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# THE CHEMISTRY AND ENGINEERING OF POLYMERIC HYDROGEL ADHESIVES FOR WOUND REPAIR: A TUTORIAL



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# The chemistry and engineering of polymeric hydrogel adhesives for wound closure: A tutorial

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The closure and repair of wounds after traumatic or surgical injury is of significant clinical and research importance. While sutures remain the common wound closure technique, they have many disadvantages. Consequently, polymeric hydrogel adhesives have emerged as essential materials for wound management and repair because of their tunable chemical and physical properties, which enable them to adhere or stick to tissues, possess sufficient mechanical strength to stay intact and be subsequently removed, provide complete wound occlusion, and act as a barrier to bacterial infection. Moreover, these materials absorb wound exudates and keep the wound moist for faster healing. This *tutorial review* summarizes the key chemical features that enabled the development and use of polymeric hydrogels as wound adhesives, sealants, and hemostats, their design requirements, synthetic routes, determination of properties, and the tests needed to evaluate their performances. This *tutorial review* is a reference and a starting point for scientists and clinicians working or interested in the field of wound management and, importantly, for the general audience who is interested in polymers for medical applications.

## 1. Why are polymeric hydrogel adhesives important for wound closure?

A wound is an externally induced injury to any of the tissues in the body (*e.g.*, skin, muscles), which results in a break and subsequent damage to organ structures, cells, blood vessels, extracellular matrices (ECM), membranes, etc. Wounds are classified either as open (*e.g.,* abrasions, avulsions, incisions, lacerations, or penetration) or closed (*e.g.,* contusions, ecchymosis, or petechiae). All wounds, including those caused by surgical procedures, require immediate closure and repair in order to prevent infection and promote healing. Sutures remain the common closure technique for wound approximation and repair because of their great tensile strength and low dehiscence rate.[1] However, they are not ideal and present the following drawbacks: 1) placement of sutures may require anesthesia; 2) sutures can induce a high rate of infection, nerve damage, inflammatory reactions, granuloma formation, and scar tissue formation; and 3) suturing is a time-consuming method and requires an acquired technical skill that can vary widely from surgeon to surgeon, thus influencing the time and success of the procedure.[2] Staples and adhesive tapes are used as alternatives to sutures. Both are fast and easily applied, with lower rates of infection than sutures. Nevertheless, staples require the use of anesthetics and can generate an imprecise wound approximation, while adhesive tapes have low tensile strength, and their application is not useful in wet wounds and hairy areas due to their lack of adhesion.[2] As a consequence, there is a clinical interest in adhesives that can replace or supplement the conventional closure techniques in the repair of wounds.

In general, a tissue adhesive is a material that can stick to a tissue (*e.g.*, epithelial, connective, muscular or neural). It can bind various tissues together to allow proper healing to occur, control or stop the bleeding, or prevent gas or fluid leakage from the tissue by acting as an adhesive, hemostat, or sealant, respectively. The interest in tissue adhesives has grown in the last decade, and a number of natural and synthetic adhesives have been commercialized for wound closure (*e.g.,* BioGlue®, DuraSeal™, CoSeal™, and Adherus™). Several literature reviews summarize the state of the art of these adhesives and describe their advantages and disadvantages.[3-5] This *tutorial review* focuses on polymeric hydrogel adhesives as exciting and promising materials for the management of wounds.

Polymeric hydrogels are three-dimensional cross-linked networks that can retain large amounts of water due to the presence of hydrophilic moieties (*e.g.*, hydroxyl, ether, amino, or carboxyl groups) in their polymeric backbones. These materials can also be tailored to exhibit adhesive properties by: 1) incorporating functional groups within their structures, which can interact and bind with the surrounding tissues (**Fig.**

**1**); or 2) forming an interpenetrating networks with the surrounding tissues to mechanically lock the material in place (**Fig. 16**). This class of biomaterials combines many advantages for wound repair such as: biocompatibility, biodegradability, ease of application, tunable mechanical properties, high water content, controlled tissue adhesion, moist environment for wound healing, barrier to bacterial penetration, and a matrix for drug delivery.[4-6] Hydrogel-based materials are being used today in the clinic, and their continued development will provide materials with new properties and treatment options for wound management and repair.

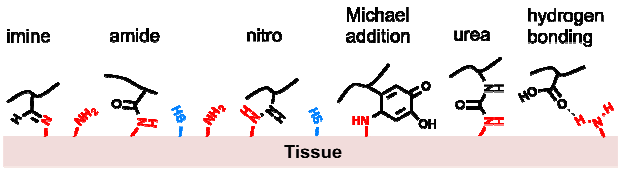


**Figure 1.** Examples of chemical/physical linkages formed between hydrogels and surrounding tissues.

## 2. What are the design requirements for polymeric hydrogel adhesives?

Polymeric hydrogel adhesives must fulfill essential design requirements for their use in the clinic.[3] Their design and development depend on the desired application, the nature and location of the wound, and its neighboring tissues and fluids. After analysis of these constraints, the hydrogel components are selected and synthesized accordingly. When the hydrogel is formed, its physical, chemical, mechanical, and adhesive properties are measured in order to evaluate its performance. For example, tuning the mechanical properties of hydrogels is advantageous because it allows control over the swelling rate of the gel as well as its mechanical strength (*i.e.*, stronger gels last longer than weaker ones).

Upon application, the hydrogel must adhere to the wound site and completely seal it to prevent bacterial penetration and infection, as well as fluid leakages. Thus, it needs to gel and adhere even in moist conditions while maintaining its mechanical strength. For example, if the hydrogel adhesive is designed as a hemostat for an arterial injury, it is required to gel upon application on the incision to instantly seal it and secure the hemorrhage, while maintaining its adhesive and cohesive strengths, under wet conditions and high fluid pressures. Hydrogels can absorb wound exudates and swell, and the swelling index depends on their structures and cross-linking densities. However, the control of the swelling index is important depending on the desired application (*e.g.*, a dural sealant *vs.* a burn wound dressing). Hydrogels may lose their mechanical strength upon swelling which can cause their breakage or eventual dissolution before complete wound healing. Moreover, highly swollen hydrogels can cause tissue compression when applied in confined areas of the body.[5]

The hydrogel components must be safe and compatible with the biological system to allow proper wound healing. Thus, the utilization of biocompatible and biodegradable polymer structures is of utmost importance in the design of hydrogel adhesives. Nevertheless, biocompatibility and biodegradability testing is required to ensure the safety of these materials before their use in the clinic. For examples, please refer to the FDA 10993 guidelines for a list of the required tests. To avoid any potential toxicity, the hydrogel formation should occur at physiological pH, buffer capacity and temperature, with ideally no generation of heat or by-products, or with by-products that are known to be biocompatible. Hydrogels should be designed to have tunable degradation rates, depending on the desired application, and degrade into safe components that are completely eliminated from or utilized by the body after the wound is healed.

Other essential requirements in the design of polymeric hydrogel adhesives are their stability and ease of storage, sterility, and relatively low cost of production. Hydrogel components must not decompose during storage, and, from a commercialization perspective, exhibit a 2-year shelf life at room temperature. The hydrogel should be sterilized after it has been loaded into the device applicator, and the sterilization process must not affect the resulting performance.

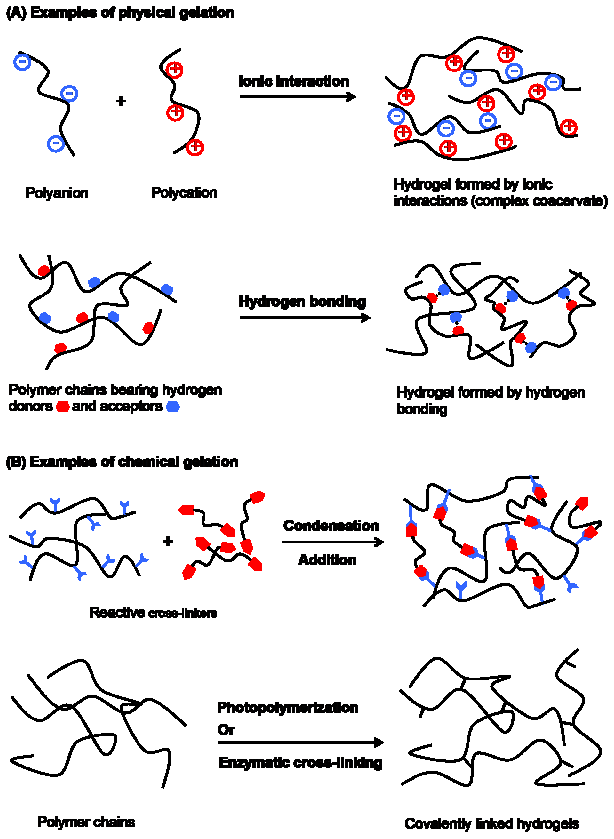


**Figure 2.** Pictorial representations of polymeric hydrogel adhesives formed by (A) physical gelation and (B) chemical gelation.

Finally, the polymeric hydrogel adhesive should be easily and controllably applied to maximize the beneficial outcomes. If used by a clinician, she/he should be able to easily and rapidly handle its preparation and application onto the wound, and, thus, its use would require minimal training. Ideally, there should be no change in the current procedure application except for an improved outcome: standard of care plus. Recently, the idea of applying a hydrogel adhesive during immediate trauma care in the field and then removing the gel during definitive treatment has been introduced.[7] Dissolving the hydrogel adhesive on demand would be a desired alternative to standard debridement methods or surgical incisions, because it would inflict less damage to the tissue, decrease pain, and allow for gradual and specific re-exposure of a complex and large wound for further treatment.[7]

## 3. How are polymeric hydrogel adhesives synthesized?

Polymeric hydrogel adhesives are synthesized by physical or chemical gelation, as depicted in **Fig. 2**, or by a combination of both.[8] Gelation occurs when water-soluble polymers cross-link together by physical or chemical linking to form a non-soluble three-dimensional network, with water filling the space voids between the polymer chains.

***Physical gelation.*** This gelation type takes place when polymers are linked together by physical interactions such as hydrogen bonding, hydrophobic interactions, ionic associations, host-guest complexation, metal coordination and molecular entanglements (**Fig. 2A**).[8] These hydrogels can be formed in aqueous media either by non-covalent interactions or by entanglements between the polymer chains, and thus do not require the use of cross-linkers, external radiation sources or additives. Due to the non-covalent and reversible nature of the junctions in the network, physical hydrogels are generally weak and they tend to break under certain physical stimuli or when stress is applied. As a result, this has limited their use as tissue adhesives for wound closure and repair, and therefore, most polymeric hydrogel adhesives developed thus far are mainly based on chemical gelation.[4,5] Nonetheless, examples of physical hydrogels based on biocompatible and biodegradable natural or synthetic polymers have been developed as adhesives for wound healing.[9-15] Their preparation is based on aqueous polymer solutions, which are either heated or cooled or left at room temperature to self-assemble (*e.g.*, helix or β-sheets formation, micelle aggregation), freeze-thawed to form microcrystal domains, or stimulated by low pHs to form hydrogen-bond cross-links within the polymer chains. Other approaches utilize polyelectrolytes of opposite charges (complex coacervates) or multivalent counter ions to form the hydrogels. Although these hydrogel often exhibit low adhesive properties and long gelation times, efforts are underway to introduce additional components or functional groups to interact more strongly with the surrounding tissues and potentially enhance the adhesion of the material.[10,16,17]

***Chemical gelation.*** The second commonly used method for the synthesis of polymeric hydrogel adhesives is the chemical gelation, which occurs when the water-soluble macromolecules are linked together by covalent bonds such as C–C, X–C or X–X bonds (where C is a carbon and X is an oxygen, nitrogen or sulfur). The covalent bonds are formed either by radical polymerization of monomers or polymer chains that yield free radicals, which recombine, cross-link and form the network, or by condensation/addition reactions of reactive macromolecules, such as amidation, esterification, thioesterification, Michael addition, Schiff base formation, or other click-type reactions, as well as by enzymatic cross-linking, as depicted in **Fig. 2B**.[8] Chemical gelation yields strong and stable hydrogels due to the permanent junctions in the network. The advantage of using chemical cross-linking to develop polymeric hydrogel adhesives is the ability to easily tune the mechanical and adhesive properties of the materials by altering and controlling the polymeric structures, the functional groups in their repeating units, the cross-linker types and the cross-linking process. In order to form the adhesive hydrogels, biocompatible and biodegradable natural or synthetic polymers are cross-linked *via* different types of chemical reactions (*e.g.*, radical polymerization, condensation/addition reactions, or enzymatic cross-linking) as described in the following paragraphs.

Radical polymerization allows the generation of free radicals due to a trigger source such as light, temperature or redox reactions, to form polymers. Photopolymerization is one class of radical polymerizations induced by light, and the most commonly used for hydrogel formation. It allows rapid network formation under physiological conditions, with efficient temporal and spatial control. Specifically, gelation by photopolymerization is driven by the use of external radiation sources such as visible light, ultraviolet (UV), gamma rays, and

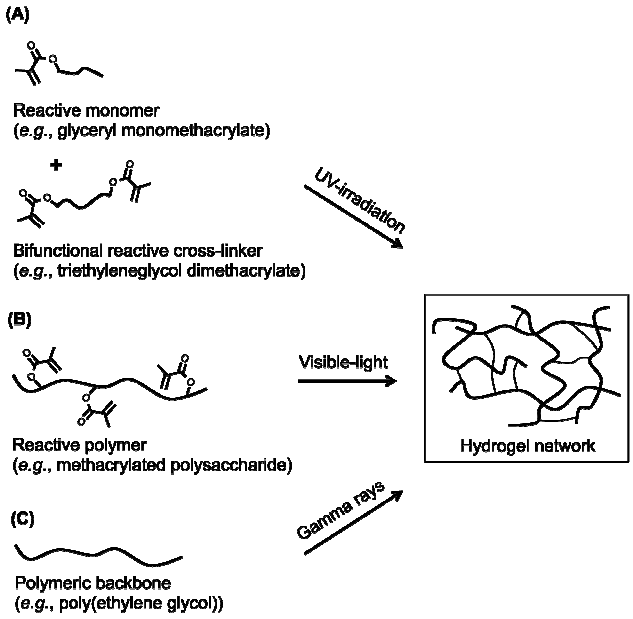


**Figure 3.** Examples of photoreactive hydrogel precursors and their subsequent cross-linking *via* photopolymerization.

electron beams and can be conducted in three ways to form hydrogels (**Fig. 3**): 1) use of hydrophilic reactive monomers and polyfunctional cross-linkers; 2) use of readily available natural or synthetic polymers functionalized with light-sensitive reactive groups, with or without reactive monomers and polyfunctional cross-linkers; and 3) use of non-functionalized polymer chains. The first two methods require the utilization of photoinitiators to initiate the formation of radicals. Examples of photoinitiators applied in the synthesis of hydrogel adhesives are the water-soluble Irgacure 2959 (1-[4-(2-hydroxyethoxy)-phenyl]-2-hydroxy-2-methyl-1-propane-1-one), Irgacure 184 (1-hydroxycyclohexylphenyl ketone), Eosin Y (disodium 2-(2,4,5,7-tetrabromo-6-oxido-3-oxo-3H-xanthen-9-yl) benzoate), and camphorquinone (CQ). In the first approach, commercially available water-soluble monomers and polyfunctional cross-linkers are mixed with photoinitiators and irradiated with a light source. Upon irradiation, the photoinitiator absorbs light of an appropriate wavelength and becomes an active species that initiates the growth of the polymer chains by reacting with the reactive monomers. Polymer chains are subsequently cross-linked with reactive polyfunctional cross-linkers to form the hydrogel network (**Fig. 3A**).[18] Acrylates, methacrylates, acrylamides, methacrylamides and vinyl pyrrolidone are the common water-soluble monomers used for hydrogel adhesive formation. However, the downside of this approach is when unreacted monomers, photoinitiators, and their reactive species are applied *in vivo*, they can be very toxic to cells and proteins in the surrounding tissues, depending on their concentrations.

Another alternative for generating hydrogel adhesives is the use of readily available hydrophilic polymers functionalized with radical polymerization-sensitive acrylate (A), methacrylate (MA) or azide ($N_3$) groups. Biocompatible and biodegradable polymer backbones such as dextran, chondroitin sulfate, hyaluronic acids, poly(glycerol succinic acids), poly(ethylene glycols) and dendrimers have been functionalized with light-sensitive groups and subsequently irradiated to form hydrogels (**Fig. 3B**),[4,5] and examples of these materials are detailed in Section 6 of the tutorial. Although the toxicity of the monomers is avoided, this method still requires the use of potentially toxic active photoinitiators.

A third less frequently explored photopolymerization technique for hydrogel adhesive formation is the use of non-functionalized water-soluble polymers and ionizing radiations such as gamma rays and electron beams. In this approach, the underlying principle is to irradiate an aqueous solution of polymers such as dextran, poly(ethylene glycols), poly(vinyl alcohols) or poly(vinyl pyrrolidones) to generate free hydroxyl radicals in water. These radicals can abstract carbon-bound H-atoms from the polymer main chains and form the macroradicals, which subsequently recombine and cross-link to form the hydrogel (**Fig. 3C**).[19] This is a process done to form the hydrogel prior to subsequent use. This technique can also be applied to irradiate monomers and polymers in dry state, however, it presents some drawbacks. When monomers are used, care must be taken to avoid the reactivity of toxic radical species generated during the process, and the effects of any unreacted monomers after the reaction. On the other hand, when polymers are used, the generation of radicals is low as compared to the irradiation in solution, and thus requires the use of higher energy doses to form the gel. Furthermore, when the irradiation is conducted without water, the cross-linking is limited by the restricted mobility of the polymer chain radicals.[19] Photopolymerization with ionizing radiation is a clean method and when used with polymer solutions, it does not require the use of monomers, photoinitiators or additional functionalization steps of the polymers. Moreover, the sterilization of the product and its subsequent gelation can be achieved in a single step. Nonetheless, photopolymerization in general uses external radiation sources that cannot be applied everywhere in the body, which can limit its *in vivo* use in certain wounded areas. Moreover, the use of photopolymerization for *in vivo* application generates reactive radical species, which can induce toxicity to cells, and damage enzymes and proteins.[20] Therefore, the effect of the monomer and the photoinitiator type on the hydrogel properties, the choice of the light source, wavelength, irradiation duration and cross-linking process need to be well evaluated and understood in order to overcome the potential toxicity problems of this technique when applied *in vivo.* Another drawback of the photopolymerization is the low adhesive properties of the generated hydrogels due to the absence of functional groups that can interact with proteins in the tissue, and the adhesion to the underlying tissue is mainly due to mechanical interlocking.

Gelation by condensation/addition reactions to form polymer networks requires the use of a water-soluble polymer that contains *at least two nucleophilic* moieties, which can cross-link with another water-soluble component, *i.e.*, low or high molecular weight molecule, that contains *at least three electrophilic* moieties, or *vice versa*. Usually, the nucleophilic moieties are amine, hydroxyl or thiol groups, whereas electrophilic moieties are activated esters, α,β-unsaturated carbonyls, aldehydes, isocyanates, nitrenes, epoxides, and X–C bonds (where X is a halogen). In general, the two components are solubilized in separate buffer solutions, and upon mixing the hydrogel is formed. During gelation, the polymeric backbones can also interact with the surrounding tissues *via* physical or chemical interactions (*e.g.*, **Figs. 1 & 4**) and/or mechanical interlocking to increase the adhesion of the

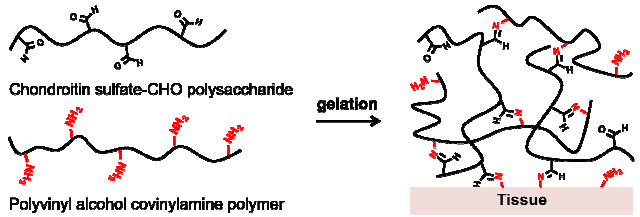


**Figure 4.** Hydrogel adhesive based on chondroitin sulfate-aldehyde (CS-CHO) polysaccharides cross-linking with polyvinyl alcohol covinylamine (PVA-A) polymers through Schiff base formation.[21] The network subsequently cross-links with proteins in the tissue (mainly through amine groups) resulting in strong adhesion between the gel and the host tissue.

hydrogel to the wound site (*e.g.*, **Fig. 16**).

Gelation with network formation by condensation/addition reactions has been widely used to develop polysaccharide, protein, poly(ethylene glycol), poly(glycerol), and dendrimer-based hydrogel adhesives for wound closure and repair, with good mechanical strength and adhesiveness.[4,5] Examples of these hydrogels and their *in vivo* applications are discussed in Section 6 of the tutorial. Nevertheless, the drawback of this technique is the limited type of reactive groups available on the cross-linkers (*e.g.*, aldehydes, activated esters), and some of these reagents (*e.g.*, formaldehyde and glutaraldehyde) have shown *in vivo* toxicity.[22]

Enzymatic cross-linking is an emerging method for the formation of covalent hydrogel adhesives (**Fig. 2B**). Horse radish peroxidase (HRP) mediated cross-linking of polysaccharide-tyramine polymers in the presence of hydrogen peroxide, is one of example based on this method.[23] Carbon atoms at the *ortho* position of phenol groups of polysaccharides react with each other or with the phenoxy oxygen *via* radical reactions and form C–C or C–O cross-linked networks. Transglutaminases have also been used as catalysts for the cross-linking of natural or synthetic polymers. One example is based on gelatin, where the network formation is triggered by the enzyme-mediated covalent cross-linking between □h□ γ–carboxamide groups of glutamine residues and□□□□ ε-amino groups of lysine residues of the gelatin backbone, and the hydrogel adhesive is formed under mild conditions.[24] The enzyme-mediated cross-linking reaction present several advantages such as rapid gelation time, use of mild aqueous reaction conditions (at neutral pH and moderate temperature), no side reactions due to the substrate specificity of the enzyme, tunable physicochemical properties, and low viscosity of precursors solutions, which facilitates their administration on the tissue site.[25] However, external enzyme sources can induce unexpected biological changes and foreign body reactions when used *in vivo,* and, thus, selection of the appropriate enzyme is critical.

## 4. How are polymeric hydrogel adhesives characterized?

Various chemical, physical and mechanical techniques have been developed to assess the properties of polymeric hydrogel adhesives. In this section, we report the most commonly used methods for the characterization of such materials. Nevertheless, other techniques such as contact angle measurements (wettability of a surface), X-ray diffraction (crystallinity), nuclear magnetic resonance spectroscopy (structure), and atomic force microscopy (surface topology) can be used along with the ones discussed below for further analysis of the material. Many of the following studies have published American Society for Testing and Materials (ASTM) methods for performing the experiment and analyzing the data, and reader is referred to their web site for details (http://www.astm.org).

***Gelation time.*** The gelation time is the period it takes for a gel to form. It can be determined either with a vial tilting method or with rheology as depicted in **Fig. 5**. The hydrogel precursors are mixed in a vial with stirring, and the time when no flow is observed after tilting the vial is recorded as the gelation time, $t_{gel}$ (**Fig. 5A**). The time required for the magnetic bar to stop stirring is another variation to measure the gelation time.

A second more quantitative method to measure the gelation time uses a rheometer. Hydrogel precursors are mixed in a precast Teflon mold and the kinetics of gelation are determined at certain frequency and stress values (**Fig. 5B**). At the beginning of the gelation, the solution is liquid-like and the gel is not yet formed ($t<t_{gel}$). With time, the solution becomes more viscous and solid-like, confirming the formation of the gel ($t>t_{gel}$). The time when the storage modulus (G') and the loss modulus (G") cross over corresponds to the gel point, $t_{gel}$. The kinetics of gelation also indicate the time at which the storage and loss moduli of the hydrogel become steady. The gelation time is an important parameter for wound closure. For example, if the hydrogel is used as a hemostat, the gelation time must be fast (preferably $t_{gel}<5$ sec) in order to instantly secure the wound and control the bleeding. However, it is also important that the hydrogel binds/adheres to the tissue in moist conditions and maintains its integrity even when it gels fast (*i.e.,* rapid gelation may lead to weaker hydrogels with low adhesive strength as not enough time is allowed for a strong network to form nor to spread and react with the tissue). Therefore, a balance between the gelation time and the mechanical and adhesive properties is required for each desired application.

***Gel fraction.*** The hydrogels are dried under vacuum at room temperature or lyophilized, until the dried weight is constant ($W_i$). The samples are then soaked in an excess of deionized water for 48 hours to rinse away all unreacted starting materials and by-products. Subsequently, the immersed hydrogels are removed from deionized water and dried under vacuum or lyophilized, until the dried weight is constant ($W_e$). The gel fraction of samples can be calculated as follows (**Eq. (1)**):

$$\text{Gel fraction (\%)} = (W_e/W_i) \times 100 \quad (1)$$

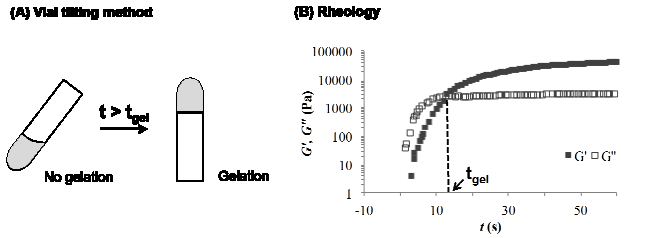


**Figure 5.** (A) Visual aspect of the material at $t<t_{gel}$ and $t>t_{gel}$. $t_{gel}$ is the gelation time; (B) Storage modulus (G') and loss modulus (G") of the hydrogel *vs.* time (*t*). The time where G' and G" cross over corresponds to the gel point, $t_{gel}$.

Where $W_i$ and $W_e$ are weights of lyophilized hydrogels before and after soaking in deionized water, respectively.

***Swelling degree.*** Hydrogels can absorb and retain large amounts of water depending on the cross-linking and charge densities of their network and the concentration of their cross-linked polymers. The determination of the absorbed quantity of water within the hydrogel is called swelling degree (SD) and is an important feature for their *in vivo* use as highly swollen hydrogels can break and dissolve before the wound is healed or damage the tissues in confined areas of the body. The swelling degree also influences the diffusion of molecules or drugs from the hydrogel. In general, hydrogels, which absorb and contain a large amount of water, exhibit high SD and diffusion rates of molecules. To determine the SD, hydrogels, once formed, are dried *via* heating or lyophilization, and then weighed ($W_d$). Subsequently, they are immersed in a sufficient amount of deionized water at room temperature, and allowed to reach equilibrium. At equilibrium, the hydrogels are filtered and weighed ($W_s$). Equilibrium is reached when the weight of hydrogels in the swollen state is steady. The equilibrium swelling degree (ESD) is thus calculated as follows (**Eq. (2)**):

$$ESD = (W_s - W_d)/W_d \quad (2)$$

Where $W_d$ and $W_s$ represent the weights of dry and swollen gels at equilibrium, respectively. The terms "swelling ratio", "water uptake", "water retention", and "fluid content" are also used in reference to swelling.

***Structural parameters of the hydrogel network.*** When designing hydrogel adhesives for wound closure, the cross-linking density ($\rho_x$), the polymer volume fraction in the swollen state ($v_{2,s}$), the molecular weight of the polymer chains between two neighboring cross-links ($M_c$) and the mesh size of the hydrogel ($\xi$) are important characteristics of the network, which have a significant influence on the material mechanical and diffusive properties once placed on a wound site. The most commonly used methods to determine these structural parameters are the equilibrium-swelling theory and the rubber-elasticity theory.[6] The former method is based on the hydrogel capacity of interacting with water and absorbing it, therefore, one can determine the structural parameters of the network from swelling degree measurements. The latter method relies on the elastic behavior of hydrogels, and their capacity to deform under stress and recover their initial dimensions once the stress is removed. The compressive or tensile measurements (*i.e.*, stress and strain values) are related to the structural parameters of the network, and allow their measurements. Different equations have been established in the literature for the calculation of these parameters, depending on the hydrogel structure (ionic *vs.* neutral) and the method applied to determine them.[6]

***Water vapor transmission rate.*** An ideal hydrogel adhesive for wound closure has to absorb wound exudates while preventing wound dehydration, and keep the wound moist for faster healing. The water vapor transmission rate (WVTR) is a measurement of the quantity of water vapor, which passes through a unit area of the material, during fixed time periods and specified temperature and humidity conditions, and is expressed in g/m$^2$.h as follows (**Eq. (3)**):

$$WVTR = G/t.A \quad (3)$$

Where G is the weight change in g, $t$ is the time in h, and A the tested area in m$^2$.[26]

For example, burn wound dressings with a WVTR of 80-100 g/m$^2$.h would retain the water in the wound and keep it hydrated for rapid healing. Typically, the hydrogel is placed on the mouth of a cylindrical plastic cup containing deionized water, and sealed across the edges to prevent any water vapor loss through the boundaries. The setup is kept at 37 °C under specified humidity conditions, and weighed at different time points. The weight loss versus time plot is then constructed and the WVTR is calculated by dividing the slope of the curve by the tested area, A.

***Scanning Electron Microscopy.*** Lyophilized hydrogel samples are examined by scanning electron microscopy (SEM) to study their morphology along with their internal structure and to determine their porosity. The porosity measurement is an important parameter for drug encapsulation and diffusion to the wound site. Environmental SEM is also being used to characterize the hydrogel state in the presence of water. Hydrogels with high porosities usually have low cross-linking densities and high swelling degrees and, therefore, are able to release the drug at faster rates.

***Fourier Transform Infrared spectroscopy.*** The chemical composition of freeze-dried polymeric hydrogels can be evaluated using Fourier Transform Infrared spectroscopy (FT-IR). This technique allows the visualization of absorption bands characteristic of functional groups within the polymer network, such as amide, ester, carboxylic acid, ether, hydroxyl, and amine groups. FT-IR can also be used to monitor the gelation by comparing the spectra of the starting materials to the hydrogel samples. For example, when photopolymerization is used to form hydrogels starting from vinyl monomers, the absence of the C=C absorption band from the hydrogel FT-IR spectrum indicates a complete polymerization of the starting materials.

***Mechanical properties.*** The mechanical strength and viscoelastic properties of polymeric hydrogels are important parameters for their use in wound closure. Ideally, hydrogels should have stiffness and flexibility comparable to the wound tissues in order to resist shear, tension or compression forces without breakage and to prevent the damage of the underlying tissues. Dynamic or oscillatory rheology is one of the common methods used to investigate the mechanical properties of hydrogels, and the underlying principle is to apply a sinusoidal

 

shear deformation in the material and measure the mechanical response to it. Typically, cylindrical hydrogel samples with defined geometries are prepared in precast Teflon molds and placed between two parallel plates of the rheometer. First, the storage (G') and loss (G") moduli are plotted as a function of the oscillatory stress or strain at a constant frequency, in order to determine the linear viscoelastic region (LVER) of the material. Afterwards, at a constant oscillatory stress or strain value within the LVER, the frequency sweep of the hydrogel is recorded. G' and G" describe the elastic (solid-like) and viscous (liquid-like) characteristics, respectively, of a hydrogel, at a given frequency range. For example, hydrogels exhibiting high G' are stiffer than those with low G'. The loss tan δ defined as G"/G' is another indicative of a hydrogel's behavior. Tan δ values of <1 indicate a more solid-like material, with δ defined as the phase angle ($0<\delta<\pi/2$). When stress is plotted as a function of strain, the slope of the linear region of the curve is the shear modulus G*. With dynamic rheology, one can also study the variation of the mechanical properties (G' and G") of the hydrogel upon heating, cooling, or exposure to various pH aqueous solutions. Similarly, the reversibility and the degradation of the material can be assessed by exposing the hydrogel to external stimuli and measuring the variation of its storage and loss moduli with time.

Another technique to evaluate the mechanical properties of an adhesive hydrogel is by determining the Young's modulus for compression, E, with compression tests using a universal testing machine (UTM). Hydrogel discs are compressed from 0 to x% strain ($x \leq 100$) or to failure, at a constant crosshead speed ($mm.min^{-1}$) and E is measured as the slope of the linear region of the stress-strain curve. Materials with high E values are stiffer than those with low E, and are usually characterized by high cross-linking densities.

Similarly, Young's modulus for tension, E, can also be measured by tension tests with UTM. However, using this method, the hydrogel discs are pulled at a constant crosshead speed, rather than compressed, and E is obtained as the slope of the linear portion of the stress-strain curve. Additionally, compressive or tensile strength (*i.e.,* the maximum stress that a material withstands before breaking) and elongation at break (*i.e.,* the maximum deformation that a material achieved before breaking) can be obtained from the strain-stress curves. Ideally, compressive or tensile moduli of adhesive hydrogels have to be comparable to those of the underlying and neighboring tissues in order to maintain their integrity and secure the wound until it is healed.

## 5. How to evaluate the performance of polymeric hydrogel adhesives for wound closure?

Prior to the application of hydrogel adhesives in animal models, one must first assess their performance *in vitro*. The following section focuses on the most relevant *in vitro* biocompatibility and biodegradability tests performed on hydrogels and their components to evaluate their potential effect on host cells. Additionally, this section discusses the required criteria for conducting *in vitro* adhesive and burst strength tests with these materials to determine their responses to shear, compression, or extension, and high pressures once the gel has adhered to the wound tissue. If the tests results are promising and conclusive, *in vivo* animal studies are warranted. These studies (as well as *in vivo* studies) should be performed in accordance with the guidelines set by the International Organization for Standardization (http://www.iso.org/, e.g., ISO-10993 biocompatibility studies).[27]

***Cytotoxicity evaluation.*** Hydrogels applied on a wound site can induce toxicity to cells, depending on their concentration, time of exposure, leachables/extracts, or degradation products. Therefore, the behaviour of the materials in the presence of live host cells is evaluated *in vitro* throughout the duration of their use. Materials that cause changes in cell morphology, adhesion or proliferation, leading to its death, are considered toxic. Various biochemical assays have been developed to assess the cytotoxicity of materials by measuring cell viability, such as MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), MTS (3-(4,5-dime-thylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium), Trypan blue, NRU (Neural red uptake), GSH (Glutathione), LDH (Lactic dehydrogenase) assays, among others.[28] Typically, hydrogels are directly or indirectly exposed to cells and incubated at 37 °C for 24/48 hours. In the latter case, cells are in contact with the hydrogel extracts. The cytotoxicity of hydrogels and their components is usually tested in a single cell line (*e.g.,* fibroblast NIH 3T3) and cell viability is quantified by light microscopy or spectrophotometry. For example, the MTT assay is based on the reduction of MTT by metabolically active cells to form an insoluble purple formazan derivative, which can be quantified at 570 nm by colorimetric spectrophotometry. The amount of formazan is directly proportional to the number of viable cells *via* a standard curve.

*In vitro* cytotoxicity methods are rapid, and allow the evaluation of the hydrogel cell toxicity. However, additional biocompatibility tests have to be conducted in order to better understand and assess how materials influence the cellular interactions, once implanted in a human body.

***Hemolysis evaluation.*** The hemolytic rate is another biocompatibility test, which determines the potential of the hydrogel to rupture red blood cells (RBC). It is important to note that when hydrogels are designed as hemostats, vascular sealants, or for any application where they are in contact with blood, this test is especially important. Experimentally, in the direct method, anticoagulated animal or human blood is exposed to hydrogels contained in phosphate buffer saline (PBS) solutions and incubated at 37 °C for one hour. Afterwards, the solutions are centrifuged to separate the supernatant from hydrogels, and the optical density (OD) of the supernatant is recorded at 545 nm in a spectrophotometer. For the negative control, blood is added to PBS without the hydrogel and incubated at 37 °C for one hour, while it was added into distilled water under similar conditions for the

positive control. In the indirect method, isotonic aqueous extracts from hydrogels are mixed with RBC suspensions and incubated at 37 °C for 24 hours, after which the optical density of the solution is measured, the isotonic aqueous solution being the negative control. The hemolysis percentage is calculated as follows (**Eq. (4)**):

$$\text{Hemolysis (\%)} = [(OD_{test}-OD_{negative})/(OD_{positive}-OD_{negative})] \times 100 \quad (4)$$

Where hemolysis ≤ 5% is considered hemocompatible.

***Platelet adhesion test.*** When hydrogels are in contact with blood, plasma proteins adsorb to their surfaces and can lead to subsequent platelet adhesion, activation and aggregation. This cascade of events results in a clot, which can obstruct the blood flow in healthy blood vessels and potentially cause serious health problems. Platelet adhesion tests are evaluated under static or dynamic conditions.[29] Briefly, under static conditions, discs of hydrogels are incubated with a specific volume of an adjusted platelet rich plasma (PRP) stock. After one hour of incubation, hydrogels are washed in PBS and stained for visualization and analysis under optical microscopy. Valuable characteristics of platelet-adhered materials can be obtained from this approach such as the platelets morphology and number per unit area. Additionally, the PBS-platelet suspension is also recovered and counted for platelets in a Coulter counter. The stock suspension of platelets is also counted. Platelet deposition on the hydrogel is estimated by quantifying depletion of platelets from the stock suspension as follows (**Eq. (5)**):

$$\text{Platelet adhesion (\%)} = 1-[(C_{stock}-C_{sample})/C_{stock}] \times 100 \quad (5)$$

Where $C_{stock}$ is the platelet count in the stock suspension (platelet #/μL), $C_{sample}$ is the platelet count measured in the stock suspension following incubation with the test.

On the other hand, in the dynamic condition, hydrogels are exposed to a circulating flow of PRP in a closed loop system. Blood samples are continuously filled in and retrieved out from the system allowing the quantification of the number of single circulating platelets. When platelets adhere to the hydrogels or circulate as aggregates, their number is decreased in the flow, whereas a constant number of single platelets circulating in the flow indicates that the cells did not adhere to the material or aggregated. By optical microscopy, one can also examine the morphology of adhered platelets and calculate their density. Additionally, other parameters involved in platelet activation process, such as markers expression, thrombin generation, and fibrin production, can be quantified and compared to a negative control (*i.e.,* silicone) to assess the hemocompatibility of hydrogels.[29]

***Macrophage activation.*** Macrophage activation assay is one of the immunotoxicity tests conducted on hydrogels to evaluate whether the material induces an immune response.[30] Experimentally, macrophage lines are exposed to hydrogels or to Lipopolysaccharide (LPS), a component of Gram-negative bacteria that elicits a strong immune response (positive control), for 24 hours. Media samples are taken and tested for a cytokine (*e.g.,* IL-1, IL-6 and TNF-α) secretion by macrophages, as a marker of macrophage activation. Cytokine levels are measured using an enzyme linked immunosorbent assay (ELISA), and samples are read using a microplate reader at specific wavelengths. A lack of cytokine presence in the hydrogel media samples demonstrates that the material does not induce an immune response.

***Hydrogel degradation.*** Degradation and degradation rate are also measured. Swollen hydrogel samples are weighed at equilibrium ($W_i$), prior to their incubation in PBS solutions at 37 °C with or without the presence of proteins, enzymes, or cells. At different time points, media are removed, the hydrogels weighed ($W_d$) and fresh media re-introduced. The weight loss percentage of the hydrogel is calculated as follows (**Eq. (6)**):

$$W_t\ (\%) = (W_i-W_d/W_i) \times 100 \quad (6)$$

Where $W_i$ and $W_d$ are the initial and degraded weights of the hydrogels, respectively.

Hydrogels can decompose in the presence of enzymes, which recognise specific functionalities on the hydrogel backbone and degrade it. Hydrogels can also decompose by cells or simply *via* hydrolysis at either acidic or basic pH conditions. The *in vitro* degradation tests can simulate the *in vivo* behaviour of hydrogels once exposed to such conditions. Hydrogel degradation studies are also important to ensure that the material degrades into safe components, which can be identified and further analyzed to assess their toxicity in cell hosts. Once these initial screening tests are completed, the *in vivo* biodegradability studies of the hydrogel are conducted to verify that its degradation products are either metabolized into harmless components or completely eliminated from the body *via* renal or hepatic pathways.

***Controlled drug release from hydrogels.*** Hydrogel adhesives can encapsulate drugs (*e.g.,* antimicrobial or anti-inflammatory agents) or growth factors within their polymeric network and deliver them to the wound site to minimize the risk of infection or expedite healing. *In vitro* drug release profiles in physiological media are important in order to understand how hydrogels behave under these conditions and how their swelling affects the rate of the drug release. For example, if hydrogel adhesives are designed as wound dressings for burns, they have to deliver the drug in a short amount of time to prevent infection (*i.e.,* in the first five hours). Therefore, the hydrogel structure is engineered to swell rapidly once in contact with the wound exudates. Tuning the hydrogel swelling properties for each application is important in order to alter the drug release profile and deliver the active molecules on site in the required amount of time. Experimentally, specific amounts of water-soluble drugs are mixed in the polymer solutions, and encapsulated within the network during the gelation process. Following, the hydrogels are placed in physiological media for

 

**Figure 6.** Schematic representation of the hydrogel adhesive tests according to the ASTM guidelines.[31] Reproduced with permission. Copyright © 2009, Elsevier.

various time periods. At each time point, the media is completely removed and then an equal amount of fresh media is replaced. Depending on the agent/drug composition, the quantification of the released amount in the media solution can be measured by atomic absorption (AA), ultraviolet-visible (UV-Vis) spectroscopy, fluorescence spectroscopy, and/or high-performance liquid chromatography (HPLC).

***Adhesion strength.*** When polymeric hydrogels are used as adhesives, hemostats, or sealants on soft tissues, adhesion tests are commonly performed according to the American Society for Testing and Materials (ASTM) methods, to assess their adhesion on animal tissues, as depicted in **Fig. 6**.[31] In general, two animal tissue sections (*e.g.*, porcine skins) are attached to rubber fixtures using cyanoacrylate glue, and the hydrogel is placed in between. A mild compressive force is applied in order to bind the hydrogel to the tissues. Afterwards, a tensile test is applied at a constant crosshead speed ($mm.min^{-1}$) to separate the sections (**Fig. 6**), and stress *vs.* strain is plotted until the separation of the hydrogel from the tissue is complete. The stress necessary to detach the hydrogel adhesive from the tissue surface is evaluated as the adhesive strength of the material. Hydrogels with high adhesive strength bind strongly to tissues. However, it is important that hydrogels remain flexible while maintaining their strong tissue adhesion in order to avoid breaking or cracking. Moreover, depending on the desired application, hydrogels may require rapid and painless removal for re-exposure of the wound and further treatment (*e.g.,* wound dressings for burns). Therefore, hydrogels exhibiting both high tissue adhesion and easy removal are needed for wound closure.

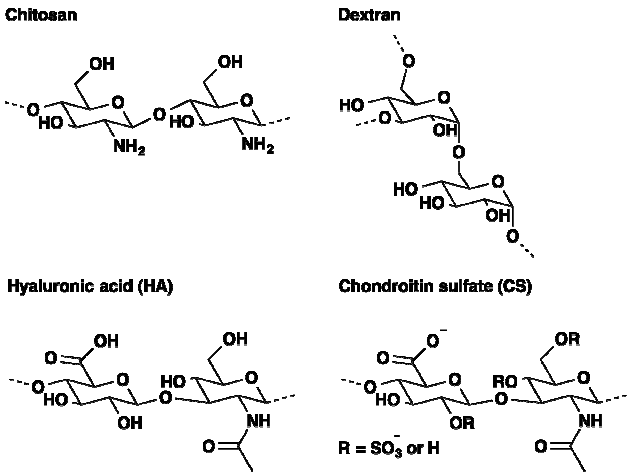


**Figure 8.** Commonly used polysaccharides in hydrogel tissue adhesives.

***Burst pressure measurement.*** The burst pressure is the maximum pressure that hydrogel adhesives can withstand before breaking with fluid leakage. When hydrogels are used as adhesives, hemostats or sealants, they are often subjected to significant pressures from underlying tissues or biological fluids. Therefore, the measurement of the burst pressure in an *ex vivo* setting allows to assess the capacity of materials to seal the incisions when exposed to such conditions *in vivo*. For example, hydrogel adhesives designed as arterial vascular sealants have to withstand pressures of about 200 mmHg before bursting, while the required burst pressure limit for hydrogels used as sealants for corneal incisions is 67 mmHg.[32,33] One example of a burst pressure-testing system is composed of a reservoir filled with fluids (*e.g.*, water, buffer solutions, biological fluids) and covered with a biological tissue (*e.g.*, artery, skin), which is tightly attached to the reservoir to avoid leaks, as depicted in **Fig. 7**. The reservoir is connected to pressure sensors that allow the monitoring and recording of the pressure in the system. Prior to the application of the gel, the pressure is increased in the system to ensure that it is leak-proof and that it could withstand pressures of ca. 250 mmHg, which is significantly greater than biological pressures (*e.g.*, normal arterial blood pressure in humans is around 120/80 mmHg). Next, incisions with different diameters are made on the tissue surface, and the pressure drops to zero. Once the hydrogel is applied on the puncture site, the pressure is increased again in the system until the hydrogel bursts and the incision leaks. The maximum pressure reached before the burst of the gel is recorded as the burst pressure. Different variations of this system have been reported for the burst pressure measurements of hydrogel tissue adhesives, which rely on similar principles as the one described herein.[7,34,35]

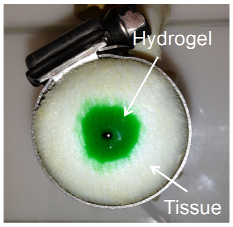


**Figure 7.** Example of a burst pressure-testing system.

## 6. Examples of polymeric hydrogel adhesives for wound closure

This section highlights specific examples of the most relevant polymeric hydrogel adhesives designed for the closure of wounds, which are composed of polysaccharide, protein and

synthetic polymers. Polysaccharide-based hydrogels are known to be biocompatible and biodegradable due to the presence of natural sugar monomers within their polymeric structures. Some of them have shown antimicrobial properties, which make them ideal materials for the treatment of wounds. Examples of these hydrogel adhesives include chitosan, dextran, hyaluronic acid, and chondroitin sulfate polymers (**Fig. 8**).[4,5] Protein-based hydrogels are another promising class of tissue adhesives due to their resemblance to natural tissues in humans. This class includes gelatin, albumin, and fibrin hydrogels and these materials have been widely used as sealants, hemostats, and adhesives in the treatment of wounds.[4,5] Synthetic polymeric hydrogel adhesives are mainly based on poly(ethylene glycol) (PEG) backbones used solely or in combination with other polymers or dendrimers.[4,5] PEG-based materials have often been developed for various biomedical applications, due to their solubility in water, biocompatibility and *in vivo* elimination routes after administration. Furthermore, their hydrophilic character

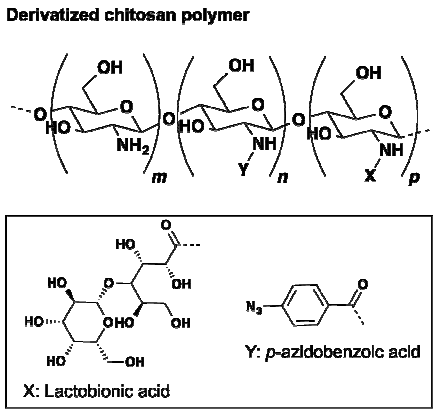


**Figure 9.** Chitosan polymer functionalized with lactobionic acid and azide moieties.[36]

reduces protein adhesion on their surfaces and recognition by the immune system.[5] Additionally to PEGs, other polyesters, polyurethanes, dendrimers and biomimetic polymers have also been used for the development of hydrogel adhesives, and many literature reviews summarize the state of the art of these adhesives and describe their advantages and disadvantages.[3-5] Therefore, in this section, we will not describe all the existing hydrogel adhesives but highlight a few examples from each polymer class, and guide the reader step by step on how the hydrogel adhesive was designed and developed and its properties evaluated to meet the requirements needed for its desired clinical application.

***Polysaccharide-based hydrogel adhesives.*** Chitosan is a linear β-(1,4)-linked polymer, composed of *D*-glucosamine and *N*-acetyl-*D*-glucosamine monomers, and derived from the partial deacetylation of chitin. Chitosan-based materials have been widely used for biomedical applications such as wound healing, tissue engineering, drug and cell delivery due to their biocompatibility, biodegradability, antimicrobial and hemostatic properties and capacity to stimulate cell proliferation.[14] In 2000, Ono *et al.* described a chitosan-based hydrogel adhesive, where chitosan polymers of 800-1000 kDa molecular weight (MW) and 80% of deacetylation were functionalized with lactobionic acid and azide moieties (**Fig. 9**).[36] The introduction of 2% of lactose groups allowed the increase of the chitosan solubility in water, at physiological pH. The cross-linking was achieved *via* the photo-mediated reduction of azides to nitrenes and their subsequent binding to amino groups of the chitosan backbones forming nitro-bridges. The hydrogel was formed in 10-60 seconds depending of the UV-light intensity. The *in vitro* binding and sealing strengths of the material were measured and compared with the commercial fibrin glue sealant commonly used in surgical procedures. The chitosan hydrogel adhesion increased with the increasing

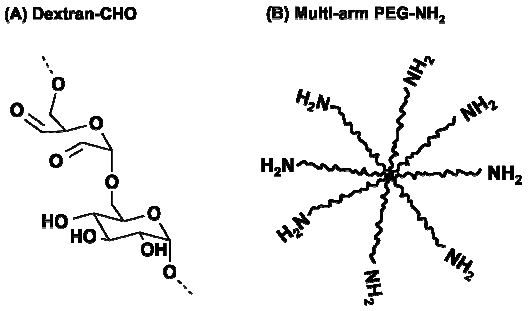


**Figure 10.** Example of a dextran-based adhesive formed from the cross-linking of (A) dextran-aldehyde and (B) multi-arm PEG-amine.[38]

concentration of the polymer, and was comparable with fibrin glue (43 g/cm$^2$ *vs.* 40 g/cm$^2$ at 50 mg/mL chitosan concentration). The hydrogel adheres strongly to the wound site *via* the interaction of nitrene groups of the polymer with proteins present in the host tissue surface. Similarly, chitosan photocrosslinked hydrogels sealed more effectively incisions made on isolated small intestine, thoracic aorta, lung and trachea than fibrin glue, and prevented air leakage with bursting pressures of 65±5 mmHg, 225±25 mmHg, 51±11 mmHg, and 77±29 mmHg, respectively. *In vitro* and *in vivo* cytotoxicity tests showed that the hydrogels and their components were not toxic to human cells and were completely degraded after one month of sub-cutaneous implantation in mice. Furthermore, the gels enhanced wound contractions, granulation tissue formation and epithelialization in an *in vivo* wound healing mouse model. The adhesive was also able to stop the bleeding faster than fibrin glue (1 min *vs.* 3 min), and prevented air leakage in an *in vivo* arterial and lung rabbit model injuries, respectively.[36]

Dextran, a branched polymer composed of α-(1,6) and α-(1,3)-*D*-glucopyranose linkages, is another widely used polysaccharide for hydrogel tissue adhesives. Kodokian *et al.* reported a dextran-based hydrogel adhesive in 2006, formed from the cross-linking of dextran-aldehyde (**Fig. 10A**) with a multi-arm polyether amine polymer (**Fig. 10B**) *via* the

 

generation of imine linkages within the network.[37] Different degrees of oxidation (*i.e.*, dialdehyde contents) were obtained upon reaction of dextrans with periodate solutions, which influenced the mechanical and adhesive properties of the materials. Hydrogels were formed in less than 30 seconds and *in vitro* burst-pressure measurements were evaluated with incisions made on different biological tissues (*e.g.*, swine uterine horn and rabbit eye) and compared to the commercial fibrin glue adhesive. Higher dextran oxidation conversions (<60%) and polymer concentrations (10-30 wt%, MW 10 kDa) generally increased the burst-pressures of the hydrogels (135-150 mmHg), which in all cases, were significantly higher than the one obtained with fibrin glue (25 mmHg), when applied on swine uterine horn incisions at equal added volumes. The hydrogels did not show any *in vivo* toxicity or observable inflammation when applied in small intestine of living rabbits and adhered well to the tissues.[37] The efficacy of the dextran-based hydrogel (50% oxidation) for sealing corneal incisions was assessed in *ex vivo* enucleated rabbit eyes.[38] The gel was able to prevent leakage from the wound up to 500 mmHg of pressure, and completely degraded after 3 days, *in vitro*, *via* the

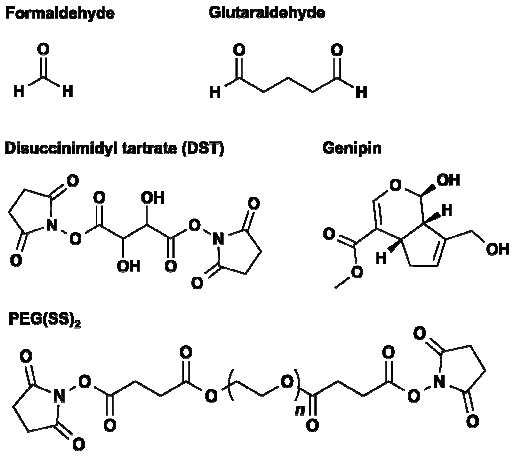


**Figure 11.** Examples of cross-linkers used in protein-based hydrogel adhesives.

hydrolysis of the reversible imine groups within the hydrogel network.

Polysaccharide-based hydrogel adhesives are biocompatible, biodegradable, and induce minimal immune responses once applied to the wound site. However, they can exhibit low solubility in aqueous solutions (*e.g.*, chitosan) and thus require further functionalization steps to introduce water-soluble moieties on their backbones. Furthermore, when cross-linked *via* imine groups, their hydrolysis is fast and their adhesion reduced before the wound is healed, which can limit their use where long adhesion times are required on the wound site.

***Protein-based hydrogel adhesives.*** The synthesis of gelatin-based hydrogel adhesives was first reported in 1966.[39,40] Gelatin is obtained by hydrolysis of collagen extracted from animals, and hydrogel formulations are mainly composed of gelatin-resorcinol solutions with formaldehyde and/or glutaraldehyde as cross-linkers (**Fig. 11**). The network is formed *via* the covalent cross-linking of lysine amino groups present in gelatin with formaldehyde or glutaraldehyde cross-linkers, and the additional electrophilic aromatic substitution reaction of resorcinol residues with aldehyde moieties. The efficacy of gelatin-resorcinol-formaldehyde-glutaraldehyde (GRFG) adhesive in sealing intestinal incisions and controlling spleen hemorrhage was assessed in *in vivo* dog model injuries.[41] All incisions sealed with GRFG adhesives healed completely and no leakage was observed. The gels were also able to control the bleeding from the spleen and their adhesion persisted for 4 months as compared to 2-3 months when applied on intestine. Furthermore, the GRFG adhesive was tested for sealing pulmonary air leaks during thoracoscopic operations in humans, and successfully stopped air leakage in patients.[42] E*x vivo* burst pressures recorded in swine lung wounds were significantly higher than fibrin glue (88.2±5.5 *vs.* 19.2±3.1 cm $H_2O$, respectively). The hydrogel applied on the wound site slowly degraded after 56 days *via* macrophage phagocytosis, however 80% of the material remained on the tissue after 188 days.[42] A major downside of commonly used gelatin-based hydrogel adhesives is the histotoxicity of formaldehyde and glutaraldehyde cross-linkers. Thus, hydrogels with alternative non-toxic cross-linkers such as genipin,[43] and disuccinimidyl tartrate (DST),[44] have been successfully developed and tested as tissue adhesives (**Fig. 11**).

Albumin is the main protein of human blood plasma, and has been commonly used to develop tissue hydrogel adhesives. Similarly to gelatin, the protein is cross-linked with glutaraldehyde to form the network and subsequent tissue binding *via* the interaction of aldehydes with the proteins in the host tissue ensures the adhesion of the material to the wound site. BioGlue®, made of bovine albumin and glutaraldehyde, is a FDA-approved surgical adhesive used in the repair of acute thoracic aortic dissection and as an adjunct to sutures and staples for sealing leaks in large blood vessels.[45] The hydrogel is formed in 20-30 seconds with a burst pressure of 300 mmHg when sealing *ex vivo* vessels of bovine hearts. Similarly to GRFG adhesives, albumin-glutaraldehyde adhesives have shown *in vivo* toxicity due to the aldehyde cross-linkers. ProGel™ is another FDA-approved albumin-based hydrogel adhesive where glutaraldehyde has been replaced with a non toxic synthetic poly(ethylene glycol) disuccinimidyl succinate (PEG-$(SS)_2$; **Fig. 11**).[46] Human serum albumin is used in this formulation and upon its cross-linking with PEG-$(SS)_2$, the hydrogel is formed within 15 seconds *via* the formation of amide linkages. The gel was successfully used as an adjunct for sealing air leaks resulting from surgical lung resection, and exhibited burst pressures significantly higher than fibrin glue (77.5±19.1 *vs.* 30.8±15.2 mmHg), when applied on incisions in a rat lung surgical model.[47] The hydrogel was biocompatible and degraded rapidly within 7 days, with renal clearance being the main elimination pathway of the material.

Protein-based hydrogel adhesives can provoke allergic reactions or contain infectious diseases when the protein is

extracted from animal or human sources, respectively. Furthermore, formaldehyde and glutaraldehyde remain the typical cross-linkers used in these materials, even though they have shown cytotoxic effects. A third class of hydrogel adhesives based on synthetic polymers has emerged to circumvent the downsides of using natural polymers. These materials are biocompatible and biodegradable, and can exhibit tunable mechanical and adhesive properties depending on the choice of their structures, cross-linkers and gelation process.

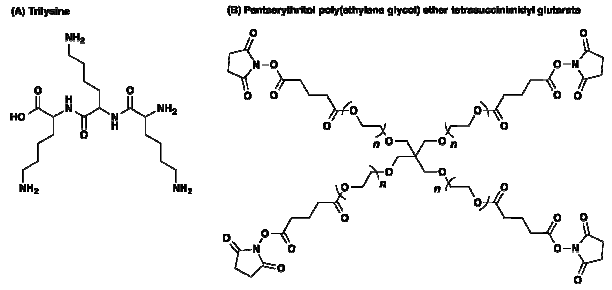


**Figure 12.** Chemical structures of (A) trilysine and (B) NHS-PEG cross-linker, precursors of DuraSeal™ hydrogel adhesive.

***Synthetic polymer-based hydrogel adhesives.*** Poly(ethylene glycol) (PEG) is one of the major classes of synthetic polymers developed for hydrogel tissue adhesives.[4,5] One of the first FDA-approved PEG-based hydrogel adhesives was DuraSeal™, indicated as an adjunct to sutured dural repair in cranial surgeries, to ensure watertight closure.[48] The hydrogel formulation consists of two aqueous solutions, a trilysine amine solution (**Fig. 12A**) and a multi-armed NHS-PEG solution (**Fig. 12B**), which upon mixing, form the hydrogel network in 3 seconds *via* a nucleophilic addition/elimination reaction and a subsequent amide linkage formation. FD&C Blue no. 1 dye is added to the system to facilitate the visualization of the hydrogel after application onto the host tissue. The hydrogel is delivered by a dual syringe applicator onto the dura mater, a thick membrane that surrounds the brain, to prevent cerebrospinal fluid (CSF) leaks, which can occur during cranial surgeries and cause serious health complications. Biocompatibility tests show that the hydrogel is non-cytotoxic, non-irritant, non-hemolytic and non-mutagenic. *In vivo* studies on patients undergoing cranial surgeries demonstrated 100% effectiveness of the system in sealing the dura and preventing CSF leaks where sutures alone were not efficient, and the wounds healed well after 3 months, postoperatively.[49] Furthermore, the hydrogel adheres strongly to the host tissue through covalent bonding between the amine groups of the surrounding proteins in tissues and the activated esters of the polymer backbones, and is able to withstand intracranial CSF pressure (15 mmHg) without any leakage. Finally, the synthetic hydrogel absorbs within 4 to 8 weeks mainly *via* hydrolysis of the ester groups within the polymer structure, and the generated PEG chains and amino acids are subsequently cleared from the body by the kidneys. The only contraindication when using DuraSeal™ is its high swelling capacity (50% after application), which restricts its use in confined spaces of the body where nerve and vessel compressions can occur.[48]

Dendrimer-based hydrogel adhesives have recently emerged as an interesting class of synthetic polymers for the treatment of wounds. Dendrimers are monodisperse macromolecules that consist of a core, branching segments (*i.e.*, generations, $G_n$) and a well-define number of peripheral groups. Dendritic macromers can be synthesized and subsequently cross-linked using light or chemical cross-linking reactions to form highly cross-linked hydrogels at low polymer concentrations. These macromers possess advantages over linear polymers such as the control of their size, architecture, density and functional end-groups, and can be easily tailored for specific applications. The first dendritic hydrogel sealant was reported in 2002 for the repair of full-thickness corneal lacerations, and is composed of biodegradable poly(glycerol-succinic acid)-poly(ethylene glycol) hybrid dendritic-linear copolymers, ([G1]PGLSA-MA)$_2$-PEG, as depicted in **Fig. 13**.[50] The glycerol hydroxyl

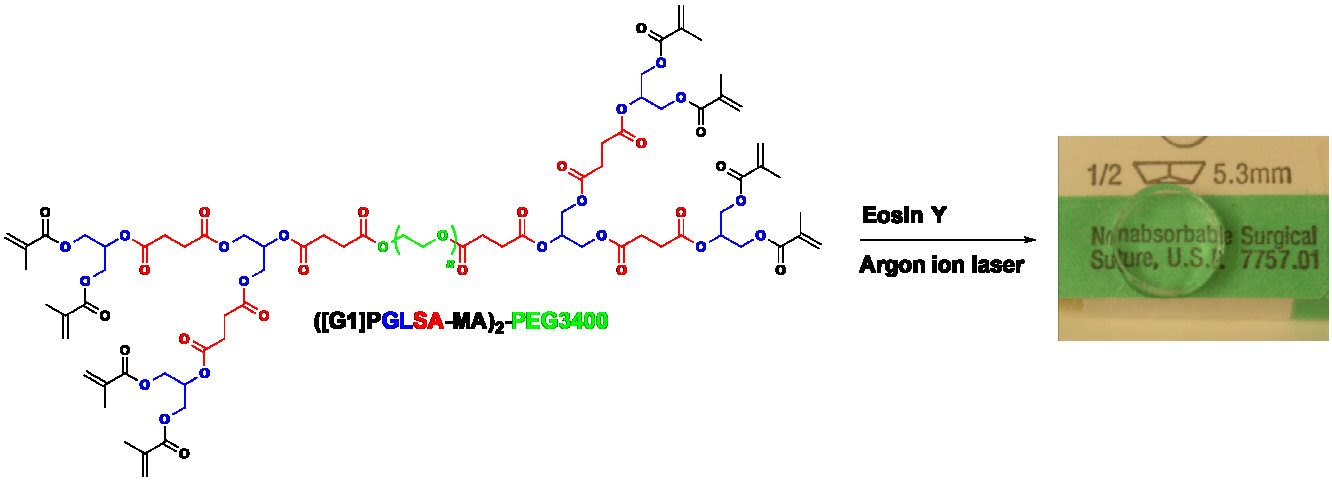


**Figure 13.** Chemical structure of dendritic-linear copolymers, ([$G_1$]PGLSA-MA)$_2$-PEG, and formation of the hydrogel adhesive by photopolymerization.[50] Glycerol moieties (GL) are represented in blue, salicylates (SA) in red, poly(ethylene glycols) (PEG) in green, and methacrylates (MA) in black.

groups on the periphery of the dendritic macromolecule are further derivatized with methacrylate groups, which upon photopolymerization, cross-link and form the hydrogel network in 20-30 seconds. The adhesion of the material to the host tissue is due to the formation of an interpenetrating network between the gel and the proteins of the surrounding extracellular matrix (ECM). The efficacy of the adhesive in sealing *ex vivo* corneal incisions of enucleated human eyes was assessed and compared to a control group where the eyes were repaired with nylon sutures. Different generations of the dendritic macromolecule (*e.g.*, $([G_0\text{-}G_3]PGLSA\text{-}MA)_2$-PEG) were tested as potential sealants for corneal lacerations and $([G_1]PGLSA\text{-}MA)_2$-PEG was the most efficient among them in ensuring a leak-tight seal of the wound. When linear incisions were made on the eye globes, mean leaking pressures of 110 mmHg and 79 mmHg were obtained with hydrogel and suture groups, respectively. Additionally, the hydrogel was reported to efficiently secure and seal laser *in situ* keratomileusis (LASIK) flaps created on human eyes *ex vivo*.[50] *In vivo* studies in a chicken model were initiated afterwards, in order to evaluate the healing response of corneal lacerations treated with the dendritic hydrogel. Therefore, 4-mm full thickness corneal wounds were made on chicken eyes onto which the dendritic hydrogel or sutures were applied, and the healing process was monitored for 28 days. The bioadhesive did not cause any evident *in vivo* toxicity, and efficiently sealed 97% of the incisions by post-operative day 1. On day 2, no leaks occurred in all wounds. Histological examinations showed that incisions sealed with the adhesive were more complete that the ones treated with sutures, by post-operative day 28, and presented a more uniform interface between epithelial and stromal layers. Finally, the hydrogel completely disappeared from the wound site by day 14, and although the degradation profile was not reported, the material is mostly composed of natural metabolites, mainly succinic acid and glycerol, as well as PEG chains, which are known to be safe and biodegradable.

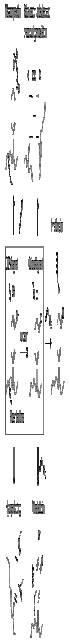

**Figure 14.** Possible adhesion and cross-linking pathways of MAPs.[51]

Recently, there has been increasing interest in the design and synthesis of naturally inspired tissue adhesives. Organisms living in intertidal regions are able to strongly adhere to underwater surfaces to circumvent strong currents, tides, and waves. For example, mussels attach to wet surfaces by producing strong and elastic byssus threads, which are composed of silky fibers made from adhesive proteins. Extraction of these proteins from mussels and their further analysis confirmed the high level of 3,4-dihydroxyphenyl-L-alanine (DOPA) fragments, which are responsible for the adhesion and cross-linking of these adhesives.[51] Although the mechanism of adhesion and curing is not yet fully understood, it has been proposed that adsorption of DOPA moieties to hydrophilic surfaces can be achieved *via* hydrogen bonding while their oxidation to quinones and their subsequent reactions with other quinones, amines, or thiols and/or complexation to ferric ions (Fe(III)), can lead to the formation of a cross-linked adhesive network (**Fig. 14**).[51] The synthesis of hydrogels that gel and stick strongly to the wound site in the presence of biological fluids remain a challenge in the development of tissue adhesives. Hence, synthetic biomimetic hydrogel adhesives offer an interesting alternative to circumvent the poor adhesion often occurring with these materials in wet conditions. While many animals and organisms such as gecko, algae, barnacles, starfish and tubeworms have been studied for potential sources of biomimetic adhesives, marine mussels remain the most explored ones.[4,5] However, the natural mussel adhesive protein (MAP) extraction and large scale production are still difficult and inefficient, often resulting in low yields despite the tremendous work conducted for their potential commercialization. As an alternative, researchers have focused their strategies on the development of synthetic bioinspired polymers (*e.g.*, copolypeptides, polystyrene and PEGs) onto which they incorporated DOPA derivatives to mimic the adhesion mechanism observed in mussels.[4,5] The synthesis of PEG-DOPA hydrogel bioadhesives is one example.[52] The gelation strategy is based on the use of sodium periodate ($NaIO_4$), as the oxidizing agent, encapsulated in phospholipid liposomes. Upon heating at 37 °C, the liposomes disassemble

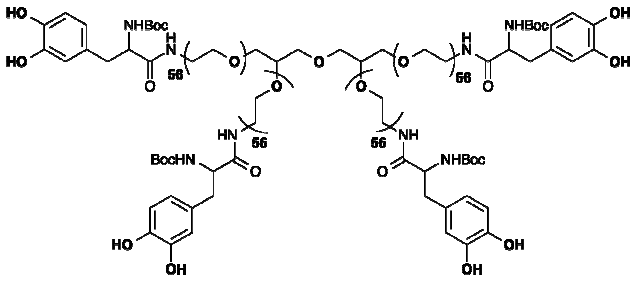


**Figure 15.** Chemical structure of PEG-$DOPA_4$ polymer.[52]

and release $NaIO_4$, which oxidizes the water-soluble PEG-

$DOPA_4$ polymers, and forms the adhesive network (**Fig. 15**). After curing for 24 hours at 37 °C, the adhesive strength of the hydrogels was evaluated on *ex vivo* porcine dermal skin surfaces, using lap-shear tensile tests, and compared to a commercial fibrin glue sealant. The gels were 5 times stronger than fibrin, with a shear strength of 35 kPa. This strong adhesion is likely due to the binding of reactive quinones generated after the oxidation of DOPA, with amines and thiols of the ECM proteins of host tissues, *via* Michael addition and/or imine formation, as depicted in **Fig. 14**. *In vivo* adhesive studies of PEG-DOHA hydrogel (DOHA: 3,4-dihydroxyhydrocinnamic acid), an analog of PEG-DOPA, were further assessed in mice for islet immobilization on extrahepatic transplant sites.[53] Histological examinations at different time points showed that the material induce minimal inflammation although toxic sodium periodate was used for the oxidation of catechol moieties. Furthermore, the hydrogel did not affect the morphology of the islets, and was still present on the tissue at one-year post-implantation, thus one can potentially use it for applications where long adhesion times are required.

Yang *et al.* have recently reported the design of a bioinspired microneedle (MN) tissue adhesive composed of a biphasic structure: an inner hydrophobic polystyrene (PS) layer and an outer amphiphilic polystyrene-*block*-poly(acrylic acid) (PS-*b*-PAA) swellable tip (**Fig. 16**).[54] The PS inner core exhibits mechanical strength whereas the outer tip swells in contact with water due to the hydrophilic poly(acrylic acid) component, thus ensuring its adhesion to tissues such as skin or intestine tissue *via* mechanical interlocking (**Fig. 16**). The MN was tested for use in skin graft fixation on muscle tissue and exhibited higher adhesion strength than stapled fixed skin grafts and non-fixed skin grafts (0.93±0.23 $N/cm^2$, 0.28±0.11 $N/cm^2$ and 0.22±0.09 $N/cm^2$, respectively). Furthermore, the MN acted as a barrier to bacterial infection due to the tight seal ensured by the interlocked swollen tip.[54] The MN was also tested as a potential sealant for tissue intestine wound closure. The adhesion was evaluated on the outer smooth serosal surface and the inner wrinkled mucosal surface that is covered by a mucin layer. The material exhibited an adhesion strength of 1.62±0.17 $N/cm^2$ to the smooth surface as compared to 3.83±1.35 $N/cm^2$ to the wrinkled surface, which was explained by the non-covalent interactions with mucin.[54]

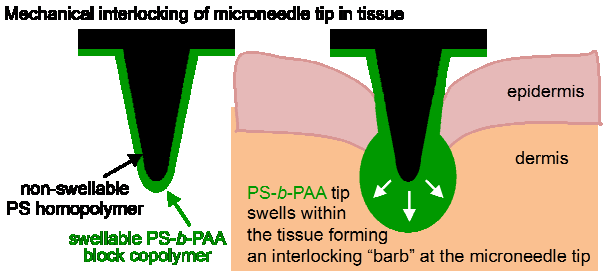


**Figure 16.** Biphasic structure of a bioinspired microneedle adhesive and its adhesion to the tissue *via* mechanical interlocking.[54]

## 7. Where are we today?

Over the past decade, hydrogel adhesives based on natural and synthetic polymers have garnered interest as promising materials for the treatment of wounds with several hydrogel compositions already approved for clinical use. Hydrogels are viscoelastic and are mechanically similar to tissues in the body. They can be easily engineered to exhibit the desired gelation time, mechanical and sealing properties, biocompatibility and biodegradability for the treatment of the wound of interest. Due to their high water content, they provide a moist environment for the wound and promote an efficient healing process. However, they generally exhibit weaker mechanical properties than common tissue adhesives such as cyanoacrylates, which can be a critical drawback for their use as external wound closure materials. Recently, the development of nanocomposite hydrogels has attracted significant interest due to the superior properties and customized functionality.[55] These hybrid hydrogels are either physically or covalently crosslinked with nanoparticles, which reinforces the hydrogel network resulting in greater mechanical strength than standard hydrogels.

Designing adhesives based on polymeric hydrogels affords additional advantages, as hydrogels are extensively used for other applications such as in the fields of drug delivery and tissue engineering. Thus, one can consider to: 1) deliver small molecule agents or drugs to the wound site to prevent infection; 2) deliver proteins, growth factors, or other biomacromolecules to promote wound healing; or 3) include specific bioactive species within the hydrogel to increase cell specific adhesion and proliferation mimicking the function of the extracellular matrix. These added features represent opportunities for new designs and hydrogel compositions. In fact, the boundaries between a traditional adhesive and a bioactive-scaffold adhesive of the future will become blurred, as materials offer more than simply to close the wound.

Several natural and synthetic hydrogels such as BioGlue®, DuraSeal™, CoSeal™, and Adherus™ are being used today as adhesives, hemostats, and/or sealants in the clinic. The synthetic hydrogels, compared to those based on natural polymers ones, are expanding the repertoire of uses for these materials in wound repair and management. They often afford better mechanical and occlusive control, reproducibility, opportunity to include active agents, and the possibility to better analyze the structure-activity relationship of the material with respect to performance. Moreover, with continued advances in the development of new synthetic materials and approaches to wound repair, specific tissue adhesive properties and features are being realized. For example, researchers describe hydrogel adhesives with improved adhesion properties in dry/wet conditions by mimicking the underlying chemistry that occurs in marine organisms, which stick to surfaces and resist tides, waves, and currents.[4,5] This bio-inspired approach is particularly elegant. An example of a recently developed feature is the on-demand dissolution of a hydrogel sealant that is applied during emergent care, then subsequently dissolved to allow for controlled gradual wound re-exposure during

definitive surgical care at a later time. The dissolution occurs *via* a thiol-thioester exchange reaction between an added thiol solution and the thioesters present within the hydrogel adhesive.[7]

Where are we today? Significant opportunities still exist for hydrogel adhesives, sealants, and hemostats, and this research area is rapidly evolving. Given the demands, and sometimes, conflicting requirements for a hydrogel adhesive for a specific application, a universal adhesive for all applications is likely to be unattainable. Thus, specific tissue adhesives are and will become the vogue - providing motivation to synthesize new chemical structures, explore new modes of cross-linking, optimize the physical and mechanical properties, and investigate new mechanisms of tissue adhesion. A critical part of this discovery and translational pathway is the identification of the unmet clinical need. We do not need yet another hydrogel adhesive. To address those needs, my group meets routinely with clinicians to discuss recent trauma cases, current standards of care, and optimal solutions. We encourage all who are working in this area to interact and communicate often with clinicians before and during the design, development, and evaluation phases of a new hydrogel adhesive, sealant, or hemostat in order to understand the requirements and performance specifications needed for each intended application.

The commercial successes of several adhesives (both natural and synthetic polymer-based) demonstrate that basic studies in this arena translate to improved patient care, and support ongoing funding of this area from governmental agencies, foundations, and industry. In summary, continued collaborative interdisciplinary efforts will afford new materials, devices, and approaches for wound management and repair.

The use of polymeric hydrogels as wound adhesives, sealants, and hemostats is summarized in this *tutorial review* along with their design requirements, key chemical features, synthetic routes, determination of properties, and tests needed to evaluate their performances. This *tutorial review* is intended to provide a foundation for those interested in this field, stimulate critical discussions, and kindle interest.

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## Notes and references

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1 J. D. Lloyd, M. J. Marque III, R. F. Kacprowicz, *Emerg. Med. Clin. N. Am.*, 2007, **25**, 73-81.
2 J. V. Quinn, *Tissue adhesives in clinical medicine*. 2nd ed. Hamilton, Ontario: Decker Inc., 2005.
3 W. D. Spotnitz, S. Burks, *Transfusion (Paris)*, 2008, **48**, 1502-1516.
4 A. P. Duarte, J. F. Coelho, J. C. Bordado, M. T. Cidade, M. H. Gil, *Prog. Polym. Sci.*, 2012, **37**, 1031-1050, and references therein.
5 P. J. M. Bouten, M. Zonjee, J. Bender, S. T. K. Yauw, H. van Goor, J. C. M. van Hest, R. Hoogenboom, *Prog. Polym. Sci.*, 2014, **39**, 1375-1405, and references therein.
6 N. A. Peppas, J. Z. Hilt, A. Khademhosseini, R. Langer, *Adv. Mater.*, 2006, **18**, 1345-1360, and references therein.
7 C. Ghobril, K. Charoen, E. K. Rodriguez, A. Nazarian, M. W. Grinstaff, *Angew. Chem. Int. Ed.*, 2013, **52**, 14070-14074.
8 A. S. Hoffman, *Adv. Drug Deliv. Rev.*, 2012, **64**, 18-23, and references therein.
9 J. L. West, J. A. Hubbell, *Biomaterials*, 1995, **16**, 1153-1156.
10 M. Kimura, M. Takai, K. Ishihara, *J. Biomater. Sci. Polymer Edn.*, 2007, **18**, 623-640.
11 E. A. Kamoun, X. Chen, M. S. Mohy Eldin, E. S. Kenawy, *Arabian J. Chem.*, 2014, DOI: 10.1016/j.arabjc.2014.07.005, and references therein.
12 Qin, Y. *Polym. Adv. Technol.*, 2008, **19**, 6-14.
13 K. Y. Lee, D. J. Mooney, *Prog. Polym. Sci.*, 2012, **37**, 106-126.
14 R. Jayakumar, M. Prabaharan, P. T. S. Kumar, S. V. Nair, H. Tamura, *Biotechnol. Adv.*, 2011, **29**, 322-337.
15. J. G. Rouse, M. E. Van Dyke, *Materials*, 2010, **3**, 999-1014, and references therein.
16 R. Bitton, H. Bianco-Peled, *Macromol. Biosci.*, 2008, **8**, 393-400.
17 B. Balakrishnan, N. Joshi, A. Jayakrishnan, R. Banerjee, *Acta Biomater.,* 2014, **10**, 3650-3663.
18 F.-J. Kao, G. Manivannan, S. P. Sawan, *J. Biomed. Mater. Res. Part B : Appl. Biomater.,* 1997, **38**, 191-196.
19 J. M. Rosiak, F. Yoshii, *Nucl. Instr. and Meth. Phys. Res. B.*, 1999, **151**, 56-64.
20 B. Baroli, *J. Chem. Technol. Biotechnol.*, 2006, **81**, 491-499, and references therein.
21 J. M. G. Reyes, S. Herretes, A. Pirouzmanesh, D.-A. Wang, J. H. Elisseeff, A. Jun, P. J. McDonnell, R. S. Chuck, A. Behrens, *Invest. Ophtalmol. Vis. Sci.,* 2005, **46**, 1247-1250.
22 S. K. Bhatia, *Biomaterials for clinical applications*, New York (NY): Springer, 2010.
23 E. Lih, J. S. Lee, K. M. Park, K. D. Park, *Acta Biomater.*, 2012, **8**, 3261-3269.
24 M. K. McDermott, T. Chen, C. M. Williams, K. M. Markley, G. F. Payne, *Biomacromolecules*, 2004, **5**, 1270-1279.
25 L. S. Moreira Teixeira, J. Feijen, C. A. van Blitterswijk, P. J. Dijkstra, M. Karperien, *Biomaterials*, 2012, **33**, 1281-1290.
26 ASTM Standard E96-00. Standard test methods for water vapor transmission of materials. Annual book of ASTM standards, vol. 4.06. Philadelphia: ASTM; 2000.
27http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM348890.pdf and references therein
28 T. J. Webster, D. A. Stout, S. Kalmodia, B. Basu, *Nano Life*, 2013, **3**, 1340008(1)-1340008(21).
29 F. Jung, S. Braune, A. Lendlein, *Clin. Hemorheol. Micro.*, 2013, **53**, 97-115, and references therein.

30 J. M. Anderson, *Annu. Rev. Mater. Res.*, 2001, **31**, 81-110.
31 S. Kull, I. Martinelli, E. Briganti, P. Losi, D. Spiller, S. Tonlorenzi, G. Soldani, *J. Surg. Res.*, 2009, **157**, e15-e21, and references therein.
32 ArteX™ surgical sealant :
http://www.accessdata.fda.gov/cdrh_docs/pdf10/P100030b.pdf
33 ReSure® sealant :
http://www.accessdata.fda.gov/cdrh_docs/pdf13/P130004b.pdf
34 M. A. Carnahan, C. Middleton, J. Kim, T. Kim, M.W. Grinstaff, *J. Am. Chem. Soc.*, 2002, **124**, 5291-5293.
35 V. M. Nivasu, T. T. Reddy, S. Tammishetti, *Biomaterials*, 2004, **25**, 3283-3291.
36 I. Masayuki, *Trends Glycosci. Glyc.*, 2002, **14**, 331-341, and references therein.
37 G. K. Kodokian, S. D. Arthur, *Polysaccharide-based polymer tissue adhesive for medical use*, U.S. Patent Application 11/244, 756, 2006.
38 S. K. Bhatia, S. D. Arthur, H. K. Chenault, G. D. Figuly, G. K. Kodokian, *Curr. Eye Res.*, 2007, **32**, 1045-1050, and references therein.
39 N. S. Braunwald, W. Gay, C. J. Tatooles, *Surgery*, 1966, **59**, 1024-1030.
40 C. J. Tatooles, N. S. Braunwald, *Surgery*, 1966, **60**, 857-861.
41 L. I. Bonchek, N. S. Braunwald, *Ann. Surg.*, 1967, **165**, 420-424.
42 H. Nomori, H. Horio, S. Morinaga, K. Suemasu, *Ann. Thorac. Surg.*, 1999, **67**, 212–216.
43 W.-H. Chang, Y. Chang, P.-H. Lai, H.-W. Sung, *J. Biomater. Sci. Polymer Edn.*, 2003, **14**, 481-495.
44 M. Matsuda, M. Ueno, Y. Endo, M. Inoue, M. Sasaki, T. Taguchi, *Colloids Surf. B : Biointerfaces*, 2012, **91**, 48-56.
45 BioGlue® surgical adhesive:
http://www.accessdata.fda.gov/cdrh_docs/pdf/P010003b.pdf
46 ProGel™ pleural air leak sealant:
http://www.accessdata.fda.gov/cdrh_docs/pdf/P010047b.pdf
47 C. Fuller, *J. Cardiothorac. Surg.*, 2013, **8**, 90-97, and references therein.
48 DuraSeal™ dural sealant :
http://www.accessdata.fda.gov/cdrh_docs/pdf4/P040034b.pdf
49 G. R. Cosgrove, J. B. Delashaw, J. A. Grotenhuis, J. M. Tew, H. van Loveren, R. F. Spetzler, T. Payner, G. Rosseau, M. E. Shaffrey, L. N. Hopkins, R. Byrne, A. Norbash, *J. Neurosurg.*, 2007, **106**, 52–58.
50 M. W. Grinstaff, *Biomaterials*, 2007, **28**, 5205-5214, and references therein.
51 J. H. Waite, *Comp. Biochem. Physiol.,* 1990, **97B**, 19-29.
52 S. A. Burke, M. Ritter-Jones, B. P. Lee, P. B. Messersmith, *Biomed. Mater.*, 2007, **2**, 203-210.
53 C. E. Brubaker, H. Kissler, L.-J. Wang, D. B. Kaufman, P. B. Messersmith, *Biomaterials,* 2010, **31**, 420-427.
54 S. Y. Yang, E. D. O'Cearbhaill, G. C. Sisk, K. M. Park, W. K. Cho, M. Villiger, B. E. Bouma, B. Pomahac, J. M. Karp, *Nat. Commun.* 2013, **4**, 1702–1710.
55 A. K. Gaharwar, N. A. Peppas, A. Khademhosseini, *Biotechnol. Bioeng.*, 2014, **3**, 441-453.

 