Acta Biomaterialia 10 (2014) 1646–1662

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Review

Injectable alginate hydrogels for cell delivery in tissue engineering ☆

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ARTICLE INFO

Article history:
Available online 12 December 2013

Keywords:
Tissue engineering
Regeneration
Cell delivery
Injectable
Alginate

ABSTRACT

Alginate hydrogels are extremely versatile and adaptable biomaterials, with great potential for use in biomedical applications. Their extracellular matrix-like features have been key factors for their choice as vehicles for cell delivery strategies aimed at tissue regeneration. A variety of strategies to decorate them with biofunctional moieties and to modulate their biophysical properties have been developed recently, which further allow their tailoring to the desired application. Additionally, their potential use as injectable materials offers several advantages over preformed scaffold-based approaches, namely: easy incorporation of therapeutic agents, such as cells, under mild conditions; minimally invasive local delivery; and high contourability, which is essential for filling in irregular defects. Alginate hydrogels have already been explored as cell delivery systems to enhance regeneration in different tissues and organs. Here, the in vitro and in vivo potential of injectable alginate hydrogels to deliver cells in a targeted fashion is reviewed. In each example, the selected crosslinking approach, the cell type, the target tissue and the main findings of the study are highlighted.

1. Injectable cell delivery systems

In many clinical scenarios, where normal physiological conditions or homeostasis are compromised, there is a need for tissue transplantation or implantation. The ideal paradigm in tissue engineering consists in introducing cells or tissues grafts, native to the injured area, to foster the regenerative process. In this context, cell-based therapeutic approaches can thus be considered a vital tool in regenerative medicine strategies. They rely on the successful delivery of living cells to the target location, where they can produce a desired therapeutic effect by paracrine delivery of biomolecules (growth factors, cytokines, hormones, etc.) or replace lost cells with donor cells that can integrate and regenerate the damaged tissues [1,2]. Cells used for cellular therapies can be previously manipulated to produce a missing substance, such as a specific protein that is absent in a metabolic disease [3].

To ensure that an adequate number of cells reach the target tissue, cell-based approaches have usually been based on the delivery of high-density single-cell suspensions to the site of injury through injection. However, such direct cell injection often has a poor outcome due to large and rapid loss of cell viability (thus requiring high cell densities, which makes it technically complex and also extremely expensive), reduced engraftment of delivered cells and limited control over cell fate, in terms of both site-specificity (with cells eventually migrating and affecting other sites) and cell differentiation [4–6]. Therefore, more effective cell transplantation methods, capable of sustaining the survival of implanted cells while maintaining their function and enhancing their incorporation into the host, are mandatory. One strategy to achieve this goal relies on the delivery of transplanted cells via a temporary support made of biocompatible materials that can be further biochemically and physically modified to improve cell delivery [7,8]. This strategy will provide cell protection, prolonged retention at the injury site and a more physiological three-dimensional (3-D) environment. Moreover, many studies have pointed to the importance of strategies that promote cell–cell and cell–matrix interactions, which impact considerably on cell morphology, viability and function [5,9]. For instance, for anchorage-dependent cells, these interactions define cell shape and organization, which in turn will regulate cell behavior, namely survival, differentiation, proliferation and migration [10]. Hydrogels are candidates of choice to mediate cell delivery and accommodate cells in a 3-D microenvironment due to their natural similarities to the extracellular matrix (ECM). One paradigmatic example is alginate, a tunable and versatile natural injectable hydrogel with huge potential as an artificial 3-D cellular matrix, which has already been explored in a myriad of studies as an injectable cell delivery system for a broad variety of biomedical applications [11].

☆ Part of the Special Issue on Biological Materials, edited by Professors Thomas H. Barker and Sarah C. Heilshorn

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http://dx.doi.org/10.1016/j.actbio.2013.12.006

For clinical applications, cell delivery through injectable materials may be a desirable method, since these systems offer specific advantages over preformed scaffold-based approaches. On the one hand, they can be applied using minimally invasive techniques, improving patient compliance and comfort, and eventually leading to faster recovery and hence lower healthcare costs. On the other hand, they present several additional appealing features, namely: (i) easy incorporation of therapeutic agents, such as proteins or cells, and their subsequent localized delivery; (ii) simplicity of implantation by injection; (iii) high contourability, providing adaptable filling of defects with irregular shapes and sizes; and (iv) site specificity as well as confined delivery [12–22]. A key requisite of cell delivery vehicles is the maintenance of cell viability throughout the injection procedure. For instance, when flowing through a syringe needle, cells can experience three types of mechanical forces that can lead to cell disruption: (i) a pressure drop across the cell; (ii) shearing forces due to linear shear flow; and (iii) stretching forces due to extensional flow [23]. Therefore, an injectable matrix is also required to exhibit adequate mechanical properties to protect injected cells and thereby ensure their survival [23,24].

Injectable cell-based systems can be prepared with different configurations, depending on the type of application. Distinct and often conflicting terms exist to designate these systems. In the present review, we propose a classification (Fig. 1) in which systems have been divided into four main categories: (1) surface attachment with a preformed microcarrier, where cells are attached to the outside surface of microcarriers or even to the surface of inner pores; (2) microencapsulation, where cells are dispersed in a liquid contained within a polymeric membrane or capsule (mainly used for immunoisolation); (3) matrix entrapment, where cells are embedded in a hydrogel matrix that creates a 3-D environment, which can be formed in situ or ex situ (as in the case of microparticles); and (4) multicellular aggregates, where cells self-aggregate spontaneously or upon induction (due to matrix properties) to present some integrity, despite sometimes being "scaffold-free", and can be manipulated as "microparticles" due to their size. These multicellular aggregates can be further injected using a hydrogel-based vehicle [5]. Although alginate hydrogels have been employed in all of these types of systems, this review will mainly focus on applications belonging to category 3, thus the term "entrapment" will be used throughout the text. The main purpose of this review is to demonstrate the alginatés potential as an adequate 3-D microenvironment for cell delivery, in which cells are kept in direct contact with this synthetic ECM and, after transplantation, might become incorporated in the host tissue and actively participate in the regeneration process. Therefore, categories 1, 2 and 4 above will be described no further in this review. In strategy 1, cells are actually in a 2-D rather than a true 3-D environment; strategy 2 aims at isolating cells from the host environment that will most likely not be incorporated in the host; and in strategy 4 the 3-D environment is a result of the natural cellular aggregation and is not necessarily provided by the matrix.

The present review focuses mainly on strategies where cell-laden alginate-based systems were designed to be delivered in a minimally invasive way and, after injection, allow the transplanted cells to be integrated in the host's damaged tissue and actively participate in the regeneration process.

2. Hydrogels in tissue engineering

Hydrogels are 3-D hydrophilic, cross-linked polymeric networks capable of absorbing a significant amount of water or biological fluids [27]. Chain crosslinking can be achieved by chemical or physical methods, and the large variety of crosslinking reaction schemes make it possible to control the gelation kinetics and the subsequent hydrogel properties [28]. Hydrogels are compliant and permeable structures, mostly constituted of water, which resemble the native ECM, providing an ideal 3-D microenvironment for culturing cells. As a consequence, hydrogels have emerged as a valuable platform for examining the effects of ECM properties on cell behavior [9,29–33]. Additionally, they can be further modified to improve their mechanical and biochemical properties, to better mimic the native ECMs, via physicochemical modifications of the gel-forming polymers and/or crosslinking

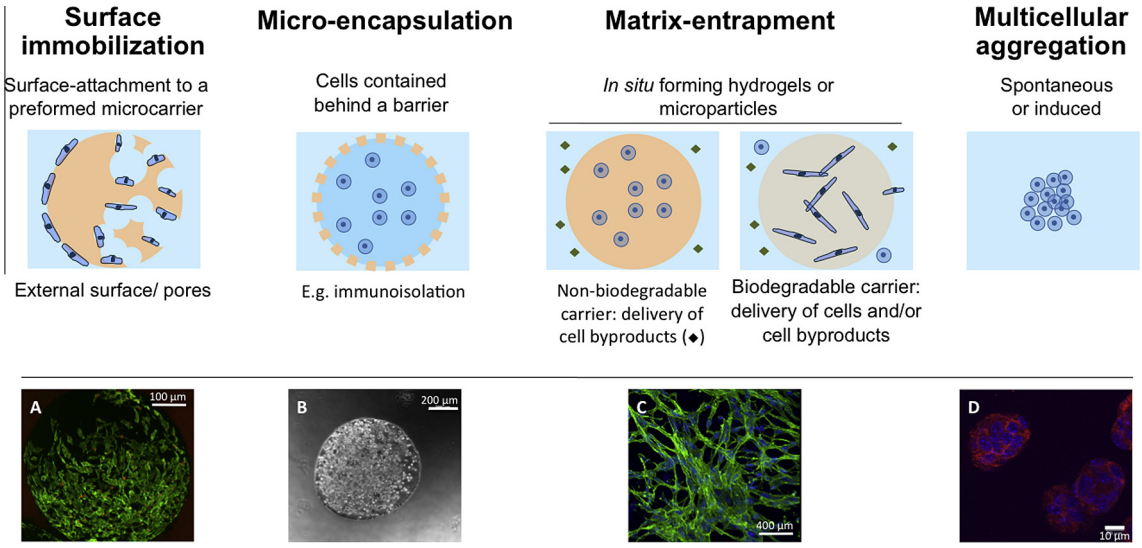

Fig. 1. Cell immobilization strategies for injectable cell-based therapies. Several strategies and carrier materials can be used for cell immobilization. These can be adapted for use in injectable cell-based therapies. Here, the different approaches were divided into four major categories: (1) surface immobilization; (2) microencapsulation; (3) matrix entrapment; and (4) multicellular aggregation. The bottom images correspond to injectable alginate-based systems incorporating cells. (A) Bone marrow stromal cells cultured for 5 days on the surface of calcium titanium phosphate microspheres, under standard osteoinductive conditions. Cells were stained with Alexa-fluor 488-phalloidin (F-actin) and counterstained with propidium iodide (DNA). (B) Human mesenchymal stem cells (hMSCs) entrapped and cultured for 21 days inside 2 wt.% RGD–alginate microspheres in basal conditions. (C) hMSCs entrapped and cultured for 8 days inside 2 wt.% PVGLIG/RGD–alginate hydrogels under basal conditions. Cells were stained with Alexa-fluor 488-phalloidin (F-actin) and counterstained with DAPI (DNA). (D) Mouse mammary epithelial cell line (EpH-4) within 2 wt.% RGD–alginate after 7 days. Cells were stained for E-cadherin (red) and counterstained with DAPI (DNA). Adapted from Refs. [25,26].

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