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Review

# Advances in hydrogel delivery systems for tissue regeneration

Wei Seong Toh [a,b,*], Xian Jun Loh [c,d,e,**]

[a] Faculty of Dentistry, National University of Singapore, 11 Lower Kent Ridge Road, Singapore 119083, Singapore
[b] Tissue Engineering Program, Life Sciences Institute, National University of Singapore, 27 Medical Drive, Singapore 117510, Singapore
[c] Institute of Materials Research and Engineering (IMRE), 3 Research Link, Singapore 117602, Singapore
[d] Department of Materials Science and Engineering, National University of Singapore, 9 Engineering Drive 1, Singapore 117576, Singapore
[e] Singapore Eye Research Institute, 11 Third Hospital Avenue, Singapore 168751, Singapore

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ABSTRACT

Hydrogels are natural or synthetic polymer networks that have high water-absorbing capacity and closely mimic native extracellular matrices. As hydrogel-based cell delivery systems are being increasingly employed in regenerative medicine, several advances have been made in the hydrogel chemistry and modification for enhanced control of cell fate and functions, and modulation of cell and tissue responses against oxidative stress and inflammation in the tissue environment. This review aims to provide the state-of-the-art overview of the recent advances in field, discusses new perspectives and challenges in the regeneration of specific tissues, and highlights some of the limitations of current systems for possible future advancements.



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## 1. Introduction

Regenerative medicine is an evolving field that involved clinical interventions to promote tissue healing with functional restoration after injury and/or disease. Early efforts of tissue engineering involving the combination of biomaterial scaffold, cells and growth factors to create tissue-like structures *in vitro* have yielded some notable success, but are hampered by difficulty in clinical translation due in part to the

* Correspondence to: W.S. Toh, Faculty of Dentistry, National University of Singapore, 11 Lower Kent Ridge Road, Singapore 119083. Tel.: +65 6779 5555x1619; fax: +65 6778 5742.
** Correspondence to: X.J. Loh, Institute of Materials Research and Engineering (IMRE), 3 Research Link, Singapore 117602, Singapore.
*E-mail addresses:* dentohws@nus.edu.sg (W.S. Toh), lohxj@imre.a-star.edu.sg, XianJun_Loh@scholars.a-star.edu.sg (X.J. Loh).

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difficulty in adaptation of technology in a clinical setting, as well as lack of understanding of the tissue injury and progression for appropriate and timely intervention.

Over the past decades, injectable hydrogels have emerged as a promising biomaterial for therapeutic delivery of cells and bioactive molecules for tissue regeneration because of their tunable tissue-like properties, controllability of degradation and release behavior, and adaptability in a clinical setting for minimally invasive surgical procedures [1,2]. Hydrogels may be made from natural and synthetic polymers. These highly-hydrated networks can be held together *via* physical or chemical crosslinks, can be made biodegradable, and responsive to specific stimuli such as pH and temperature, and can be engineered to deliver therapeutic cells and soluble factors in a sustained and controlled fashion.

Successful implementation of hydrogel-based cell delivery systems for tissue regeneration will largely depend on biomimetic design and engineering, harnessing cell–material interactions and material influences of cell fate and functions, incorporation of tissue elements from our understanding of the cellular processes in the tissue environment of normal and injured/diseased tissue, and ease of adaptation in a clinical setting. Some of the tissue and environment elements that have impact over the outcome of the biomaterials-based regenerative therapies include oxidative stress and inflammation. This review will focus on the advances in these areas, with emphasis on the various hydrogel-based cell delivery systems. More specifically, this paper reviews the state-of-the-art in the design and development of injectable hydrogel systems for tissue regeneration, with respect to their control of cell fate and modulation of the tissue environment.

## 2. Injectable hydrogel systems for cell delivery

As society ages, there is a greater demand for improved organ functions and repair of damaged tissues. This has led to the use of synthetic materials in different parts of our body. Traditional covalent chemistry has served us well in terms of the design of materials. The question is what lies ahead for the future of biomaterials? The future of soft biomaterials demands easy synthesis, the ability to respond to multi-stimuli, safety and efficacy.

Polymeric hydrogels can be categorized in numerous ways depending on the type of polymer and their structural characteristics. Chemically crosslinked hydrogels are formed by polymer chains linked permanently by non-reversible covalent bonds. This causes the hydrogels to be brittle, at times opaque and not having the self-healing property when the network is disrupted. These covalent bonds can be made using various reactions such as Michael type addition, Schiff base formation, thiol-ene photopolymerizations, free radical photopolymerization, enzyme-triggered reactions and “click” reactions. Chemical crosslinking can be modulated in order to sufficiently modify the mechanical properties of hydrogels and it has been frequently used when tough and stable hydrogels are desired. Unlike traditional chemistry which relies on covalent interactions, supramolecular chemistry focuses on weaker and reversible non-covalent interactions between molecules [3,4].

Supramolecular hydrogels are the next-generation materials to enter the biomedical arena [3,4]. These materials are three-dimensional (3D) entities built from crosslinking agents which bond non-covalently (*via* hydrogen bonds, π–π stacking and van der Waals interactions) to produce fibers and crosslinking among fibers. The properties of these materials are vastly different from their covalent counterparts. The use of injectable supramolecular hydrogels as tissue engineering scaffolds is promising owing to their ability to deliver therapeutics in a sustained and controlled manner. Drugs and cells can be easily encapsulated within the hydrogel matrix. The ideal injectable hydrogel needs to be carefully designed, taking into consideration the hydrogel's physical, chemical and biological properties. Enormous efforts have been put into the development of injectable hydrogels for the support and repair of the body tissues. Ideally, an injectable hydrogel should mimic the role of the extracellular matrix (ECM) found in tissues. The biomaterials reported up to date do not meet all the design parameters simultaneously (*e.g.*, lifetime, compatibility with the body or mechanical strength). It can be expected that research into the development of injectable hydrogels will have a huge impact on the progress of tissue engineering and regenerative medicine.

### 2.1. Natural hydrogels

Natural hydrogels are often used for delivery of cells for tissue regeneration, due to their innate biological characteristics and resemblance to the native ECM. Some of the natural biopolymers commonly used include collagen, fibrin, hyaluronic acid (HA), gelatin, chitosan, cellulose, alginate and agarose.

In reconstructive dental and orthopedic surgeries, bone grafts are always in high clinical demand. Combining injectable hydrogels with cells have potential for minimally invasive reparative procedures for bone repair. Recently, an injectable scaffold based on oxidized alginate microbeads encapsulating periodontal ligament stem cells (PDLSCs) and gingival mesenchymal stem cells (MSCs) was developed [5]. The encapsulated stem cells remained viable 4 weeks after culturing in osteogenic induction medium. Apatitic mineral was observed to be deposited by the stem cells. Ectopic mineralization was observed inside and around the implanted microbeads containing the immobilized stem cells in *in vivo* studies. These results show that immobilization of PDLSCs and gingival MSCs in alginate microbeads is a promising approach for bone tissue engineering.

Genetically modified bone marrow-derived MSCs cultured for the delivery of neurotrophic factors to the brain is promising as a neuroprotective strategy for neurodegenerative diseases [6]. In order to improve on the cell survival rate at post-transplantation, biomaterial scaffolds can provide a supportive matrix for transplanted cells which may assist in the grafting process. An *in situ* gelling type I collagen hydrogel was evaluated as an intracerebral transplantation matrix for delivery of glial cell line-derived neurotrophic factor (GDNF)-overexpressing MSCs (GDNF-MSCs) to the rat brain (Fig. 1). The collagen hydrogel did not affect the viability of the GDNF-MSCs nor did it prevent GDNF secretion into the surrounding medium. The collagen hydrogel did not negatively impact on the survival of the cells and permitted GDNF secretion into the striatal parenchyma *in vivo*. The transplantation of GDNF-MSCs in a collagen hydrogel significantly diminished the host brain's response to the cells by reducing the recruitment of both microglia and astrocytes at the site of delivery. Overall, this material was a well-tolerated cell delivery platform technology which could be modified to further aid cell support and graft integration.

In recent years, there is also a surge of interest in creating composite hydrogel systems based on natural biopolymers [7–9]. For example, Naderi-Meshkin et al. describes the synthesis of the biocompatible and biodegradable chitosan-beta glycerophosphate-hydroxyethyl cellulose (CH-GP-HEC) as an injectable gel scaffold. Chondrogenic factors or MSCs can be included in the CH-GP-HEC, and injected into the site of injury to fill the cartilage tissue defects with minimal invasion and pain. The MSCs have very good survival and proliferative rates within CH-GP-HEC hydrogel during the 28-day study period. Such a hydrogel system also has the capability to sustain the release of an encapsulated bioactive component over a period of a week. The interior of the hydrogel is also suitable for chondrogenic differentiation of the encapsulated human MSCs [9].

Ischemic cardiomyopathy can be treated by the transplantation of cardiac stem cells (CSCs) which are proliferated *ex vivo*. This therapy is however limited by modest engraftment efficiency and poor long-term survival [10]. A method of single cell microencapsulation is explored for the enhancement of CSC engraftment and survival after myocardial injection. Human CSCs were suspended in media and mixed with agarose. The agarose was supplemented with human