Cell Therapies and Hydrogels:

What is Crosslinking?

The Choice of a Hydrogel is Only Part of a Cell Therapy's Success.

Stephen Harrington, PhD
Director of Cell and Hydrogel Formulation, Likarda

Hydrogels start out as liquid solutions of individual polymer chains dissolved in an aqueous (water-based) solvent. To transform this liquid solution into a solid hydrogel, the polymer chains have to be connected together through a process called crosslinking, also known as "gelation". Upon crosslinking, the liquid polymer solution transforms into a solid interconnected three-dimensional polymer network. The resulting hydrogel's physical properties are highly dependent on the number and nature of these newly formed crosslinks.

An easy way to visualize this process is to imagine the frame of a house being built, where hundreds of loose, individual boards are connected together with hammers and nails to create a unified, solid structure. Describing the nature of these crosslinks (the nails) and the methods used to create them (hammering) are additional and useful ways of classifying hydrogels beyond the source of their polymeric backbones (natural vs. synthetic).

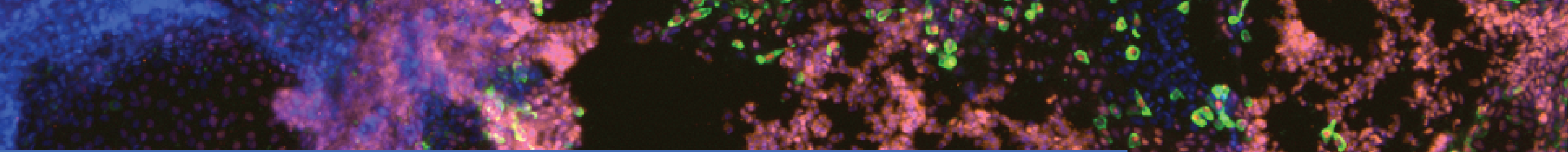
Classification of Hydrogels by Crosslink Chemistry and Gelation Method

One way of describing hydrogels is according to the chemistry of their crosslinks, which are broadly classified as covalent, physical, or ionic. Classification of crosslink chemistry is useful for understanding the physical properties of a hydrogel, such as its stiffness, durability, and degradation mechanics.

Hydrogels can also be characterized according to their method of gelation, which includes chemical, photo (light) initiated, and thermal processes. This type of classification is useful when considering how the hydrogels might be manufactured or used clinically. The following table provides a general summary of these classification schemes for easy reference.

Hydrogel Classifications

By Gelation Method Describes the process used to cause gelation and transform a liquid solution to a solid gel.	**By Crosslink Chemistry** Describes the chemical and physical nature of the crosslinks that connect the hydrogel polymers.
Chemical Gelation occurs upon the physical mixing of a hydrogel polymer with a reactive chemical agent. The reactive agent connects two nearby polymer chains (i.e. forms a crosslink), incorporating itself into the gel. Common crosslinks formed: covalent, ionic, or physical	**Covalent** Strong, non-reversible bonds involving electrons shared between atoms, resulting from a chemical reaction. Generally associated with high hydrogel strength and durability.
Photoinitiated Gelation is initiated by exposing the hydrogel precursor (pre-gelled solution) to specific wavelengths of light. Gelation rate is controlled by exposure time and light intensity. Common crosslinks formed: covalent	**Ionic** A non-covalent bond resulting from electrostatic attraction between two oppositely charged ions. Ionic crosslinked hydrogels are often weaker than covalently crosslinked gels. Ionic crosslinks may be reversed by displacement with monovalent ions or chelation of the crosslinking ion.
Thermal Gelation is achieved by increasing or decreasing the temperature of the hydrogel precursor beyond a critical value. Gelation can typically be reversed in the same way. Common crosslinks formed: physical	**Physical** Neither covalent nor ionic, physical crosslinks are formed by weak molecular forces such as Van der Waals forces and hydrophobic/hydrophilic interactions. Physical crosslinks are typically reversible via application of external force or changes in temperature.



Cell Therapies and Hydrogels:

What is Crosslinking?

The Choice of a Hydrogel is Only Part of a Cell Therapy's Success.

Chemical Gelation

Chemical gelation occurs when the hydrogel polymer solution is mixed with a reactive chemical agent that initiates the formation of crosslinks (hence why these agents are often referred to as "crosslinkers") that connect neighboring polymers together. In these hydrogels, the chemical agent itself becomes incorporated into the hydrogel network. Chemical gelation may be initiated by the addition of ions, peptides, or synthetic chemical species to participate in the gelation process[1]. Chemically gelled hydrogels may have covalent, physical, or ionic crosslinks, or combinations thereof.

A classic example of a chemical gelation process is the preparation of the widely popular calcium alginate hydrogel microspheres, which are formed by ionic crosslinks between the negatively charged alginate polymers and divalent calcium cations. When the calcium cations interact with alginate polymer chains, nearly instantaneous gelation occurs, making it easy to manufacture microspheres with great precision. This is achieved by simply dripping alginate droplets into a solution of calcium chloride. While calcium is the most commonly utilized cation when crosslinking sodium alginate, it has been shown that different divalent cations, such as barium or strontium, can be utilized to influence the stiffness and durability of the alginate hydrogel[2].

Photo-Crosslinking

Photo-initiated crosslinking (photo-crosslinking, for short) utilizes a catalytic photo-initiator species that generates free radicals when exposed to specific wavelengths of light, typically in the UV range[3]. These free radicals initiate a chain reaction between the hydrogel polymer chains, forming covalent crosslinks between them, resulting in gelation. Because gelation occurs only when and where the UV light is applied, the process can be controlled and is very rapid, making it useful for 3D printing and cell encapsulation applications. However, prolonged exposure to UV light can damage the encapsulated cells during the manufacturing process by damaging DNA, leading to cell death[3].

Thermal Gelation

Thermal gelation utilizes physical crosslinking via non-covalent bonds such as hydrophilic/hydrophobic interactions or van der Waals forces, and is induced by changes in temperature[4]. Thermal gelation can be achieved by either increasing or decreasing temperature, depending on the nature of the polymer, and can be reversed by adjusting the temperature in the opposite direction. An interesting application of thermal gelation is the ability to create a polymer solution that is liquid at room temperature but forms a hydrogel once it reaches body temperature, enabling facile injection and gelation in vivo. Additionally, no additional crosslinking agent, initiator, or UV light is required for thermal gelation. However, the final product's mechanical properties are often weaker than covalent or ionically crosslinked hydrogels, which is also true for other physically crosslinked hydrogels.

Serious Implications for Crosslinking

When characterizing hydrogels too often the focus is on the base polymer such as alginate or polyethylene glycol. However, the crosslinking strategy is equally, if not more, important in determining the physical properties of the final product. A full appreciation of the nuances of crosslinking chemistry is often lacking, which can lead to ultimate product failure.

If you have questions about crosslinking strategies, please connect with our hydrogel engineers at Likarda.

Have a Crosslinking Issue?

If you believe you have a crosslinking issue with a cell therapy currently in development, or you're planning on developing a new cell therapy and want to avoid crosslinking issues, learn more about how Likarda's targeted delivery system can help you:
https://likarda.com/biologic-and-cell-solutions/

Or book a call today with the Likarda team at **816.605.6440.**

References

1. M. Bahram, N. Mohseni, M. Moghtader. An introduction to hydrogels and some recent applications, in: S. Majee (Ed.), Emerging Concepts in Analysis and Applications of Hydrogels. Intech Open, https://doi.org/10.5772/61692, 2016.

2. B. Harper, S. Barbut, L. Lim, M. Marcone. Effect of various gelling cations on the physical properties of "wet" alginate films. J. Food Sci 79 (2014) E562-E567.

3. C. Lee, C. O'Connell, C. Onofrillo, P. Choong, C. DiBella, S. Duchi. Human articular cartilage repair: Sources and detection of cytotoxicity and genotoxicity in photo-crosslinkable hydrogel bioscaffolds. Stem Cells Transl Med 9 (2020) 302-315.

4. S. Jo, J. Kim, S. W. Kim. Reverse thermal gelation of aliphatically modified biodegradable triblock copolymers. Macromol Biosci 6(11) (2006) 923-929.

