Biomimicry of microbial polysaccharide hydrogels for tissue engineering and regenerative medicine – A review

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ARTICLE INFO

ABSTRACT

Hydrogels as artificial biomaterial scaffolds offer a much favoured 3D microenvironment for tissue engineering and regenerative medicine (TERM). Towards biomimicry of the native ECM, polysaccharides from Nature have been proposed as ideal surrogates given their biocompatibility. In particular, derivatives from microbial sources have emerged as economical and sustainable biomaterials due to their fast and high yielding production procedures. Despite these merits, microbial polysaccharides do not interact biologically with human tissues, a critical limitation hampering their translation into paradigmatic scaffolds for *in vitro* 3D cell culture. To overcome this, chemical and biological functionalization of polysaccharide scaffolds have been explored extensively. This review outlines the most recent strategies in the preparation of biofunctionalized gellan gum, xanthan gum and dextran hydrogels fabricated exclusively *via* material blending. Using inorganic or organic materials, we discuss the impact of these approaches on cell adhesion, proliferation and viability of anchorage-dependent cells for various TERM applications.'

1. Introduction

TERM involves the repair, replacement or regeneration of damaged tissues which are difficult to heal (Gomes, Rodrigues, Domingues, & Reis, 2017; Liu et al., 2017). Current practice for tissue repair is achieved primarily through transplantation of tissues obtained from a healthy donor (an allograft) or patient's own body (an autograft). However, these techniques are constrained by the lack of donor tissue, potential infection, high risk of tissue rejection and poor graft survival (Hsieh et al., 2017). Therefore, the use of innovative techniques to form new tissues from a very small number of recipients' own cells is archetypical of modern TERM.

The *in vitro* fabricated tissue is usually composed of a tissue scaffold, host cells, and animal-derived growth factors. Flat and hard plastic surfaces are not putative of the cellular environment found in organisms. This is because cellular interactions with the extracellular matrix (ECM) play a critical role in tissue homeostasis by establishing a three dimensional (3D) communication network (Pampaloni, Reynaud, & Stelzer, 2007). Thus, in TERM, the scaffold is required to both accommodate the host cells and provide environmental cues to guide their adhesion and proliferation (Goetzke et al., 2018; Huang et al., 2017). Apart from such basal cellular activities, the 3D scaffold also supports cell communication and complex events such as cell differentiation (Azoidis et al., 2017; Goetzke et al., 2018). These processes are regulated by structural organizing principles (Tibbitt & Anseth, 2009). Previously, natural ECMs had been intuitively used as 3D scaffolds, but poor mechanical behaviour and unpredictable biodegradation propelled the development of alternative biomimetic materials such as hydrogels.

Hydrogels are 3D cross-linked networks of hydrophilic polymers that are capable of holding a large amount of water without being solvated. This aqueous environment qualifies hydrogel-based scaffolds to be ideal 3D matrices in which cells can be cultured to create tissues *in vitro* (Liu et al., 2010). Numerous studies have demonstrated hydrogels' unique efficacy in recapitulating aspects of the native cellular microenvironment for 3D *in vitro* cell culture (Geckil, Xu, Zhang, Moon, & Demirci, 2010; Huang et al., 2017; Trappmann et al., 2012). As the major structural component of hydrogels, polysaccharides represent a class of biomaterial of particular interest (Fig. 1).

Polysaccharides are carbohydrate polymers linked by glycosidic bonds. Hydrolytic cleavage of these linkages generates the polymers' constituent subunits. Polysaccharide-based hydrogels are derived from living tissues that are either components of or have macromolecular properties similar to the natural ECM (Upadhyay, 2017). Therefore, they are inherently biodegradable and biocompatible (Matricardi, Di Meo, Coviello, Hennink, & Alhaique, 2013; Upadhyay, 2017). They also display unique properties such as stimuli-responsive characteristics and bio-responsive functions, making them materials of choice for diverse TERM applications (Gentilini et al., 2018). Natural polysaccharides can be derived from renewable biomass like algae or plants, or from the fermentation of bacterial or fungal cultures which are harvested as microbial polysaccharides (Moscovici, 2015). Compared to algal or plant sources, microbial sources are increasingly favoured for their high yielding commercial production procedures (Shih, 2010).

The ECM in the body provides a milieu of cell binding ligands that connect the cellular cytoskeletons to the ECM microenvironment (Hamel, Gimble, Jung, & Martin, 2018; Muncie & Weaver, 2018; Niklason, 2018). These binding ligands are located on physically entrapped ECM proteins, such as collagen, laminin, or fibronectin, in the ECM network (Hay, 2013). A wide range of nature-inspired protein-based hydrogels have thus been developed as scaffolds for TERM (Schloss, Williams, & Regan, 2016). Intuitively, they are appealing due to their inherent cell adhesivity as conferred by the presence of integrin-recoginizing peptide sequences (Jabbari, 2019). However, sustained use of proteins as hydrogel scaffold materials is impeded by multiple challenges such as their high cost and non-renewability, complex purification procedures as well as demanding storage conditions (Hinderer, Layland, & Schenke-Layland, 2016). In contrast, microbial polysaccharides are more economical, easy to handle and less sensitive chemical entities with relatively facile production and storage requirements (Guillen & Tezel, 2019).

However, polysaccharides as a hydrogel material lack bioactivity and are devoid of integrin-binding domains (da Silva et al., 2018 ; Diekjürgen & Grainger, 2017; Hunt et al., 2017). As such, modifications

Fig. 1. Various types of hydrogel-forming natural polysaccharides and their respective sources.

of the polysaccharide molecule *via* attachment of chemical moieties that can facilitate cell adhesion become important (Y. Hu, Li, & Xu, 2017; Huettner, Dargaville, & Forget, 2018; Kirschning, Dibbert, & Dräger, 2018; Varaprasad, Raghavendra, Jayaramudu, Yallapu, & Sadiku, 2017). Unfortunately, covalent crosslinking of bio-functional chemical groups often requires toxic crosslinking agents and harsh chemical conditions and results in the formation of toxic by-products. This in turn necessitates an extensive cleansing strategy before the materials could be harvested for biomedical applications (Crescenzi, Cornelio, Di Meo, Nardecchia, & Lamanna, 2007; Kirschning et al., 2018; K. Y. Lee & Mooney, 2001).

As an alternative, a number of physical approaches have been employed by various groups (Bacelar, Silva-Correia, Oliveira, & Reis, 2016; Köpf, Campos, Blaeser, Sen, & Fischer, 2016; Matricardi et al., 2013; Schütz et al., 2017; H. Shin, Olsen, & Khademhosseini, 2012; Tytgat et al., 2018; Vishwanath, Pramanik, & Biswas, 2017). Among the multitude of strategies employed, direct blending of bioactive molecules into the hydrogels' network presents as a straightforward method for biological modification. This is especially pertinent for already FDA-approved materials, material blending as a process to improve bioactivity of hydrogels holds the advantage of accelerating the development of innovative hydrogels with synergistic bioactive features for TERM. The main aim of this review is to highlight recent strategies for improving the cellular proliferation and attachment of polysaccharide-based hydrogels through direct blending. We provide a brief overview of gellan gum, xanthan gum and dextran: the three most widely used microbial polysaccharides. Thereafter, we summarize recent reports on direct blending by comparing strategies that incorporate organic and inorganic materials into microbial polysaccharide-based hydrogels. Finally, we discuss the potential use of these polymers in TERM.

Table 1 shows the sources, structures and U.S. Food and Drug Administration (FDA)-approved excipient applications of aforementioned polysaccharides. The difference in their monomeric structures confers significant difference in their resultant hydrogel applications. These differences burgeon with the introduction of other bioactive materials. A preface of each microbial polysaccharide followed by an introductory general discussion will help achieve a better understanding of their gelation process and niche in the biomedical bearing. With this knowledge, this review aims to present an organized view of current approaches on how both inorganic and organic bioactive substances blended into their hydrogel matrices can improve microbial polysaccharide hydrogel bio-functionality.

1.1. Gellan gum, xanthan gum and dextran hydrogels for biomedical applications

1.1.1. Gellan gum

Gellan gum is an anionic extracellular microbial fermentation product secreted primarily by the bacterium, *Sphingomonas elodea* (ATCC 31461) (Banik, Santhiagu, & Upadhyay, 2007; Kang & Pettitt, 1993). It is a linear polysaccharide comprising a repeating tetrasaccharide unit of two D-glucose, one L-rhamnose and one D-glucuronic acid (Fig. 2A). Gellan gum is commercially available in two forms: high acyl (acetylated) gellan gum and low acyl (deacetylated) gellan gum. Both forms of gellan gum are capable of gelation. However, the native acetylated gellan gum produces translucent elastic gels whereas, the deacetylated form produces transparent rigid gels which are more suitable for TERM applications (Deasy & Quigley, 1991; Miyoshi, Takaya, & Nishinari, 1996).

The gelation process of gellan gum involves a distinct two-step mechanism (Grasdalen & Smidsrød, 1987; Moritaka, Fukuba, Kumeno, Nakahama, & Nishinari, 1991; Morris, Nishinari, & Rinaudo, 2012). The initial step is a temperature-dependent process. When an aqueous solution of gellan gum is heated above 80 °C for 20 to 30 minutes and subsequently cooled, the linear polymers of gellan gum undergo a bimolecular association from randomly coiled chains to highly ordered double helices. Next, the addition of cations crosslinks the helices to form a stable hydrogel. Gels formed by divalent cations are stronger as compared to monovalent cations because divalent cations form a direct electrostatic bridge between the carboxylate groups on the gellan backbone whereas, monovalent cations merely provide a screening effect of the electrostatic repulsion between them (Grasdalen & Smidsrød, 1987).

Gellan gum hydrogels possess attractive characteristics such as biocompatibility (Smith, Shelton, Perrie, & Harris, 2007), mild conditions of gelation (Oliveira et al., 2010; Takata, Tosa, & Chibata, 1977), structural similarity with native glycosaminoglycans found in the body (Geckil et al., 2010; Oliveira et al., 2010), and tunable mechanical properties (Berti et al., 2017; Bonifacio, Gentile, Ferreira, Cometa, & De Giglio, 2017; Carvalho et al., 2018; Manda et al., 2018; Tsaryk et al., 2017). A mild condition of gelation facilitates the incorporation of cells, which allows gellan gum-based hydrogels to be studied for various TERM applications. However, gellan gum lacks specific cell adhesion sites (da Silva et al., 2014), which limits their use for the culture of anchorage-dependent cells.

1.1.2. Xanthan gum

Xanthan gum is an extracellular microbial polysaccharide fermentation product produced by bacteria of the genus *Xanthomonas* (Petri, 2015). The c*ampestris* species is the most common variant employed for industrial production of xanthan gum (Palaniraj & Jayaraman, 2011; Tao et al., 2012). Xanthan gum is a branched polysaccharide composed of a repeating pentasaccharide unit of D-glucose, D-mannose and D-glucuronic acid in the molar ratio of 2:2:1 (Fig. 2**B**) (Jansson, Kenne, & Lindberg, 1975). It was approved by the FDA (Fed. Reg. 345376) in 1969 as a nontoxic and safe polymer (Kennedy, 1984). Traditionally, xanthan gum plays an important role in food and pharmaceutical applications as binder, thickener and emulsion stabilizer (Katzbauer,

Table 1

The main producing microbe(s) of the respective microbial polysaccharide, their definitive repeating units, and major applications in the food and pharmaceutical industries.

Polysaccharide	Microbe	Structure	Application[a]
Gellan gum	*Sphingomonas elodea*	Composed of a tetrasaccharide repeating unit, consisting of two residues of d-glucose, one residue of l-rhamnose and one residue of d-glucuronic acid..	Gelling agent Thickener Emulsifier Stabilizer
Xanthan gum	*Xanthomonas campestris*	Composed of a pentasaccharide repeating unit, consisting of D-glucose, D-mannose and D-glucuronic acid the molar ratio of 2:2:1.	Food additive Binder Thickener Stabilizer
Dextran	*Leuconostoc mesenteroides*, *Streptococcus mutans*	Consist of α-1,6 glycosidic linkages between D-glucose monomers, with branches from α-1,3 linkages	Antithrombotic Volume expander Lubricant

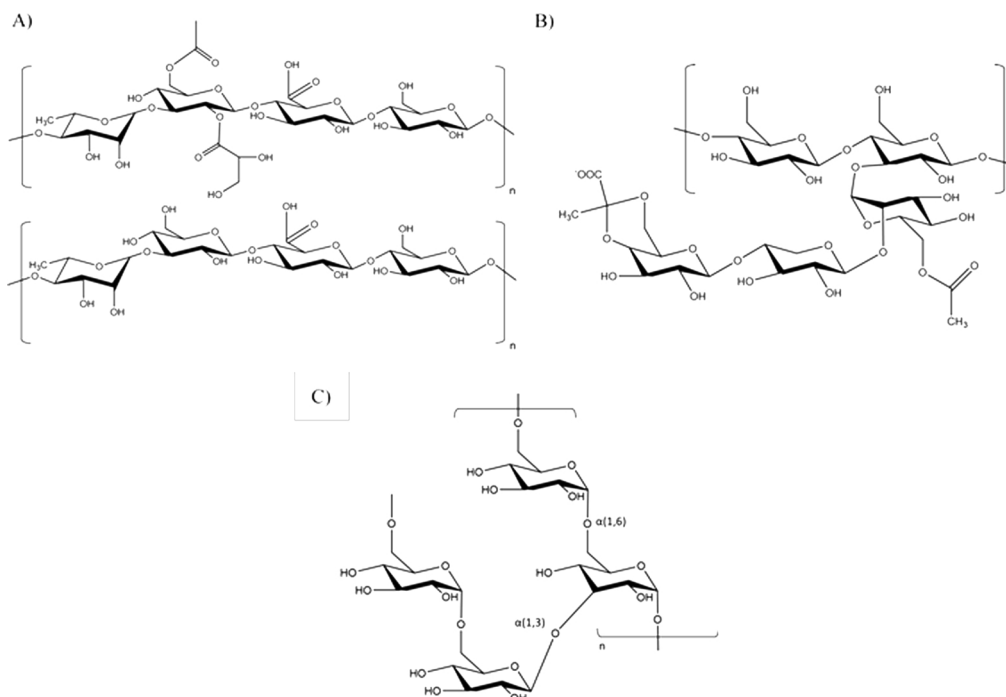[a] Based on FDA's inactive ingredient database.

Fig. 2. Chemical structures of the repeating unit of A) high-acyl (top) and low-acyl (bottom) gellan gum, B) xanthan gum and C) Dextran.

1998). More recently, due to its innocuous nature and shear-thinning properties, xanthan gum hydrogels have been explored as injectable scaffold for cartilage tissue engineering purposes (Kumar, Rao, & Han, 2018).

Xanthan gum undergoes a single-step temperature-dependent gelation process. A colloidal heterogeneous suspension, comprised of pockets of molecular assemblies, forms when xanthan gum polymers are dispersed in water at room temperature. When the heterogeneous suspension is heated to above sol-gel transition temperature (T_{g-s}) of 40 °C for 3 h, annealing occurs, and homogeneity is achieved. Firm hydrogels are subsequently formed upon cooling of the homogeneous solution (Yoshida, Takahashi, Hatakeyama, & Hatakeyama, 1998). Although the biocompatibility of xanthan gum hydrogels is well established (Kumar et al., 2018), drawbacks such as harsh gelation conditions, poor mechanical performance and lack of cell attachment moieties are depriving its widespread used in TERM applications (Bueno, Bentini, Catalani, Barbosa, & Petri, 2014).

1.1.3. Dextran

Dextran is the first commercially available microbial polysaccharide and is produced by *Leuconostoc mesenteroides* and *streptococcus mutans* bacteria (Doman Kim & Day, 1994). Its structure consists of linear α-1,6 and branch α-1,3 glycosidic linkages between glucose monomers (Fig. 2C). The branching distinguishes dextran from dextrin which have a branch α-1,4 glycosidic linkages (Heinze, Liebert, Heublein, & Hornig, 2006). Dextran is an essential medicine, widely used as an antithrombotic and volume expander in the clinical setting (Sun & Mao, 2012). Unfortunately, dextran does not form hydrogels in its native state but composite dextran-based hydrogels have been successfully formulated for TERM purposes (McCann, Behrendt, Yan, Halacheva, & Saunders, 2015; Nikpour et al., 2018). However, the exhaustive potential of manipulating dextran with precisely tuned signalling cues for large-scale tissue regenerative scaffolds has yet to be fully developed and remains a significant challenge in TERM.

Cell adhesion to matrix is critical for cellular homeostasis for anchorage-dependent cells and disruption of such interaction leads to anoikis (Chiarugi & Giannoni, 2008; Gilmore, 2005). The poor cell adhesivity of gellan gum, xanthan gum (Bueno et al., 2014) and dextran

(Massia, Stark, & Letbetter, 2000) hydrogels could be attributed to the lack of integrin recognition site (da Silva et al., 2014). Moreover, the hydrophilic nature of natural polysaccharides repels the hydrophobic cell surface (Barbosa, Granja, Barrias, & Amaral, 2005; Hoffman, 2012). To overcome this, researchers have adopted various strategies of incorporating cell adhesion sites within the polysaccharide hydrogel network to alter their surface or mechanical properties and improve bioactivity. This is the first review that particularly focuses on material blending with microbial polysaccharide for the development of novel cell-conducive hydrogels with enhanced cell adhesion and proliferation. Different materials and fabrication methods are discussed. Finally, perspectives on novel materials that can be used to formulate advanced hydrogels for TERM applications are also discussed.

2. Biofunctionalization of microbial polysaccharide hydrogels using inorganic materials

Composite hydrogel materials or hydrogel blends are physical mixtures of two or more materials (Bae & Kim, 1993; (Jones and Division, 2009)). At least one of the components must be able to form a continuous network, enabling gelation to occur. If there are two or more polymers capable of forming networks (copolymer systems), individual constituents should not be covalently crosslinked with one another *i.e.* they are at least partially interlaced but not chemically bonded to each other (Wool & Sun, 2011; Work, Horie, Hess, & Stepto, 2004). Microscopically, hydrogel blends are akin to metal alloys whereby the combination create "new" materials with a complete different set of physical properties (Parameswaranpillai, Thomas, & Grohens, 2015). In some instances, incorporation of particle, polymer or nanomaterial reinforcements permits the fabrication of cell-adhesive hydrogel matrices, which may also be characterized by high mechanical performance and/or other biocompatible functionality (Anjum et al., 2016; Crosby & Lee, 2007; Y. Guo et al., 2016) (Fig. 3).

Various methods such as direct blending of materials during gelation (Moxon et al., 2019; Vuornos et al., 2019), enzymatic incorporation as well as electrospinning or electropolymerization have been reported (Douglas, 2016; Pham, Sharma, & Mikos, 2006; Rauner, Meuris, Zoric, & Tiller, 2017). The latter two methods focus on precise control
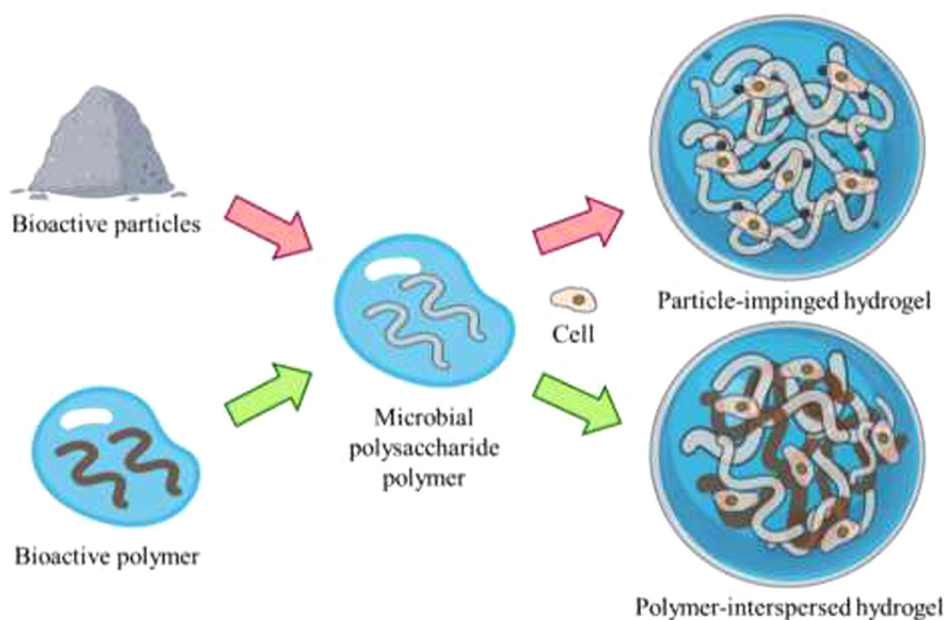
Fig. 3. Schematic representation of the material blending of microbial polysaccharide with bioactive particles or polymer to form cell-adhesive hydrogel scaffolds.

of the physiochemical properties of resultant matrices by manipulating the enzymatic or electrospinning parameters (Manoukian et al., 2017; Wang et al., 2010). However, these approaches are usually more complicated and require extensive tuning before they can meet the requirements of specific TERM application(s).

In recent years, the types of materials that could be incorporated into a hydrogel matrix have considerably broadened. The following sections discuss the use of both organic and inorganic materials in the fabrication of hydrogel blends with improved biocompatibility and bio-functionality. Emphasis will be placed on scaffolds with the abilities to promote cell adhesion, proliferation and/or migration as they are crucial characteristics of man-made TERM matrices. Scaffolds with improved mechanical properties, gelation requirements or other features resulting in an improved biological response will also be inspected.

2.1. Enhancement of cell attachment and proliferation of microbial polysaccharide hydrogel scaffolds

2.1.1. Direct incorporation of inorganic materials

The incorporation of inorganic materials is pivotal in the construction of bone tissue biomimicry. A highly regulated blend of the organic (collagen) and inorganic (hydroxyapatite) phases (Hessle et al., 2002) of bone ECM produces the environmental cues required for homeostasis of osteoblasts (Chatterjee et al., 2010). In turn, the bone ECM is continuously modulated by the osteoblasts in a two-way signalling cascade. To re-create these complex microenvironment, various materials were employed for the assembly of composite scaffolds. They are composed of a polymeric scaffold blended with at least one other inorganic material, through a process known as hydrogel mineralization. The inorganic materials partake in the modulation the hydrogels' pore structure and surface topography, which ultimately affect host bone cells' behaviour (Chen et al., 2018). In some instances, the inorganic minerals behave as a bioactive component of the hydrogels, serving as epitopes that bind to cell surface receptors which triggers cell signalling pathways to direct cell survival, adhesion, and/or differentiation (Kattimani, Kondaka, & Lingamaneni, 2016; Le et al., 2018; Pourmollaabbassi, Karbasi, & Hashemibeni, 2016). Therefore, the incorporation of inorganic materials is an essential strategy to design biomaterials from microbial polysaccharides that can direct deliberate cell fate(s) for bone TERM.

Besides, inorganic materials are often introduced to strengthen the

mechanical properties of resultant hydrogels for bone and cartilage tissue engineering whereby the synthetic tissues will be subjected to repetitive weight compression upon implantation (Bittner et al., 2019). In this aspect, microbial polysaccharides are suitable candidates as their tunable nature work synergistically with inorganic materials to produce sufficiently strong tissue scaffolds. Specifically, hydrogels of varying mechanical similarity to native human bone ECM can be achieved by fine-tuning the interplays of the polymers' and inorganic materials' concentrations (Douglas et al., 2014; Izawa et al., 2014; Nikpour et al., 2018; Oliveira et al., 2010; Osmałek, Froelich, & Tasarek, 2014). In addition, given their ductile nature, a myriad of minerals and fabrication methods have been successfully developed, and reported, to form composite hydrogels of their origin for TERM purposes.

Amongst the strategies employed, direct incorporation of inorganic materials such as bioactive glass (BAG) during the gelation process appears to be the most popular approach. BAG is a ceramic-based biomaterial that is capable of bonding to living bone and stimulate osteogenesis (J. R. Jones, Brauer, Hupa, & Greenspan, 2016). In a recent article by Vuornos et al. (2019), BAG-infused gellan gum hydrogels significantly increased the cell viability of encapsulated human adipose-derived stem cells (ADSC). A higher expression of osteogenic markers and mineralization of the matrix were also observed after 21 days of culture.

Intuitively, mineralization of hydrogels can also be achieved with the direct addition of bone mineral (hydroxyapatite). Manda et al. (2018) developed a gellan gum–hydroxyapatite (HAp) spongy-like hydrogel through repeated freeze-drying and re-hydration. HAp powder was mixed into the freeze-dried gellan gum before reconstitution. The combination of enlarged pore size (spongy-like) and HAp deposition influenced cell activity, including adhesion, proliferation and formation of cytoskeleton. Scanning electron microscope (SEM) imaging confirmed the enrichment of the entire surface of spongy-like gellan gum hydrogel with HAp. The altered microenvironment of the resultant hydrogel enabled encapsulated ADSC to attach, spread and proliferate for up to 21 days of culture.

In a more recent paper, Kim et al. (2020) prepared a scaffold using demineralized bone powder (DBP) extracted from Gallus var domesticus (GD), and gellan gum for osteochondral (OC) tissue regeneration. DBP incorporated scaffolds allowed adhesion of chondrocytes which extended into a fibroblastic morphology by day 4, indicating cell spread. In addition, using RT-PCR, enhanced expression of osteogenic
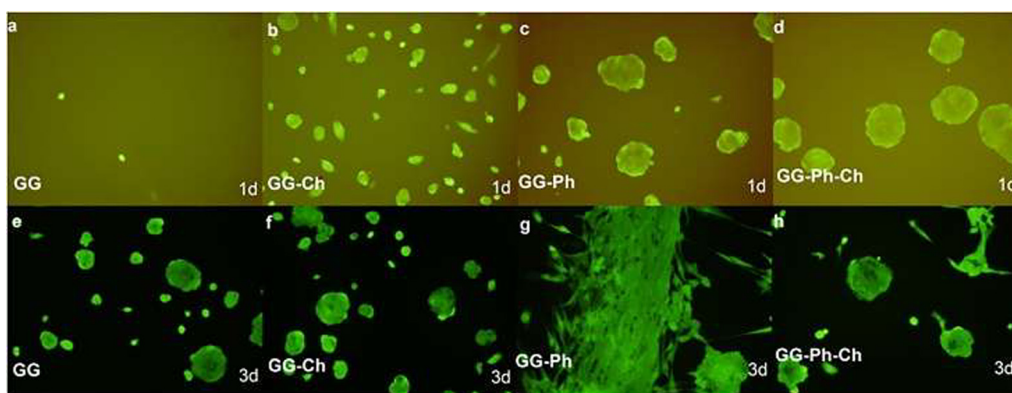
Fig. 4. Reference (Lišková et al., 2018). ADSC morphology. a-d: after 1 d. e-h: after 3 d. GG: gellan gum only (no chitosan or phytase). GG-Ch: with chitosan (1.5 %). GG-Ph: with phytase: GG-Ph-Ch: with phytase and chitoaan. Reproduced with permission.

and chondrogenic marker genes were observed after 14 days of culture of chondrocytes on the hydrogel scaffolds. Cartilage and subchondral bone formation were accelerated by implanting the DBP/GG scaffolds in rabbit OC defects for 6 weeks.

Native cartilage ECM is comprised mainly of type-II collagen and glycosaminoglycans (Gong et al., 2015; Hutmacher, 2006). The presence of one glucuronic acid for every repeating tetrasaccharide unit of gellan gum bears structural resemblance to native cartilage glycosaminoglycans such as chondroitin sulfate and hyaluronan as they contain at least one uronic acid in their repeating disaccharide unit (Colley, Varki, & Kinoshita, 2017). However, adult hyaline cartilage is continuously mineralized at the interface with bone tissues (Freeman, 1979). This process is necessary to confer cartilage with sufficient mechanical strength to withstand contact load and shear stress (Bhosale & Richardson, 2008). Hence, cartilage-mimetic gellan gum hydrogels are often formulated with the direct blend of inorganic materials that are able to rearrange their micro- and nanostructural topology for mechanical conditioning.

Bonifacio et al. (2017) reported the preparation and characterization of a tri-component hydrogel, based on gellan gum, glycerol and halloysite nanotubes (HNT) for cartilage tissue engineering. An aqueous suspension of HNT was mixed into a pre-heated solution of gellan gum and glycerol to obtain the composite material, which was subsequently cooled and crosslinked with $CaCl_2$ to form the hydrogel. Glycerol is a popular biocompatible molecular spacer; it increases the porosity of gellan gum hydrogels through a process known as porogenesis (Aoki et al., 2006). On the other hand, HNT belongs to a class of nanoclay materials which, when impinged onto the surface of gellan gum hydrogel, led to a reduction in hydrophilicity of gellan gum hydrogel. The enhanced pore size and hydrophobicity of the resultant gellan gum hydrogel remarkably improved the cell viability of encapsulated human dermal fibroblasts (HDFs) for up to 7 days of culture.

Rao, Kumar, and Han (2018)) prepared a polyelectrolyte complex hydrogel made up of xanthan and chitosan reinforced with HNT. The electrostatic interactions between the two biopolymers and HNT formed a dense network, allowing significant level of HNT deposition. Cell viability of MC3T3-E1 osteoblasts increased along with higher amount of HNT impinged.

2.1.2. Enzymatic incorporation of inorganic materials

Enzymatic mineralization is an alternative strategy to enrich microbial polysaccharide hydrogels with bone minerals. In comparison to direct blending, the specificity and controllable rates of enzymatic reactions promote uniform distribution of inorganic materials within the hydrogel matrix (Colaço et al., 2020). Reactions that generate positively charged cations further provide the ingredient for an in-situ gelling system with the anionic gellan gum. Similarly, the enzymatic deposition of inorganic materials enhanced the mechanical and surface topography of resultant hydrogels. Certain enzymatic reactions have also facilitated the coating of hydrogel matrix with bone salts such as calcium and magnesium which further provided chemical cues to direct bone cell fates (Z. Du et al., 2020).

In the first report of its kind, using alkaline phosphatase (ALP), an enzyme involved in mineralization of native bone by cleaving phosphate group from organic compounds, Douglas et al. (2014) were able to induce mineralization of gellan gum with calcium phosphate (CaP). The incorporation of CaP not only enabled mechanical reinforcement, but also supported osteoblast adhesion and proliferation. In a more recent paper, by adding a small amount of zinc in the mineralization medium, the same group (Douglas, Pilarz et al., 2017) endowed CaP-laced gellan gum hydrogel with antibacterial activity against methicillin-resistant *staphylococcus aureus* (MRSA). Moreover, the presence of zinc improved the adhesion and early proliferation of MC3T3-E1 osteoblast-like cells.

The carboxylate groups on gellan gum act as nucleation sites for CaP crystal growth. As a result, CaP inadvertently becomes a competitive inhibitor of ionic crosslinking. Therefore, supplementary calcium ions are often required to overcome the reduction in crosslinking potential. A strategy using a more reactive type of inorganic particle, alpha-tricalcium phosphate (α-TCP), was adopted to react with water to form calcium-deficient HAP and excess calcium ions (Douglas et al., 2018), Gelation was achieved without the need for calcium supplementation. Furthermore, gelation was completed only after 30 min of incubation in mineralization medium, allowing injectability of the pre-gelation mixture. Microcomputed tomography (μCT) characterization revealed that the slower rate of crystallization has enabled CaP crystals to be more evenly distributed throughout the hydrogel network.

Interestingly, in a more recent paper, Lioková et al. (2018) showed that a plant-derived phosphatase known as phytase could also be used for the enzymatic mineralization of gellan gum hydrogels. Pre-formed gellan gum discs were incubated in solution containing phytase, chitosan and calcium glycerophosphate (CaGP). The enzyme catalysed the conversion of CaGP to CaP. Phytase-mineralized gellan gum supported both MG63 osteoblast and ADSC cell adhesion and proliferation (Fig. 4). While the same assays showed that ADSC adhesion and proliferation was poor without phytase-mediated mineralization.

Another inorganic material which has been widely and successfully applied in bone regeneration is calcium carbonate ($CaCO_3$). $CaCO_3$ exists either as amorphous calcium carbonate (ACC) or in three different crystalline polymorphs, namely calcite, aragonite and vaterite (Aizenberg, Weiner, & Addadi, 2003; Andersen & Brecevic, 1991; Vallet-Regí & González-Calbet, 2004). Bone regeneration has been demonstrated for calcite (Barrère, van Blitterswijk, & de Groot, 2006; Obata, Hotta, Wakita, Ota, & Kasuga, 2010). A strategy to promote the deposition of magnesium calcite in gellan gum hydrogel was proposed by Douglas, Łapa et al. (2017) In this work, gellan gum was modified

using urease-mediated mineralization with calcium carbonate, magnesium-enriched calcium carbonate and magnesium carbonate for bone regeneration applications. Hydrogels were mineralized when the components were incubated in mineralization media containing urease, urea and different ratios of calcium and magnesium ions. Urease catalysed the conversion of urea and water to bicarbonate ions and ammonia. Bicarbonate ions further underwent spontaneous deprotonation to form carbonate ions, which subsequently reacted with calcium ions to form $CaCO_3$. The generation of ammonia raised the pH of the mineralization media, promoting $CaCO_3$ precipitation and deposition. The presence of magnesium in the mineralization media promoted the conversion of magnesium carbonate to magnesium calcite. Confocal laser scanning microscopy (CLSM) images of MC3T3-E1 osteoblast-like cells seeded onto the surface of the functionalized hydrogel showed an extended morphology indicating good adhesion. Although magnesium is a minor toxic metal (Hollinger, 1996), the viability of MC3T3-E1 osteoblast-like cells seeded onto the mineralized hydrogel was comparable to that of unmineralized hydrogel after 7 days of culture.

Lopez-Heredia et al. (2017) further enhanced the urease-mediated mineralization of gellan gum hydrogels by introducing a second enzyme.. The rate-limiting step of mineralization – deprotonation of bicarbonate to carbonate ions, can be accelerated by carbonic anhydrase. Dry mass percentage changes and inductively coupled plasma optical emission spectrometry (ICP-OES) demonstrated that hydrogel precursor solution containing both urease (U) and carbonic anhydrase (CA) were mineralized with more calcite than solution containing only urease. SEM imaging revealed that MC3T3-E1 osteoblast-like cells attached to hydrogel surface containing both U + CA displayed a flatter morphology (Fig. 5).

2.1.3. Nano-inorganic materials

Beside granular form of inorganic materials, nano-sized counterparts with stronger affinity to materials have recently garnered considerable interest in TERM (Pepla, Besharat, Palaia, Tenore, & Migliau, 2014). The usage of nano-inorganic materials significantly increases the surface-to-volume ratio, and thus the aspect ratio, of impinged materials. As a result, the areas of interface between nano-inorganic materials, the matrix, and cells are at least an order of magnitude higher than conventional composite materials mentioned above (Mostafavi, Quint, Russell, & Tamayol, 2020). This in turn implies that a relatively lower, and often less toxic concentration of nano-inorganic materials is required to impart predetermined biological effects (Conte et al., 2019). In the examples given below, nano-inorganic materials were shown to influence structural, chemical, and even magnetic properties of microbial polysaccharide hydrogels that eventually resulted in their enhanced biomimicry.

The process of incorporating nano-inorganic materials into microbial polysaccharide hydrogels was recently described by Razali, Ismail, Zulkafli, and Amin (2018)), whereby freeze-drying was used to fabricate titanium oxide (TiO$_2$) nanoparticles-gellan gum scaffold. A suspension of TiO$_2$ nanoparticles was stirred into a heated solution of gellan gum, glycerol and KCl. The homogeneous mixture was then subsequently cooled and freeze-dried. When seeded on the surface of reconstituted hydrogels, fluorescent images of the MC3T3 mouse fibroblasts showed enhanced time-dependent spread as compared to pristine gellan gum hydrogels. The authors postulated that the presence of TiO$_2$ stimulated the expression of growth factors like fibroblast growth factor through upregulation of reactive oxygen species (ROS).

Nanoparticles were also incorporated into xanthan gum hydrogels as a strategy to biofunctionalize the material. Certain inorganic nanomaterials are capable of altering the architectural topology of matrices which could promote its interaction with cells (Engin et al., 2017). For example, Kumar, Rao, and Han (2017)) prepared a highly porous xanthan/silica glass hybrid scaffold reinforced with cellulose nanocrystals. The incorporation of silica glass and cellulose nanocrystals significantly increased the adhesion and proliferation of pre-osteoblast MC3T3-E1 cells.

Neuronal cells are sensitive to external electromagnetic stimulation (Sensenig, Sapir, MacDonald, Cohen, & Polyak, 2012). By incorporating magnetite nanoparticles into xanthan gum hydrogel, Glaser, Bueno, Cornejo, Petri, and Ulrich (2015)) enhanced neuronal cell attachment, proliferation and differentiation could be achieved. It was postulated
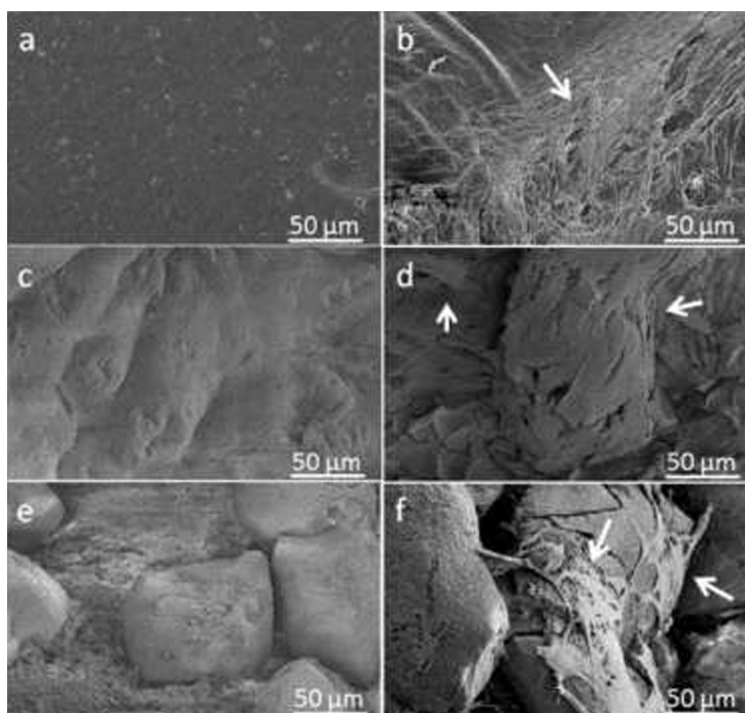

Fig. 5. Reference (Lopez-Heredia et al., 2017). SEM images of samples without (left) and with (right) MC3T3-E1 osteoblast-like cells on enzyme-free GG hydrogels (a and b), hydrogels containing U (c and d) and hydrogels containing U and CA (U + CA, e and f). Cells are indicated by arrows. Reproduced with permission.

that the electromagnetic fields generated from the highly charged magnetic nanoparticles led to these processes. Rao, Kumar, and Han (2018)) also used xanthan, chitosan and iron oxide magnetic nanoparticles to form magnetically responsive polyelectrolyte complex hydrogels. In the presence of a magnetic field, SEM imaging showed that cell adhesion of NIH3T3 fibroblasts was stronger, with obvious clustering of cells. Correspondingly, the fibroblasts exhibited significantly increased cell viability. Under the influence of a magnetic field, magnetic nanoparticles are able to alter the microenvironment of resultant hydrogels, making them more suitable for receptive cells.

2.1.4. Synthetic inorganic materials

Blending of gellan gum hydrogels with biocompatible synthetic inorganic materials has also been explored. Synthetic inorganic materials possess a wide spectrum of tailor-designed properties thus, organic-inorganic composite hydrogels made from these materials have significantly expanded biological applications (J. Du et al., 2015). In the examples shown below, extraordinary properties such as dual functionality of cell adhesivitiy and electrical conductivity, as well as mesoporous microarchitecture can be imbued by integrating synthetic inorganic materials into the hydrogels' matrices.

One of such example is given by Zargar, Mehdikhani, and Rafienia (2019)) where a gellan gum/reduced graphene oxide (rGO) composite hydrogel was assembled for the growth of rat myoblasts (H9C2). Apart from improved porosity and mechanical properties, the incorporation of reduced graphene oxide instilled electrical conductivity, which is not an intrinsic property of anionic hydrogels such as gellan gum. At 2% rGO concentration, the resultant hydrogels mimicked the native myocardium conductivity and enabled the growth of embryonic cardiomyocte H9C2. Overall, the data provided evidence for the potential application of gellan gum/reduced graphene oxide hydrogels as myocardial tissue engineering scaffolds.

By infusing synthetic inorganic clays such as mesoporous silica, sodium-calcium bentonite, or halloysite nanotubes, Bonifacio et al. (2020) prepared gellan gum/manuka honey-based composite hydrogels for articular cartilage repair. The void area, pore area and pore diameter of all clay-containing scaffolds lowered dramatically in comparison to the bare polymeric matrix. The altered hydrogel microarchitectures were considered important to promote cell attachment, proliferation, and colonization. More specifically, gellan gum/manuka honey hydrogels incorporated with mesoporous silica were effective in enabling hMSC 3D culture and supporting chrondrogenesis for cartilage tissue engineering applications.

2.2. Enhancement of other biological and/or mechanical properties of microbial polysaccharide hydrogel scaffolds

2.2.1. Improvement of mechanical properties

As mentioned briefly above, physiologically, the ECM's mechanical properties influence many cellular functions, including migration, growth, differentiation, and even cell survival (Schwartz, Schaller, & Ginsberg, 1995). Alteration of the mechanical properties of hydrogel scaffold can tweak the cell mechanosensing process, providing a more conducive microenvironment for cell growth (Humphrey, Dufresne, & Schwartz, 2014). Pristine gellan gum hydrogels have inadequate mechanical strength to facilitate cell adhesion (Yeung et al., 2005) and induce osteogenesis (Tozzi, De Mori, Oliveira, & Roldo, 2016). Often, extensive tuning is required before they become suitable for motion-intensive bone and intervertebral fibrocartilage tissue engineering (Kumar et al., 2018; Osmałek et al., 2014; Silva-Correia et al., 2011, 2012; Sun & Mao, 2012). Beside changing the polymer and/or cross-linker concentrations, addition of certain inorganic materials can also foster strengthening of the resultant hydrogels.

In an attempt to overcome the abovementioned shortcomings, Hu et al. (2018) prepared a hydrogel that is composed of gellan gum-poly (ethylene glycol) diacrylate (GG-PEGDA) and poly(lactic acid) (PLA).

PLA is a biocompatible synthetic polymer that is commonly used to increase the mechanical strength of composite hydrogels (Cai et al., 2009; Drury & Mooney, 2003; Gentile, Chiono, Carmagnola, & Hatton, 2014). Using 3D bioprinting technologies, 3D cell-laden constructs containing a physical blend of GG-PEG and PLA were fabricated. Compressive stress tests revealed that the resultant hydrogel can tolerate multiple cycles of loading (0.1–3 MPa) at high magnitudes with strain under 3 MPa and stress less than 5%. Bone marrow stromal cells (BMSCs) encapsulated within the GG-PEGDA-PLA hydrogel maintained a high cell proliferation rate with viability above 90 % during the 7 days of culture time. Further F-actin immunostaining confirms that the actin cytoskeleton of BMSCs is dynamic and cells are spreading in rapid division.

Polycaprolactone (PCL) is another synthetic polymer that has received a great deal of attention for its use as a sturdy implant material (Low, Ng, Yeo, & Chou, 2009; Nisbet, Rodda, Horne, Forsythe, & Finkelstein, 2009). Being highly compatible with other resin materials, it is often used as an additive to enhance mechanical properties (Kashanian et al., 2010). A hybrid scaffold based on gellan gum, gelatin and PCL was developed by Vashisth and Bellare (2018) when they exploited this advantageous trait. Electrospun sheets of gelatin and PCL were woven into the gellan gum scaffold forming core-sheath layers. PCL altered the nanotopography of the hydrogel scaffold by providing a niche mimicking bone ECM. SEM imaging, MTT assay and DNA quantification assay confirmed the existence of specific physical cues on hybrid hydrogel for improved bone cell growth. CLSM illuminated the formation of distinct bone cell colonies that expanded in a 3D manner throughout the scaffold after 14 days of culture.

It can also be observed that the incorporation of nanoparticles presents another approach to strengthen the mechanical features of hydrogels (Zaragoza, Fukuoka, Kraus, Thomin, & Asuri, 2018). This strategy was recently applied on gellan gum by Sahraro, Barikani, and Daemi (2018)). In their work, cationic polyurethane soft nanoparticles (CPUN) were used as reinforcing agent to improve the mechanical properties of methacrylated gellan gum (GGMA) hydrogels. The cationic nanoparticles function as "molecular glues" that connect the anionic carboxylate groups through ionic interactions. The entropy-driven tendency of CPUN to aggregate *via* hydrogen bonds and hydrophobic interactions further assists the reinforcing mechanism by pulling the crosslinking sites closer to each other. To formulate the nanocomposite hydrogel (NCH), different amounts of CPUN dispersion were separately mixed with 1% w/v of gellan gum macromers before photocrosslinking. Compression analysis and rheological measurements proved that the incorporation of CPUNs into GGMA networks substantially improved the mechanical performance of the resulting hydrogels. *In vitro* MTS cell viability tests demonstrated the cytocompatibility and non-toxicity of NCHs. Seeded HDFs retained more than 90 % cell viability after 7 days of incubation.

2.2.2. Improvement of other biological properties

Xanthan gum hydrogels were also conferred with fortuitous properties when nanoparticles were incorporated into their meshwork. Using gold nanoparticles, Pooja, Panyaram, Kulhari, Rachamalla, and Sistla (2014)) prepared xanthan gum nanohydrogel that exhibited colloidal stability in a wide range of pH as well as electrolyte and serum concentrations. The optimized concentration of gold nanoparticles was non-toxic and biocompatible with human cells. In another work, Bueno et al. (2014) prepared xanthan gum hydrogel incorporated with HAp's strontium substituted nanoparticles. Although the nanocomposite hydrogel did not enable significant proliferation of osteoblasts, the cells' ALP activity improved. The authors posit a nanoparticle-mediated osteogenic differentiation phenomenon.

Raafat, El-Sawy, Badawy, Mousa, and Mohamed (2018)) prepared nanocomposite hydrogels composed of xanthan gum, PVA and zinc oxide nanoparticles. The embedded nanoparticles improved the hydrogel's swelling capacity, fluid uptake ability, water retention and

water vapour transmission properties. In addition, the presence of zinc further imparted broad spectrum antimicrobial activity to the resultant hydrogel. Rao, Kumar, Haider, and Han (2016)) incorporated silver nanoparticles into polyelectrolyte hydrogel consisting of xanthan and chitosan. The nanoparticle-laced hydrogel also exhibited strong antibacterial activity, specifically against *Escherichia coli* and *Streptococcus aureus*. Although extensive toxicological studies have shown that silver nanoparticles are toxic (Vazquez-Muñoz et al., 2017), cell proliferation and cell attachment of NIH3T3 fibroblast cells were not compromised.

Fernandez-Piñeiro et al. (2018) (Fernandez-Piñeiro et al., 2018) incorporated sorbitan monooleate nanoparticles into xanthan gum, forming a stable complex nanohydrogel for gene-targeting to endothelial cells. The authors investigated the hydrogels' biocompatibility in both *in vitro* and *in vivo* systems. Human umbilical vein endothelial cell (HUVEC) viability remained unchanged until an effective nanoparticle concentration of 384 µg/mL. No significant toxicity was observed in major organs including kidney, liver, lung and spleen after similar concentration of nanoparticles were administered intravenously to mice model.

El-Meliegy et al. (2018) prepared nanocomposite scaffolds based on dicalcium phosphate nanoparticles, dextran and carboxymethyl cellulose. Using simple lyophilization technique of the frozen dispersions, they were able to fabricate a more physically stable scaffold with good cytotoxicity profile. By regulating the amount of dicalcium phosphate nanoparticles, porosity of the composite hydrogel could also be precisely controlled.

3. Biofunctionalization of microbial polysaccharide hydrogels using organic materials

Nature offers an amazing repository of organic materials yet unearthed for their potential in biomedical applications. Since time immemorial, nature-derived organic products have been the source of traditional bioactive materials. The use of these materials in preparations that have been concocted for medical purposes dates back hundreds, even thousands, of years ago (Harvey, 2008; Koehn & Carter, 2005; J. W.-H. Li & Vederas, 2009). Fast forward to contemporary biomaterial landscape, even though chemical modifications allow the precise tuning of hydrogels' biological properties, their safety and efficacy have always remained questionable. As a result, many recent researches turn towards nature for a rich source of biotic materials possessing innate propensity to form bioactive composite hydrogels.

3.1. Enhancement of cell attachment and proliferation of microbial polysaccharide hydrogel scaffolds

3.1.1. Nature-derived organic materials

A broad range of natural organic materials have been applied for cartilage TERM. These organic materials behave like biological factors, capable of instructing cell fate. For example, phytochemical saponins which have cartilage-protective effects (Wang, Xiang, Yi, & He, 2017; Wu et al., 2017; Xie et al., 2018; Xu, Zhang, Diao, & Huang, 2017) were recently used for cartilage tissue engineering by Jeon et al. (2018). Saponins were physically entrapped within the gellan gum hydrogel network during its gelation process. The presence of saponins had a positive effect on the cell viability of chrondrocytes. Saponins also stimulated the encapsulated chrondrocytes to express higher levels of specific cartilage related genes such as type-1 & -2 collagen as well as aggrecan. These preliminary data suggested saponin-infused gellan gum hydrogel as a promising cartilage implant material.

In another study, Bonifacio et al. (2018) described the incorporation of manuka honey as a molecular spacer for the preparation of cartilage-mimicking gellan gum composite hydrogel. Apart from improving the compressive moduli of the unmodified gellan gum hydrogel from 116 up to 143 kPa, human mesenchymal stem cells (hMSC) seeded on the hydrogel surface proliferated. Gene expression assays further validated the resultant hydrogel's ability to support chondro-like matrix formation. Moreover, according to reverse transcription-polymerase chain reaction (RT-PCR), there were higher expression of collagen-II, glycosaminoglycans (GAGs) and proteoglycans by hMSC cultivated on said hydrogel.

Da-Lozzoa et al. (2013) (Da-Lozzo et al., 2013) prepared curcumin/xanthan-galactomannan hydrogel and investigated its *in vivo* biocompatibility using chick embryo chorioallantoic membrane assay. The hydrogels were completely absorbed after 1 week of incubaton, no significant tissue damage was observed. Kuo, Chang, Wang, Tang, and Yang (2014)) prepared hydrogel comprising of various formulation of xanthan, gellan and hyaluronam and evaluated their ability in preventing premature adhesion of post-excision tendons.

3.1.2. Polymeric organic materials

An interpenetrating polymer network comprising of a secondary bioactive polymer could also greatly enhance cell-matrix interaction (Matricardi et al., 2013). In particular, organic polymers with native cell-adhesive ligands are able to bestow integrin-recognizing moiety on resultant hydrogels (Cerqueira et al., 2014; (da Cunha et al., 2014) Liu & Chan-Park, 2009). In many other cases, topological constraint due to the presence of a secondary network also further augments poor mechanical properties through a phenomenon known as entanglement enhancement effect (Myung et al., 2007, 2008).

In an interesting article, Sant et al. (2017) formulated a self-assembling fibrous hydrogel comprising of GGMA and chitosan, omitting the need for ionic crosslinking completely. GGMA and chitosan are oppositely charged macromolecules that can form hydrogel *in situ*. Individual components flowed through two spatially separated polydimethylsiloxane (PDMS) channels, gelation was observed when the negatively charged gellan gum come in contact with the positively charged chitosan at a junction. The resultant hydrogel displayed a hierarchical fibrous network with characteristic periodic light/dark bands similar to native collagen at both the nano- and microscale. Other than being a structural mimicry of collagen, the presence of carboxyl- (in gellan gum) or amino- (in chitosan) moieties further allowed the hydrogel to be functionalized with RGD groups. Overall, the collagen-mimetic hydrogel system exhibits vast potential as a scaffold for tissue engineering applications.

Hyaluronan (HA) is one of the chief components of the extracellular matrix that contributes significantly to cell adhesion and migration (Hay, 2013; Toole, 2004). It is an anionic, nonsulfated glycosaminoglycan distributed widely throughout the connective and epithelial tissues. Three main groups of cells receptors have been isolated and amongst which, CD44 is recognized as the main cell surface receptor. Cells with CD44 recognition ligand such as keratinocytes are widely distributed throughout the body. Karvinen, Koivisto, Jönkkäri, and Kellomäki (2017)) recognized this utilitarian feature and constructed a hydrogel based on an optimized blend of HA and gellan gum. Rheological measurements confirmed the successful gelation of HA-gellan gum composite hydrogel. Mechanical compressive tests showed that the composite hydrogels have similar stiffness to soft tissues, and together with inherent cell adhesive properties of HA, highlighted its potential in soft tissue engineering.

Agar is a mixture of agarose/agaropectin and is a common congealed substrate for microbiological research (Buil et al., 2017). Chemically, agar is a polymer made up long chains of D-galactose subunits (W.-K. Lee et al., 2017). It exhibits good biocompability and shear-thinning properties (Liu, Xue, Zhang, Yan, & Xia, 2018; Tonda-Turo et al., 2017). Baek et al. (2019) blended different concentrations of agar into gellan gum hydrogels. The presence of agar enabled cell adhesion and proliferation of embedded chondrocytes. Besides, rheological examinations further proved that increasing concentrations of agar improved the injectability of the formulae. As a result, the chondrocyte-loaded gellan gum-agar hydrogel exhibited potential as an injectable TERM scaffold for cartilage regeneration purposes.

Silk fibroin is a mixture of insoluble proteins produced by the larvae of *Bombyx mori*. Previous studies have demonstrated its superior biocompatibility and ability to promote chondrocyte proliferation as a scaffold material (Wang, Kim, Vunjak-Novakovic, & Kaplan, 2006). Shin et al. (2019) prepared silk fibroin/gellan gum (SF/GG) hydrogels in combination with miR-30a, a miRNAs (MicroRNAs), to further induce chondrogenic differentiation of encapsulated bone marrow mesenchymal stem cells (BMSC). Cell viability assay and histological analysis demonstrated the suitability of the SF/GG hydrogel for cells adhesion, ingrowth and nutrients perfusion. Results of quantitative polymerase chain reaction (qPCR) corroborated the ability of the hydrogel to carry and expose miR-30a for the chondrogenic differentiation of BMSC isolated from rats.

Wang, Wen, and Bai (2017)) attempted to incorporate polyvinyl alcohol (PVA) into gellan gum hydrogel network. Due to its ability to form tubular microporous structure that enhances cell adhesion and spread, PVA have been extensively recognized as a potential material in tissue engineering, especially for cartilage repair (M. F. Cutiongco et al., 2016; Hassan & Peppas, 2000). A mixture of pre-heated PVA and gellan gum was subjected to repeated freeze-thaw cycles and finally cross-linked with aluminium ions (Al^{3+}). Subsequent SEM imaging confirmed the reorganization of the hydrogel's porous structure. The authors also attributed this phenomenon to the strong electrostatic interaction between Al^{3+} and carboxylate groups of gellan gum, which further altered the network structure and enhanced mechanical properties of the composite hydrogel. The improved porosity and stiffness of the resultant hydrogels was touted to meet the requirement of a synthetic articular cartilage.

Hybrid hydrogels composed of xanthan gum (XG) and PVA as potential nucleus pulposus (NP) substitutes were synthesized by Leone et al. (2019). NP are soft tissues with peculiar mechanical properties. In this work, optimized PVA and XG in the molar ratio 4:1 showed mechanical, swelling, and thermal properties which make it a good candidate as a potential NP substitute. More importantly, NIH3T3 fibroblast cells, in contact with the hydrogel, were able to grow and proliferate normally over 7 days of incubation period.

Xanthan gum has also been formulated with chitosan to form hydrogel blends with significantly improved properties. As xanthan gum and chitosan are also oppositely charged polyelectrolytes, they have a tendency to associate in aqueous solvents into macroporous polyelectrolyte complex. Chellat et al. (2000) showed that the complexation of xanthan and chitosan did not cause cytotoxic effects in an *in vitro* model with L929 mouse fibroblast cell line as well as an *in vivo* mouse model.

Aguiar, Silva, Rodas, and Bertran (2019)) prepared mineralized layered films composed of xanthan and chitosan. *In vitro* cell adhesion test with MG63 cells revealed that the films could be further interweaved with calcium phosphate (CaP), enhancing cell attachment on the material surface (Fig. 6). The formation of hydroxyapatite by the addition of calcium and phosphate ions also promoted cell growth. The films appear to be promising candidates for bone tissue regeneration.

Beside calcium phosphate ions, other materials have also been incorporated into the xanthan gum-chitosan blend scaffold. de Souza et al. (2019) added a surfactant (Kolliphor P188, K) to generate pores and silicon rubber (Silpuran 2130A/B, S) to increase mechanical properties of the xanthan-chitosan matrix. When HDF cells were exposed to the extracts of the materials, they remained viable and no cytotoxicity effect was observed. ADSC seeded on the scaffolds retained metabolic activity as consistent amount of lactate dehydrogenase (LDH) was released.

Other than chitosan, other polymers could also be employed as a secondary material for blending with xanthan gum. Juris et al. (2011) investigated the biocompatibility of a hydrogel blend made up of a mixture of xanthan gum, konjac, k-carrageenan and I-carrageenan. Human fibroblasts seeded onto the composite hydrogels showed greater than 90 % viability after 7 days of culture. The fabricated hydrogel is non-toxic to mammalian cells.
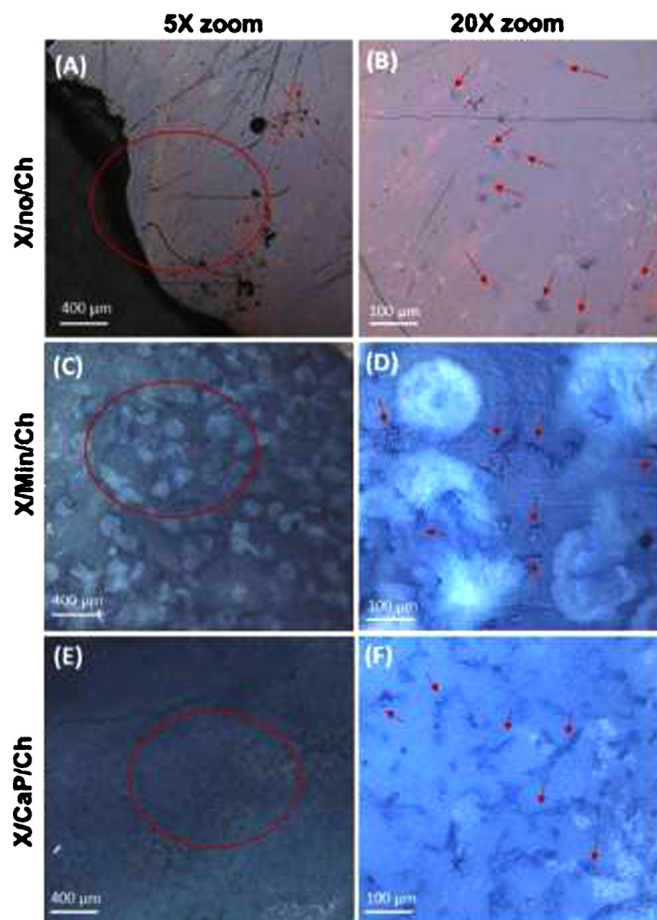

Fig. 6. Reference (Aguiar et al., 2019). Images obtained with a confocal microscope for the *in vitro* cell adhesion test with the culture of MG63 cells in the X/No/Ch, X/Min/Ch and X/CaP/Ch films. The images were obtained with a magnification of 5X (A, C, E), 20X (B, D, F). Reproduced with permission.

Liu et al. (2015) (Liu and Yao, 2015) prepared injectable thermoresponsive hydrogel composed of xanthan and methylcellulose. Its *in vivo* biocompatibility was examined in rats. Xanthan/methylcellulose solution was injected into the rats and gelation was achieved *in situ*. The hydrogel swelled from day 1 to day 7 and degraded completely after 36 days. Although inflammatory cells were observed around the implanted hydrogel, but their amount decreased rapidly with time. The material was injectable, biodegradable and biocompatible

Mendes et al. (2012) (Mendes et al., 2012) used self-assembled peptide-polysaccharide microcapsules as 3D environments for cell culture. Cells encapsulated in the xanthan-peptide matrix with the highest peptide concentration were able to reduce AlamarBlue significantly over the 21 days of culture, indicating a higher cell viability as compared to matrix formulated with the lowest peptide concentration. The cells remained viable up to 21 days of culture, demonstrating the ability of this matrix to support cell viability over a prolonged period of time.

Alves et al. (2020) formulated a thermo-reversible hydrogel composed of xanthan gum–konjac glucomannan blend for wound healing applications. In this work, the combination of two polysaccharides, xanthan gum and konjac glucomannan, produced a hydrogel film dressing that is hydrophilic, possesses the ability to provide a moist local wound environment and absorbs excess exudate to promote proper wound healing. Besides, the resultant hydrogel was able to improve human fibroblasts migration, adhesion and proliferation, thereby promoting the cells' secretion of ECM components to accelerate the granulation process.

Dextran has been blended with other polymers to enhance its cell

attachment and proliferation. Cutiongco, Tan, Ng, Le Visage, and Yim (2014)) modified pullulan-dextran scaffolds with interfacial polyelectrolyte complexation fibers to improve their ability to support adherent cell growth. There was an increase in the no. of cells seeded on the composite scaffolds incorporated with fibronectin as compared to the plain pullulan-dextran scaffolds.

Zhu et al. (2018) fabricated a dextran-hyaluronic acid hydrogel enriched with sanguinarine-incorporated gelatin microspheres. Hyaluronic acid was incorporated *via* Schiff reaction which avoided the possible cytotoxicity caused by the free radical crosslinking agent by traditional methods. Enhanced NIH3T3 fibroblast proliferation could be observed when exposed to culture media extract of the hydrogels for up to 4 days of incubation. Moreover, the hydrogels inhibited the growth of common wound bacteria such as MRSA and *Escherichia coli*.*In vivo* burn infection model showed that the hydrogel improved re-epithelialization and enhanced extracellular matrix remodeling. Wound proinflammatory cytokines of TGF-β1 and TNF-α were lower than the other groups, while TGF-β3 expression was increased. Overall, the composite hydrogel served as a potential material to treat infected burn wounds.

Kulikouskaya et al. (2018) formed multi-layer films with oppositely charged components. Polyethyleneimine (PEI) and chitosan were used as polycations. Dextran sulfate (DexS), pectin citrus, sodium salt of carboxymethyl cellulose (CMC) were used as polyanions. A mono-layer cell culture of mesenchymal stem cells was seeded on all chitosan-containing films. (PEI/DexS)$_4$ and mixed positively charged PEI-terminated films were more favourable for mesenchymal stem cell (MSC) adhesion as compared to other PEI-containing films. This phenomenon may be attributed to the cell-resistant properties of DexSwhich affected the physiochemical and mechanical properties of the films. DexS lowered the surface roughness and stiffness of the films, resulting in greater cell adhesion and number of viable cells.

More recently, Guo, Qu, Zhao, and Zhang (2019)) synthesized a series of injectable electroactive biodegradable hydrogels with rapid self-healing ability composed of N-carboxyethyl chitosan (CECS) and dextran-graft-aniline oligomers. Dynamic Schiff base bonds between the formylbenzoic acid and amine group from N-carboxyethyl chitosan conferred the hydrogels with self-regenerating properties. As the hydrogels were formed at physiological conditions, C2C12 myoblasts could be successfully encapsulated. In addition, skeletal muscle regeneration was observed when the myoblast-laden hydrogels were examined in an *in vivo* volumetric muscle loss injury model.

Grenier et al. (2019) prepared a blend hydrogel between dextran and pullulan. Delving deep into the mechanism of pore formation during freeze-drying, the group found a method to control the porous structure of hydrogel scaffolds by adjusting the cooling rate. With an optimal formula, pores in the freeze-dried scaffold became adequately interconnected to allow homogenous cell distribution of MC3T3-E1 pre-osteoblasts into spheroids (Fig. 7). Since cell clustering and spheroid growth are important to promote cell-cell interactions in bone tissue engineering (Walser et al., 2013), the blend hydrogel couldbe further developed for this purpose.

3.1.3. Cell-adhesive materials

Certain organic materials, especially ECM-derived, such as fibronectin and laminin possess innate cell adhesive proeprties. Consequently, an adequate degree of cell viability and cell spread could be derived from these materials as tissue scaffolds. In comparison to bulk hydrogels formed directly from these organic cell adhesive materials, their conjugation to polymers forming protein-polymer composites have greatly reduced fabrication cost as well as improved enzymatic stability (da Silva et al., 2014).

In a recent example, Gering et al. (2019) developed modular gellan gum hydrogels functionalized with avidin and biotinylated adhesive ligands such as RGD or fibronectin for cell culture applications. By exploiting the highly selective avidin-biotin binding system, stable non-covalent conjugation of RGD and fibronectin to gellan gum polysaccharide structure was achieved. The conjugation did not affect gellan gum's ability to form ionically crosslinked hydrogels and, in fact, promoted cell adhesion and growth for human fibroblasts and BMSC for over 3 weeks of culture.

A thiolated gellan gum (TGG) hydrogel with binding sites for laminin was developed by Yu et al. (2020). In this study, non-covalent binding of laminin to thiolated gellan gum enabled the sustained release of laminin peptides for the 3D cell culture of encapsulated human neural stem cells (HNSCs) for up to 14 days. It was postulated that the thiolation introduced sulfhydryl groups to form a double network structure that binds the laminin peptides. Altogether, the results illustrated the use of TGG in combination with laminin for neural tissue engineering applications.

3.1.4. Nano-organic materials

Nanoparticles can also be prepared from organic molecules such as chitosan (Mohammed, Syeda, Wasan, & Wasan, 2017). Chitosan nanoparticles are widely favoured as a carrier for drug delivery (Nagpal, Singh, & Mishra, 2010). As a polymer, chitosan chains form diffusion barrier making it difficult for drug molecules to diffuse through the interior of a polymeric matrix (Singh & Lillard, 2009). Applying this feature, Dyondi, Webster, and Banerjee (2013)) prepared xanthan-gellan gum hydrogel incorporated with chitosan nanoparticles, basic fibroblast growth factor and bone morphogenetic protein 7. When exposed to the hydrogels, cell viability was found to be greater than 95 % for L929 cells and greater than 80 % for human fetal osteoblast cells.
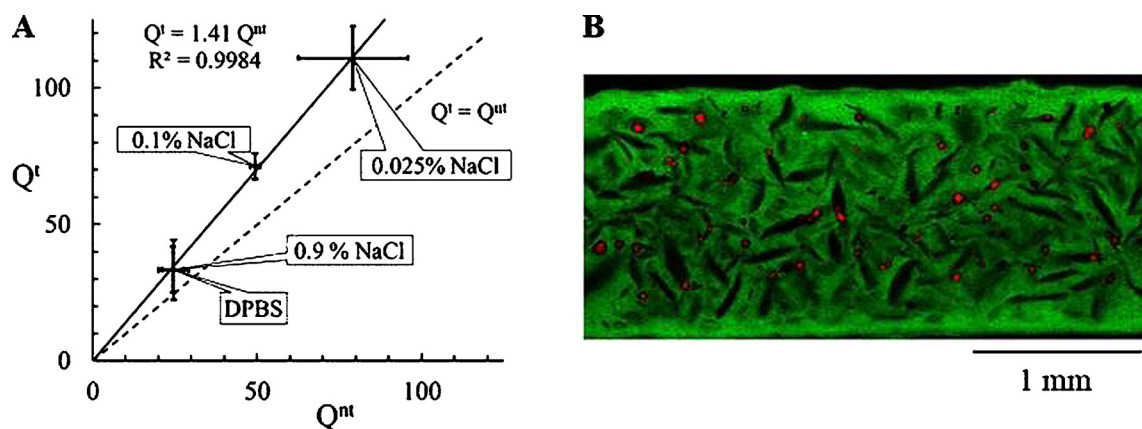
Fig. 7. Reference (Grenier et al., 2019). Fate of the porous structure after swelling and 3D cell culture. A: Swelling volume ratio for textured (Q_t) and non-textured (Q_{nt}) scaffolds swollen in 0.025 % NaCl, 0.1 % NaCl, 0.9 % NaCl and DBPS. The linear regression model is fitted to the data without the intercept term. B: CLSM XZ cross-section of a freeze-dried scaffold (7.2 mm diameter, 1.4 mm height) 24 h after the seeding of 100,000 MC3T3-E1 cells. Reproduced with permission.

The hydrogel enabled significant improvement in cell growth and differentiation of osteoblast cells due purportedly to the sustained release of growth factors.

Bioactive nanocomposite hydrogel fabricated from xanthan, chitosan and cellulose nanocrystals by Rao, Kumar, and Han (2017)) also displayed superior biocompatibility with NIH3T3 mouse embryo fibroblasts. More importantly, the group also showed that cell viability was positively correlated to concentration of cellulose nanocrystals used. In another paper, Kumar, Rao, Han et al. (2017) prepared sodium alginate-xanthan gum based scaffold reinforced with cellulose nanocrystals and/or halloysite nanotubes. A significant increase in cell viability of MC3T3-E1 osteoblasts was obtained for the scaffold reinforced with both types of nanoparticles. The authors proposed that the combined effect of cellulose nanocrystals and halloysite nanotubes provided mechanical stability and in turn led to improved cell adhesion and proliferation.

3.1.5. Synthetic electroactive organic material

Prior studies have documented the use of electroactive organic polymers to influence cell behaviour. Specfically, polypyrrole (PPy) films were shown to induce differentiation of neural stem cells under electrical stimulation. This is particularly relevant in neural tissue engineering when targeted differentiation of encapsulated neural stem cells allows controlled generation of neurons and glial cells. Functional tissue replacement hinges on an optimal cell population of these cells. PPy are amongst the few non-toxic conjugated polymers with higher electrical conductivity. By blending PPy into spongy-like gellan gum hydrogel precursor, Berti et al. (2017) synthesized an electroactive scaffold for skeletal muscle tissue engineering applications. Electrical conductivity of the resultant hydrogels was measured and confirmed with a four-probe standard method. Both L929 mouse fibroblasts and C2C12 myoblast encapsulated within the PPy-gellan gum hydrogels were able to adhere and spread better as compared to pristine gellan gum hydrogels. Successful synthesis of this elecrtroactive scaffold may provide an alternative platform to analyze the influence of electrical stimulation on skeletal muscle cells.

In another work, Bueno, Takahashi, Catalani, de Torresi, and Petri (2015)) electro-polymerized PPy into xanthan hydrogel network to produce a hybrid functional scaffold. Due to the increased roughness and hydrophobicity of the gel topology, greater cell proliferation and attachment could be observed on the xanthan/PPy hydrogel when placed under an electromagnetic field. Elongated cells were noted on the hydrogel when viewed under SEM..

3.2. Enhancement of other biological and/or mechanical properties of microbial polysaccharide hydrogel scaffolds

3.2.1. Improvement of mechanical properties

Certain organic biomolecules derived from structural ECM serve the principal role of providing mechanical support (Frantz, Stewart, & Weaver, 2010; Humphrey et al., 2014; Hynes & Naba, 2011). Incorporation of these biomolecules can thus alter the structural framework of microbial polysaccharide hydrogels. This allows their structural attributes to be tuned and certain deficient properties such as brittleness to be improved. This in turn improves the biological performance of the hydrogels. Furthermore, conferment of therapeutic properties could be achieved if these structural biomolecules also has some form of interaction with cells. Assimilating these molecules into the hydrogel network may endow beneficial biological and/or pharmacological functions.

For example, apart from its antibacterial properties *in vivo* (Lusby, Coombes, & Wilkinson, 2002; Visavadia, Honeysett, & Danford, 2008), manuka honey has unique viscosity-enhancing features that could be exploited to enhance hydrogels' mechanical features. A procedure to incorporate manuka honey as a composite hydrogel material with gellan gum was reported by Azam and Amin (2017). Different concentrations of manuka honey were stirred with a mixture of gellan gum and glycerine at an elevated temperature of 70 °C. After which, the mixture was casted on petri dish at 50 °C for 24 h to form films. Water vapour transmission rate and the tensile strength of the resultant hydrogel films were increased to values within the range of commercial wound dressing products, substantiating their potential use in treating infected wounds.

In an interesting study, a stable tricomposite hydrogel comprised of xanthan gum, gellan gum and pullulan was formed by Yasin and Yousaf (2019). Xanthan gum and pullulan do not form hydrogel in aqueous solvents but by incorporating them into the gellan gum's network, synergistic effects on viscoelastic properties and flow behaviour were observed. In addition, higher water retention ability and swelling ratio as compared to gellan gum hydrogel alone were imparted. Since the composite hydrogels further displayed higher responsiveness when subjected to environment with acidic pH of 3 and an alkaline pH of 10, the authors proposed that they may have eventual utility as intraabdominal drug delivery systems.

3.2.2. Improvement of other biological properties

Possession of a biological activity may also refer to bioresponsiveness, in which the hydrogel's physical properties change in response to biological cues (Ulijn et al., 2007). In some cases, carefully selected biological sites may provide the necessary cues to trigger a desired response (Berti et al., 2017). A "smart" ion sensitive hydrogel (ISH) composed of 88 % w/v low-acyl gellan gum and 12 % w/v kappa-carrageenan was recently prepared by Luaces-Rodríguez et al. (2017). When exposed to ocular tissues, the liquid gelling formulation hardens *in situ* upon contact with tear fluid, rapidly converting from liquid to gel-like consistency. Images of the treated cornea showed presence of hydrogels for a long duration, up to 8 h post application. A more specific quantitative positron emission tomography (PET) scan elucidated that after 1 h of contact, 83.5 % of the ISH remained; further proving the increased dwell time of the formulation. Moreover, cytotoxicity assays showed no irritation on the treated ocular surface. These results confirmed high potential of the developed hydrogel system for prolonged ophthalmic drug administration.

In the context of tissue engineering, gelation temperature ($T_{gelation}$) of a cell encapsulating hydrogel material is another critical point of consideration. Gelation temperature should fall within the physiological range of 36 °C–37 °C for living cells to be viably encapsulated. $T_{gelation}$ of 42 °C for unmodified gellan gum is too high for cell encapsulation purposes (Bacelar et al., 2016). Fortunately, $T_{gelation}$ of gellan gum can be adjusted to physiological range *via* blending with another gelling macromolecule. In a recent report, Zheng et al. (2018) successfully assembled a gelatin/gellan gum hydrogel blend with an optimized gelation temperature of 37 °C. The blend contains 10 % w/v of gelatin and 0.3 % w/v of gellan gum. Rheological experiments confirmed stable gel-like viscoelastic properties at 37 °C. The authors proposed that the intermolecular complexation between gelatin and gellan forged another physically cross-linked network, which is distinct from the network of gellan gum alone. Cell viability assays of L929 mouse fibroblast cells seeded on the surface of the blend hydrogels suggested good biocompatibility. Furthermore, CLSM revealed cell adhesion after 24 h of culture.

In another instance, blending may reduce the concentration of polymer required to achieve gelation. GGMA can be ionically cross-linked to form hydrogels with impressive mechanical properties, making them excellent material for soft-tissue tissue engineering (Coutinho et al., 2010; Silva-Correia, Gloria et al., 2013, 2011; Silva-Correia, Zavan et al., 2013). However, the acrylation of carboyxlate groups on gellan gum reduced the crosslinking potential of GGMA. As a result an increased concentration of 2% w/v GGMA is required for physical crosslinking to occur (Coutinho et al., 2010). Hydrogels developed from higher concentration of GGMA often exhibit poorer biocompatibility (Coutinho et al., 2010). Pereira et al. (2018) attempted to
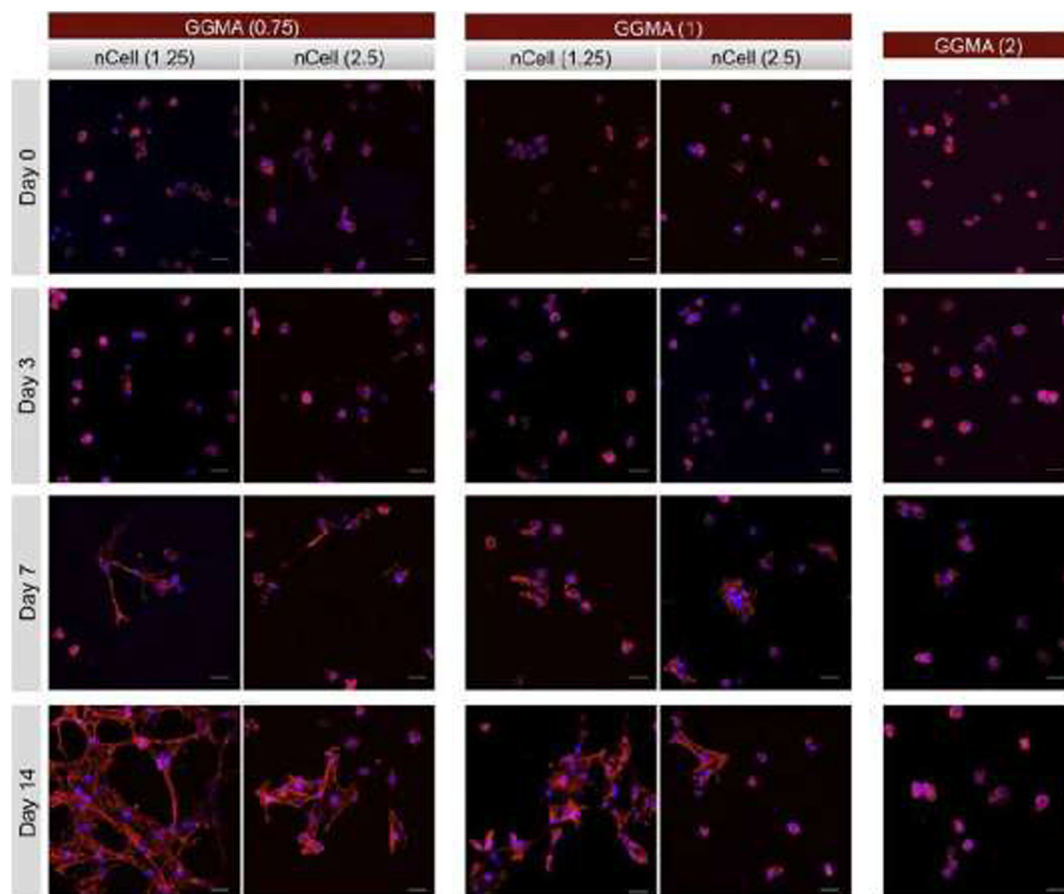
Fig. 8. Reference (Pereira et al., 2018). DAPI (blue)/Rhod-Phall (red) staining of AF cells for a period of 14 days in culture, labelling nuclei and F-actin respectively. Scale bar = 30 μm. Reproduced with permission (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

reduce the gelation concentration of GGMA by reinforcing the matrix with nanocellulose crystals. The entanglement of nanocellulose in between gellan gum helices resulted in molecular bridging that increased the packing of GGMA chains. The increased proximity of carboxylate groups of GGMA enabled gelation to occur at a lower GGMA concentration of 0.5 % w/v. On top of that, cell culture studies with encapsulated bovine annulus fibrous (AF) cells indicated that nanocomposite constructs promoted cell viability and cell attachment for up to 14 days of *in vitro* cell culture (Fig. 8).

Hydrogels are also ideal polymeric wound dressing membrane materials. However, single-component hydrogels are mechanically too weak to withstand wear and tear (Kamoun, Kenawy, & Chen, 2017). Recent trends offer composite hydrogel materials as means to achieve typical wound dressing requirements. Bellini et al. (2015) prepared dense and porous xanthan-chitosan membranes capable of supporting cell growth of multipotent mesenchymal stromal cells. More than 98 % of mesenchymal stromal cells in the culture adhered to membranes after 3 h and cell growth was observed for up to 96 h of cultivation. Under the SEM, cells appeared to be growing over the surface of as well as within the pores of the porous membranes. When treated with the membranes, a group of rats subjected to surgical wounds showed significantly faster rates of healing than the ones not covered by any dressings.

Li, Tan, Liu, and Li (2018)) investigated the optimal combination of three anionic polymers (alginate, xanthan and k-carrageenan) with three cationic polymers (chitosan, gelatin and gelatin methacrylate) for the best 3D printability with strong interface bonding. They found that 6% w/v of xanthan gum exhibited good shape fidelity with 8% w/v of gelatin and 10 % w/v of gelatin methacrylate, but not with chitosan.

4. Conclusion and future perspectives

Natural hydrogels and their derivatives have quickly become mainstays in TERM as they are inherently biocompatible and safe for implantation. Microbial polysaccharides, which have been extensively utilized in the food and pharmaceutical excipient industries, hold great potential in the biomedical field as our understanding of the chemistry and manipulation of the material improves.

Materials for TERM need to enable cell adhesion, proliferation, and differentiation much like the body's ECM. The interfacial phenomena between cell and scaffold depends largely on the presence of ligands that are immobilized within the hydrogel matrix. However, microbial polysaccharides are mostly exopolysaccharides that bacteria secrete for structural purposes hence are naturally devoid of these ligands and do not elicit biological response from cells. To overcome this problem, scientist have introduced bioactive materials into the matrices of high utility microbial polysaccharides such as gellan gum, xanthan gum, and dextran *via* physical and chemical strategies. In this review, we have summarized the physical blending approaches used to incorporate bioactive materials into hydrogels for the requirements of TERM for different tissues (Tables 2 and 3). We conclude that many successful systems based on microbial polysaccharide-bioactive material composite hydrogels have been developed thus far.

Until now, raw bioactive materials with intrinsic bioactivity were frequently interrogated as additive to biofunctionalize natural hydrogels. Recently, researchers have begun to delve into the field of synthetic material chemistry where the capacity to engineer molecules with properties of interest is enabled. As an alternative to natural bioactive materials, semi-synthetic materials such as cell-adhesion

Table 2
Biofunctionalization of microbial polysaccharide hydrogels by blending with inorganic bioactive materials.

Microbial polysaccharide	Bioactive material	Cell	Bioresponse	Reference
Gellan gum	Bioactive glass (BAG)	ADSC	Increased cell viability	(Vuornos et al., 2019)
	Calcium carbonate (CaCO$_3$).	MC3T3-E1 osteoblast-like cells	Improved cell adhesion	((Douglas, Łapa et al., 2017) Lopez-Heredia et al., 2017)
	Calcium phosphate (CaP)	MC3T3-E1 osteoblasts	Improved cell adhesion & viability	(Douglas, Pilarz et al., 2017, 2018; Douglas et al., 2014)
		MG63 human osteosarcoma fibroblasts & ADSC	Improved cell adhesion & viability	(Lišková et al., 2018)
	Cationic polyurethane soft nanoparticles (CPUN)	HDF	Improved mechanical properties & cell viability	(Sahraro et al., 2018)
	Gallus var domesticus (GD) derived demineralized bone powder (DBP)	Chondrocytes	Improved cell adhesion, viability & osteogenic differentiation of chondrocytes	(Kim et al., 2020)
	Hydroxyapatite (HAp)	ADSC	Improved cell adhesion & viability	(Manda et al., 2018)
	Halloysite nanotubes (HNT)	HDF	Increased cell viability	(Bonifacio et al., 2017)
	Inorganic clays (silica, bentonite, or halloysite)	hMSC	Improved cell viability & chondrogenic differentiation	(Bonifacio et al., 2020)
	Poly(lactic acid) (PLA)	BMSC	Improved cell adhesion & spread	(D. Hu et al., 2018)
	Polycaprolactone (PCL)	MG63 human osteosarcoma fibroblasts	Improved cell viability	(Vashisth & Bellare, 2018)
	Reduced graphene oxide (rGO)	Rat myoblasts (H9C2)	Improved cell viability	(Zargar et al., 2019)
	Titanium oxide (TiO$_2$)	MC3T3 mouse fibroblasts	Improved cell spread	(Razali et al., 2018)
Xanthan gum	Gold nanoparticles	A549 human lung cancer cells.	Improved cell viability	(Pooja et al., 2014)
	Halloysite nanotubes (HNT)	MC3T3-E1 osteoblasts	Improved cell viability	(Rao et al., 2018b)
	HAp's strontium substituted nanoparticles	OFCOLL II osteoblasts	Improved osteogenic differentiation	(Bueno et al., 2014)
	Iron oxide magnetic nanoparticles	NIH3T3 fibroblasts	Improved cell adhesion & viability	(Rao et al., 2018a)
	Magnetite nanoparticles	Mouse embryonic stem cells	Improved cell adhesion, spread & differentiation	(Glaser et al., 2015)
	Silica glass and cellulose nanocrystals	MC3T3-E1 osteoblasts	Improved cell adhesion & viability	(Kumar, Rao, Kwon, Lee, & Han, 2017)
	Silver nanoparticles	NIH3T3 fibroblasts	Improved cell adhesion & viability	(Rao et al., 2016)
	Sorbitan monooleate nanoparticles	Human umbilical vein endothelial cell (HUVEC)	Improved gene delivery to endothelial cells	(Fernandez-Piñeiro, Alvarez-Trabado, Márquez, Badiola, & Sanchez, 2018)
Dextran	Zinc oxide nanoparticles	Ehrlich ascites carcinoma (EAC)	Improved cell viability	(Raafat et al., 2018)
	Dicalcium phosphate nanoparticles	Human hepatocellular carcinoma cell	Improved cell viability	(El-Meliegy et al., 2018)

Table 3
Biofunctionalization of microbial polysaccharide hydrogels by blending with organic bioactive materials.

MICROBIAL POLYSACCHARIDE	BIOACTIVE MATERIAL	CELL	BIORESPONSE	REFERENCE
Gellan gum	Agar	Chondrocytes	Improved cell adhesion & viability	(Baek et al., 2019)
	Biotinlyated RGD or fibronection	Human fibroblasts and BMSC	Improved cell adhesion & viability	(Gering et al., 2019)
	Chitosan	MSC	Improved cell adhesion & spread	(Sant et al., 2017)
	Gelatin	L929 mouse fibroblasts	Improved cell adhesion & viability	(Zheng et al., 2018)
	Hyaluronan	–	Improved material stiffness to match soft tissues'	(Karvinen et al., 2017)
	Kappa-carrageenan	–	In situ gelation of hydrogel with tear fluid	(Luaces-Rodríguez et al., 2017)
	Laminin	Human neural stem cells (HNSC)	Improved cell viability	(Yu et al., 2020)
	Manuka honey	hMSC	Improved cell viability & chondrogenic differentiation	(Bonifacio et al., 2018)
		–	Improved mechanical properties for hydrogel film	(Azam & Amin, 2017)
	Nanocellulose crystals	Bovine annulus fibrous cells	Improved cell adhesion & viability	(Pereira et al., 2018)
	Phytochemical saponins	Chondrocytes	Improved expression of cartilage-related genes (type 1 & 2 collagen, aggrecan)	(Jeon et al., 2018)
	Polypyrrole (PPy)	L929 mouse fibroblasts and C2C12 myoblast	Improved cell adhesion & spread	(Berti et al., 2017)
	Polyvinyl alcohol (PVA)	–	Improved cell adhesion	(Wang, Wen et al., 2017)
	Silk fibroin	BMSC	Improved cell adhesion, viability & chondrogenic differentiation	(Shin et al., 2019)
	Xanthan gum & pullulan	–	Improved responsiveness of hydrogel to pH 3 and pH 10 for intraabdominal drug delivery	(Yasin & Yousaf, 2019)
Xanthan gum	Anionic polymers (alginate, xanthan and k-carrageenan) & cationic polymers (chitosan, gelatin and gelatin methacrylate)	–	Improved 3D printability for biomedical applications	(H. Li et al., 2018)
	Cationic multidomain peptide	ATDC5 cells	Improved cell viability	(Mendes, Baran, Lisboa, Reis, & Azevedo, 2012)
	Chitosan	L929 mouse fibroblasts	Improved cell viability	(Chellat et al., 2000)
		MG63 human osteosarcoma fibroblasts	Improved cell adhesion and viability	(Aguiar et al., 2019)
		Multipotent mesenchymal stromal cells	Improved mechanical properties	(Bellini et al., 2015)
	Chitosan nanoparticles	L929 mouse fibroblasts & human fetal osteoblast cells	Improved cell viability & osteogenic differentiation	(Dyondi et al., 2013)
	Chitosan & cellulose nanocrystals	NIH3T3 fibroblasts	Improved cell viability	(Rao et al., 2017)
	Chitosan, Kolliphor & Silpuran	HDF &ADSC	Improved cell viability	(de Souza et al., 2019)
	Curcumin	Heterogeneous human epithelial colorectal adenocarcinoma cells (Caco-2)	Improved cell viability	(Da-Lozzo et al., 2013)
	Gellan gum & hyaluronan	L929 mouse fibroblasts	Improved cell viability	(Kuo et al., 2014)
	Konjac, kappa-carrageenan and iota-carrageenan	HDF	Improved cell viability	(Juris et al., 2011)
	Konjac Glucomannan	Human fibroblasts	Improved cell adhesion & viability	(Alves et al., 2020)
	Methylcellulose	Injected into rats' body	Improved in vivo biocompatibility	(Liu & Yao, 2015)
	Polypyrrole (PPy)	HDF	Improved cell viability	(Bueno et al., 2015)
	Polyvinyl alcohol (PVA)	NIH3T3 fibroblasts	Improved cell viability	(Leone et al., 2019)
	Sodium alginate, cellulose nanocrystals & halloysite	MC3T3-E1 osteoblasts	Improved cell viability	(Kumar, Rao, Han et al. (2017))
Dextran	Hyaluronic acid & sanguinarine	NIH3T3 fibroblasts	Improved cell adhesion & viability	(Zhu et al., 2018)
	Polyethyleneimine & chitosan	MSC	Improved cell adhesion	(Kulikouskaya et al., 2018)
	Pullulan	L929 mouse fibroblasts	Improved cell adhesion & viability	(Cutiongco et al., 2014)
		MC3T3-E1 pre-osteoblasts	Improved spheroid formation	(Grenier et al., 2019)
	N-carboxyethyl chitosan (CECS)	C2C12 myoblasts	Improved cell viability & impart hydrogel self-healing ability	(B. Guo et al., 2019)

peptides (CAPs) have the propensity to make tremendous impact on the design of 3D cell culture platforms (Huettner et al., 2018). We suspect there will be considerable efforts moving forward in this area to expand the offerings of composite hydrogel scaffolds for TERM applications.

Author contributions

JYN conceptualized, designed and wrote the paper. **SO** and **MLC** wrote and reviewed the paper. **CZ, SH, YK** reviewed and edited the paper. **RJ** and **PLRE** conceptualized, edited and supervised the work.

Notes

The authors declare no competing financial interest.

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