and the side-chains amino acids), the most common being alcohol,^{110,111} amine^{110,112,113} and carboxylic acid^{114,115} functions. To enable silylation, organic functions displayed by the polymer can also be chemically transformed to gain reactivity and/or selectivity. As an example, alkene functions can be introduced on the polymer backbone to further react with amine,^{107,116} sulfhydryl-¹¹⁷ (Michael reactions) or hydrosilane-(hydrosilylation)^{118,119} containing silylating reagents.

c. Interpenetrating networks

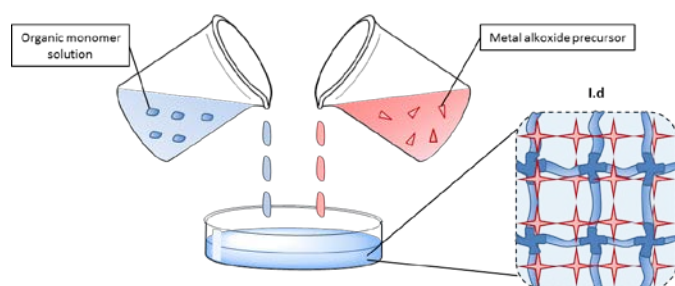


Figure 12: Synthesis of class I.d hydrogels.

Hybrid interpenetrating polymer network (IPN) hydrogels are composed of independent but intertwined organic and inorganic networks (Figure 12). Noteworthy, inorganic polymerization proceeds chemoselectively over organic polymerization, allowing the one-pot synthesis of the hybrid IPN.⁹⁸ By comparison with

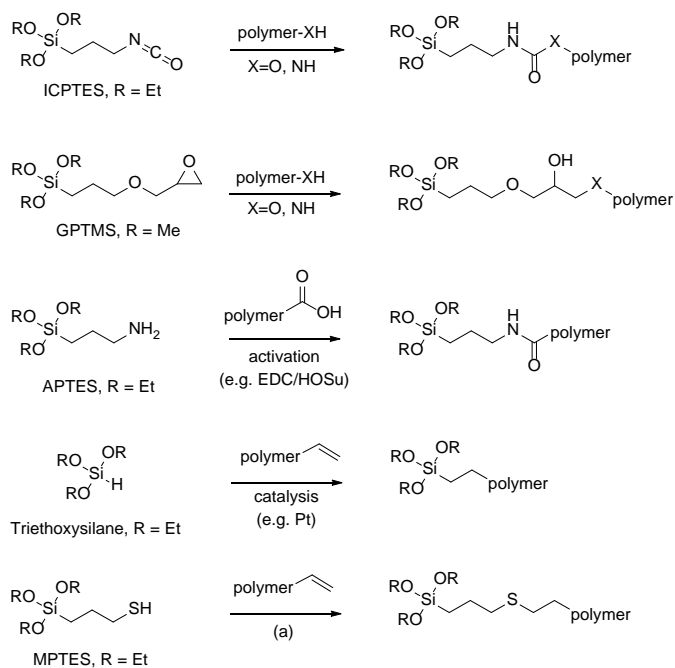


Figure 13: Examples of functionalization of polymers with trialkoxysilyl reagents. ICPTES: 3-isocyanatopropyl triethoxysilane; GPTMS: 3-glycidoxypropyl trimethoxysilane; APTES: 3-aminopropyl triethoxysilane; MPTES: 3-Mercaptopropyl trimethoxysilane. (a) Michael addition reaction may only proceed with activated unsaturated double bond.

An alternative to silylation is the direct synthesis of a hybrid polymer from monomers bearing an inorganic moiety, leading to a polymer with inorganic pendant groups. The most widely used monomer is 3-methacryloxypropyltrimethoxysilane (MAPTS), in which the acrylate moiety can undergo free radical polymerization. This monomer can be copolymerized with other organic monomers to lead to hybrid copolymers of poly(MAPTS) (PMAPTS) (Figure 14).^{120,121}

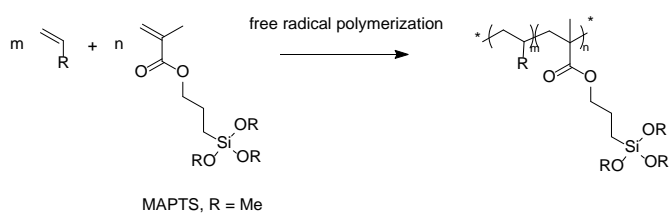


Figure 14: Hybrid copolymer synthesis by copolymerization of a vinyl monomer and MAPTS.

a. Inorganic nanoparticles as cross-linkers

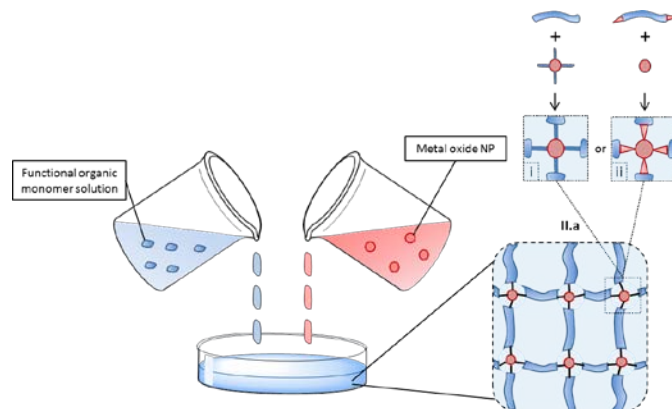


Figure 15: Synthesis of class II.a hydrogels containing metal oxide NPs. Subclass i: organic covalent binding. Subclass ii: inorganic covalent binding.

The specificity of this hydrogel category lies in the presence of metal oxide nanoparticles, most of the time silica nanoparticles (SiNPs), acting as reticulation nodes (Figure 15). Unlike class I.b hybrid hydrogels in which NPs are added as non-covalent doping agents, here NPs are covalently bound to the chains constituting the three-dimensional network. Depending on the nature of the reaction linking particles and chains, two types of class II.a hydrogels could be distinguished: subclass II.a-i (organic reaction) and subclass II.a-ii (inorganic reaction). An elegant example of subclass II.a-i was recently reported by Huang *et al.* who used vinyltriethoxysilane as precursor for the sol-gel synthesis of NPs covered by vinyl groups.¹²² These functionalized NPs were added to a solution of acrylamide and involved in a free radical polymerization. Acrylamide acted as the main monomer for the chain growth, while NPs constituted cross-links between the chains. The resulting hydrogels were found to be very stretchable and compressible, and were used as supercapacitors for electronic applications.

Subclass II.a-ii hydrogels are obtained by reaction between silicon alkoxide hybrid precursors and silanols displayed on the surface of SiNPs, resulting in Si-O-Si bonds. To do so, macromolecules are silylated and engaged in the sol-gel process along with SiNPs. Ashraful *et al.* described the synthesis of a copolymer constituted of PNiPAAm and PMAPTS; the inorganic polymerization of this hybrid copolymer in the presence of silica NPs yielded a hybrid thermosensitive hydrogel.¹²⁰

b. Co-hydrogels

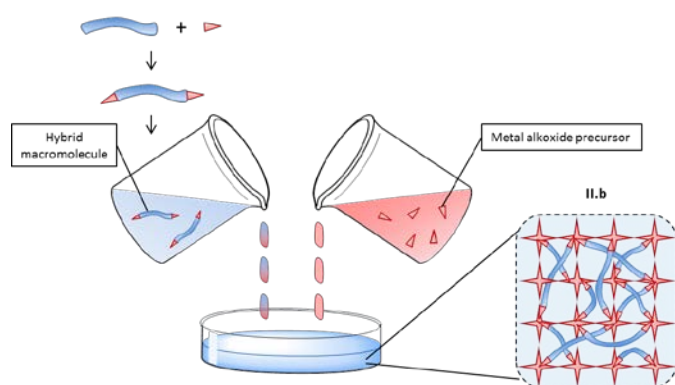


Figure 16: Synthesis of class II.b hydrogels.

As already pointed out, the sol-gel inorganic polymerization proceeds chemoselectively in the presence of organic functional groups. More interestingly, when organic precursors are silylated (e.g. hybrid macromolecules, hybrid peptides, hybrid dyes), it allows the formation of covalent bonds between organic and inorganic components of the hydrogel (Figure 16). On the one hand, the resulting hydrogels can be mainly composed of inorganic domains with organic chains linked to the network through inorganic reticulation nodes. The inorganic network can be synthesized from simple metal alkoxide precursors (mainly TEOS), and covalently modified at the same time by hybrid silylated organic compounds, chosen for their physicochemical and/or biological properties. As an example, Beltrán-Osuna *et al.* conferred antifouling properties to silica hydrogels by adding an antifouling polymer i.e. silylated poly(carboxybetaine methacrylate) to the silica precursor during the sol-gel process.¹²³ On the other hand, when hybrid multi-silylated polymers were used instead of monosilylated organic compounds, the resulting hydrogels could be considered as IPNs covalently bound together. This strategy was described using chitosan,¹²⁴ (poly(2-hydroxyethylsartamide),¹²⁵ and poly(oxazoline)¹¹⁸. In order to increase the cross-link density, a double-polymerization can be performed. It consists in the organic polymerization of silylated organic monomers (e.g. free radical polymerization of methacrylate monomers bearing an alkoxysilyl group).^{107,116} The organic polymerization occurs at the same time as the inorganic polymerization of metal alkoxide precursors. Hybrid monomers take part in both polymerizations. As expected, the resulting network is composed of both organic and inorganic cross-links.

c. Nanostructured hydrogels (II.c and II.d)

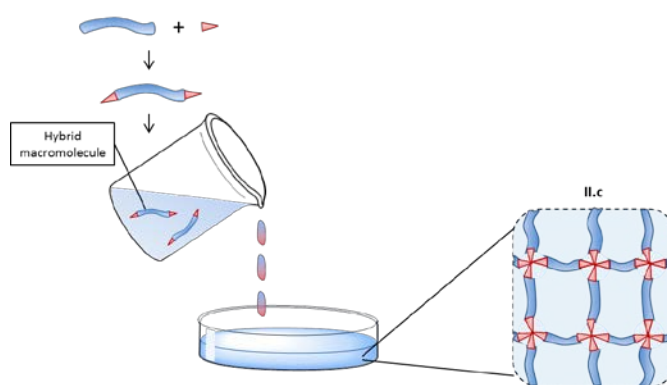


Figure 17: Synthesis of class II.c hydrogels.

Nanostructured hydrogels (II.c) (Figure 17) share the following distinctive features: i) only one hybrid organic/inorganic building block is required to create the network; ii) the network is covalently assembled only by inorganic sol-gel polymerization; iii) in terms of molecular composition, nanostructured hydrogels are considered as monophasic since the organic and the inorganic parts cannot be separated from each other.¹²⁶ Interestingly, a large variety of organic polymers can be used as long as they are previously silylated.

i. Nanostructured hydrogels as intermediates

Despite their attractiveness, class II.c hydrogels have been scarcely described in the literature. They first appeared at the end of the 90s. Table 1 gathers the most significant examples, but it is worth noting that most of these studies did not aim at obtaining a hydrogel as a final material. Indeed, after the sol-gel process, additional treatments were used to obtain other hybrid materials such as thin films,¹¹¹ xerogels¹¹⁴ and membranes.¹²⁷ Nonetheless, in all cases a nanostructured hydrogel was obtained as an intermediate. This is why they are described in this review.

As an example, Jo *et al.* have first silylated PEG and Pluronic (a triblock copolymer constituted of PEG and polypropylene) by reacting the hydroxyl groups at the ends of the polymer chains, with 3-isocyanatopropyl triethoxysilane (ICPTES), forming carbamate bonds (Table 1-1).¹¹¹ To initiate the hydrolysis of ethoxysilanes and the condensation, silylated precursors were poured into a mixture of water and ethanol, under acidic conditions (pH 2). The solution was casted and the resulting hydrogel was dried to obtain a hybrid thin film. Molina *et al.* silylated amino-PEG and amino-poly(propylene) with 3-glycidoxypropyl trimethoxysilane (GPTMS) (Table 1-3), to obtain a hybrid film with tunable hydrophilic/hydrophobic properties, in the perspective to developing stimuli-responsive delivery devices.¹¹³

Biopolymers were also silylated, to afford the biocompatibility required for biomedical applications. Primary amines of chitosan

Table 1: Examples of class II.c hybrid hydrogels, silylation methods, and parameters of the sol-gel process.

Entry #	Hybrid precursor functionalization			Sol-gel process parameters			Hybrid material	Ref.
	Polymer or biomolecule	Reactive function	Silylating reagent	Solvent	Catalysis	Further treatments		
1	PEG / Pluronic	-OH	ICPTES	Water + ethanol	Acidic	Solvent evaporation	Thin film	111
2	PEG	-OH	ICPTES	Cell culture medium	Nucleophilic	/	Hydrogel	110,128
3	PEG / Pluronic	-NH ₂	ICPTES	Water + ethanol	Acidic	Freeze drying	Xerogel	113
4	Peptide	-NH ₂	ICPTES	Cell culture medium	Nucleophilic	/	Hydrogel	129
5	Chitosan	-NH ₂	GPTMS	Water	Acidic	Freeze drying	Xerogel	130,131
6	Chitosan	-NH ₂	GPTMS	Water	Acidic	Solvent evaporation	Membrane	127
7	Gelatin	-OH / -NH ₂	GPTMS	Water	Acidic	Solvent evaporation	Membrane Xerogel	132,133 133,134
8	Cellulose	-OH	GPTMS	Water	Basic followed by neutralisation	/	Hydrogel	112,135,136
9	Alginate	-CO ₂ H	APTES	Water	Acidic	Freeze drying	Xerogel	114
10	PNiPAAm	Copolymerization	MAPTS	Water	Acidic	/	Hydrogel	121

were reacted with GPTMS under acidic conditions (pH 2 to 4) (Table 1-5 and 1-6). The silylated polymer was not isolated, and the resulting colloidal solution was either directly freeze-dried to get hybrid porous scaffolds for tissue engineering,^{130,131} or allowed to evaporate to prepare hybrid membranes.¹²⁷ In this latter case, the authors demonstrated the cytocompatibility of this membrane for osteoblastic cells culture.

Similarly, Ren *et al.* studied the silylation of gelatin, which displays both hydroxyl and amine functions (Table 1-7). The silylation was performed with GPTMS and the colloidal solution was directly polymerized without any purification of the hybrid precursor.¹³² The hydrogel was then either dried or freeze-dried, leading respectively to hybrid membranes¹³² and hybrid porous xerogel scaffolds.^{133,134} These hybrids materials were presented as bioactive and biodegradable scaffolds for bone tissue engineering.

When carboxylic acids are present on the polymer backbone, they can be activated and coupled to amine-containing silylating reagents such as 3-aminopropyl triethoxysilane (APTES). As an example, amide bond formation can proceed via carboxylic acid activation with the water soluble 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), in the presence of N-hydroxysuccinimide (HOSu) as auxiliary nucleophile. Hosoya *et al.* used this method to silylate alginate (Table 1-9).¹¹⁴ The hybrid alginate was not isolated, and gelation occurred immediately after silylation. Subsequent washings allowed removal of the urea by-product and HOSu. The resulting hydrogels were then freeze-dried. These authors showed that modification of alginate with silanol groups induced gel formation, and that the resulting hydrogel was able to promote apatite formation, opening applications for bone repair.

Nevertheless, this strategy was probably even more attractive to envision biocompatible hydrogels for health applications. Lutecki *et al.* described the fabrication of a heat-sensitive hydrogel from a PNiPAAm-co-PMAPTS copolymer (Table 1-10).¹²¹ Alkoxysilane pendant chains of PMAPTS were used to establish the covalent network via inorganic polymerization, while PNiPAAm was chosen for its thermosensitive properties. The resulting hybrid hydrogel showed improved swelling/shrinkage behaviour and improved mechanical properties, compared to a non cross-linked PNiPAAm hydrogel (physical hydrogel). No application was described, but such thermosensitive hybrid hydrogels could be useful as excitable carriers for the controlled release of active drugs.

Recently, Zou *et al.*¹³⁷ reported the first synthesis of an hybrid hydrogel from titanium alkoxyde. Perylene tetracarboxylate (PTC) derivative was modified by titanium isopropoxide (-Ti(OⁱPr)₃) by a one-pot synthesis in toluene at moderate temperature. The resulting hybrid monomer (Figure 18) was obtained in the form of an orange crystal. Then, sol-gel was initiated by adding water only, without the need of any catalyst, and Ti-O-Ti reticulation nodes ensure the network formation. The resulting hydrogel showed a green fluorescence when irradiated by a UV light. Finally, films were prepared from the hydrogel to be used as a visual fluorescence sensor for aromatic amines and phenols (Figure 18).

ii. Nanostructured hydrogels as biomaterials

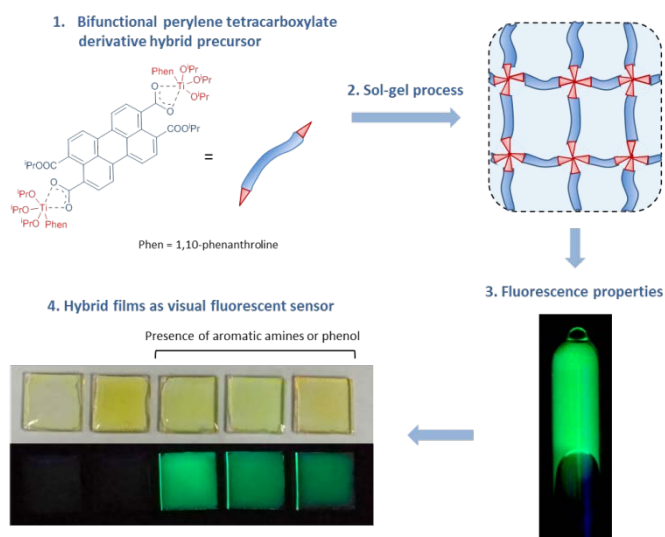


Figure 18: PTC-TiO₂ hybrid precursor and preparation of hybrid films as fluorescent sensors. Adapted from Zou *et al.*¹³⁷

Weiss and co-workers studied silylation of cellulose derivatives to develop hybrid hydrogels for biomedical applications. They functionalized hydroxyethylcellulose (HEC) with GPTMS and 3-glycidopropylmethyldiethoxysilane (GPDMS), *via* a Williamson reaction between the epoxide of the silylating reagent and the hydroxyl groups of the biopolymer (Table 1-8).^{135,138} They was found that GPTMS was a better choice than GPDMS because GPDMS molecules might react together, thereby decreasing the silylation yield. Difficulties in HEC solubilisation led this group to work on hydroxypropyl methylcellulose (HPMC).¹¹² Nonetheless, HPMC solubility remained limited in organic solvents. Silylation was performed in heterogeneous medium with an organic solvent (hexane,¹¹² propanol¹³⁵), and in homogeneous medium by using ionic liquids.¹³⁹

Silylated HPMC (HPMC-Si) was dissolved in basic medium (pH 13) to hydrolyse the ethoxysilyl groups and form the sodium silanolate derivative of HPMC-Si. Then, the solution was neutralized either with acids¹¹² or buffer solutions¹³⁶ to induce gelation. Below pH 12.4, polycondensation of silanols and self-hardening of the gel can occur. These authors showed that pH, temperature, as well as the percentage of silyl groups on HPMC had an impact on the gelation kinetics.^{112,140}

HPMC-Si hydrogels could encapsulate chondrocytes and be injected for cartilage tissue regeneration.¹³⁶ They were also used for other applications such as bone repair¹⁴¹ and substitution,¹⁴² cell-based tissue engineering^{143–145} and intramyocardial cell delivery.¹⁴⁶ (Figure 19).

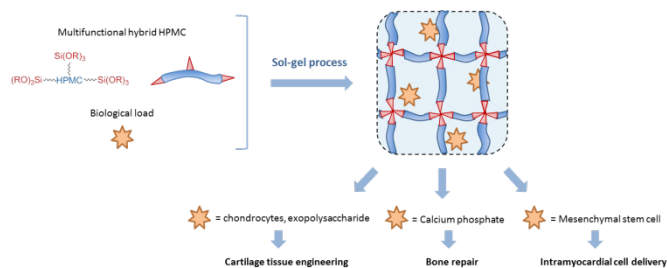


Figure 19: Preparation of hybrid hydrogel from silylated HPMC precursors and various applications in biomedical field.

As an example, Zhang *et al.* described a strategy to prepare injectable macroporous calcium phosphate cements (CPCs).¹⁴¹ He used HPMC-Si hydrogel as foaming agent which confer to CPCs good handling properties such as injectability and cohesion. The resulting foamed CPCs was comparable to cancellous bone in term of mechanical properties. Moreover, in a preliminary *in vivo* study, the authors showed the biofunctionality of the cement and its efficiency by demonstrating the evidence of formed bone in the cement implantation zone (Figure 20).

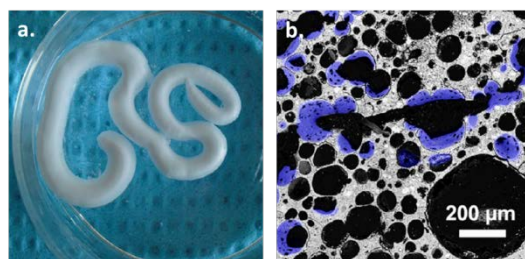


Figure 20: (a) Extruded HPMC-Si foamed CPC and (b) SEM image of the central zone of defect, the areas of newly mineralized bone were coloured with dark blue. Adapted from Zhang *et al.*¹⁴¹

Rederstorff *et al.* described the use of HPMC-Si as scaffolds for cartilage regeneration. They doped the hydrogel with a marine exopolysaccharide (GY785) in order to stimulate the *in vitro* chondrogenesis of adipose stromal cells.¹⁴³ The resulting HPMC-Si hydrogel/GY785 construct was transplanted into nude mice and the production of cartilaginous tissue by rabbit articular chondrocytes was evaluated. The results showed that the scaffolds enriched with GY785 improve the production of cartilage-like extracellular matrix containing glycosaminoglycans and type II collagen in comparison with pure HPMC-Si hydrogels (Figure 21). These results indicates clearly that doped HPMC-Si hydrogels are good candidates for cartilage tissue engineering.

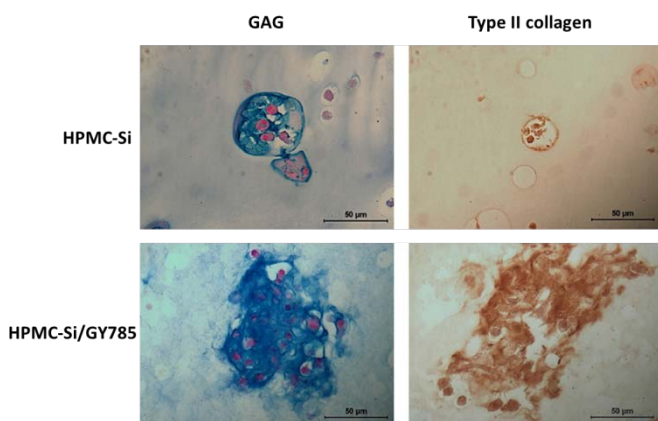


Figure 21: Cartilaginous matrix production by rabbit articular chondrocytes in 3D culture in Si-HPMC scaffolds. Blue : glycosaminoglycan production ; orange : type II collagen production. The samples were observed with a light microscope; scale bar = 50 µm. Adapted from Rederstorff *et al.*¹⁴³

More recently, Zayed *et al.* described the synthesis of HPMC hybrid microgels, by an emulsion templating process.¹⁴⁷ They showed that hydrophobic microdomains (made of sesame oil) could be incorporated during the synthesis. These materials could be used as carriers for hydrophobic drugs.

Since a few years ago, our group has developed a straightforward bottom-up strategy to get multifunctional hybrid hydrogels. By contrast with the two step sol-gel procedure described above, which requires a careful monitoring of pH and neutralization prior to cell seeding, we work at neutral pH, in cell culture buffer. For that purpose, the sol-gel process is catalysed by a nucleophile. As a proof of concept, instead of a multi-silylated biopolymer, a relatively short bi-silylated polyethylene glycol (MW 2000 g/mol) was used as building block (Table 1-2). The hydrogel synthesis was carried out at 37°C, in a phosphate buffer (DPBS) at pH 7.2.¹¹⁰ A concentration of 10 wt % in hybrid PEG was found to be the more relevant in terms of gelation time and mechanical properties of the resulting hybrid hydrogel. Sodium fluoride (NaF) was investigated as catalyst. As expected, an increase in the NaF concentration could shorten the gelation time, but special attention had to be paid to cytotoxicity.¹¹⁰ Hence, the maximum concentration to guarantee cell viability on the surface of the hydrogel was 0.3 wt % NaF. At this concentration, gelation occurred within two hours. Noteworthy, when cell encapsulation was not involved, NaF could be used at a higher concentration and removed afterwards by extensive washings of the hydrogel.

Like some other synthetic polymers, PEG is biocompatible. However it is also bio-inert, it cannot replicate the complexity and the bioactivity of natural tissues. On the contrary, biopolymers such as collagen extracts, are able to mimic the specific environment of extracellular matrices, which is a prerequisite for tissue engineering applications. However, they suffer from several drawbacks, such as high cost of production, weak batch-to-batch reproducibility, and potential immunogenicity. In this context, our group described the preparation of a synthetic covalent hydrogel obtained by sol-gel polymerization of a silylated peptide inspired from collagen sequences (Table 1-4).¹²⁹ Indeed, as an alternative to PEG, we

designed a short undecapeptide sequence derived from the collagen consensus sequence [Pro-Hyp-Gly] repeated three times and flanked by two lysine residues. Silylation of lysine side chains by ICPTES afforded the hybrid peptide block used for the hydrogel network. The hybrid peptide was polymerized by sol-gel at 37°C and pH 7.4, with 0.3 wt% NaF as catalyst. Cell adhesion and proliferation properties were assayed at the surface of the hydrogel, highlighting the cytocompatibility of such materials. More interestingly, the physiological-like synthetic conditions enabled the straightforward embedment of living cells during the formation of the hybrid peptide gel, before the gel point.¹²⁵

The chemoselectivity of the sol-gel process was also exploited to propose a modular strategy leading to functional hybrid hydrogels (Class II.d, Figure 22) from a bis-silylated building block (e.g. PEG) used for the network establishment, and other alkoxyisilane hybrid precursors¹⁴⁸ (e.g. silylated dyes, silylated integrin ligands for cell adhesion and silylated antibacterial peptides). These blocks could be combined and mixed in an appropriate ratio to get covalent functional hybrid hydrogels.^{110,128,147} Virtually, any type of hybrid silylated blocks could be introduced covalently in the hydrogel during the sol-gel process, as long as they can be modified by alkoxyisilyl groups. Fluorescent, antibacterial and cell adhesive PEG-based hydrogels were obtained that way.

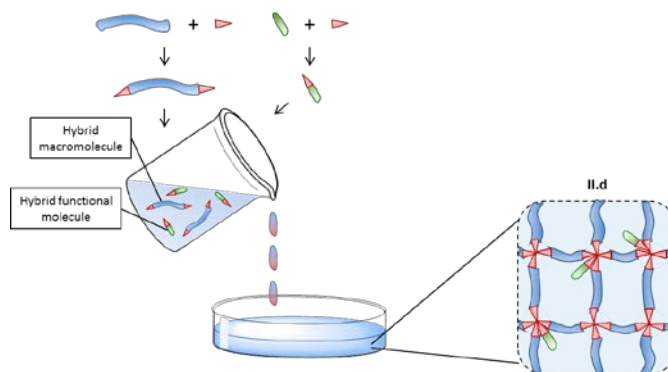


Figure 22: Schematic synthesis route to class II.d hydrogels.

The easy preparation of class II hybrid hydrogels by sol-gel was recently exploited for 3D fabrication. Echaler *et al.* prepared hybrid hydrogel scaffolds by extrusion-based 3D printing, using a colloidal solution of hybrid precursors as bioink (Figure 23).¹²⁸ A good monitoring of the sol-gel kinetics allowed to select the ideal time window when the viscosity of the bioink was ready for printing: liquid enough to be extruded through the syringe, but also viscous enough not to flow and spread upon deposition. Beyond this first example, the combination of sol-gel chemistry and 3D printing paves the way to the design of tailor-made biocompatible and biomimetic scaffolds, from unlimited range of silylated precursors, including synthetic and natural polymers, biomolecules, contrast agents, and even drugs.

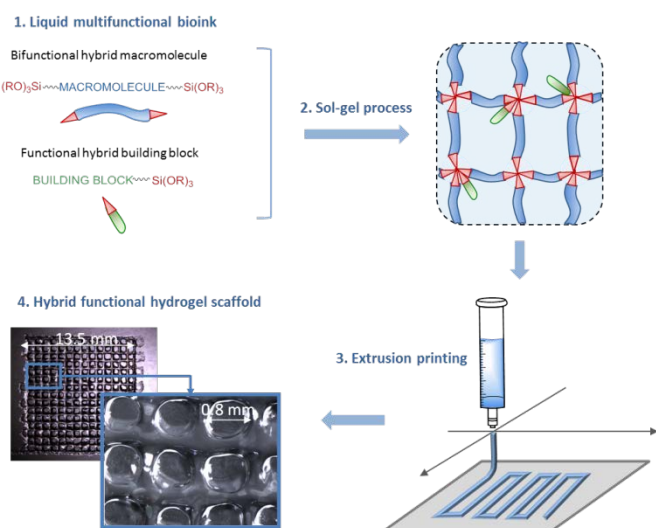


Figure 23: Preparation of hybrid hydrogel scaffolds using sol-gel polymerization and 3D printing. Adapted from Echaliar *et al.*¹²⁸

5. Conclusion and perspectives

The inorganic sol-gel polymerization process has definitely a lot of attractive features for scientists involved in the field of biomaterial design. Simple, it only requires one type of chemical function (i.e. alkoxyisilanes) to obtain a covalent bond, and the establishment of a network. Biocompatible, it can proceed in water, at room temperature and at physiological pH. The classification we propose in this review brings together all types of hybrid hydrogels, presenting their similarities, their structural and chemical specificities. Class I hydrogels are the most studied, but they display a strong lack of homogeneity since organic and inorganic phases are not covalently bound together. On the contrary, class II hydrogels have their organic and inorganic domains linked through covalent bonds, leading to more homogeneous materials with tunable properties. The next improvement has been to afford properties to such materials by incorporation of compounds of interest inside the network, first in a non-covalent way, then covalently. The most recent innovations deal with the design of complex biomimetic materials, suitable for the synthesis of cell-laden scaffolds and bioinks, for tissue engineering purposes. Noteworthy, a careful control of the mechanical properties is necessary, since mechanical environment strongly impacts cell behaviour (migration, differentiation, etc.).

Although the sol-gel process proceeds chemoselectively, it is not the case for the silylation reaction, which has to be carefully controlled to avoid undesired modifications of organic groups displayed by bioactive molecules or polymers. For example, silylation of a bioactive peptide has to be performed at a suitable position on the molecule to preserve the biological activity. This implies that protecting groups have to be introduced during the preparation of the hybrid building blocks. This problem could be even worse when larger biomolecules, such as proteins, have to be modified with a silyl moiety. Thus, a wide range of conjugation and chemoselective chemistries are still to be developed to push

forward the use of hybrid bioorganic/inorganic building blocks. Combined with different fabrication techniques, such strategies could pave the way to the biofabrication of multifunctional biomimetic organoids, and artificial tissues, with a control of the macroscopic shape, but also of the molecular bioactive content resulting from the polymerization of biomimetic blocks through the sol-gel chemistry.

Conflicts of interest

In accordance with our policy on [Conflicts of interest](#) please ensure that a conflicts of interest statement is included in your manuscript here. Please note that this statement is required for all submitted manuscripts. If no conflicts exist, please state that “There are no conflicts to declare”.

Acknowledgements

The acknowledgements come at the end of an article after the conclusions and before the notes and references.

Notes and references

‡ Footnotes relating to the main text should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

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