View metadata, citation and similar papers at core.ac.uk

brought to you by CORE
provided by Universidade do Minho: RepositoriUM

Critical Reviews in Biotechnology

http://informahealthcare.com/bty
ISSN: 0738-8551 (print), 1549-7801 (electronic)

Crit Rev Biotechnol, Early Online: 1–14
© 2014 Informa Healthcare USA, Inc. DOI: 10.3109/07388551.2014.889079

informa healthcare

REVIEW ARTICLE

Seaweed polysaccharide-based hydrogels used for the regeneration of articular cartilage

Elena Geta Popa[1,2], Rui Luís Reis[1,2], and Manuela Estima Gomes[1,2]

[1]3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Guimarães, Portugal and [2]ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

Abstract

This manuscript provides an overview of the *in vitro* and *in vivo* studies reported in the literature focusing on seaweed polysaccharides based hydrogels that have been proposed for applications in regenerative medicine, particularly, in the field of cartilage tissue engineering. For a better understanding of the main requisites for these specific applications, the main aspects of the native cartilage structure, as well as recognized diseases that affect this tissue are briefly described. Current available treatments are also presented to emphasize the need for alternative techniques. The following part of this review is centered on the description of the general characteristics of algae polysaccharides, as well as relevant properties required for designing hydrogels for cartilage tissue engineering purposes. An in-depth overview of the most well known seaweed polysaccharide, namely agarose, alginate, carrageenan and ulvan biopolymeric gels, that have been proposed for engineering cartilage is also provided. Finally, this review describes and summarizes the translational aspect for the clinical application of alternative systems emphasizing the importance of cryopreservation and the commercial products currently available for cartilage treatment.

Keywords

Cartilage regeneration, clinical and commercial products, hydrogels, *in vitro* – *in vivo* applications, polysaccharide

History

Received 29 July 2013
Revised 11 October 2013
Accepted 26 October 2013
Published online 19 March 2014

Critical Reviews in Biotechnology Downloaded from informahealthcare.com by Mrs Claire Summerfield on 04/08/14
For personal use only.

Introduction

Biological structures can be defined as open dynamic systems which interact and respond accordingly to changes in the environment. Therefore, to obtain a specific response from a target biological structure, it is necessary to create appropriate environments. A lot of work is being conducted to unravel the puzzle around structural and biochemical functions of the natural extracellular milieu that directs cell fate. Understanding the mechanisms of cell function, the typical response to generate matrix development and tissue growth, is critical for further advances in regenerating any damaged biological component. Also, a great deal of research has been focusing on the development of biological constructs that support cell proliferation based on specific interactions between the biomaterial interface domains and the cell receptors to mimic the physiological environment. The natural extracellular matrix (ECM) is a hydrogel-like structure itself, comprised of several different biopolymers, encompassing a wide range of biological, chemical, and mechanical properties (Aizawa et al., 2012). Generally, hydrogels are used for cell growth and delivery, with the goal of developing de novo tissues and ultimately regenerating and integrating the functional engineered tissue equivalent within the body. In the last few years, a vast range of different hydrogels that mimic more closely the native ECM have been proposed for regeneration strategies, produced by different methodologies and materials, with varying properties and composition. The knowledge obtained to this date, indicates that there is not a single ideal hydrogel available that can meet the requirements for all possible applications and thus, one must select a specific matrix with unique properties akin to target regenerative purposes. The use of polysaccharides as supportive systems for tissue formation reveals an increasing tendency in the biomedical field (Tirtsa et al., 2005). Among all the naturally derived polymers, the carbohydrate based polysaccharides, composed of sugar-ring building blocks, are emerging as a front runner in cartilage tissue engineering (TE) applications (Guarino et al., 2012; Ko et al., 2010). The polysaccharides of marine algae origin, especially the ones of seaweed source, such as alginate, agarose and recently κ-carrageenan or ulvan, which will be presented in this review, play important roles in biomedical applications, contributing with specific properties. In cartilage tissue, the cells are anchored into a matrix network that hydrogel design intends to mimic. Once embedded in hydrogels, commonly used as encapsulation systems (Hunt & Grover, 2010), the cells renew and specialize due to spatial organization

Address for correspondence: Manuela E. Gomes, 3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, 4806-909 Taipas, Guimarães, Portugal. Tel: +35 1253510906. Fax: +35 1253510909. E-mail: megomes@dep.uminho.pt

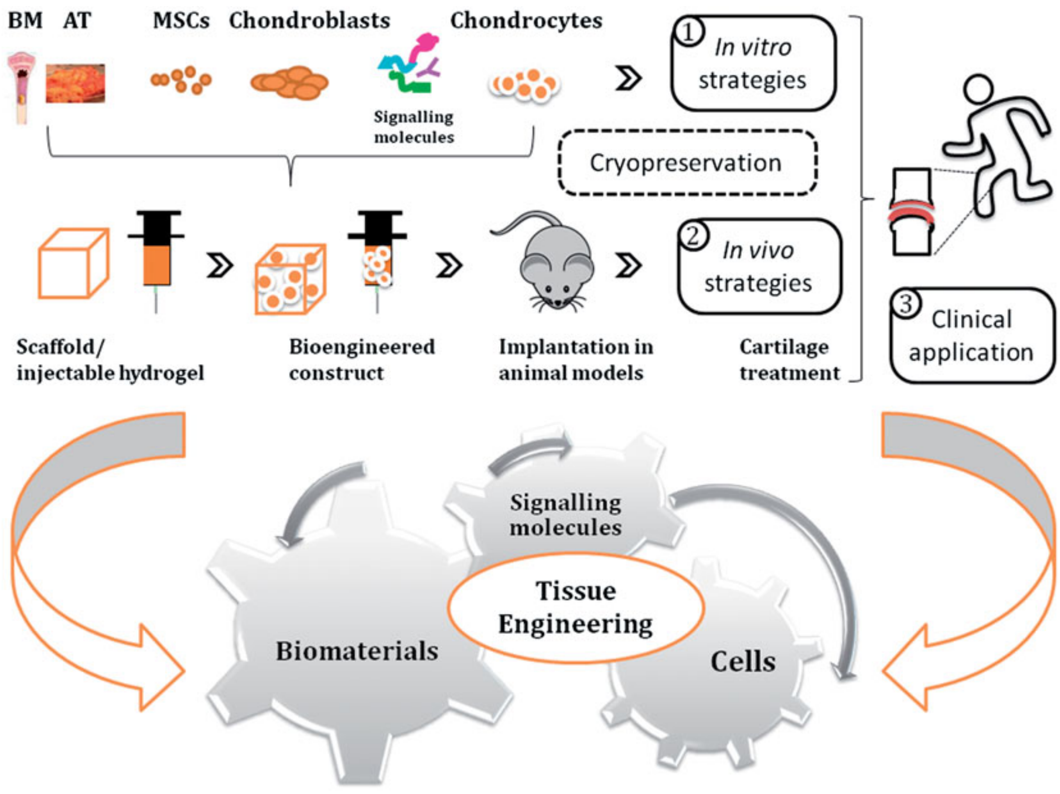

Figure 1. Schematic diagram showing multidisciplinary approach of cartilage tissue engineering (BM-bone marrow; AT-adipose tissue; MSCs-mesenchymal stem cells).

Critical Reviews in Biotechnology Downloaded from informahealthcare.com by Mrs Claire Summerfield on 04/08/14
For personal use only.

maintaining the round shape that characterizes specific cartilage cells phenotype (Abbott, 2003).

This review addresses the recent developments on the use of the above mentioned marine origin materials in studies envisioning the treatment and repair of cartilage defects. Several aspects are considered, namely the polysaccharide main characteristics/properties, *in vitro* and *in vivo* research and clinical/biomedical applications, with emphasis in cartilage TE (Figure 1).

Cartilage structure, pathologies and tissue engineering therapies

In order to design adequate therapies for the regeneration of damaged cartilage tissue it is essential to know and understand its structure, function and properties, so as to mimic as closely as possible its native environment. Cartilage tissue is a stiff, dense and inflexible connective tissue typically deprived of blood vessels, aneural and with few cells (Vunjak-Novakovic & Freed, 1998). Chondrocyte specific characteristics include no cell-to-cell contacts, spherical shape, high individual metabolic activity and the ability to synthesize type II collagen. Furthermore, the cells in articular cartilage receive nutrition through a double diffusion barrier, survive on low oxygen concentration and hence depend on anaerobic metabolism (Bhosale & Richardson, 2008). Details about composition with the corresponding ultra-structure can be found in Supplementary Figure S1. In fact, considering the low cellularity and proliferative capacity of chondrocytes and due to specific characteristics such as being bound in lacunae with low migrating abilities to damaged areas (Chhavi et al., 2011), cartilage tissue underlies an intrinsic inability to repair.

Damage of cartilage can be traumatic or degenerative and emerge as a result of a wide range of injuries or as an effect of another treated injury, having a great impact on the quality of life of thousands of people. Defects can be associated to partial and full thickness extension to the underlying bone and, based on the macroscopic changes of the articular cartilage, are categorized in grade I, II, III and IV (Suh et al., 1995). More detailed information on cartilage structure, diseases and treatment can be found elsewhere (Buckwalter & Mankin, 1998; Nesic et al., 2006).

Current strategies in the clinical field of cartilage repair progressed to what might be called the 3 ''R'' paradigm: reconstruction, repair and replacement (Haleem & Chu, 2010). Cartilage regeneration strategies have evolved from marrow stimulation–based techniques to osteochondral transplantation and to cell-based repair techniques. Microfracture, autogenic (mosaicplasty) and allogenic tissue transplantation techniques show positive results for short term but low outcomes for longer times (Berthiaume et al., 2011; Gross et al., 2005; Lattermann & Romine, 2009). The four generations of the therapeutic approach consisting in the autologous chondrocyte implantation (ACI) intend to preserve the chondrogenic phenotype, maintain cellular viability and function (Gikas et al., 2009; Peterson et al., 2002). The 3rd generation of ACI matrix-induced autologous chondrocyte implantation (MACI) uses 3-dimensional (3-D) supports to achieve such requirements. Alternative cell sources have been investigated, particularly allogenic adult mesenchymal stem cells (MSCs) (Marquass et al., 2011). A summary description

Critical Reviews in Biotechnology Downloaded from informahealthcare.com by Mrs Claire Summerfield on 04/08/14
For personal use only.

of these cartilage repair techniques is detailed in Supplementary Table S1. Although these approaches offer good opportunities for the regeneration of cartilage defects, all current treatment options inflict some degree of tissue destruction before any therapeutic effect can be achieved. Consequently, the current available treatments based on ''damage to heal approaches'' (Rodrigues et al., 2011), have limitations, which might be overcome through emerging TE strategies (Figure 1). The potential advantages include the formation of a more reliable hyaline cartilage tissue, through the delivery of appropriate cell types embedded in a suitable hydrogel (Tuli et al., 2003) promoting enhanced integration with surrounding tissues (Hardingham et al., 2002). However, to achieve further improvements, minimally invasive procedures and innovative cell carrier concepts should be refined (Sittinger et al., 2004).

Furthermore, there is a lot of debate over the use of most suitable cell source in cartilage cell-based therapies. Ideally, a cell source should be easily available, enabling an excellent yield number, high proliferation capacity, stable phenotype/genotype, with no issues of immunogenicity or disease transmission risks, and no donor site morbidity. For cartilage TE, most of the published studies refer to the use of primary autologous chondrocytes (Brittberg et al., 1994; Wang et al., 2006) and adult stem cells (Noth et al., 2008; Raghunath et al., 2005). There are a number of pros and cons arguments in applying stem cells versus somatic, pre-committed cells for cartilage tissue engineering as summarized in Supplementary Table S2. Disadvantages associated with the use of chondrocytes are mostly related to the collection site, limited number of cells with low renewal capacity and the tendency to dedifferentiate Supplementary Table S2 (Jakob et al., 2001; Von Der Mark et al., 1977). Bone marrow-derived stem cells (BMSCs) (Kuroda et al., 2007) and adipose tissue-derived cells (ASCs) (Rada et al., 2009; Varma et al., 2007) have been frequently considered top candidates for cartilage TE, due to their ability to create functional cartilaginous tissues. Advantages of the two stem cell sources may be clearly identified in terms of collection procedures, cell number, proliferation capacity, while the major disadvantage include the need of growth factors supplementation (Supplementary Table S2). Some studies described a higher chondrogenic potential of BMSCs when compared to ASCs (Niemeyer et al., 2010) likely due to their role in bone formation, including endochondral ossification.

Another important issue is to determine the optimal number of cells (chondrocytes or stem cells) for a successful *in vivo* application. Although this is still under consideration, several studies suggest that higher cellular content induces better tissue repair (Gulotta et al., 2012; Iwasa et al., 2003; Watt, 1988). Commercially available sources of chondrocyte suspensions recommend a dose between 0.5 and $2.0 \times 10^6/\text{cm}^2$ and 2 to 3×10^6 for a $4\,\text{cm}^2$ collagen membrane have been used in the clinical practice (Steinwachs et al., 2012). Moreover, the chondrocytes density in native cartilage tissue is around 1.4×10^7 cells/cm^3, i.e. about 5–10% of the cartilage volume, but such cell density is difficult to replicate *in vitro* due to slow growth rates that differ depending on the cell type (Buschmann et al., 1992). The *in vitro* experiments have confirmed that growth factor supplementation (Holland & Mikos, 2003) enhances the production of cartilage in tissue-engineered constructs (Blunk et al., 2002; Stoop, 2008). Clearly chondrogenesis is a complex process which involves not only biological growth factors, but also a carefully controlled time dependency (Bobick et al., 2009; Csaki et al., 2008). Furthermore, this process can be dramatically influenced by the 2D and 3D (two and three dimensional) environment in which cells are delivered/cultured. The fact that hydrogels provide 3D cellular microenvironments that can be tailored to stand physical, chemical and biological signals, has encouraged the development of engineered functional tissue equivalents based on such systems. In addition, the hydrogel structure protects the entrapped cells against the immune system of the host, simultaneously allowing the unhindered passage of nutrients, oxygen and secreted therapeutic factors or proteins (Zimmermann et al., 2007). The hydrogels design can vary from injectable systems to solid structure like discs, fibers, cylinder and capsules ranging from micro to macro dimensions.

Several natural origin hydrogels sources including proteins (collagen, elastin, silk fibroin, fibrin), polysaccharides (chitosan, chondroitin sulphate, hyaluronic acid) and seaweed source (alginate, agarose, carrageenan, ulvan) have been extensively studied for cartilage repair (Malafaya et al., 2007). According to data collected from the literature, the reasons for using seaweed polysaccharides in regeneration applications lie in their intrinsic features, such as chemical similarity with native tissue components, non-harsh processing, variable degrees of hydrophilicity and biocompatibility (Oliveira & Reis, 2010). Natural materials obtained from algae are often preferred for biological applications since they are believed to elicit low immune response when choosing a potential biological application (Gomes et al., 2008). Most of them offer advantages concerning biocompatibility, which is of extreme importance for the integration with the surrounding tissues. Moreover, they are readily available, inexpensive and easy to fabricate into hydrogels, making them appealing choices for different biomedical application (Ko et al., 2010).

Algae polysaccharides

Detailed characteristics

Some algae polysaccharide based hydrogels, namely alginate and agarose have already been extensively studied for TE applications, but others such as carrageenan and ulvan are just starting to be investigated (Table 1). Even though hardly applied in the field of cartilage repair, these algae polysaccharides have recently registered increased attention in the biomedical research field. Such consideration is owed to the intriguing feature of the amounts of sulphate groups found in their structure, whose beneficial biological properties prompt scientists to focus on their use in the biomedical field (Silva et al., 2012). Certainly, the presence of sulphate groups in their structure/composition and the chemical affinity with mammalian glycosaminoglycans (GAGs) due to the similar chemical content, play important roles in the antiviral, anticoagulant, antioxidant and anticancer activity of these polysaccharides (Vera et al., 2011).

Table 1. Important properties of algae polysaccharide.

Polysaccharide	Seaweed type	Gelation	Degradation	Cell interaction	Ref.
Alginate	Brown seaweed	Ionic (Ca^{2+})	Ion exchange; others	Low	Augst et al. (2006)
Agarose	Red seaweed	Thermal	Non-degradable	Low	Varoni et al. (2012)
Carrageenan	Red seaweed	Thermal and ionic (Ca^{2+}/K^{+})	Ion exchange	Low	Campo et al. (2009)
Ulvan	Green seaweed	Ionic (boric acid and divalent cations)	Enzymatic degradation	n.d.	Alves et al. (2013)

Modified from Hunt & Grover (2010) and Tan & Marra (2010).
n.d. – not determined.

Critical Reviews in Biotechnology Downloaded from informahealthcare.com by Mrs Claire Summerfield on 04/08/14
For personal use only.

The carrageenans are temperature dependent materials since they are soluble in water above 60 °C and gel upon cooling to temperatures between 30 and 40 °C, being designated as a physical hydrogels. The gelation of carrageenan is induced by the reversible temperature sensitive formation of intermolecular hydrogen bonds and involves a coil to helix conformational transition followed by helix aggregation (Mangione et al., 2003). The thermo-responsive solubility behavior and the gelation promoted by monovalent cations such as potassium ions, open perspectives to develop systems that gel at body temperature (Núñez-Santiago et al., 2011). They are characterized as high molecular weight polysaccharides with high sulfate-ester content, meaning higher levels of solubility and lower gel strength (Stanley, 1987). Ionically cross-linked hydrogels, such as carrageenan, normally undergo slow dissolution that can be shifted through chemical modification and the stiffness can be altered as well, allowing tuning of the mechanical properties (Daniel-da-Silva et al., 2009). Ulvan structure shows great complexity and variability as evidenced by the numerous oligosaccharide repeating structural units identified (Ray & Lahaye, 1995). The main constituents of ulvan are sulfated rhamnose residues linked to uronic acids, resulting in a repeated disaccharide unit β-D-glucuronosyl-(1,4)-α-L-rhamnose 3-sulfate, called aldobiouronic acid (Jaulneau et al., 2010). In aqueous solution, ulvan tends to form micro-aggregates, and the limited number of functional groups available for chemical modifications hampers its potential versatility (Chiellini & Morelli, 2011).

The friendly gelation mechanism induced by temperature or ions clearly demonstrates the relevance of the use of such biopolymers in the field of TE. In particular, sulphated polysaccharides (carrageenan and ulvan) present a real potential for delivering products for therapeutic applications providing a valid alternative to mammalian glycosaminoglycans.

More details regarding seaweed type, gelation or degradation mechanism and the interactions of alginate, agarose, carrageenan and ulvan with cells are summarized in Table 1. These polymers are cell friendly due to the ionic nature of the cross linking process. Nevertheless, the cell interaction is low and the degradation is still not fully studied. For complete description of chemical structure, gelation process or other characteristics of algae polysaccharide, we recommend the reader to other existing publications (Gomes et al., 2013; Lahaye & Robic, 2007; Silva et al., 2012).

However, not every characteristic comes only as an advantage. Supplementary Table S3 summarizes pros and cons features reported for the polysaccharide hydrogels. As advantages, the cheap source and the easy gelling characteristic stand out, although the ionic degradation, low mechanical properties and low cellular interaction might be difficult

to overcome. For example, the lack of manufacture reproducibility on a large scale, batch-to-batch variability and also the presence of impurities in the final processed material due to the extraction process, constitute major concerns (Yang et al., 2001). These are time- and money-consuming processes and introduce variation in the biological response. For example, recent data suggests that contaminants during extraction processing are likely to cause an immune response but this issue is still being disputed, although such molecules can be removed by purification methods (Klöck et al., 1997; Orive et al., 2006). Other associated common weakness of the seaweed-based hydrogels are related to inadequate mechanical properties, which is a common trend (Drury & Mooney, 2003). Nonetheless, it is expected that a hydrogel structure will partially tolerate shock absorption and deformation, mimicking articular cartilage characteristics. Enhanced mechanical properties can be achieved by adjusting various parameters, including the concentration, the cross-linking density and the possibility of mixing such hydrogels with composite. In addition, introducing photo-cross-linkable parts, appropriate to the chemical structure of the hydrogels, can modify the stiffness of the structure but simultaneously compromise the viability of the cells (Jeon et al., 2009).

In terms of cellular response, in these natural algae polysaccharides, like many hydrogels, there is no integrin interaction between cells and the hydrogels matrix. Therefore, cells retain their rounded shape, likely to enhance chondrogenesis (Steward et al., 2011). On the other hand, long-term integrin binding can lead to dedifferentiation and formation of fibrocartilage (Steward et al., 2011). However, being hydrophilic and binding to water, hydrogels provide few sites for the cells to attach, directly affecting cell viability and proliferation.

In vitro applications of hydrogels based on algae polysaccharide

Considering all the above mentioned polysaccharide hydrogels characteristics, this section will review some of the most extensively studied algae polysaccharide-based hydrogels, such as alginate and agarose and some new candidates, like carrageenan and ulvan, for the culture of chondrocytes and differentiation of stem cells. In what follows, it will be provided an overview of different approaches and parameters including hydrogel concentration, different cell source and cell number, as well as the stimulation with various growth factors, envisioning application in cartilage regeneration. Table 2 summarizes the most recent studies concerning cartilage TE applications using algae polysaccharide hydrogels.

Alginate

Alginate continues to be the most widely used hydrogel for *in vitro* studies due to its easy production, effectiveness and low cost (Tables 1 and 2). Applications of alginate in the

Table 2. A summary of key studies from the current literature describing polymers hydrogels, cell types and cell densities used in laboratory cartilage tissue engineering applications.

Polymer concentration	Cell source	Signaling molecules	Cell density (cells/mL)	Ref.
Alginate				
1.2% (w/v)	Human chondrocytes	–	2×10^6	Chia et al. (2005); Choi et al. (2006); Lee et al. (2007)
20 mg/mL; 2% (w/v)	Non-human chondrocytes	IGF-1, TGF- β1	$25–50 \times 10^6$	Coates & Fisher (2011); Gleghorn et al. (2007); Lee et al. (2007); Stevens et al. (2004b)
20 mg/mL	Human bone marrow MSCs	TGF-β3	20×10^6	Ma et al. (2012); Xu et al. (2008)
2%; 1.5% (w/v)	Non-human bone marrow MSCs	TGF-β1 or Dex;	20×10^6; $1–2 \times 10^6$	Bosnakovski et al. (2006); Coleman et al. (2007)
2%; 1.2% (w/v)	Human adipose tissue MSCs	TGF-β1; BMP-6	10×10^6; 5×10^6	Awad et al. (2004); Estes et al. (2006)
Agarose				
2% or 3% (w/v)	Non-human chondrocytes	TGF-β3	$30–60 \times 10^6$	Beris et al. (2005); Lima et al. (2007); Rada et al. (2009)
2% (w/v)	Non-human bone marrow MSCs and chondrocytes	TGF-β3 or -	$10–60 \times 10^6$	Bian et al. (2010); Kelly et al. (2006); Mauck et al. (2003); Mauck et al. (2006)
2% (w/v)	Human bone marrow MSCs	TGF-β3	3, 6, and 9×10^6	Charles Huang et al. (2004); Finger et al. (2007); Pelaez et al. (2009)
2% (w/v)	Human adipose tissue MSCs	TGF-β1	10×10^6	Awad et al. (2004)
2% (w/v)	Non human bone marrow & adipose MSCs	TGF-β1; TGF-β3	$10–15 \times 10^6$	Kisiday et al. (2008); Niemeyer et al. (2010); Sheehy et al. (2012); Steward et al. (2012); Thorpe et al. (2008)
Carrageenan				
0.8% and 1.2%	Human chondrocytes	–	2×10^6	Pereira et al. (2009)
1.5% (w/v) 2.5% (w/v)	Human adipose tissue MSCs; Human nasal chondrocytes;	TGF- β1	5×10^6	Popa et al. (2012); Rocha et al. (2011)
2% (w/v)	ATDC5-chondrocytic cell line	–	1×10^6	Popa et al. (2011)
Ulvan				
5%; 8% (w/v)	L929-mouse fibroblasts	–	5×10^5 cells/structure	Alves et al. (2012c)

GF – Growth factor; TGF – Transforming growth factor; BMP-6/BMP-2 – Bone morphogenetic protein – BMP-6; Dex – dexamethasone; IGF-1 – Insulin-line growth factor; ES – embryonic stem; ATDC5 – mouse teratocarcinoma AT805-derived cell line.

Critical Reviews in Biotechnology Downloaded from informahealthcare.com by Mrs Claire Summerfield on 04/08/14
For personal use only.

cartilage TE field revealed that after an initial cell loss, chondrocyte maintained their typical chondrocyte phenotype (van Susante et al., 1995). To maintain the chondrocytic phenotype and the synthesis of ECM proteins, alginate has been used *in vitro* as a matrix for the three-dimensional culture of human articular chondrocytes from elderly patients (Carossino et al., 2007). Similar performance was reported in another study where human articular chondrocytes, embedded in alginate beads, showed enhanced collagen type II and aggrecan expression (Gründer et al., 2004; Vinatier et al., 2009). Moreover, the 3D alginate culture system was proven to be efficient in keeping high viability, chondrogenic phenotype and promoting the redifferentiation of articular chondrocytes (Choi et al., 2006; Lin et al., 2009). A different work showed that chondrocytes encapsulated in alginate maintain their viability and function due to the addition of microchannels to the polymeric hydrogels (Choi et al., 2007). In another study, the supportive alginate-based hydrogels provided an adequate environment to deliver chondrocytes and, when compared with monolayer culture, stimulated the deposition of sulphated glycosaminoglycans (sGAG) and collagen type II, but without promoting redifferentiation (Chia et al., 2005). Furthermore, alginate hydrogels have been used to expand and induce stem cell differentiation (Tortelli & Cancedda, 2009). Human MSCs encapsulated in alginate beads undergo chondrogenesis, demonstrated by the cells assuming a rounded morphology with lacunae. Another report showed the development of hyaline cartilage-like tissue since it was, positively stained for Safranin-O and other typical chondrogenic markers, namely COL2A1 and COL10A1 (Ma et al., 2003). Human MSCs showed a time-dependent accumulation of sGAG, aggrecan and type II collagen in this type of hydrogel (Xu et al., 2008). In other study, bone marrow-derived stem cells (BMSCs) encapsulated in alginate evidences an enhanced cartilaginous matrix accumulation over agarose (Coleman et al., 2007). Alginate has been demonstrated to improve the stability of the system, supporting sGAG and collagen II production as well as chondrogenic gene expression, when human BMSCs were entrapped in a blend that included fibrin in addition to alginate (Ma et al., 2012). Induction of chondrogenesis of stem cells, isolated from adipose tissue in alginate hydrogels, has also been achieved. After 2 weeks of *in vitro* culture the adipose-derived stem cells produced cartilage matrix proteins which show dependency of the physical environment and the culture conditions (Erickson et al., 2002). To overcome weaknesses like low mechanical and uncontrollable degradation properties, ionically cross-linked alginate hydrogels have been modified, with no toxic effects on the encapsulated MSCs, supporting chondrogenic differentiation (Ghahramanpoor et al., 2011). Recently, investigators have produced hybrid constructs mixing alginate with other materials or tailored alginate with synthetic adhesion peptides as ways of improving its properties (Bidarra et al., 2011; Connelly et al., 2007; Wayne et al., 2005).

Agarose

Agarose has also been widely investigated in cartilage TE strategies (Table 2). For example, encapsulation within agarose hydrogels has shown to support rabbit articular chondrocytes redifferentiation (Benya & Shaffer, 1982). Articular chondrocytes seeded on agarose hydrogels demonstrated enhanced chondrogenic matrix elaboration when cultured under physiological deformational loading, accelerating the formation of a cartilage-like tissue (Mauck et al., 2000). Compressive mechanical forces are transmitted to the embedded chondrocytes which respond by producing extracellular matrix proteins leading to increased stiffness of the newly developed engineered tissue (Bougault et al., 2008). The culture of murine chondrocytes embedded within agarose hydrogels has been shown to maintain the chondrocytic phenotype, allowing matrix deposition around chondrocytes and showing that chondrocytes sense and respond to mechanical forces (Bougault et al., 2009). The concept of developing functional TE systems centered on the use of bioreactor enabling physiologic-like loading, has also reported successful results with immature bovine primary chondrocytes but did not produce the same outcome when using adult canine primary chondrocytes (Lima et al., 2007). Moreover, studies suggest that the age of the cells plays an important role, showing that unpassaged cells can elaborate an inferior matrix as compared to passaged mature chondrocytes. The continuous supplementation of TGF-β3 in combination with mechanically loading prior to implantation also seems to result in an improved engineered tissue substitute (Bian et al., 2010). Agarose hydrogels have also been investigated in combination with mesenchymal stem cells, including human adipose-derived stem cells and bovine mesenchymal stem cells, for a variety of applications, including cartilage repair, as shown in Table 2. Agarose hydrogels are noted for their ability to promote and maintain the chondrogenic phenotype of bone marrow stem cells (BMSCs), with deposition of cartilage-specific biomacromolecules (Huang et al., 2009). Another work showed that the chondrogenesis of the stem cells was directly correlated with the number of cells used, meaning that higher cell density led to enhanced expression of cartilage-specific gene (Charles Huang et al., 2004). It has also been reported that BMSCs produced an ECM with lower mechanical properties than that produced by differentiated articular chondrocytes embedded in agarose hydrogel (Erickson et al., 2009; Mauck et al., 2006). Findings reported in other publications such as the induction of chondrogenesis in hMSC-seeded agarose constructs without TGF-β, suggest that the application of hydrostatic pressure may initiate chondrogenesis faster than lower pressure (Finger et al., 2007). Although the exclusion of TGF-β3 from culture conditions has been reported to have a superior effect on the mechanical properties and also on the biochemical content (Huang et al., 2009), the functionality of cartilaginous tissues using MSCs from joint infrapatellar fat pad, encapsulated in agarose hydrogels, has resulted in robust chondrogenesis with TGF-β3 supplementation, as shown by another study (Buckley et al., 2010).

Carrageenan

Carrageenan hydrogels have been mostly used as drug or growth factor delivery systems (Daniel-da-Silva et al., 2011; Rocha et al., 2011; Santo et al., 2009,), immobilization of enzymes (Desai et al., 2004) and in pharmaceutical

Critical Reviews in Biotechnology Downloaded from informahealthcare.com by Mrs Claire Summerfield on 04/08/14
For personal use only.

Critical Reviews in Biotechnology Downloaded from informahealthcare.com by Mrs Claire Summerfield on 04/08/14
For personal use only.

formulations (Sipahigil & Dortunc, 2001). However, the characteristics and specific properties of these natural derived hydrogels concerning their potential for cartilage regeneration are poorly exploited in the literature (Pereira et al., 2009). Upon adding cations, the carrageenan solution rapidly forms a gel and can be used as *in situ* cell matrix delivery material, due to its mild cross-linking properties (Popa et al., 2011) which makes it extremely interesting to be applied in the biomedical field (D'Ayala et al., 2008). Nevertheless, carrageenan has been used for the encapsulation of human-adipose-derived stem cells (hASCs), human nasal chondrocytes (hNCs), and a chondrocytic cell line (ATDC5) showing potential for cartilage regeneration strategies (Popa et al., 2012). The findings from this study showed that hASCs embedded in k-carrageenan hydrogels exhibited higher expression of typical cartilage markers (collagen type II and aggrecan) than human chondrocytes, providing further evidence for the advantageous use of hASCs as an alternative cell source in cartilage treatments (Table 2). In a subsequent study, hASCs encapsulated in κ-carrageenan hydrogel and cultured with medium supplemented with chondrogenic growth factors appeared to increase the stiffness and the viscoelastic properties of the hydrogel construct providing a versatile platform for cartilage TE (Popa et al., 2013a).

Ulvan

Ulvans are the major constituents of green seaweeds cell walls (Ulvales, Chlorophyta) and are composed of rhamnose, glucuronic acid, iduronic acid, xylose and sulphate (Lahaye & Robic, 2007). These green algae can be prepared into different structure designs such as membranes (Alves et al., 2012b, 2013), particles (Alves et al., 2012a), hydrogels (Morelli & Chiellini, 2010) and 3D porous structures (Alves et al., 2012b). Due to the difficulties in identifying the chemical structure of algal sulfated polysaccharides, the relation between their structures and biological activities is not completely understood (Jiao et al., 2011). Despite ulvan chemical variability, biological properties have been frequently reported like antioxidant effects (Qi et al., 2010), antitumoral activity (Jiao et al., 2010), immunostimulating ability (Leiro et al., 2007). Antihyperlipidemic activities (Sathivel et al., 2008) or antiviral effects (El-Baky et al., 2009) have also been studied. Subsequent to all of these findings, ulvan polysaccharides are considered to have great potential for biomedical applications (Table 2). Nevertheless, relevant studies on ulvan are limited and their applicability may range from drug delivery (Alves et al., 2012b) to wound dressing or TE (Alves et al., 2012a, 2012c).

From the studies discussed above, it is possible to conclude that the redifferentiation of chondrocytes has been more successful using agarose hydrogels than alginate, although stem cells encapsulated in alginate displayed enhanced cartilaginous matrix accumulation over agarose (Chia et al., 2005; Coleman et al., 2007). Furthermore, these studies also showed that the addition of growth factors promoting stem cell chondrogenic differentiation increases the mechanical properties and the ECM content when using both alginate or agarose hydrogels construct. Moreover, the ECM produced by stem cells has lower mechanical characteristics compared to the ECM synthesized by articular chondrocytes in agarose hydrogels (Erickson et al., 2009; Mauck et al., 2006).

When comparing alginate and agarose hydrogels as cell encapsulation systems, it is suggested that the gelation mechanism influence the interaction with cells, considering that the cells interfere with the hydrogen bond formation required for agarose gelation (Shoichet et al., 1996). The addition of cells did not decrease alginate strength as much as for agarose gels, indicating that the cells have an impact over the mechanical properties of the hydrogels. The chemical structure is also responsible for different cellular behavior, since alginates with high-M or G and intermediate-G content produce distinct cell growth. Also, the protein diffusion profile and the presence of ligands for cell attachment will affect the proliferation and chondrogenic differentiation of the cells when using alginate or agarose constructs. Overall, the studies presented suggest that agarose is more biologically active when compared to the inert nature of alginate (Awad et al., 2004).

Some advantages of carrageenan hydrogels over current systems include the thermo-reversible and ionic nature enabling mild condition for cell encapsulation, versatility of processing them into various shapes/formats or use as injectable systems administrated under physiological conditions. However, a significant temporal weight loss of the 3D carrageenan hydrogel was observed when exposed to environments rich in electrolytes, ions and cations, a behavior that has been commonly reported for other ionic hydrogels (Nicodemus & Bryant, 2008). Such behavior might be overcome through chemical modification. Ulvan hydrogel exposed versatility, enabling processing in different shapes; in addition, the presence of functional groups allow them to be easily chemically modified, although the unusual chemical composition and structure did not allow a standardized commercial form of this polysaccharide. Nevertheless, the potential of the ulvan hydrogel to be used in TE and regenerative medicine is still barely explored during in vivo studies on algae polysaccharide based hydrogels.

In vivo studies on algae polysaccharide based hydrogels

As a first step in demonstrating the *in vivo* chondrogenic potential of tissue engineered constructs, researchers have used heterotopic animal models, typically consisting on the implantation of the bioengineered cartilage-like tissue in dorsal subcutaneous pouches of immuno-compromised nude mice (Reinholz et al., 2004). Although these experiments may indicate the biological construct performance under *in vivo* conditions, different results may be observed when the cartilage substitute is implanted into a cartilage defect.

Alginate

Several *in vivo* studies have been conducted aiming at assessing the potential of alginate as a supportive matrix for different relevant cell types, including primary chondrocytes and stem cells from different origins, in various animal models (Table 3). Alginate seems to stimulate chondrogenesis as suggested by studies where chondrocytes were seeded and stimulated to produce a cartilage-like matrix, being

Critical Reviews in Biotechnology Downloaded from informahealthcare.com by Mrs Claire Summerfield on 04/08/14
For personal use only.

Table 3. A summary of key studies describing polymers hydrogels, cell types, densities and implantation time register in experimental animal models for cartilage tissue engineering applications.

Polymer concentration	Cell type	Formulation cells density	Animal model (defect)	Follow up time	Ref.
Alginate					
2% sodium alginate; 1.2%; 0.75%	Chondrocytes	10–50 × 10^6 cells/mL	Subcutaneous implantation, mice; defect in rabbits;	8 weeks; 30 weeks; 6 months	Chang et al. (2001); Marijnissen et al. (2002)
2% alginate; 1.2% alginate beads	Chondrocytes	50 × 10^6 cells/mL; 10^7/mL; 4 × 10^6 cells/mL	Subcutaneous implantation, sheep/nude mice; defects 4 mm diameter mini-pigs	1 week; 5 weeks; 6 months;	Chang et al. (2003); Chawla et al. (2007); Kaps et al. (2002)
1.2%; 2% sodium alginate	Bone marrow MSCs	1 × 10^6 cells/defect	Ø3 × 6 mm defects in rabbits; Ø4 × 5 mm defects in rabbit	3 months	Diduch et al. (2000); Lopiz-Morales et al. (2010)
2.2% barium alginate; 1.25% sodium alginate	Human and mouse ESCs	10 × 10^6 cells/mL	SCID and BALB/c nude mice		Bai et al. (2010); Dean et al. (2006)
Agarose					
2%	Chondrocytes	–	Rabbit	18 Months	Rahfoth et al. (1998)
2%	Chondrocytes	30 × 10^6 cells/mL.	Canine knee	12 Weeks	Ng et al. (2010)
0.6–1%	Periosteum	–	Rabbit knee	6 Weeks	Stevens et al. (2004a)
0.6%	Human bone marrow/ adipose tissue MSCs	10 × 10^6 cells/mL	Injection, muscles of athymic mice	5 Weeks	Xu et al. (2008)
2%	Bone marrow MSCs	20 × 10^6 cells/mL	Subcutaneous implantation, nude mice	28 Days	Vinardell et al. (2012)

MSCs – mesenchymal stem cells; Ø – diameter; SCID – albino mouse strain with spontaneous severe combined immunodeficiency mutation; BALB/c – albino, immunodeficient inbred mouse strain; ESCs – embryonic stem cells.

subsequently implanted subcutaneously in nude mice for 8 weeks (Marijnissen et al., 2002). The same study suggests that the addition of alginate provided retention of the cartilage graft shape without any influence on the amount of cartilage matrix proteins produced per tissue wet weigh. The implantation of alginate with allogenic rabbit chondrocytes, (followed up for 6 months), provided complete *in vivo* reconstruction of a full-thickness articular cartilage defect (Fragonas et al., 2000). After 6 months, the implantation of alginate alone developed only a fibrous cartilage while the suspension of chondrocytes led to the recovery of a normal tissue structure. On the other hand, transplanted chondrocytes in an alginate gel implanted into full-thickness osteochondral defects in rabbits did not form repaired tissue and cell numbers decreased with time (Mierisch et al., 2003). The evaluation of chondrogenesis of human adipose tissue-derived stromal cells when cultured in alginate hydrogels and implanted subcutaneously in nude mice for up to 20 weeks (Beris et al., 2005), reveled significant production of cartilage matrix proteins, suggesting the beneficial use of alginate systems for cartilage regeneration, as they maintain stable cartilaginous phenotype with no sign of hypertrophy during 20 weeks. Also, using a rabbit model, full-thickness defects filled with alginate beads containing rabbit stromal cells remained viable, showing chondrogenic phenotype embedded in a positively stained proteoglycans matrix and occupying the defects within regenerated tissue (Diduch et al., 2000).

Agarose

Agarose hydrogels laden with different cells types have been studied to assess their ability to induce the *in vivo* development of cartilage tissue (Table 3). One of these studies showed that, 18 months after the transplantation of allogenic chondrocytes in agarose hydrogels in rabbits, neo cartilage tissue was formed, exhibiting higher type II collagen and proteoglycan contents when compared to untreated defects. Additionally, control implants of agarose alone produced poor fibrous substitute tissue, insufficient healing and incomplete filling of the cartilage defects (Rahfoth et al., 1998). In another study, the implantation of chondral and osteochondral constructs based on primary or passaged (using growth factors) canine chondrocytes encapsulated in agarose, showed no gross or histological signs of rejection and excellent integration with surrounding cartilage and subchondral bone (Ng et al., 2010). However, when agarose constructs were seeded with stem cells and subcutaneously implanted in nude mice, they showed a significant decrease of sGAG content, while no significant change was observed using primary chondrocytes, indicating that the *in vitro* generated chondrocyte-like phenotype was transient (Vinardell et al., 2012).

Laboratory to clinical application

Although the *in vivo* trials involving cell laden hydrogels have registered different outcomes, overall, the use of hydrogels in cartilage regeneration strategies produce positive results.

The clinical approach to any cartilage repair technique should be customized based on both patient-specific and lesion-specific factors (Haleem & Chu, 2010).

Critical Reviews in Biotechnology Downloaded from informahealthcare.com by Mrs Claire Summerfield on 04/08/14
For personal use only.

Table 4. Commercial products in cartilage tissue engineering.

Product name	Company	Product composition	Regulatory status	Form and Use	Ref.
Cartipatch	Tissue Bank of France, Lyon, France	Agarose-alginate hydrogel & autologous chondrocytes	Clinical trial phase is unclear	Chondral and osteochondral	Selmi et al. (2008)
Carticel, MACI	Genzyme Biosurgery, Cambridge, Massachusetts, USA	Type I/III collagens (porcine) and autologous chondrocytes	Completed phase III clinical trials; product not available	Sheet- articular cartilage injury	Bahuaud et al. (1998); Bartlett et al. (2005); Cherubino et al. (2003)
Atelocollagen	Koken Co. Ltd, Tokyo, Japan	Type I collagen	Clinical trial phase is unclear		Adachi et al. (2007); Adachi et al. (2006)
CaReS	Arthro Kinetics, Esslingen, Germany	Type I collagen (rat-tail) and autologous chondrocytes	2007 Germany;	3D discs –articular cartilage injury	Maus et al. (2008)
Novocart 3D	TETEC Tissue Engineering Technologies AG, Reutlingen, Germany	Collagen-chondroitin-sulfate based membrane and autologous chondrocytes	Clinical trial phase is unclear		Ochs et al. (2007)
Hyalograft® C	Fidia Advanced Biopolymers, Abano Terme, Italy	Benzylic ester of hyaluronic acid (roosters) and autologous chondrocytes	Commercial sale began in 2000; completed phase III clinical trials	3D discs –articular cartilage injury	Kon et al. (2009); Manfredini et al. (2007); Vijayan et al. (2012)
BioSeed-C	Biotissue Technologies, Freiburg, Germany	Polymer of polyglycolic/polylactic acid and polydioxanone and autologous chondrocytes	First clinical trials were in 2001; completed phase II clinical trials	3D discs –articular cartilage injury	Kreuz et al. (2009)

Adapted from Ahmed & Hincke (2010); Jacobi et al. (2011); Place et al. (2009). Status and descriptions of the clinical trials are available from the web site of U.S. National Institutes of Health, http://clinicaltrials.gov/.

The engineered graft has to fit completely into the damaged area, providing support and biological cues to restore the tissue function. A well-designed clinical model to regenerate cartilage should always consider the safety, efficiency and simplicity of the developed system, even if it is necessary to compromise the quality of the regenerated tissue (Chiang & Jiang, 2009). Additionally, to translate the research-scale production into routine clinical use, biological cartilage substitutes will need to demonstrate cost-efficiency ratios that are beneficial over other existing treatments and excellent reproducible results in order to perform reliable quality control and standardization (Pelttari et al., 2009).

One important development for the translation of TE products into the clinical/industrial scenario would be the production of ''off the shelf'' products, eliminating the waiting time and reducing the patient's incapacity period, relying on the availability of the engineered grafts upon immediate clinical need. This could be achieved by common cryopreservation approaches, (Bhakta et al., 2009; Kuleshova et al., 2007) specifically designed and scaled up to generate ready-to-use engineered tissue substitutes. However, few studies have focused on tissue substitutes banking and storage technologies, addressing the impact of such process on hybrid constructs that contain a scaffold/carrier material and cells (Bhakta et al., 2009; Chen et al., 2011). Yet, there are studies that have reported the effect of cryopreservation in cell encapsulating systems (hydrogels) (Miyamoto et al., 2009; Popa et al., 2013b) using several different cryoprotectants, (Campbell & Brockbank, 2007; Thirumala et al., 2010,) and vitrification solutions (Kuleshova et al., 2007; Lawson et al., 2011), predicting that tissue-engineered products can be cryopreserved without prejudice of their functionality

It is already possible to find several hydrogels approved or under clinical trials for cartilage treatments (Table 4). The most well-known commercial product based on polysaccharides is a hybrid medical grade agarose-alginate hydrogel, named Cartipatch® (Company/Responsible – TBF Genie Tissulaire, Mions, France). This product was tested for implantation of autologous chondrocytes in chondral and osteochondral human defects. After a minimum follow-up of two years, patients with lesions larger than $3\,cm^2$ improved significantly more than those with smaller lesions. Also, in 8 out of 13 patients, hyaline cartilage-like repaired tissue was predominantly observed (Selmi et al., 2008). In a phase III clinical trial, an alginate gel suspension (Curis Inc., Lexington, MA) was used, injecting autologous chondrocytes harvested from the ear cartilage for the treatment of pediatric patients with vesicoureteral reflux. As seen in Table 4 the majority of commercial tissue engineering products developed for cartilage regeneration are collagen-based hydrogels, since collagen is a major component of ECM and due to the presence of bioactive domains in its structure, which regulates important processes during chondrogenesis (Ahmed & Hincke, 2010). However, other clinical products are being used to promote cartilage repair namely, hyaluronate (hyaluronic acid) or synthetic polymers. Although there is an extensive range of products being studied for cartilage treatments, only few of them are approved for clinical trials.

Ultimately, the essential biopolymers properties that prevent the industrial scale-up and clinical applications of these hydrogels are the existing batch-to batch differences, the inferior mechanical characteristics and low cell interaction.

Summary and future directions

Research and knowledge on polysaccharide-based hydrogels for TE application is continuously growing and there are many questions that instigate the attempts to use them in cartilage regeneration therapies. To mimic the structural (biochemical and histological characteristics) and functional (compression properties) complexity of native cartilage is a challenging objective to accomplish. Consequently, every attempt to achieve this aim brings scientists closer in the process of assembling the necessary knowledge to repair damaged cartilage tissue.

The knowledge and research, so far, supports that natural cell-carrying matrices based on algae polysaccharide are suitable for engineering cartilage. Many of these hydrogels can be combined well with the cells and can be designed as injectable systems, setting *in situ* and filling any shape and size of the recipient defect. All of these available data need to be compiled in order to develop clinical relevant solutions for cartilage regeneration, considering that the functionality of these hydrogels in the laboratory and animal experiments has been established.

For *in vitro* applications, the developed tissue engineered constructs show promising results, the potential value for the *in vivo* treatment of cartilage defects remains uncertain, as set-up parameters of the performed *in vitro* and *in vivo* studies vary to a great extent. Furthermore, *in vitro* manipulation of either chondrocytes or stem cells used is required in most of the currently available approaches. In fact, culturing the cells prior to *in vivo* implantation allows creating a mature cartilage and the presence of matrix around the cells is known to enhance donor cell retention at the repair site (Berthiaume et al., 2011). However, the manipulation of the cells *in vitro* increases concerns on how cells may be affected and increases the complexity of the treatment, compromising the safety of the procedure and the associated cost. Despite the advantages of using polysaccharide hydrogels in cartilage TE, some concerns remain which results mainly from insufficient characterization of the cell hydrogel construct or the level of repaired tissue formation and from too short follow-up periods. Findings have demonstrated that cell density is likely a key parameter to consider in TE design, since cells could not develop cell-cell contacts or express cartilage-like tissue component without some minimal cell density which can vary with the source of cells (Troken et al., 2007). Also, cells respond differently to substrate rigidity. Therefore, the increase in polymer concentration or stiffness involves consequences on the cell morphology, cytoskeletal structure and on stem cell differentiation (Engler et al., 2006; Yeung et al., 2005). Although current cartilage TE strategies investigate a wide range of hydrogel materials in combination with a large variety of cell sources, the existing data demonstrates that the optimal hydrogel type has not been determined so far, while outcomes also depend on the cell type – stages of differentiation and culture conditions (Renth & Detamore, 2012). Nevertheless, hydrogels based on marine origin polysaccharides are still commonly used and each individual natural polymer has strengths and weaknesses to its use and results can vary depending on the application.

Besides these comments, consideration should be given to the lesion location and damage size, activity level and patients' age. These are influencing parameters in choosing the right cartilage repair techniques and controlling the outcome of the treatment. More clinical trials are needed to find definitive answers and to develop procedures that relieve patient pain and produce a durable replacement for damaged cartilage. Until the present day, no treatment has been shown to completely regenerate or restore articular cartilage/subchondral bone to its normal status, therefore articular cartilage repair remains under intense investigation and the cure is yet to be defined. The newer generation of repair/regeneration techniques has shown some promise, but the long-term outcome is still unknown.

Furthermore, the extended variety of existing polysaccharides and their inherent features opens a wide range of opportunities for synergistic fabrications of new multifunctional biomaterials (Shull, 2012; Sun et al., 2012). The development of strategies aiming to overcome hydrogels limitations is gaining increasing interest. Such alternatives encompass the fabrication of blended systems, or chemically and/or physically modification of their original structure. Modifying the hydrophobic properties of the polysaccharides to self-associate, reducing the molecular weight by depolymerization and changing the sulfation content are ways to improve polysaccharide-hydrogels properties (Lawson et al., 2011). The addition of interest signaling molecules is another way to confer a better outcome performance to the overall tissue engineered constructs (Freyria & Mallein-Gerin, 2012).

The opportunities and trends regarding the use of natural based hydrogels are currently directed towards strategies for chemical modification through photopolymerization, allowing temporal and spatial control of various features (Jeon et al., 2009) and to technologies to conjugate peptides (Yamada et al., 2010).

Final remarks

The purpose of this review is to detail a range of the most commonly used biomaterial hydrogels based on algae polysaccharides for transplantation of chondrocytes or stem cells in a cartilage regeneration strategy approach. The progress from the *in vitro* experimental cartilage TE research, using relevant cells types, to the *in vivo* scenarios was described throughout the demonstration of applicable results. Ultimately, some hydrogels have already been submitted through clinical trials, showing that it is possible to maximize the generated knowledge and attain the fabrication of medical products that make use of these systems.

With regard to their prevalence in the cartilage TE field, it is easily identified that alginate is the most commonly used polysaccharide followed by agarose, whereas polysaccharide κ-carrageenan and ulvan have only recently been proposed, reflected by the few found published reports. It is clear that these biopolymers provide a versatile class of hydrogels that

Critical Reviews in Biotechnology Downloaded from informahealthcare.com by Mrs Claire Summerfield on 04/08/14
For personal use only.

may found widespread application in the field of regenerative medicine.

Declaration of interest

Authors report no declarations of interest. Authors thank the Portuguese Foundation for Science and Technology (FCT) for the PhD fellowship of Elena G. Popa (SFRH/BD/64070/2009) and research project (MIT/ECE/0047/2009). The research leading to these results has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° REGPOT-CT2012-316331-POLARIS.

Critical Reviews in Biotechnology Downloaded from informahealthcare.com by Mrs Claire Summerfield on 04/08/14
For personal use only.

References

Abbott A. (2003). Cell culture: biology's new dimension. Nature, 424, 870–2.

Adachi N, Ochi M, Deie M, et al. (2007). Osteonecrosis of the knee treated with a tissue-engineered cartilage and bone implant a case report. J Bone Joint Surg, 89, 2752–7.

Adachi N, Ochi M, Deie M, et al. (2006). Lateral compartment osteoarthritis of the knee after meniscectomy treated by the transplantation of tissue-engineered cartilage and osteochondral plug. Arthroscopy, 22, 107–12.

Ahmed TA, Hincke MT. (2010). Strategies for articular cartilage lesion repair and functional restoration. Tissue Eng Part B Rev, 16, 305–29.

Aizawa Y, Owen SC, Shoichet MS. (2012). Polymers used to influence cell fate in 3D geometry: new trends. Prog Polym Sci, 37, 645–58.

Alves A, Duarte ARC, Mano JF, et al. (2012a). PDLLA enriched with ulvan particles as a novel 3D porous scaffold targeted for bone engineering. J Supercrit Fluids, 65, 32–8.

Alves A, Pinho ED, Neves NM, et al. (2012b). Processing ulvan into 2D structures: cross-linked ulvan membranes as new biomaterials for drug delivery applications. Int J Pharm, 426, 76–81.

Alves A, Sousa R, Reis R. (2013). A practical perspective on ulvan extracted from green algae. J Appl Psychol, 25, 407–24.

Alves A, Sousa RA, Reis RL. (2012c). Processing of degradable ulvan 3D porous structures for biomedical applications. J Biomed Mater Res A, 101A, 998–1006.

Andersen T, Berit LS, Formo K, et al. (2012). Alginates as biomaterials in tissue engineering. Carbohydr Chem, 37, 227–58.

Augst AD, Kong HJ, Mooney DJ. (2006). Alginate hydrogels as biomaterials. Macromol Biosci, 6, 623–33.

Aust L, Devlin B, Foster SJ, et al. (2004). Yield of human adipose-derived adult stem cells from liposuction aspirates. Cytotherapy, 6, 7–14.

Awad HA, Quinn Wickham M, Leddy HA, et al. (2004). Chondrogenic differentiation of adipose-derived adult stem cells in agarose, alginate and gelatin scaffolds. Biomaterials, 25, 3211–22.

Bahuaud J, Maitrot RC, Bouvet R, et al. (1998). Implantation of autologous chondrocytes for cartilagenous lesions in young patients. A study of 24 cases. Chirurgie, 123, 568–71.

Bai HY, Chen GA, Mao GH, et al. (2010). Three step derivation of cartilage like tissue from human embryonic stem cells by 2D-3D sequential culture in vitro and further implantation in vivo on alginate/PLGA scaffolds. J Biomed Mater Res A, 94A, 539–46.

Bartlett W, Skinner JA, Gooding CR, et al. (2005). Autologous chondrocyte implantation versus matrix-induced autologous chondrocyte implantation for osteochondral defects of the knee. J Bone Joint Surg Br, 87-B, 640–5.

Benya PD, Shaffer JD. (1982). Dedifferentiated chondrocytes reexpress the differentiated collagen phenotype when cultured in agarose gels. Cell, 30, 215–24.

Beris AE, Lykissas MG, Papageorgiou CD, Georgoulis AD. (2005). Advances in articular cartilage repair. Injury, 36, S14–23.

Berthiaume F, Maguire TJ, Yarmush ML. (2011). Tissue engineering and regenerative medicine: history, progress, and challenges. Annu Rev Chem Biomol Eng, 2, 403–30.

Bhakta G, Lee KH, Magalhaes R, et al. (2009). Cryopreservation of alginate-fibrin beads involving bone marrow derived mesenchymal stromal cells by vitrification. Biomaterials, 30, 336–43.

Bhosale AM, Richardson JB. (2008). Articular cartilage: structure, injuries and review of management. Br Med Bull, 87, 77–95.

Bian L, Fong JV, Lima EG, et al. (2010). Dynamic mechanical loading enhances functional properties of tissue-engineered cartilage using mature canine chondrocytes. Tissue Eng Part A, 16, 1781–90.

Bidarra SJ, Barrias CC, Fonseca KB, et al. (2011). Injectable in situ crosslinkable RGD-modified alginate matrix for endothelial cells delivery. Biomaterials, 32, 7897–904.

Blunk T, Sieminski AL, Gooch KJ, et al. (2002). Differential effects of growth factors on tissue-engineered cartilage. Tissue Eng, 8, 73–84.

Bobick BE, Chen FH, Le AM, Tuan RS. (2009). Regulation of the chondrogenic phenotype in culture. Birth Defects Res C Embryo Today, 87, 351–71.

Bosnakovski D, Mizuno M, Kim G, et al. (2006). Chondrogenic differentiation of bovine bone marrow mesenchymal stem cells (MSCs) in different hydrogels: influence of collagen type II extracellular matrix on MSC chondrogenesis. Biotechnol Bioeng, 93, 1152–63.

Bougault C, Paumier A, Aubert-Foucher E, Mallein-Gerin F. (2008). Molecular analysis of chondrocytes cultured in agarose in response to dynamic compression. BMC Biotechnol, 8, 71.

Bougault C, Paumier A, Aubert-Foucher E, Mallein-Gerin F. (2009). Investigating conversion of mechanical force into biochemical signaling in three-dimensional chondrocyte cultures. Nat Protoc, 4, 928–38.

Brittberg M, Lindahl A, Nilsson A, et al. (1994). Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N Engl J Med, 331, 889–95.

Buckley CT, Vinardell T, Thorpe SD, et al. (2010). Functional properties of cartilaginous tissues engineered from infrapatellar fat pad-derived mesenchymal stem cells. J Biomech, 43, 920–6.

Buckwalter JA, Mankin HJ. (1998). Articular cartilage: tissue design and chondrocyte-matrix interactions. Instr Course Lect, 47, 477–86.

Buschmann MD, Gluzband YA, Grodzinsky AJ, et al. (1992). Chondrocytes in agarose culture synthesize a mechanically functional extracellular matrix. J Orthop Res, 10, 745–58.

Campbell LH, Brockbank KG. (2007). Serum-free solutions for cryopreservation of cells. In Vitro Cell Dev Biol Anim, 43, 269–75.

Campo VL, Kawano DF, Silva Jr DBd, Carvalho I. (2009). Carrageenans: biological properties, chemical modifications and structural analysis - A review. Carbohydr Polym, 77, 167–80.

Carossino A, Recenti R, Carossino R, et al. (2007). Methodological models for in vitro amplification and maintenance of human articular chondrocytes from elderly patients. Biogerontology, 8, 483–98.

Chang SCN, Rowley JA, Tobias G, et al. (2001). Injection molding of chondrocyte/alginate constructs in the shape of facial implants. J Biomed Mater Res, 55, 503–11.

Chang SCN, Tobias G, Roy AK, et al. (2003). Tissue Engineering of Autologous Cartilage for Craniofacial Reconstruction by Injection Molding. Plast Reconstr Surg, 112, 793–9.

Charles Huang CY, Reuben PM, D'Ippolito G, et al. (2004). Chondrogenesis of human bone marrow-derived mesenchymal stem cells in agarose culture. Anat Rec A Discov Mol Cell Evol Biol, 278A, 428–36.

Chawla K, Klein TJ, Schumacher BL, et al. (2007). Short-term retention of labeled chondrocyte subpopulations in stratified tissue-engineered cartilaginous constructs implanted in vivo in mini-pigs. Tissue Eng, 13, 1525–37.

Chen F, Zhang W, Wu W, et al. (2011). Cryopreservation of tissue-engineered epithelial sheets in trehalose. Biomaterials, 32, 8426–35.

Cherubino P, Grassi FA, Bulgheroni P, Ronga M. (2003). Autologous chondrocyte implantation using a bilayer collagen membrane: a preliminary report. J Orthop Surg, 11, 10–15.

Chhavi S, Sneh G, Amit KD, Narayan CM. (2011). Cartilage tissue engineering: current scenario and challenges. Adv Mat Lett, 2, 90–9.

Chia SH, Homicz MR, Schumacher BL, Thonar EJMA, et al. (2005). Characterization of human nasal septal chondrocytes cultured in alginate. J Am Coll Surg, 200, 691–704.

Chiang H, Jiang C-C. (2009). Repair of articular cartilage defects: review and perspectives. J Formos Med Assoc, 108, 87–101.

Chiellini F, Morelli A. (2011). Ulvan: a versatile platform of biomaterials from renewable resources. In: Prof. Rosario P, ed. Biomaterials - Physics and Chemistry, Rijeka: InTech, DOI: 10.5772/24901.

Choi BH, Woo J-I, Min B-H, Park SR. (2006). Low-intensity ultrasound stimulates the viability and matrix gene expression of human articular

Critical Reviews in Biotechnology Downloaded from informahealthcare.com by Mrs Claire Summerfield on 04/08/14
For personal use only.

chondrocytes in alginate bead culture. J Biomed Mater Res A, 79A, 858–64.

Choi NW, Cabodi M, Held B, et al. (2007). Microfluidic scaffolds for tissue engineering. Nat Mater, 6, 908–15.

Coates E, Fisher JP. (2011). Gene expression of alginate-embedded chondrocyte subpopulations and their response to exogenous IGF-1 delivery. J Regen Med Tissue Eng, 6, 179–92.

Coleman RM, Case ND, Guldberg RE. (2007). Hydrogel effects on bone marrow stromal cell response to chondrogenic growth factors. Biomaterials, 28, 2077–86.

Connelly JT, García AJ, Levenston ME. (2007). Inhibition of in vitro chondrogenesis in RGD-modified three-dimensional alginate gels. Biomaterials, 28, 1071–83.

Csaki C, Schneider PRA, Shakibaei M. (2008). Mesenchymal stem cells as a potential pool for cartilage tissue engineering. Ann Anat, 190, 395–412.

D'Ayala GG, Malinconico M, Laurienzo P. (2008). Marine derived polysaccharides for biomedical applications: chemical modification approaches. Molecules, 13, 2069–106.

Daniel-da-Silva AL, Fateixa S, Guiomar AJ, et al. (2009). Biofunctionalized magnetic hydrogel nanospheres of magnetite and kappa-carrageenan. Nanotechnology, 20, 355602.

Daniel-da-Silva AL, Ferreira L, Gil AM, Trindade T. (2011). Synthesis and swelling behavior of temperature responsive κ-carrageenan nanogels. J Colloid Interface Sci, 355, 512–17.

Daniel-Da-Silva AL, Lopes AB, Gil AM, Correia RN. (2007). Synthesis and characterization of porous κ-carrageenan/calcium phosphate nanocomposite scaffolds. J Mater Sci, 42, 8581–91.

Dean SK, Yulyana Y, Williams G, et al. (2006). Differentiation of encapsulated embryonic stem cells after transplantation. Transplantation, 82, 1175–84.

Desai PD, Dave AM, Devi S. (2004). Entrapment of lipase into K-carrageenan beads and its use in hydrolysis of olive oil in biphasic system. J Mol Catal B Enzym, 31, 143–50.

Diduch DR, Jordan LCM, Mierisch CM, Balian G. (2000). Marrow stromal cells embedded in alginate for repair of osteochondral defects. Arthroscopy, 16, 571–7.

Drury JL, Mooney DJ. (2003). Hydrogels for tissue engineering: scaffold design variables and applications. Biomaterials, 24, 4337–51.

El-Baky HHA, Baz FKE, Baroty GSE. (2009). Potential biological properties of sulphated polysaccharides extracted from the macroalgae *Ulva lactuca* L. Acad J Cancer Res, 2, 1–11.

Engler AJ, Sen S, Sweeney HL, Discher DE. (2006). Matrix elasticity directs stem cell lineage specification. Cell, 126, 677–89.

Erickson GR, Gimble JM, Franklin DM, et al. (2002). Chondrogenic potential of adipose tissue-derived stromal cells in vitro and in vivo. Biochem Biophys Res Commun, 290, 763–9.

Erickson IE, Huang AH, Chung C, et al. (2009). Differential maturation and structure-function relationships in mesenchymal stem cell- and chondrocyte-seeded hydrogels. Tissue Eng Part A, 15, 1041–52.

Estes BT, Wu AW, Guilak F. (2006). Potent induction of chondrocytic differentiation of human adipose-derived adult stem cells by bone morphogenetic protein 6. Arthritis Rheum, 54, 1222–32.

Finger AR, Sargent CY, Dulaney KO, et al. (2007). Differential effects on messenger ribonucleic acid expression by bone marrow-derived human mesenchymal stem cells seeded in agarose constructs due to ramped and steady applications of cyclic hydrostatic pressure. Tissue Eng, 13, 1151–8.

Fragonas E, Valente M, Pozzi-Mucelli M, et al. (2000). Articular cartilage repair in rabbits by using suspensions of allogenic chondrocytes in alginate. Biomaterials, 21, 795–801.

Freyria A-M, Mallein-Gerin Fdr. (2012). Chondrocytes or adult stem cells for cartilage repair: the indisputable role of growth factors. Injury, 43, 259–65.

Ghahramanpoor M, Hassani Najafabadi S, Abdouss M, et al. (2011). A hydrophobically-modified alginate gel system: utility in the repair of articular cartilage defects. J Mater Sci Mater Med, 22, 2365–75.

Gikas PD, Bayliss L, Bentley G, Briggs TW. (2009). An overview of autologous chondrocyte implantation. J Bone Joint Surg Br, 91, 997–1006.

Gleghorn JP, Jones ARC, Flannery CR, Bonassar LJ. (2007). Boundary mode frictional properties of engineered cartilaginous tissues. Eur Cell Mater, 14, 20–28.

Gomes M, Azevedo H, Malafaya P, et al. (2008). Chapter 6. Natural polymers in tissue engineering applications. In: Clemens van B and others, eds. Tissue engineering. Burlington: Academic Press, 145–92.

Gomes M, Azevedo H, Malafaya P, et al. (2013). Chapter 16. Natural polymers in tissue engineering applications. In: Sina E, ed. Handbook of biopolymers and biodegradable plastics. Boston: William Andrew Publishing, 385–25.

Grenha A, Gomes ME, Rodrigues M, et al. (2009). Development of new chitosan/carrageenan nanoparticles for drug delivery applications. J Biomed Mater Res A, 92A, 1265–72.

Gross AE, Shasha N, Aubin P. (2005). Long-term followup of the use of fresh osteochondral allografts for posttraumatic knee defects. Clin Orthop Relat Res, 435, 79–87.

Gründer T, Gaissmaier C, Fritz J, et al. (2004). Bone morphogenetic protein (BMP)-2 enhances the expression of type II collagen and aggrecan in chondrocytes embedded in alginate beads. Osteoarthritis Cartilage, 12, 559–67.

Guarino V, Gloria A, Raucci MG, Ambrosio L. (2012). Hydrogel-based platforms for the regeneration of osteochondral tissue and intervertebral disc. Polymers, 4, 1590–612.

Gulotta LV, Chaudhury S, Wiznia D. (2012). Stem cells for augmenting tendon repair. Stem Cell Res Ther, 2012. DOI: 10.1155/2012/291431.

Haleem AM, Chu CR. (2010). Advances in tissue engineering techniques for articular cartilage repair. Oper Tech Orthop, 20, 76–89.

Hardingham T, Tew S, Murdoch A. (2002). Tissue engineering: chondrocytes and cartilage. Arthritis Res, 4, S63–8.

Holland TA, Mikos AG. (2003). Advances in drug delivery for articular cartilage. J Control Release, 86, 1–14.

Huang AH, Stein A, Tuan RS, Mauck RL. (2009). Transient exposure to transforming growth factor beta 3 improves the mechanical properties of mesenchymal stem cell-laden cartilage constructs in a density-dependent manner. Tissue Eng Part A, 15, 3461–72.

Hunt N, Grover L. (2010). Cell encapsulation using biopolymer gels for regenerative medicine. Biotechnol Lett, 32, 733–42.

Iwasa J, Ochi M, Uchio Y, et al. (2003). Effects of cell density on proliferation and matrix synthesis of chondrocytes embedded in atelocollagen gel. Artif Organs, 27, 249–55.

Jacobi M, Villa V, Magnussen R, Neyret P. (2011). MACI - a new era? Sports Med Arthrosc Rehabil Ther Technol, 3, 10.

Jakob M, Demarteau O, Schafer D, et al. (2001). Specific growth factors during the expansion and redifferentiation of adult human articular chondrocytes enhance chondrogenesis and cartilaginous tissue formation in vitro. J Cell Biochem, 81, 368–77.

Jaulneau V, Lafitte C, Jacquet C, et al. (2010). Ulvan, a sulfated polysaccharide from green algae, activates plant immunity through the jasmonic acid signaling pathway. J Biomed Biotechnol, 2010. DOI: 10.1155/2010/525291.

Jeon O, Bouhadir KH, Mansour JM, Alsberg E. (2009). Photocrosslinked alginate hydrogels with tunable biodegradation rates and mechanical properties. Biomaterials, 30, 2724–34.

Jiao G, Yu G, Zhang J, Ewart H. (2011). Chemical structures and bioactivities of sulfated polysaccharides from marine algae. Mar Drugs, 9, 196–223.

Jiao L, Jiang P, Zhang L, Wu M. (2010). Antitumor and immunomodulating activity of polysaccharides from *Enteromorpha intestinalis*. Biotechnol Bioprocess Eng, 15, 421–8.

Kaps C, Bramlage C, Smolian H, et al. (2002). Bone morphogenetic proteins promote cartilage differentiation and protect engineered artificial cartilage from fibroblast invasion and destruction. Arthritis Rheum, 46, 149–62.

Kelly T-AN, Ng KW, Wang CCB, et al. (2006). Spatial and temporal development of chondrocyte-seeded agarose constructs in free-swelling and dynamically loaded cultures. J Biomech, 39, 1489–97.

Kino-Oka M, Maeda Y, Yamamoto T, et al. (2005). A kinetic modeling of chondrocyte culture for manufacture of tissue-engineered cartilage. J Biosci Bioeng, 99, 197–207.

Kisiday JD, Kopesky PW, Evans CH, et al. (2008). Evaluation of adult equine bone marrow- and adipose-derived progenitor cell chondrogenesis in hydrogel cultures. J Orthop Res, 26, 322–31.

Klöck G, Pfeffermann A, Ryser C, et al. (1997). Biocompatibility of mannuronic acid-rich alginates. Biomaterials, 18, 707–13.

Ko H-F, Sfeir C, Kumta PN. (2010). Novel synthesis strategies for natural polymer and composite biomaterials as potential scaffolds for tissue engineering. Philos Trans R Soc A, 368, 1981–97.

Kon E, Gobbi A, Filardo G, et al. (2009). Arthroscopic second-generation autologous chondrocyte implantation compared with

Critical Reviews in Biotechnology Downloaded from informahealthcare.com by Mrs Claire Summerfield on 04/08/14
For personal use only.

microfracture for chondral lesions of the knee. Am J Sports Med, 37, 33–41.

Kreuz P, Muller S, Ossendorf C, et al. (2009). Treatment of focal degenerative cartilage defects with polymer-based autologous chondrocyte grafts: four-year clinical results. Arthritis Res Ther, 11, R33.

Kuleshova LL, Gouk SS, Hutmacher DW. (2007). Vitrification as a prospect for cryopreservation of tissue-engineered constructs. Biomaterials, 28, 1585–96.

Kuroda R, Ishida K, Matsumoto T, et al. (2007). Treatment of a full-thickness articular cartilage defect in the femoral condyle of an athlete with autologous bone-marrow stromal cells. Osteoarthritis Cartilage, 15, 226–31.

Lahaye M, Robic A. (2007). Structure and functional properties of ulvan, a polysaccharide from green seaweeds. Biomacromolecules, 8, 1765–74.

Lattermann C, Romine SE. (2009). Osteochondral allografts: state of the art. Clin Sports Med, 28, 285–301.

Laurienzo P. (2010). Marine polysaccharides in pharmaceutical applications: an overview. Mar Drugs, 8, 2435–65.

Lawson A, Ahmad H, Sambanis A. (2011). Cytotoxicity effects of cryoprotectants as single-component and cocktail vitrification solutions. Cryobiology, 62, 115–22.

Lee CSD, Gleghorn JP, Won Choi N, et al. (2007). Integration of layered chondrocyte-seeded alginate hydrogel scaffolds. Biomaterials, 28, 2987–993.

Leiro JM, Castro R, Arranz JA, Lamas J. (2007). Immunomodulating activities of acidic sulphated polysaccharides obtained from the seaweed *Ulva rigida* C. *Agardh*. Int Immunopharmacol, 7, 879–88.

Lima EG, Bian L, Ng KW, et al. (2007). The beneficial effect of delayed compressive loading on tissue-engineered cartilage constructs cultured with TGF-Î²3. Osteoarthritis Cartilage, 15, 1025–33.

Lin Y-J, Yen C-N, Hu Y-C, et al. (2009). Chondrocytes culture in three-dimensional porous alginate scaffolds enhanced cell proliferation, matrix synthesis and gene expression. J Biomed Mater Res Part A, 88A, 23–33.

Lopiz-Morales Y, Abarrategi A, Ramos V, et al. (2010). In vivo comparison of the effects of rhBMP-2 and rhBMP-4 in osteochondral tissue regeneration. Eur Cell Mater, 20, 367–78.

Ma H-L, Hung S-C, Lin S-Y, et al. (2003). Chondrogenesis of human mesenchymal stem cells encapsulated in alginate beads. J Biomed Mater Res A, 64A, 273–81.

Ma K, Titan AL, Stafford M, et al. (2012). Variations in chondrogenesis of human bone marrow-derived mesenchymal stem cells in fibrin/alginate blended hydrogels. Acta Biomater, 8, 3754–64.

Malafaya PB, Silva GA, Reis RL. (2007). Natural-origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications. Adv Drug Deliv Rev, 59, 207–33.

Manfredini M, Zerbinati F, Gildone A, Faccini R. (2007). Autologous chondrocyte implantation: a comparison between an open periosteal-covered and an arthroscopic matrix-guided technique. Acta Orthop Belg, 73, 207–18.

Mangione MR, Giacomazza D, Bulone D, et al. (2003). Thermoreversible gelation of κ-Carrageenan: relation between conformational transition and aggregation. Biophys Chem, 104, 95–105.

Marijnissen WJ, van Osch GJ, Aigner J, et al. (2002). Alginate as a chondrocyte-delivery substance in combination with a non-woven scaffold for cartilage tissue engineering. Biomaterials, 23, 1511–17.

Marquass B, Schulz R, Hepp P, et al. (2011). Matrix-associated implantation of predifferentiated mesenchymal stem cells versus articular chondrocytes. Am J Sports Med, 39, 1401–12.

Mauck RL, Soltz MA, Wang CCB, et al. (2000). Functional tissue engineering of articular cartilage through dynamic loading of chondrocyte-seeded agarose gels. J Biomech Eng, 122, 252–60.

Mauck RL, Wang CCB, Oswald ES, et al. (2003). The role of cell seeding density and nutrient supply for articular cartilage tissue engineering with deformational loading. Osteoarthritis Cartilage, 11, 879–90.

Mauck RL, Yuan X, Tuan RS. (2006). Chondrogenic differentiation and functional maturation of bovine mesenchymal stem cells in long-term agarose culture. Osteoarthritis Cartilage, 14, 179–89.

Maus U, Schneider U, Gravius S, et al. (2008). Clinical results after three years use of matrix-associated ACT for the treatment of osteochondral defects of the knee. Z Orthop Unfall, 146, 31–7.

Mehrban N, Hunt N, AM S, LM G. (2009). A comparative study of iota carrageenan, kappa-carrageenan and alginate hydrogels as tissue engineering scaffolds. In: Williams PA, Phillips GO, eds. Gums and stabilisers for the food industry 15. Cambridge: Royal Society of Chemistry, 407–13.

Mierisch CM, Wilson HA, Turner MA, et al. (2003). Chondrocyte transplantation into articular cartilage defects with use of calcium alginate: the fate of the cells. J Bone Joint Surg, 85, 1757–67.

Miyamoto Y, Enosawa S, Takeuchi T, Takezawa T. (2009). Cryopreservation in situ of cell monolayers on collagen vitrigel membrane culture substrata: ready-to-use preparation of primary hepatocytes and ES cells. Cell Transplant, 18, 619–26.

Morelli A, Chiellini F. (2010). Ulvan as a new type of biomaterial from renewable resources: functionalization and hydrogel preparation. Macromol Chem Phys, 211, 821–32.

Nesic D, Whiteside R, Brittberg M, et al. (2006). Cartilage tissue engineering for degenerative joint disease. Adv Drug Deliv Rev, 58, 300–22.

Ng KW, Lima EG, Bian L, et al. (2010). Passaged adult chondrocytes can form engineered cartilage with functional mechanical properties: a canine model. Tissue Eng Part A, 16, 1041–51.

Nicodemus GD, Bryant SJ. (2008). Cell encapsulation in biodegradable hydrogels for tissue engineering applications. Tissue Eng Part B Rev, 14, 149–65.

Niemeyer P, Fechner K, Milz S, et al. (2010). Comparison of mesenchymal stem cells from bone marrow and adipose tissue for bone regeneration in a critical size defect of the sheep tibia and the influence of platelet-rich plasma. Biomaterials, 31, 3572–9.

Noth U, Steinert AF, Tuan RS. (2008). Technology insight: adult mesenchymal stem cells for osteoarthritis therapy. Nat Clin Pract Rheum, 4, 371–80.

Núñez-Santiago MC, Tecante A, Garnier C, Doublier JL. (2011). Rheology and microstructure of κ-carrageenan under different conformations induced by several concentrations of potassium ion. Food Hydrocoll, 25, 32–41.

Ochs BG, Müller-Horvat C, Rolauffs B, et al. (2007). Treatment of osteochondritis dissecans of the knee: one-step procedure with bone grafting and matrix-supported autologous chondrocyte transplantation. Z Orthop Unfall, 145, 146–51.

Oliveira JT, Reis RL. (2010). Polysaccharide-based materials for cartilage tissue engineering applications. J Tissue Eng Regen Med, 5, 421–36.

Orive G, Tam SK, Pedraz JL, Hallé J-P. (2006). Biocompatibility of alginate-poly-L-lysine microcapsules for cell therapy. Biomaterials, 27, 3691–700.

Pawar SN, Edgar KJ. (2012). Alginate derivatization: a review of chemistry, properties and applications. Biomaterials, 33, 3279–305.

Pelaez D, Charles Huang CY, Cheung HS. (2009). Cyclic compression maintains viability and induces chondrogenesis of human mesenchymal stem cells in fibrin gel scaffolds. Stem Cells Dev, 18, 93–102.

Pelttari K, Wixmerten A, Martin I. (2009). Do we really need cartilage tissue engineering? Swiss Med Wkly, 139, 602–9.

Pereira RC, Scaranari M, Castagnola P, et al. (2009). Novel injectable gel (system) as a vehicle for human articular chondrocytes in cartilage tissue regeneration. J Tissue Eng Regen Med, 3, 97–106.

Peterson L, Brittberg M, Kiviranta I, et al. (2002). Autologous chondrocyte transplantation: biomechanics and long-term durability. Am J Sports Med, 30, 2–12.

Place ES, Evans ND, Stevens MM. (2009). Complexity in biomaterials for tissue engineering. Nat Mater, 8, 457–70.

Popa E, Reis R, Gomes M. (2012). Chondrogenic phenotype of different cells encapsulated in κ-carrageenan hydrogels for cartilage regeneration strategies. Biotechnol Appl Biochem, 59, 132–41.

Popa EG, Caridade SG, Mano JF, et al. (2013a). Chondrogenic potential of injectable κ-carrageenan hydrogel with encapsulated adipose stem cells for cartilage tissue-engineering applications. J Tissue Eng Regen Med. DOI: 10.1002/term.1683.

Popa EG, Gomes ME, Reis RL. (2011). Cell delivery systems using alginate-carrageenan hydrogel beads and fibers for regenerative medicine applications. Biomacromolecules, 12, 3952–61.

Popa EG, Rodrigues MT, Coutinho DF, et al. (2013b). Cryopreservation of cell laden natural origin hydrogels for cartilage regeneration strategies. Soft Matter, 9, 875–85.

Critical Reviews in Biotechnology Downloaded from informahealthcare.com by Mrs Claire Summerfield on 04/08/14
For personal use only.

Qi H, Liu X, Ma J, et al. (2010). In vitro antioxidant activity of acetylated derivatives of polysaccharide extracted from *Ulva pertusa* (Cholorophta). J Med Plant Res, 4, 2445–51.

Rada T, Reis RL, Gomes ME. (2009). Adipose tissue-derived stem cells and their application in bone and cartilage tissue engineering. Tissue Eng Part B Rev, 15, 113–25.

Raghunath J, Salacinski HJ, Sales KM, et al. (2005). Advancing cartilage tissue engineering: the application of stem cell technology. Curr Opin Biotechnol, 16, 503–9.

Rahfoth B, Weisser J, Sternkopf F, et al. (1998). Transplantation of allograft chondrocytes embedded in agarose gel into cartilage defects of rabbits. Osteoarthritis Cartilage, 6, 50–65.

Ray B, Lahaye M. (1995). Cell-wall polysaccharides from the marine green alga *Ulva ''rigida''* (ulvales, chlorophyta): extraction and chemical composition. Carbohydr Res, 274, 251–61.

Reinholz GG, Lu L, Saris DBF, et al. (2004). Animal models for cartilage reconstruction. Biomaterials, 25, 1511–21.

Renth AN, Detamore MS. (2012). Leveraging ''raw materials'' as building blocks and bioactive signals in regenerative medicine. Tissue Eng Part B Rev, 18, 341–62.

Rocha PM, Santo VE, Gomes ME, et al. (2011). Encapsulation of adipose-derived stem cells and transforming growth factor-β1 in carrageenan-based hydrogels for cartilage tissue engineering. J Bioact Compat Polym, 26, 493–507.

Rodrigues MrT, Gomes ME, Reis RL. (2011). Current strategies for osteochondral regeneration: from stem cells to pre-clinical approaches. Curr Opin Biotechnol, 22, 726–33.

Santo VE, Frias AM, Carida M, et al. (2009). Carrageenan-based hydrogels for the controlled delivery of pdgf-bb in bone tissue engineering applications. Biomacromolecules, 10, 1392–401.

Sathivel A, Raghavendran HRB, Srinivasan P, Devaki T. (2008). Anti-peroxidative and anti-hyperlipidemic nature of *Ulva lactuca* crude polysaccharide on D-galactosamine induced hepatitis in rats. Food Chem Toxicol, 46, 3262–7.

Schuh E, Hofmann S, Stok K, et al. (2011). Chondrocyte redifferentiation in 3D: the effect of adhesion site density and substrate elasticity. J Biomed Mater Res A, 100A, 38–47.

Selmi TAS, Verdonk P, Chambat P, et al. (2008). Autologous chondrocyte implantation in a novel alginate-agarose hydrogel. J Bone Joint Surg Br, 90-B, 597–604.

Sheehy EJ, Buckley CT, Kelly DJ. (2012). Oxygen tension regulates the osteogenic, chondrogenic and endochondral phenotype of bone marrow derived mesenchymal stem cells. Biochem Biophys Res Commun, 417, 305–10.

Shoichet MS, Li RH, White ML, Winn SR. (1996). Stability of hydrogels used in cell encapsulation: an in vitro comparison of alginate and agarose. Biotechnol Bioeng, 50, 374–81.

Shortkroff S, Spector M. (1998). Isolation and In Vitro Proliferation of Chondrocytes, Tenocytes, and Ligament Cells. Methods Mol Med, 18, 195–203.

Shull KR. (2012). Materials science: a hard concept in soft matter. Nature, 489, 36–7.

Silva TH, Alves A, Popa EG, et al. (2012). Marine algae sulfated polysaccharides for tissue engineering and drug delivery approaches. Biomatter, 2, 278–89.

Sipahigil O, Dortunc B. (2001). Preparation and in vitro evaluation of verapamil HCl and ibuprofen containing carrageenan beads. Int J Pharm, 228, 119–28.

Sittinger M, Hutmacher DW, Risbud MV. (2004). Current strategies for cell delivery in cartilage and bone regeneration. Curr Opin Biotechnol, 15, 411–8.

Stanley N (1987). Production, properties and uses of carrageenan. In: McHugh DJ, ed. Production and utilization of products from commercial seaweeds. FAO Fisheries Technical Paper 288, 116–46.

Steinwachs M, Peterson L, Bobic V, et al. (2012). Cell-seeded collagen matrix-supported autologous chondrocyte transplantation (ACT-CS). Cartilage, 3, 5–12.

Stevens MM, Marini RP, Martin I, et al. (2004a). FGF-2 enhances TGF-β1-induced periosteal chondrogenesis. J Orthop Res, 22, 1114–19.

Stevens MM, Qanadilo HF, Langer R, Prasad Shastri V. (2004b). A rapid-curing alginate gel system: utility in periosteum-derived cartilage tissue engineering. Biomaterials, 25, 887–94.

Steward A, Liu Y, Wagner D. (2011). Engineering cell attachments to scaffolds in cartilage tissue engineering. JOM, 63, 74–82.

Steward AJ, Thorpe SD, Vinardell T, et al. (2012). Cell-matrix interactions regulate mesenchymal stem cell response to hydrostatic pressure. Acta Biomaterialia, 8, 2153–9.

Stoop R. (2008). Smart biomaterials for tissue engineering of cartilage. Injury, 39, 77–87.

Suh J-K, Scherping S, Mardi T, et al. (1995). Basic science of articular cartilage injury and repair. Oper Tech Sports Med, 3, 78–86.

Sun J-Y, Zhao X, Illeperuma WRK, et al. (2012). Highly stretchable and tough hydrogels. Nature, 489, 133–6.

Tan H, Marra KG. (2010). Injectable, biodegradable hydrogels for tissue engineering applications. Materials, 3, 1746–67.

Thirumala S, Gimble JM, Devireddy RV. (2010). Evaluation of methylcellulose and dimethyl sulfoxide as the cryoprotectants in a serum-free freezing media for cryopreservation of adipose-derived adult stem cells. Stem Cells Dev, 19, 513–22.

Thorpe SD, Buckley CT, Vinardell T, O Brien FJ, Campbell VA, Kelly DJ. (2008). Dynamic compression can inhibit chondrogenesis of mesenchymal stem cells. Biochem Biophys Res Commun, 377, 458–62.

Tirtsa EK, Abraham D, Jacob G. (2005). Polysaccharide Scaffolds for Tissue Engineering. In: Peter XM, Jennifer E, eds. Scaffolding in tissue engineering. Boca Raton: CRC Press, 27–44.

Tortelli F, Cancedda R. (2009). Three-dimensional cultures of osteogenic and chondrogenic cells: a tissue engineering approach to mimic bone and cartilage in vitro. Eur Cell Mater, 17, 1–14.

Troken A, Marion N, Hollister S, Mao J. (2007). Tissue engineering of the synovial joint: the role of cell density. Proc Inst Mech Eng H, 221, 429–40.

Tuli R, Li WJ, Tuan RS. (2003). Current state of cartilage tissue engineering. Arthritis Res Ther, 5, 235–8.

van Susante JLC, Buma P, van Osch GJVM, et al. (1995). Culture of chondrocytes in alginate and collagen carrier gels. Acta Orthop, 66, 549–56.

Varma MJ, Breuls RG, Schouten TE, et al. (2007). Phenotypical and functional characterization of freshly isolated adipose tissue-derived stem cells. Stem Cells Dev, 16, 91–104.

Varoni E, Tschon M, Palazzo B, et al. (2012). Agarose gel as biomaterial or scaffold for implantation surgery: characterization, histological and histomorphometric study on soft tissue response. Connect Tissue Res, 1–7.

Vera J, Castro J, Gonzalez A, Moenne A. (2011). Seaweed polysaccharides and derived oligosaccharides stimulate defense responses and protection against pathogens in plants. Mar Drugs, 9, 2514–25.

Vijayan S, Bartlett W, Bentley G, et al. (2012). Autologous chondrocyte implantation for osteochondral lesions in the knee using a bilayer collagen membrane and bone graft. J Bone Joint Surg Br, 94-B, 488–92.

Vinardell T, Sheehy EJ, Buckley CT, Kelly DJ. (2012). A comparison of the functionality and in vivo phenotypic stability of cartilaginous tissues engineered from different stem cell sources. Tissue Eng Part A, 18, 1161–70.

Vinatier C, Bouffi C, Merceron C, et al. (2009). Cartilage tissue engineering: towards a biomaterial-assisted mesenchymal stem cell therapy. Curr Stem Cell Res Ther, 4, 318–29.

Von Der Mark K, Gauss V, Von Der Mark H, Muller P. (1977). Relationship between cell shape and type of collagen synthesised as chondrocytes lose their cartilage phenotype in culture. Nature, 267, 531–2.

Vunjak-Novakovic G, Freed LE. (1998). Culture of organized cell communities. Adv Drug Deliv Rev, 33, 15–30.

Wang Y, Blasioli DJ, Kim HJ, et al. (2006). Cartilage tissue engineering with silk scaffolds and human articular chondrocytes. Biomaterials, 27, 4434–42.

Watt FM. (1988). Effect of seeding density on stability of the differentiated phenotype of pig articular chondrocytes in culture. J Cell Sci, 89, 373–8.

Wayne JS, McDowell CL, Shields KJ, Tuan RS. (2005). In vivo response of polylactic acid-alginate scaffolds and bone marrow-derived cells for cartilage tissue engineering. Tissue Eng, 11, 953–63.

Xu J, Wang W, Ludeman M, et al. (2008). Chondrogenic differentiation of human mesenchymal stem cells in three-dimensional alginate gels. Tissue Eng Part A, 14, 667–80.

Yamada Y, Hozumi K, Katagiri F, et al. (2010). Biological activity of laminin peptide-conjugated alginate and chitosan matrices. Peptide Sci, 94, 711–20.

Yang S, Leong KF, Du Z, Chua CK. (2001). The design of scaffolds for use in tissue engineering. Part I. Traditional factors. Tissue Eng, 7, 679–89.

Yeung T, Georges PC, Flanagan LA, et al. (2005). Effects of substrate stiffness on cell morphology, cytoskeletal structure, and adhesion. Cell Motil Cytoskeleton, 60, 24–34.

Zimmermann H, Ehrhart F, Zimmermann D, et al. (2007). Hydrogel-based encapsulation of biological, functional tissue: fundamentals, technologies and applications. Appl Phys A, 89, 909–22.

Critical Reviews in Biotechnology Downloaded from informahealthcare.com by Mrs Claire Summerfield on 04/08/14
For personal use only.