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# Granular hydrogels for endogenous tissue repair

Taimoor H. Qazi, Jason A. Burdick*

*Department of Bioengineering, University of Pennsylvania, Philadelphia, PA 19104, USA*

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ABSTRACT

Granular hydrogels, formed by the packing of hydrogel microparticles (microgels), are emerging to support the endogenous repair of injured tissues by guiding local cell behavior. In contrast to traditional pre-formed scaffolds and bulk hydrogels, granular hydrogels offer exciting features such as injectability, inherent porosity, and the potential delivery of biologics. Further, granular hydrogel design allows for the tuning of constituent microgel properties and the mixing of discrete microgel populations. This modularity allows the creation of multifunctional granular hydrogels that promote cell recruitment, guide extracellular matrix deposition, and stimulate tissue growth to drive endogenous repair.

## Principles of endogenous tissue repair

Traditional tissue engineering approaches combine cells, growth factors, and biomaterials to produce implantable constructs that can replace lost or damaged tissue. These approaches require extensive time, effort, and resources to facilitate cell and tissue growth on supportive scaffolds prior to implantation. In comparison, endogenous tissue engineering strategies rely on acellular biomaterials to stimulate innate healing mechanisms for tissue repair. Tissue repair through endogenous mechanisms depends on the availability of host cells, including tissue-resident stem cells. Whereas scar tissue that forms as a natural response to injury can limit the reparative capacity of these cells, biomaterials can present signaling cues and physical microenvironments that mobilize and guide cell function to accelerate repair. For example, decellularized extracellular matrices (dECMs) are naturally-derived biomaterials that possess intrinsic bioactivity and have been used as acellular scaffolds to promote tissue repair [1]. Acellular scaffolds like dECMs assume the role of a microenvironmental niche in vivo that recruits cells and manipulates their fate via cell-matrix interactions, growth factor signaling, and immunomodulatory mechanisms [2]. However, dECMs may carry risk of disease transmission owing to their xenogeneic origins, are prone to having large batch variability, and offer little control over physicochemical properties, motivating the development of engineered biomaterials.

## Engineered hydrogels for endogenous repair

Hydrogels are water-swollen polymer networks derived from either natural sources (e.g. hyaluronic acid, collagen) or synthetic routes (e.g. poly(vinyl alcohol), poly(ethylene glycol)) and are attractive for a variety of biomedical applications including tissue engineering. Numerous materials have been processed into bulk hydrogels that mimic features of native tissues, such as mechanics, cell-adhesion, degradability, and growth factor presentation. Bulk hydrogels typically exhibit a nanoporous mesh that prevents the infiltration of cells and blood vessels within its structure without extensive degradation, leaving the entire hydrogel volume typically devoid of any biological activity and hindering endogenous healing. Moreover, the majority of bulk hydrogels exhibit homogeneous properties throughout their volume, making it near impossible to incorporate diverse signaling cues to stimulate cellular populations in vivo. These drawbacks limit the utility of bulk hydrogels in endogenous tissue repair strategies.

As an alternative, granular hydrogels overcome many limitations of bulk hydrogels and build in additional functionalities that make them particularly well-suited to promote endogenous tissue repair (**Fig. 1A**) [3]. This includes inherent porosity that supports cell and vessel invasion, whereas traditional injectable bulk hydrogels may result in fibrous encapsulation without rapid degradation. Granular hydrogels are composed of packed assemblies of hydrogel microparticles (or microgels). Microgels can be made from the same polymers and with the same properties as bulk hydrogels, just processed into the micro-scale using methods such as water-in-oil microfluidics, batch emulsions, mechanical fragmentation of bulk hydrogels, or lithography. These methods either convert polymer precursor solutions into micro-scale droplets that are crosslinked using various chemistries to form microgels, or break down already crosslinked bulk hydrogels into micro-scale fragments with the application of mechanical force. Some of these methods (e.g. microfluidic emulsions) allow precise tuning of microgel size (few to hundreds of micrometers), whereas others (e.g. photolithography) permit the generation of microgels with user-defined shapes and topographies. The physicochemical properties of individual microgels (e.g. size, shape,

* Corresponding author.
*E-mail address:* burdick2@seas.upenn.edu (J.A. Burdick).

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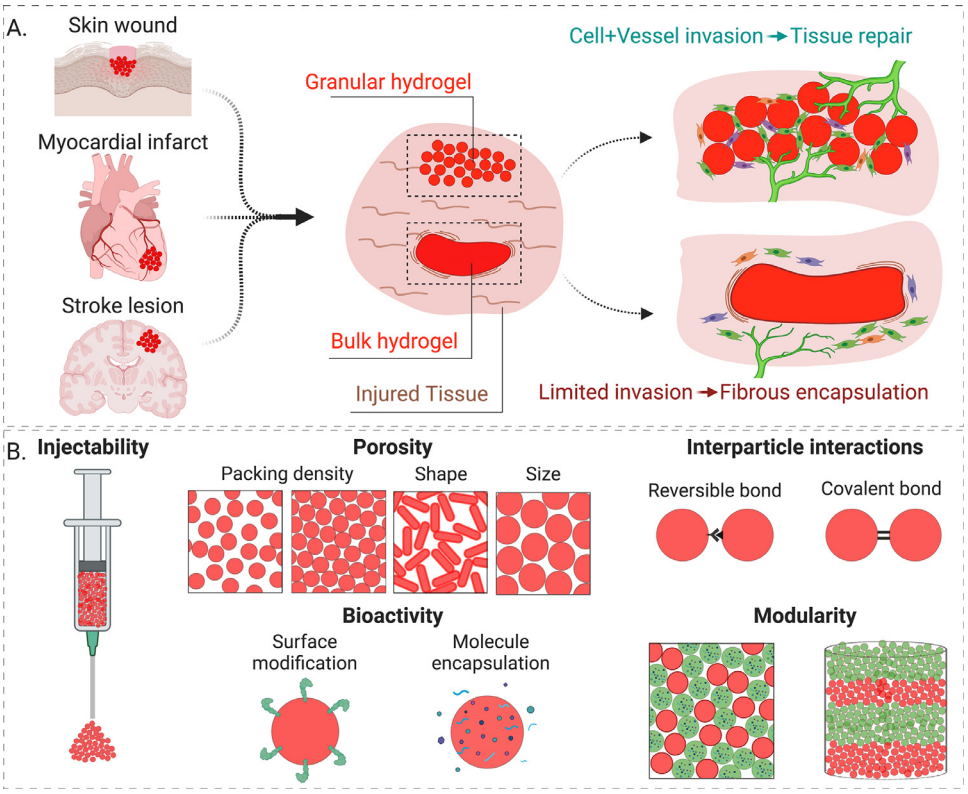


**Fig. 1. Endogenous tissue repair is accelerated due to the unique functional properties of granular hydrogels. A.** Examples of granular hydrogel delivery to injured tissues where the inherent porosity of granular hydrogels supports cell and vessel invasion, which can be hindered by more traditional bulk hydrogels. **B.** Granular hydrogels are highly tunable, and their properties can be engineered for application in endogenous tissue repair, including: **Injectability** through microgel flow within granular hydrogels; **Porosity** through changes in microgel packing density, shape, or size; **Bioactivity** via microgel surface modification with bioactive peptides or encapsulation of biologics; **Interparticle interactions** to control granular hydrogel properties through reversible or covalent interactions between microgels; **Modularity** through the combination of discrete populations of microgels to create multifunctional granular hydrogels.

mechanics, degradability) impact the macro-scale behavior of granular hydrogels, allowing for numerous degrees of freedom to achieve desirable hydrogel characteristics. Microgel packing to obtain granular hydrogels can be achieved by various means (e.g. centrifugation, vacuum-driven removal of the liquid phase from microgel suspensions, gravity-assisted sedimentation).

## Functional granular hydrogel properties for endogenous repair

### *Injectability*

The relatively small size of microgels allows easy injection of granular hydrogels into tissues via syringe needle or catheter (**Fig. 1B**). Granular hydrogels are held together through simple microgel packing, as well as optional non-covalent interactions (e.g. reversible guest-host bonds, electrostatics) between constituent microgels [4]. Under applied stress, for example during syringe injection, granular hydrogels show viscous flow behavior, allowing their minimally-invasive injection into tissues. Upon removal of this stress, granular hydrogels show almost complete recovery of their mechanical properties. Injectability not only allows granular hydrogels to be used for filling arbitrarily-shaped volumetric defects, but also expands their applicability to dense tissues such as the myocardium where minimally-invasive interventions are desirable. Fang et al. recently reported that the intramyocardial injection of acellular drug-carrying granular hydrogels after ischemia-reperfusion injury improved cell infiltration, angiogenesis, and tissue function [5].

### *Porosity*

A central tenet of endogenous tissue repair is the recruitment of cells to a repair site, including into a scaffold microenvironment that presents appropriate signals to modulate cell behavior. Unlike traditional bulk hydrogels, granular hydrogels are better suited for cell recruitment and invasion due to their microporous structure. Porosity has been a central design feature of biomaterial scaffolds and its critical role is illustrated in injuries such as bone osteotomy defects and volumetric muscle loss, where the application of rigid non-porous hydrogels physically inhibits defect bridging and wound closure, whereas porous hydrogels guide cell invasion, matrix deposition, and growth of new tissue. Granular hydrogels exhibit an interconnected microporous structure owing to the interstitial pores that are formed when microgels are packed together. Pore geometry and overall hydrogel porosity can be altered through the use of microgels of different shapes (e.g. high aspect ratio rods, polygonal fragments) and sizes, by incorporating a degradable microgel population that creates space over time, or by varying the packing density (**Fig. 1B**) [6]. The microporous structure of granular hydrogels results in numerous paths for surrounding cells to infiltrate, migrate, spread, and proliferate within the hydrogel structure for tissue repair. Hsu et al. recently reported that granular hydrogels with interconnected porosity markedly enhanced axonal outgrowth, leading to defect bridging and functional repair in peripheral nerve defects when compared to non-porous control gels [7]. Bulk hydrogels can also be programmed to allow cell infiltration for example by incorporating enzymatically-degradable crosslinkers, but this is usually at the expense of slow cellularization and compromised mechanical properties as the bulk matrix degrades. In contrast, granular hydrogels allow decoupling of porosity from degradability such that the invasion of cells and blood vessels does not challenge overall structural integrity [8].

### *Bioactivity*

The bioactivity of acellular biomaterials plays a significant role in driving endogenous tissue repair and the introduction of a bioactive matrix can improve structural and functional outcomes [9]. There are a number of ways in which granular hydrogels can stimulate endogenous cells to adopt a regenerative phenotype. Bioactivity can be derived from the delivery of therapeutic molecules and drugs, from the recognition of peptide ligands and similar bioactive cues presented on the microgel surface, or from the sensing of physicochemical properties of individual microgels (**Fig. 1B**). When microgels are prepared from naturally-

derived polymers (e.g. hyaluronic acid), by-products of in vivo polymer degradation can also be recognized by endogenous cells to elicit a biological response. Griffin et al. recently demonstrated that the degradation behavior of granular hydrogels can evoke a pro-healing adaptive immune response, resulting in accelerated and complete regeneration of skin wounds [10].

*Interparticle interactions*

The application of granular hydrogels in mechanically active tissues such as myocardium and skeletal muscle may cause microgel dispersion and structural disintegration at the site of injury. Although free-standing granular hydrogels can be held together by physical interactions, additional interparticle interactions can be used to form crosslinks between adjacent microgels to enhance stabilization under mechanical loads. This may be accomplished through the reaction of functional groups present on microgels that facilitate either covalent or non-covalent crosslinking (**Fig. 1B**). Besides imparting structural integrity, interparticle crosslinking can modulate the rate of infiltration of biological structures such as blood vessels and restrict excessive deposition of ECM by invading cells such as activated myofibroblasts. In other injury contexts, it may be sensible to disperse functional microgels within large defects to maximize endogenous cell response over large volumes. Thus, the ability to control these properties of granular hydrogels helps to evoke the appropriate host response.

*Modularity and multifunctionality*

Advances in single cell profiling have revealed the previously underappreciated cellular diversity that exists in tissues, highlighting the complex mechanisms of tissue repair that involve the coordinated action of multiple cell types. In this regard, multifunctional biomaterials that can appropriately stimulate diverse cells may be advantageous. In contrast to traditional bulk hydrogels, the modularity of granular hydrogels makes it possible to mix multiple microgel populations into a single injectable hydrogel (**Fig. 1B**). This provides exciting prospects towards simultaneously targeting various biological processes (e.g. via delivery of multiple drugs) and cell types (e.g. via presenting cell-type specific signaling cues).

## Summary

There is compelling evidence to indicate that acellular granular hydrogels can stimulate endogenous repair in tissues with diverse healing mechanisms including myocardium, peripheral nerve, and skin. The modular nature of granular hydrogels in combination with their injectability and tunable structural characteristics presents virtually limitless permutations in which they can support tissue repair. We anticipate that upcoming years will show efficacy of granular hydrogels in larger animal models, that new applications for granular hydrogels will be revealed, and that complementary advances in microfluidic technologies will enable the scale-up of microgel production to pave a path to their eventual translation to the clinics.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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