## Overview

Hydrogels can retain a large quantity of water within their network without disturbing their original structure. This imparts flexibility and swelling properties to the hydrogel structures. These properties of hydrogels, along with the polar functional groups they bear such as amines, amides, carboxylic acids, hydroxyls, and sulfonic acids, enable hydrogels for many promising biomedical applications, such as 3D printing for tissue engineering and cell graft injections for in vitro screenings and in vivo organ-function regeneration.

## Existing hydrogel chemistry (for future references)

* Polymerization Crosslinking: Polymerization, including free radical polymerization, polyester condensation, Michael addition, and click chemistry. Examples include the polymerization of acrylate or methacrylate monomers with crosslinkers such as poly(ethylene glycol) diacrylate (PEGDA) or poly(ethylene glycol) dimethacrylate (PEGDMA). (https://onlinelibrary.wiley.com/doi/full/10.1002/smtd.202300553)
* Chemical Crosslinking: Chemical crosslinking includes amine-reactive groups (EDC, NHS-ester, carbodiimide), thiol-reactive groups and aldehyde-functionalized polymers. These reactions enable precise control over crosslinking density and network architecture, they can also serve as secondary chemistry for conjugation of functional groups.
* Physical Crosslinking: Physical crosslinking involves reversible interactions between polymer chains or segments to form transient crosslinks, such as hydrogen bonding, hydrophobic interactions, or host-guest interactions. Physical hydrogels exhibit stimuli-responsive behavior and tunable mechanical properties based on environmental cues such as temperature, pH, or ionic strength. Examples include polysaccharide-based materials and many naturally derived materials, such as collagen type I. (Schmaljohann D. Thermo- and pH-responsive polymers in drug delivery. Advanced Drug Delivery Reviews. 2006;58(15):1655–1670.)
* Ionic Crosslinking: Ionic crosslinking includes alginate hydrogels crosslinked with calcium ions and chitosan hydrogels crosslinked with tripolyphosphate. Ionic crosslinking offers simplicity and versatility in hydrogel synthesis but may exhibit limited mechanical stability and control over degradation.
* Enzymatic Crosslinking: Enzymatic crosslinking involves the use of enzymes as catalysts to mediate the crosslinking of polymer chains or peptides, such as fibrinogen can generate fibrin hydrogels with tunable mechanical properties.
* Photo-Crosslinking: Photo-crosslinking relies on the activation of photoinitiators or photosensitizers by light irradiation to initiate polymerization or crosslinking reactions. Photo-crosslinking enables spatiotemporal control over hydrogel formation and is widely applied for 3D bioprinting, i.e. GELMA, ColMA. They offer high level compatibility with cell encapsulation and in situ gelation approaches.

The combination of these approaches could be used to result a desired hydrogel formulation.

## Desired properties for applications related to tissue engineering and 3D bioprinting

* Shear-thinning properties:

The hydrogel experiences high shear rates during extrusion, causing a temporary reduction in viscosity due to the disruption of polymer chain interactions. This reduction in viscosity facilitates smooth and uniform extrusion of the hydrogel, allowing for the deposition of intricate structures with high resolution and fidelity. The hydrogel rapidly recovers post printing, which ensures the printed structure maintains the shape and desired structures after upper layer depositions. With changes in polymer composition, molecular weight and crosslink density, fine tuning is possible for the printing resolution and desired porosity.

* Viscoelastic properties

Viscoelasticity allows hydrogels to mimic the mechanical behavior of native tissues more accurately. Biological tissues typically exhibit viscoelastic behavior, meaning they can both deform under stress (elasticity) and flow over time (viscosity). By replicating this behavior, hydrogels provide a more realistic microenvironment for cells, influencing their behavior and responses. This is particularly interesting on the cellular mechanosensing for physiological recapitulation in vitro

* Mechanical Strength:

Tissue mimicking mechanical strength ensures structural integrity and stability for long term cell retention, interaction and protection during 3D printing a necessary for supporting cells and maintaining the overall shape of tissue constructs. Ranges for tissue mechanical properties ranges from 10kpa (regular connective tissues) to 10GPa (Bone).

* Biocompatibility:

The selected polymer and crosslinker requires cell-friendly and can facilitate cellular attachment. The hydrogel would prefer to have secondary crosslinking sites for functional group modification to promote various desired properties for cell attachment, drug delivery encapsulation, etc...

## Current limitation in hydrogel synthesis and optimization

Hydrogel properties depend on numerous factors such as polymer composition, crosslinking density, and processing conditions. Given the wide range of applications and used chemical reactions, developing systemic, consistent, and reproducible approaches for synthesis, evaluation, and optimization can be greatly challenging.

Rationale for self-driving lab utilizing ML

Self-driving lab could revolutionize hydrogel synthesis and optimization by automating experimentation processes, conducting high-throughput screening, and implementing real-time monitoring and control to execute synthesis protocols with precision and reproducibility, eliminating human error and increasing throughput. More importantly, by systematically exploring the effects of various formulation parameters and iteratively refining synthesis protocols using machine learning algorithms, researchers can accelerate the discovery of optimal hydrogel formulations to achieve a series of targeted properties. This streamlined approach not only reduces experimental variability but also enables dynamic adjustments covering to cover wide ranges of parameter exploration.