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Chapter

The Use of Alginate Hydrogels for the Culture of Mesenchymal Stem Cells (MSCs): In Vitro and In Vivo Paradigms

Michail E. Klontzas, Hicham Drissi and Athanasios Mantalaris

Abstract

Alginate hydrogels have been widely used in stem cell cultures due to their biocompatibility, malleable nature, high water content, enhanced mass transport properties, and their functionalization with bioactive molecules providing cues that modulate cell proliferation and differentiation. Mesenchymal stem cells (MSCs) are extensively utilized in clinical cellular therapies because of their differentiation efficiency, their immunosuppressive properties, and them not being tumorigenic when implanted in vivo. MSCs are isolated from numerous fetal and adult tissues, suitable for both autologous and allogeneic applications. Consequently, alginate hydrogels/MSCs have been applied in vivo for the treatment of a wide variety of musculoskeletal, cardiac, neural, and endocrine disorders. This chapter will review the use of alginate hydrogels (physical properties and functionalization) for MSC culture in vitro (various culture systems) and the application of alginate/MSC implants (animal models and human applications) for cellular therapy purposes in vivo.

Keywords: alginate, mesenchymal stem cells, MSCs, in vivo, in vitro, hydrogels

1. Introduction

Alginate has been extensively used for tissue engineering and regenerative medicine purposes [1]. Its ability to form hydrogels under mild gelation conditions in the presence of ions such as Ca^{2+}, Ba^{2+}, and Sr^{2+} renders it suitable for cell-based applications where exposure to harsh crosslinking buffers can lead to cell damage. When alginate is exposed to a crosslinking solution, L-guluronic residues of adjacent polysaccharide strands are connected forming a hydrogel [2, 3]. Alginate hydrogels possess the advantages of natural biomaterials such as excellent biocompatibility and abundance in nature with a low cost, properties which render it an excellent candidate for cell-based regenerative medicine applications [4]. However, the lack of alginate bioactivity requires functionalization with a wide variety of molecules promoting adhesion and modulation of stem cell fate. The purpose of this chapter is to provide an overview of the use of alginate hydrogels with mesenchymal stem cells (MSCs) which represent one of the most widely used stem cell type and the only stem cell type currently in clinical use.

2. Mesenchymal stem cells (MSCs) for tissue engineering

MSCs are multipotent stem cells with the ability to proliferate and differentiate into a variety of mature cells, mainly osteocytes, chondrocytes, and adipocytes [5].

MSCs can be isolated from a multitude of adult and fetal tissues including but not limited to the bone marrow, adipose tissue, peripheral blood, synovial tissue, placenta, Wharton's jelly, and umbilical cord blood. Importantly, it has been shown that MSCs isolated from different tissue sources possess differential proliferation and differentiation capacity toward various lineages [6] (**Figure 1**). Since their description by Friedenstein et al. [7], MSCs have been evolved as the stem cell type with the most regenerative medicine applications and the only stem cell type used in clinic to date.

MSCs represent attractive stem cell candidates for the use in tissue engineering and regenerative medicine applications for a variety of reasons. Firstly, they have the ability to proliferate and differentiate producing tissues, which are clinically relevant for regenerative medicine purposes such as musculoskeletal and neural tissues. In addition, they offer the possibility of autologous use, which can avoid adverse immune responses to allogeneic cells while also possessing an immunomodulatory capacity being able to regulate the immune environment even when implanted in an allogeneic fashion. Finally, the use of MSCs avoids the ethical shortcomings of embryonic stem cell (ESC) use and is not associated with the formation of teratomas which is a characteristic of pluripotent stem cell implantation (ESCs and induced pluripotent stem cells—iPSCs) [8–10]. Recently, protocols for the derivation of MSCs from iPSCs have also enabled the production of unlimited MSC

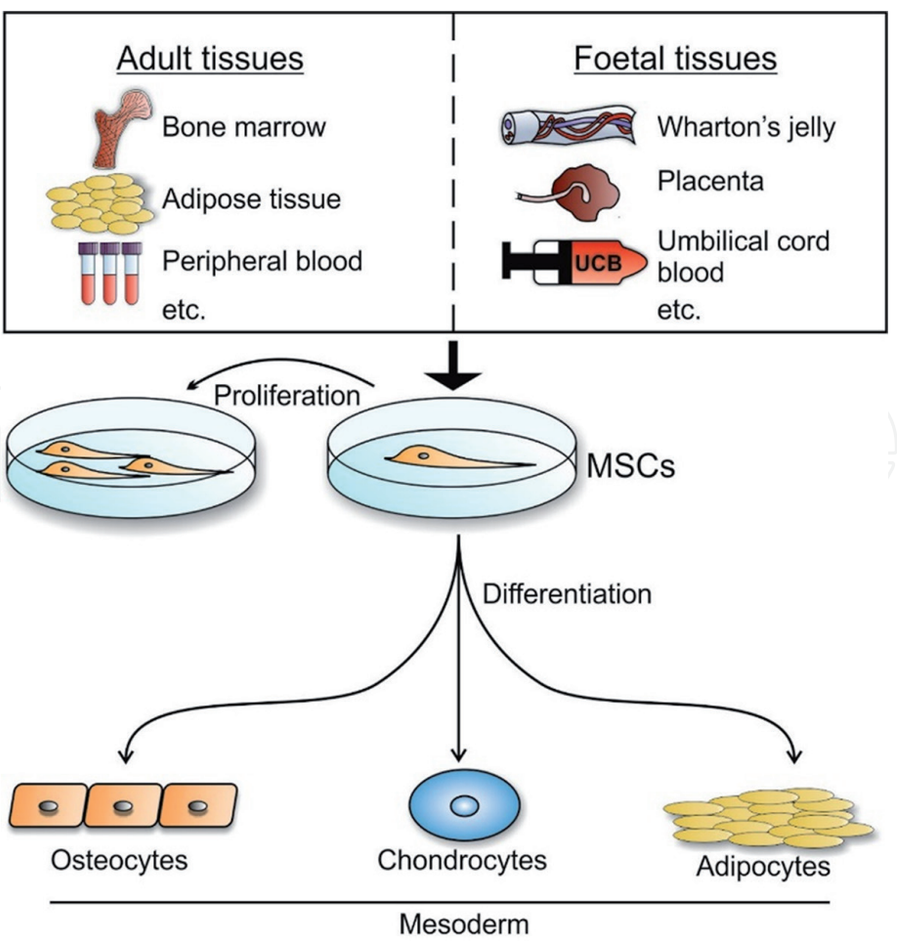

Figure 1.
Tissue sources and properties of mesenchymal stem cells.

numbers by exploiting the unlimited proliferation capacity of iPSCs prior to their differentiation to MSCs [11–13].

The use of MSCs for tissue engineering and regenerative medicine purposes requires the robust characterization of MSCs at several levels. At the moment, the International Society for Cell and Gene Therapy (ISCT) has posed the minimal criteria that need to be fulfilled so that a cell population is characterized as MSCs. These include the adherence to plastic; the presence (≥95%) of surface markers including CD73, CD90, and CD105; and the absence (≤2%) of hematopoietic markers (CD34, CD45, CD79a or CD19, CD14 or CD11b, and HLA II). Finally, cells characterized as MSCs should possess the capacity to differentiate to osteoblasts, chondroblasts, and adipocytes in vitro [14]. Other surface markers have been utilized over the years for the characterization of MSCs including Stro-1, CD271, CD146, and MSCA-1, but their use has not yet been established as a routine for MSC research [15–18]. Recently, omics strategies have emerged as promising alternatives for the comprehensive evaluation of MSC quality at the undifferentiated and differentiated states [11, 19–23].

3. The use of alginate hydrogels and MSCs in tissue engineering and regenerative medicine applications

Due to the lack of bioactive molecules on the alginate structure, alginate hydrogels used for cell-based applications require functionalization with molecules which can aid cell adhesion, increase cellular proliferation, and/or guide stem cell differentiation toward the desired cell lineages. In an attempt to increase cell adhesion on alginate hydrogels, a wide variety of extracellular matrix proteins or protein fragments have been employed. The most commonly used molecules include collagen, gelatin (product of collagen hydrolysis), and arginylglycylaspartic acid (RGD) peptide, which is the functional adhesion sequence in several extracellular matrix (ECM) proteins. Gelatin has been widely utilized for the enhancement of cell adhesion and differentiation in alginate hydrogels [24] either mixed [25] or crosslinked with alginate [26]. It has also been shown that crosslinking of alginate with gelatin reduces gelatin leak over prolonged culture while enhancing cell adhesion and vascular endothelial growth factor (VEGF) secretion compared to natural alginate and RGD-alginate [27].

Oxidized alginate has been widely used for tissue engineering purposes. Alginate can be oxidized with the use of agents including sodium permanganate ($KMnO_4$) and periodate, to produce two free aldehyde groups on the alginate backbone, offering enhanced in vitro and in vivo. Alginate oxidation is necessitated by the lack of natural alginate degrading enzymes in mammals, which is translated to a slower biodegradation of alginate hydrogels [28, 29]. Additionally, free aldehyde groups offer sites for possible crosslinking with amine group-containing molecules, which can be used for the robust functionalization of hydrogels used for tissue engineering [29, 30]. Similarly to natural alginate, a wide range of biomolecules have been used for the functionalization of oxidized alginate. The most commonly used are gelatin and RGD, which have been shown promote cell adhesion and viability [31] .

3.1 In vitro paradigms of alginate/MSC constructs

Culture of MSCs in alginate hydrogels has been attempted for applications ranging from the regeneration of bone, cartilage, and tendon to the repair of damaged myocardium and trachea. Most of the initial data on the use of alginate/MSC constructs have been obtained in vitro (**Table 1**).

Functionalization of alginate has been achieved with molecules mimicking the ECM, the most commonly used of which are RGD and gelatin. RGD has been used to increase adhesion in photo cross-linked alginate hydrogels which were found to maintain viability and promote proliferation of bone marrow MSCs [32] and muscle differentiation of umbilical cord MSCs in alginate-fibrin hydrogels [33]. Tyramine has been also cross-linked to alginate to increase MSC adhesion [34]. In hydrogels functionalized with RGD, it has been shown that high cell density favors cell-cell contact and promotes osteogenic differentiation [35] as well as increasing survival and VEGF secretion from MSC spheroids [36]. The combination of RGD with a matrix metalloproteinase cleavable peptide (proline-valine-glycine-leucine-isoleucine-glycine) in alginate has been shown to promote adhesion and allow better

First author [reference]	Year	Type of hydrogel	MSC type
Park Y [43]	2005	Alginate	Synovial MSCs
Coates EE [42]	2013	Methacrylated alginate-HA	Bone marrow MSCs
Tohamy KM [45]	2018	Sodium alginate (SA)/hydroxyethylcellulose (HEC)/hydroxyapatite (HA)	Bone marrow MSCs
Yeatts A [60]	2011	Alginate	Bone marrow MSCs
Wang M [61]	2016	Alginate-HA	Bone marrow MSCs
Chen B [56]	2013	Strontium crosslinked alginate	Bone marrow MSCs
Weber M [41]***	2002	Alginate	C3H10T1/2 MSC cell line
Hsu S [50]	2011	Alginate/nano-sized calcium-deficient hydroxyapatite/RGD	Placental MSCs and bone marrow MSCs
Schütz K [58]	2017	Alginate/methylcellulose	Bone marrow MSCs
Kolambkar Y [64]	2007	Alginate	Amniotic fluid MSCs
Liu J [33]	2012	Alginate-fibrin-RGD	Umbilical cord MSCs
Du W-J [65]	2016	Alginate-HA	Bone marrow and adipose MSCs
Straccia M [66]	2015	Alginate-chitosan	Bone marrow MSCs
Maia F [35]	2014	Alginate-RGD	Bone marrow MSCs
Huang J [59]	2016	Alginate-gelatin-carboxymethyl chitosan	Bone marrow MSCs
Karunanithi P [38]	2016	Alginate-fucoidan	Bone marrow MSCs
Klontzas ME [20]	2019	Oxidized alginate-GHK	Umbilical cord blood MSCs
Jose S [39]	2014	Alginate-GHK	Bone marrow MSCss
Sarker B [53]	2017	Oxidized alginate-gelatin	Adipose tissue MSCs
Bernhardt A [46]	2009	Alginate-gelatin-HA	Bone marrow MSCs
Wang Y [47]	2014	Oxidized alginate-gelatin-N-succinyl chitosan	Bone marrow MSCs
Zhao L [48]	2010	Alginate-calcium phosphate	Umbilical cord MSCs
Zhou H [49]	2011	Alginate-fibrin	Umbilical cord MSCs

Table 1.
Representative in vitro studies combining alginate-based hydrogels with MSCs.

elongation of MSCs than RGD alginate [37]. Increased chondrogenesis has been also demonstrated with the incorporation of fucoidan (a heparan sulfate analogue) in alginate hydrogels seeded with bone marrow MSCs [38]. Glycine-histidine-lysine (GHK), a tripeptide fragment 0f osteonectin (a bone ECM protein), has been cross-linked with natural alginate and oxidized alginate achieving enhanced VEGF secretion from bone marrow MSCs [39] and increased osteogenic differentiation of umbilical cord blood MSCs compared to oxidized alginate with gelatin [20]. Finally, functionalization of alginate with RGD has been shown to promote adipose tissue MSC chondrogenesis via integrin-dependent transforming growth factor (TGF)-β3 activation [40].

One of the most common applications of alginate/MSC constructs is for cartilage tissue engineering. It has been shown that cells differentiated to chondroblasts in alginate hydrogels produce more collagen type II than in monolayer where they predominantly produce collagen type I [41]. In addition, photocrosslinked alginate/hyaluronic acid injectable hydrogels have been shown to support the chondrogenic differentiation of bone marrow MSCs for cartilage tissue engineering [42]. Alginate hydrogels have been also combined with synovial MSCs showing chondrogenic gene expression and collagen type II deposition under the effect of bone morphogenetic protein-2 (BMP-2). However, the authors noted that full progression of chondrogenesis was not feasible [43]. Interestingly enough when applied to bone marrow MSCs in RGD-alginate hydrogels, BMP-2 has promoted osteogenic differentiation showing that it favors osteogenic differentiation [44].

Several studies have demonstrated the suitability of alginate hydrogels in combination with MSCs for bone tissue engineering. Sodium alginate (SA)/hydroxyethylcellulose (HEC)/hydroxyapatite (HA) hydrogels have been combined with bone marrow MSCs for bone tissue engineering maintaining high cell viability and proliferation [45]. Alginate-gelatin-hydroxyapatite [46] and oxidized alginate-gelatin-N-succinyl chitosan hydrogels [47] have been shown to promote the osteogenic differentiation of bone marrow MSCs. Injectable hydrogels have been also tested for the repair of bone defects such as alginate-calcium phosphate [48] and alginate-fibrin hydrogels [49] combined with umbilical cord MSCs. Such materials enable the direct injection of the hydrogel paste in a bone defect and have been shown to promote osteogenic differentiation of MSCs facilitating fracture healing. Hydroxyapatite (calcium-deficient) and RGD have also been combined with alginate for cartilage regeneration showing that placental MSCs could perform better chondrogenesis than bone marrow MSCs [50]. However, RGD-functionalized alginate has been also shown to enhance osteogenic differentiation, mineralization, and viability [51, 52]. Oxidized alginate hydrogels have been also widely utilized for bone tissue engineering. It has been cross-linked with fibrin achieving high cell viability and osteogenic differentiation of Wharton's jelly MSCs compared to plain natural and oxidized alginate [49]. Sarker and co-workers have described the crosslinking of oxidized alginate with gelatin hydrogels for bone regeneration, demonstrating enhanced osteogenesis of adipose tissue and increase of VEGF secretion from MG-63 osteosarcoma cells compared to plain alginate and RGD-functionalized alginate [27, 53]. Other groups have also confirmed the suitability of oxidized alginate for the osteogenic differentiation of adipose-derived MSCs [53] and muscle differentiation of Wharton's jelly MSCs [54].

Apart from bone and cartilage regeneration, alginate hydrogels have found a limited number of other applications such as the regeneration of nucleus pulposus of the intervertebral disk, the cryopreservation of MSCs, and the three-dimensional printing of cellularized structures. Specifically, alginate hydrogels outperform chitosan hydrogels in glycosaminoglycan deposition and the production of collagen type II for nucleus pulposus engineering [55]. In addition, they have been

used for the cryopreservation of MSCs avoiding minimizing the effects of freezing and thawing on stem cell viability [56], and various formulations of alginate such as oxidized alginate-gelatin [57], alginate/methylcellulose [58], and alginate-gelatin-carboxymethyl chitosan [59] have been found to be suitable for 3D printing applications.

Finally, it should be mentioned that there is a constantly increasing use of dynamic bioreactor cultures for the cultivation of alginate/MSC constructs. For example, dynamic perfusion bioreactor cultures of bone marrow MSCs in alginate hydrogels have been shown to enhance early in vitro osteogenic commitment and late osteogenesis [60, 61], and dynamic cultures incorporating compression forces have been used for chondrogenic differentiation purposes [62].

Despite the encouraging in vitro results, it needs to be noted that in vitro data do not necessarily correlate to the efficiency of hydrogels in vivo. As shown by Yang et al. who performed a direct in vitro-in vivo comparison of differentiation in alginate-gelatin hydrogels with MSCs, subcutaneous implantation in mice inhibits tri-lineage differentiation despite the efficient in vitro differentiation [63]. These results highlight the fact that caution is needed when extrapolating in vitro results to the in vivo setting.

3.2 In vivo paradigms of alginate/MSC constructs

Various types of alginate hydrogels have been shown to promote bone healing in animal models (**Table 2**). Injectable materials such as chitosan-alginate-BMP-2 and alginate-hydroxyapatite (HA)-mineralized microsphere combinations have been used in conjunction with MSCs to promote bone healing in vivo, demonstrating the efficient formation of trabecular bone [67, 68]. When used for bone tissue engineering, alginate hydrogels are usually seeded with MSCs and are allowed to gradually obtain higher mechanical stability as a result of ECM deposition and mineralization. However, tough alginate hydrogels have been also developed in order to achieve high mechanical stability which has been shown to promote bone healing [69]. Additionally, animal experiments have shown that when RGD is used for alginate modification, faster stress relaxation of alginate hydrogels [70] and high peptide density are linked to more efficient osteogenic differentiation than low peptide density which was linked to cell migration [71]. This correlates with results showing that increasing RGD concentrations inhibit chondrogenic differentiation in vitro [72]. Rottensteiner et al. utilized oxidized alginate-gelatin-nano-Bioglass hydrogels for bone regeneration identifying evidence of in vivo vascularization without adverse reactions, despite the cytotoxic action of Bioglass in vitro [73]. Additionally, Paul et al. successfully treated critical size calvarial defects with serum-loaded oxidized alginate-gelatin-biphasic calcium phosphate hydrogels with rat BM MSCs [74]. Importantly, encapsulation of MSCs in oxidized and natural alginate hydrogels increases vascularization which is of utmost importance in bone tissue engineering and the repair of vascular lesions [75] such as hind limb ischemia [76].

The ability of alginate hydrogels with MSCs to repair cartilage defects in animal models has been demonstrated in a variety of studies with various MSC types and hydrogel formulations. Chung et al. have compared a variety of hydrogel formulations including alginate, HA, chitosan, pluronic, and combinations of them seeded with umbilical cord blood MSCs. Their results demonstrated that even though alginate mixed with pluronic and chitosan achieved a certain degree of healing in rat knee cartilage defects, it was 4% hyaluronic acid which resulted in the optimal cartilage repair with macroscopic and microscopic appearance of adjacent healthy cartilage [77]. High-quality repair of in vivo rabbit cartilage defects has been shown with the use of

First author [reference]	Year	Type of hydrogel	MSC type	Application
Zhang F [81]	2012	Alginate	Co-culture of synovial MSCs with transgenic chondrocytes	Cartilage regeneration
Yu J [85]	2010	Alginate-RGD	Bone marrow MSCs	Myocardial regeneration
Yang C [63]	2009	Alginate-gelatin porous scaffolds	Bone marrow MSCs	Regeneration of multiple tissues
Leijs M [91]	2017	Alginate	Bone marrow MSCs	Inflammatory diseases
Steiner D [92]	2018	Oxidized alginate-gelatin	Bone marrow MSCs	Vascularization
Wang S [90]	2016	Alginate	Umbilical cord MSCs	Skin wound healing
Rottensteiner [73]	2014	Oxidized alginate with nano-Bioglass®	Bone marrow MSCs	Bone regeneration
Chung J [77]	2014	Alginate combined with pluronic, HA, and chitosan	Umbilical cord blood	Cartilage regeneration
Re'em T [84]	2012	Alginate with TGF-β1	Bone marrow MSCs	Cartilage regeneration
Sondermeijer H [86]	2018	Alginate-cyclic RGD	Bone marrow MSCs	Cardiac regeneration
Park D [67]	2005	Alginate-chitosan-BMP-2	Bone marrow MSCs	Bone regeneration
Schon LC [88]	2014	Alginate	Bone marrow MSCs	Tendon regeneration
Hashemibeni B [80]	2012	Alginate	Adipose MSCs and chondrocytes	Tracheal repair
Ho SS [93]	2016	Oxidized methacrylated alginate-RGD	Bone marrow MSCs	Bone regeneration
Moshaverinia A [89]	2014	RGD-alginate with TGF-β3	Gingival and periodontal MSCs	Tendon regeneration
Ingavle GC [68]	2019	Alginate-HA-mineralized microspheres	Bone marrow MSCs	Bone regeneration

Table 2.
Representative in vivo studies combining alginate-based hydrogels with MSCs.

bone marrow MSCs and natural alginate [78]. Alginate has been also combined with polylactic acid to promote in vivo cartilage repair with bone marrow MSCs [79]. In vivo cartilage differentiation in alginate hydrogels has been also attempted for the repair of tracheal tissue with the combination of adipose tissue MSCs and chondrocytes [80]. Synovial MSCs have also been co-cultured with chondrocytes transgenic for TGF-β3 in alginate hydrogels, demonstrating that TGF-β3 release can induce synovial MSC chondrogenesis [81]. The simultaneous activation of TGF-β3 and BMP-2 genes in MSC laden alginate hydrogels showed superior chondrogenesis compared to the isolated delivery of each one of the factors where cells progressed to endochondral osteogenesis instead of chondrogenesis [82]. Interestingly, alginate was found more capable in promoting endochondral osteogenesis than chondrogenesis when compared to chitosan [83].

Finally, TGF-β1-releasing alginate hydrogels have been used to promote chondrogenesis of MSCs, demonstrating in vitro increase of chondrogenic markers and healing of articular cartilage defects in mice [84].

Another important application of alginate/MSC constructs is the treatment of myocardial lesions. Yu et al. combined RGD-functionalized alginate hydrogels with human bone marrow MSCs showing that they could improve left ventricular function after myocardial infarction in a rat acute myocardial infarction model [85]. Cyclic RGD in alginate hydrogels has been also shown to promote neoangiogenesis and cardiac neovascularization, improving cardiac function in animals post-myocardial infarction [86]. Finally, when alginate hydrogels are used for cardiac regeneration, it has been shown that G-type alginates possess properties suited for the regeneration of cardiac tissue [87].

MSCs have been combined with alginate hydrogels for tendon repair purposes in animal model of tendon tears. For example, rat Achilles tendon lesions have been treated with hydrogels loaded with MSCs [88] showing healing of higher quality than surgical meshes and sutures. In addition, RGD-functionalized hydrogels loaded with TGF-β3 and loaded with periodontal and gingival MSCs were found to efficiently produce tendon tissue when implanted subcutaneously in mice [89].

Alginate hydrogels have also been widely utilized as wound dressings either alone or in combination with MSCs. For this application, various types of MSCs have been used including umbilical cord MSCs [90] and bone marrow MSCs in alginate-chitosan hydrogels with antibacterial properties [66].

Finally, alginate hydrogels have been used to protect MSCs from the local immune response elicited when allogeneic cells are implanted in vivo. They have been shown to provide protection from the immune system increasing the survival of MSCs in the hostile environment of the host-releasing immunomodulatory factors [91].

4. Conclusions

In conclusion, alginate/MSC constructs have been used for a wide variety of regenerative medicine applications, ranging from musculoskeletal to cardiac tissue repair. MSCs isolated from adult and fetal tissues have been combined with alginate hydrogels functionalized with extracellular matrix components, minerals, and other natural polymers and evaluated in vitro and in vivo. In vitro studies demonstrated the ability of alginate hydrogel at different formulations to support MSC growth and differentiation toward several lineages, whereas in vivo data have shown that when alginate-based materials are combined with MSCs, they can achieve successful healing of bone and cartilage defects, myocardial tissue after myocardial infarction, tendon tears, and skin wound. Nonetheless, evaluation of safety and efficacy of the constructs is required prior to clinical use. Existing in vitro and in vivo data demonstrate the potential of alginates to play an important future role in regenerative medicine, reaching the bedside and achieving regeneration of damaged tissues.

Conflict of interest

The authors declare no conflict of interest.

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