Bioactive Materials 3 (2018) 389–400

Contents lists available at ScienceDirect

Bioactive Materials

journal homepage: http://www.keaipublishing.com/en/journals/bioactive-materials/

Therapeutic neovascularization promoted by injectable hydrogels

Amrita Pal, Brent L. Vernon**, Mehdi Nikkhah*

School of Biological and Health Systems Engineering, Arizona State University, Arizona 85281, USA

ARTICLE INFO

Article history:
Received 1 November 2017
Received in revised form
27 February 2018
Accepted 2 May 2018

Keywords:
Injectable hydrogels
Neovascularization
Tissue regeneration
Angiogenic factors
Cell-therapy

ABSTRACT

The aim of therapeutic neovascularization is to repair ischemic tissues via formation of new blood vessels by delivery of angiogenic growth factors, stem cells or expansion of pre-existing cells. For efficient neovascularization, controlled release of growth factors is particularly necessary since bolus injection of molecules generally lead to a poor outcome due to inadequate retention within the injured site. In this regard, injectable hydrogels, made of natural, synthetic or hybrid biomaterials, have become a promising solution for efficient delivery of angiogenic factors or stem and progenitor cells for *in situ* tissue repair, regeneration and neovascularization. This review article will broadly discuss the state-of-the-art in the development of injectable hydrogels from natural and synthetic precursors, and their applications in ischemic tissue repair and wound healing. We will cover a wide range of *in vitro* and *in vivo* studies in testing the functionalities of the engineered injectable hydrogels in promoting tissue repair and neovascularization. We will also discuss some of the injectable hydrogels that exhibit self-healing properties by promoting neovascularization without the presence of angiogenic factors.

1. Introduction

During the past few decades, scholars have extended significant effort to develop minimally invasive injectable remedies, using catheters or syringes, for targeted cell therapy and drug delivery [1]. At the same time, hydrogels have gained importance as suitable scaffolding biomaterials for cell/drug delivery and regeneration of damaged tissues or organs [2]. Hydrogels are two-component semi-solid system, composed of polymer and water, where the polymer forms an extensive network and water is trapped in it. Based on their rheological properties, hydrogels are viscoelastic in nature. Injectable hydrogels belong to a class of biomaterial that are frequently injected in liquid state, and then form a solid gel *in situ*. These types of hydrogels can be categorized based on their chemical or physical crosslinking property. The polymer networks can be formed either by the physical interactions between the polymer molecules or by forming a covalent chemical bond between one or more polymers or by a combination of these modes [3–5] Structural, mechanical, and biological properties of injectable hydrogels are strongly influenced by the material cross-linking (type and concentration of physical or covalent bonding) properties. Injectable hydrogels should be porous, nontoxic, biocompatible (not harmful to living tissue), and have proper mechanical stability and biodegradability. Injectable hydrogels with higher mechanical stability exhibit improved cell-based tissue regeneration [6] and serve as better delivery platforms due to their ability to control the diffusion of entrapped growth factors, nutrients or metabolites [7]. The gelation kinetics of injectable hydrogels also plays an important role in its efficiency as a delivery scaffold. If the gel forms too fast, then it is difficult to inject and if it forms too slowly, then the network formation, adaption, and recovery may be too slow for effective 3D cell encapsulation as the solution can flow away from the injected site [8]. Moderate to rapid rates of gel formation is desirable for most tissue engineering or drug/cell delivery applications as it facilitates a homogeneous cell distribution during encapsulation by preventing cell sedimentation or aggregation [8]. Additionally, it prevents undesirable diffusion of the polymers away from the injection site before the gel formation [9].

A newer approach of regenerative tissue engineering, involving injectable hydrogels, is based on shape memory polymers [10–14]. Shape memory polymers are able to recover their original shapes after large deformation when subjected to external stimuli such as temperature, magnetism, moisture, or light [10]. Previous reports have demonstrated that shape memory polymers can be

* Corresponding author.
** Corresponding author.
E-mail addresses: Brent.Vernon@asu.edu (B.L. Vernon), mehdi.nikkhah@asu.edu (M. Nikkhah).
Peer review under responsibility of KeAi Communications Co., Ltd.

https://doi.org/10.1016/j.bioactmat.2018.05.002

promisingly used as injectable scaffolds in cell therapy [12], cardiac tissue engineering [13], bone tissue engineering [14] etc. Another alternate strategy to enhance the therapeutic efficacy of injectable hydrogels is based on the use of shear thinning materials [15,16]. In a shear thinning hydrogel, the gel has lower viscosity and flows under shear of injection. Once the shear stress is removed, gel restores its rigidity and remains localized at the point of injection [15,16]. Colloid gels, derived from colloid particles, also displays shear thinning behavior [17–19].

In the past few years, injectable hydrogels have gained notable attention for a wide range of clinical therapeutic applications, among them cardiovascular, endovascular, orthopedic, and dental [20]. In this regard, one of the significantly important applications of injectable hydrogels is in neovascularization of ischemic tissues/organs such as ischemic heart, musculoskeletal, etc. [21,22]. This specific application of injectable hydrogels is critically important, as vascularization remains to be one of the major bottlenecks in regenerative medicine. Neovascularization can take place by three processes: angiogenesis, vasculogenesis and arteriogenesis [23]. Angiogenesis is the formation of new blood vessels from the existing blood vessels via migration, growth, and differentiation of endothelial cells (ECs) inside wall of blood vessels, whereas vasculogenesis is the formation blood vessel via *de novo* assembly of ECs [23]. On the other hand, arteriogenesis is the increase in the diameter of existing arterial vessels. Injectable hydrogels derived from natural polymers such as fibrin and gelatin can promote neovascularization without incorporation of angiogenic factors or stem cells, due to their natural binding sites for solubilized growth factors and cellular integrin receptors [21]. Neovascularization can be also triggered by incorporation of growth factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet derived growth factor (PDGF), angiopoitin-1 [23], as well as VEGF mimetic synthetic peptides. Therapeutic neovascularization is expected to take place for several weeks or months with persistent stimulation of growth factors [24]. However, the delivery of angiogenic growth factors alone based on a bolus injection results in a burst and short period of biological activity within the injured zone [21]. With respect to cell-based therapies, in the majority of clinical trials, stem cells are injected directly in the ischemic area, which result in around 90% cell loss during injection [22], and poor viability. Therefore, to prohibit cell loss and achieve prolonged and sustained release of angiogenic factors, injectable hydrogels have been at the center of attention for efficient delivery of growth factors or cells. In this review article, we report recent advancements in the development of injectable hydrogels, derived from natural and synthetic biomaterials, with an emphasis on their application in neovascularization via controlled delivery of angiogenic factors or cells (see Table 1).

2. The classes of injectable hydrogels

Injectable hydrogels are derived from biomaterials, that can be natural or synthetic or a combination of these referred to as hybrid. Chemical structures of a few of these natural and synthetic polymers have been presented in Fig. 1(i–iv) and 2(i-iii). These biomaterials are used alone or in mixtures, can give rise to the formation of polymers with various properties [25]. The choice of biomaterial for the injectable hydrogel is a critical aspect for a specific target application. The biomaterial should be non-toxic, biodegradable and able to integrate with the host tissue. Natural polymers such as chitosan, gelatin, fibrinogen, hyaluronic acid and collagen have advantages over synthetic polymer as they promote cellular adhesion and infiltration due to the presence of cell adhesion moieties. Natural polymers also contain specific bioactive molecular domains that can enhance biological interactions between the material and the host [26–28]. These polymers, including injectable hydrogels, have already been used in clinical applications [27,28].

On the other hand, synthetic hydrogels have several advantages over natural hydrogels. Synthetic hydrogels readily allow precise control over critical material properties including polymerization, degradation and mechanical stiffness. Synthetic polymers have been hybridized with natural biopolymers to engineer native like extracellular matrix (ECM) with controlled biophysical and biochemical properties. In the following sections we will cover different types of natural and synthetic injectable hydrogels, which have been used for neovascularization application [28].

2.1. Natural injectable hydrogels for neovascularization

2.1.1. Chitosan

Chitosan (CS) is a linear polysaccharide composed of D-glucosamine and *N*-acetyl-D-glucosamine. It is prepared by deacetylation of chitin from crustacean shells [29] with an alkaline substance like sodium hydroxide. CS has been widely used in several biomedical applications [30–33] because of its biocompatibility, biodegradability, antimicrobial property, nontoxicity, and proper cell binding capability. However, the poor solubility of chitosan in physiological solvents, such as phosphate buffer saline (PBS) makes it challenging for the use in the form of an injectable hydrogel scaffold [34]. To address this problem, investigators have produced hydrogels by mixing chitosan with other natural polymers [28] for the formation of bio-hybrid injectable scaffolds. Apart from poor solubility, fast biodegradation *in situ* and possible allergic reactions of the residual proteins during chitosan preparation are some of the other major disadvantages of chitosan-based biomaterials [29].

To date, several chitosan-based hydrogels have been developed for delivery of growth factors and stem cells for neovascularization. For example, an injectable chitosan/heparinoid hydrogel was used by Fujita et al. for entrapment and sustained release of FGF-2 for neovascularization in a mouse model [35]. Significant increase in the formation of capillary networks after the injection of FGF-2 loaded hydrogel as compared to the injection of FGF-2 alone confirmed that the promotion of neovascularization was due to controlled growth factor release (Fig. 1v and vi).

Several acellular neovascularization approaches have also explored the use of chitosan based hydrogels. For instance, Deng et al. studied vasculogenesis *in vitro* by human umbilical vein endothelial cells (HUVECs) using collagen-chitosan injectable hydrogel [36]. In this study, chitosan was added in different ratio to a collagen matrix via 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide (EDC-NHS) chemistry. Several studies on the physical properties including injectability, denaturation temperature, and the mechanical strength measurements indicated that the gel strength enhanced with the increased ratio of chitosan, leading to better spreading and assembly of HUVECs. Specifically, the authors suggested that a higher ratio of chitosan in the gel enhanced the tubular network formation by HUVECs by increasing 'total capillary tube length' and 'total area covered'. The result of the *in vitro* study was supported by *in vivo* study, performed by acellular implantation of the hydrogel scaffold via subcutaneous injection, where branching of capillary networks was observed only in collagen:chitosan with 1:1 ratio. In another study, Deng et al. used an injectable chitosan-based hydrogel to study the tissue regeneration in the defected abdominal wall of a rat model [34]. The gel was formed via Schiff base reaction of N,O-carboxymethyl chitosan (NOCC) and aldehyde hyaluronic acid (A-HA). The gelation time of this hydrogel was tunable by altering the ratio of NOCC and A-HA and hence it could be optimized for injection in the affected tissue zone. Considerable increase of M2