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REVIEW

Methyl Cellulose based *in situ* Hydrogel for Controlled Drug Delivery and Tissue Engineering Application

MITALI DEWAN

Department of Chemistry, Shahid Matangini Hazra Government General Degree College for Women, Chakshrikrishnapur, Kulberia, Purba Medinipur-721649, India

Corresponding author: E-mail: mitalichem@gmail.com

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Methyl cellulose is an attractive smart biomaterial for its thermoreversible gelation nature. When subjected to temperature stimuli, an aqueous solution containing methyl cellulose at a critical concentration exhibits the ability to form stable physically cross-linked hydrogels. These hydrogels alter the physico-chemical and mechanical properties of the methyl cellulose. This thermo-responsive nature become quite appealing when the physiological temperature functions as a trigger to regulate the thermogelation of methyl cellulose. Regarding this, exciting inventions have been explored in the drug delivery and tissue engineering field which make methyl cellulose an attractive and versatile biomaterial. In this review, we have covered traditional and advanced applications of methyl cellulose as thermoreversible *in situ* gelling system. Three main types of applications are presented: (i) *in situ* gelling system for controlled drug delivery; (ii) fabrication of injectable scaffolds and cell sheet for tissue engineering application; and finally (iii) developing methyl cellulose based bioink for 3D bioprinting by applying *in situ* thermogelation property of methyl cellulose.

Keywords: Methyl cellulose, *In situ* gel, Drug delivery, Tissue engineering, 3D bioprinting.

INTRODUCTION

Methyl cellulose (MC) is widely recognized as one of the most prominent cellulose ethers employed in the commercial applications. It is the simplest derivative of cellulose, where hydroxyls groups of anhydro-D-glucose units are partially substituted by methyl groups (–CH_3) at C-2, C-3 and/or C-6 positions (Fig. 1) [1] and contains 27.5-31.5% of methoxy groups [2]. Unlike cellulose, methyl cellulose is soluble in water. Hydrogen bonding interaction between the -OH groups of cellulose molecule results in a highly ordered and crystalline cellulose structure that prevents water molecules from entering into it [3]. When these hydrogen bond forming –OH groups are replaced by the methoxy groups in methyl cellulose then the ordered crystalline structure is destroyed, which allow water molecules to penetrate the methyl cellulose structure and therefore interact to the hydrophilic side chains by electrostatic force [4]. Due to non-polar nature of methyl groups, water solubility of methyl cellulose eventually reduces by a rise in the value of

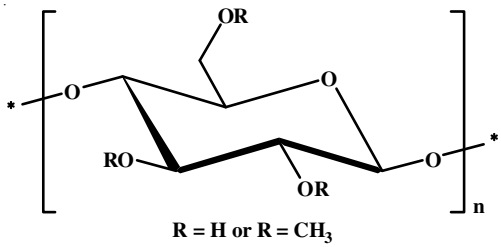

Fig. 1. Repeating unit of methylcellulose: –OH or –OCH_3 at 2, 3 and 6 positions of the anhydro-D-glucose

degree of substitution (DS). The DS value in between 0 to 3 is appropriate for methyl cellulose to become soluble in water or organic solvent [1]. Methyl cellulose with DS in the range of 1.4 to 2.5 is soluble in water and when it exceeds 2.5, it become soluble in various organic solvents in addition to water [5]. Aqueous solution of methyl cellulose at a critical concentration has the property to construct a stable physically cross-linked hydrogel under temperature stimuli [6]. The occurrence of this

physical cross linking is due to the development of hydrophobic zones at higher temperature [7-9]. The gelation mechanism is shown in Fig. 2.

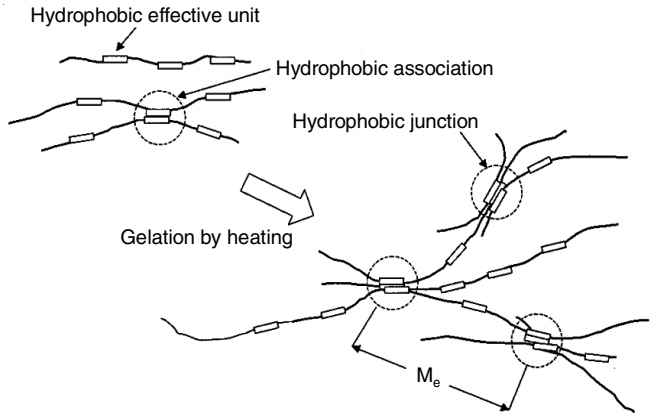

Fig. 2. Schematic drawing indicating gel formation of methyl cellulose are taking place *via* the hydrophobic effective units

This thermo-reversible gelation nature of MC has made this polysaccharide a smart biomaterial. In most of the biomedical application of methyl cellulose based *in situ* hydrogel, its gelation temperature is tuned to near physiological temperature and used it as a stimulus to trigger their activity. In particular, *in situ* gel formulations are fabricated to initiate the gelation upon injection or instillation into human body during drug delivery and tissue engineering applications, whereas in case of bioprinting applications, it is required to heat the bioink (*i.e.*, methyl cellulose hydrogel loaded with cells) near 37 ºC to achieve the sol-gel transition. Moreover, smart culture surfaces are developed by taking advantage of the sol-gel transformation of methyl cellulose near 37 ºC, which enables a specific cell adhesion and expulsion on their surface [10].

Methyl cellulose is considered to be an attractive material for drug delivery and tissue engineering application due to its capability to support in cell regeneration and also due to its approval by the Food and Drug Administration (USA) [1,11]. However, poor gel structure caused by the inadequate mechanical strength of physically cross-linked methyl cellulose and the higher gelation temperature (50-70 ºC) of pure methyl cellulose solution beyond the physiological temperature (37 ºC) render it unsuited as injectable product for *in vivo* gelation in biomedical applications. According to different studies, gel forming temperature of methyl cellulose is significantly influenced by different salts [12-14]. By adding metal salts, the gelation temperature of methyl cellulose is lowered as a consequence of alteration of water structure by salting out effect [15]. When salt is introduced to aqueous solution of methyl cellulose, the water molecules will cluster around the salt and thus inhibit the intermolecular hydrogen-bonding interaction between water molecules and the hydroxyl groups of methyl cellulose. This may result in an increase in the interaction between hydrophobic zone of methyl cellulose molecules and a reduction in the gelation temperature of methyl cellulose [16]. The gelation temperature is reduced to a more suitable level (*i.e.* to below body temperature) by the addition of metal salts, citrate, sugars like sucrose, fructose, sorbitol and glycerol [16-24].

Another crucial tactic for enhancing the thermogelation capabilities of the methyl cellulose gel and so expanding its application range is to combine it with other natural or synthetic polymers. In this review, three main families of applications of methyl cellulose based *in situ* hydrogel are discussed, (i) *in situ* gelling system for controlled drug delivery; (ii) fabrication of injectable scaffolds and cell sheet for tissue engineering application; and lastly (iii) developing methyl cellulose based bioink for 3D bioprinting by applying *in situ* thermogelation property of methyl cellulose.

Methyl cellulose based *in situ* gel for controlled drug delivery application: Numerous studies have proved that methyl cellulose-based *in situ* hydrogel system may perform extremely well as a vehicle for controlled drug delivery to get around many of the challenges associated with traditional drug delivery systems. Due to *in vivo* swelling and dissolution, removal of methyl cellulose gel is not crucial after entire drug release [11]. Methyl cellulose is used as sustained drug delivery vehicle for its thermogelation and adhesion property as well as its ability to enhance viscosity [25,26]. The gel forming temperature of drug loaded methyl cellulose based *in situ* gel formulations are adjusted in such a manner that it can maintain fluid state while being applied to the human body, making it simple to use and administer but upon instillation into intended spot, it can readily transforms to semi-solid gel *in situ*. *In situ* gel formation is triggered by stimulation to the physiological circumstances of pH 7.4 and at 37 ºC in the body. Such systems will reside in the human body for an increased period, which enables them to reserve any incorporated drug for extended period of time. Hence, drug release occurs in sustained manner and long lasting effect of drug can be accomplished which limit systemic absorption, lower the requirement for repeated drug administration and promote improved patient compliance [27]. Sustain drug release is achieved because of cross linked gel network, higher viscosity and better gel strength of *in situ* gel, which resist diffusion of drug through the gel matrix [28-30]. Combining methyl cellulose with other stimuli responsive polymers has been found to enhance the gelling capabilities, reduce overall polymer content and bring the gelation temperature closer to body temperature. Methyl cellulose based *in situ* hydrogel has been applied in the following route to achieve controlled drug release rate.

Ophthalmic drug delivery: One of the most exciting challenges that pharmaceutical scientists have been dealing with recently is ocular drug delivery. Conventional ophthalmic formulations like eye drops face several difficulties during instillation due to lacrimal secretion and nasolacrimal drainage. This causes the drug to be drained quickly in precorneal part, which recognizes the need for repeated drug instillation to get the intended therapeutic effect [30,31]. In the realm of ophthalmology, *in situ* gelling systems exist as sol before being applied, but change to a gel once applied to the eye surface. *In situ* gel-based drug delivery vehicle appear to be a promising candidate to extend the drug residence period and improve drug penetration into the surface of eye. Because of its high biocompatibility

[32,33], methyl cellulose is employed to develop ophthalmic formulations. The application of methyl cellulose-based solution for dry eye issues was patented by Haddad & Loucas [34]. To get longest possible precorneal retention period of ofloxacine, Ahuja *et al*. [35] utilized methyl cellulose as thickening agent. A methylcellulose, polypropylene glycol and citric acid containing *in situ* gel based ophthalmic vehicle is commercialized in Japan as trade name RysmonR TG for long term impact of timolol maleate [36-38]. By blending methyl cellulose with carbopol, Kumar *et al*. [39] have designed an *in situ* gel-based formulation that can be utilized to deliver ocular drugs. A stable gel was created by combining 1.5% methyl cellulose and 0.3% carbopol. This gel exhibited a regulated drug release, with a duration of 10-12 h and the release mechanism involved both diffusion and dissolution processes. According to Deardorff & Mueller [40], methyl cellulose does not cause any ocular damage or discomfort and utilized 1% methyl cellulose for formulating ophthalmic solution. Bain *et al*. [18,22,30,41] developed a series of 1% methyl cellulose based ophthalmic *in situ* gel formulation by blending it with fructose-salt mixture, PEG-salt mixture and PVA-salt mixture to optimize gelation temperature and improve gel quality. They proved that all these formulations were very effective at extending the release period of ketorolac tromethamine. El-Kamel *et al*. [25] combined different derivatives of cellulose as theckning agent with poloxamer 407 to construct ophthalmic formulation. They have demonstrated that 3% methyl cellulose containing formulation with highly viscous gelation property was capable of releasing timolol maleate at slowest rate in comparison to other cellulose derivative-poloxamer 407 based formulations. Dewan *et al*. [26] demonstrated that higher molecular weight methyl cellulose was significantly more successful than lower molecular weight methyl cellulose at sustaining KT release from methyl cellulose-poloxamer 407 based ophthalmic formulation due to its greater capacity to improve viscosity and gel strength.

A methylcellulose based *in situ* gelling ocular formulation of pilocarpine hydrochloride had been developed by Bhowmik *et al*. [42]. It contains various ratios of i-carrageenan and KCl. The sol-gel transition temperature of pure methyl cellulose solution was successfully brought down from 60 ºC to the physiological temperature range using i-carrageenan and KCl of specific weight percentages. Each of their designed formulations possess adequate ocular bioavailability, displaying a useful prolonged drug release feature. For a lengthy residence period of drug at the ocular surface, Nagai *et al*. [43] combined methyl cellulose and tranilast nanoparticles to create an *in situ* gel ophthalmic formulation. 0.5 to1.5% of methyl cellulose solution was capable to enhance the contact time of tranilast containing gel in the cornea and conjunctiva.

Oral drug delivery: Conventional forms of oral dosage, like tablets and capsules, are difficult for dysphagic and geriatric patients to handle and swallow however compliance increases with viscous liquid preparations since they are typically simple to swallow [44,45]. However, traditional liquid dosage forms usually have uncertain bioavailability, mostly due to the unpredictability of gastric emptying time. *In situ* gelling oral liquid preparations based on methyl cellulose offer a unique and intriguing way of achieving prolonged drug release by lengthening residence duration. Itoh *et al*. [46] fabricated an oral *in situ* gelling sustained drug delivery vehicle for dysphagic patients by blending methyl cellulose and pectin. Suitable gelling temperature, rheological behaviour and improved long lasting release behaviour was achieved by properly optimizing the formulations. The added pectin (containing complexed calcium ions) reduced the gelation temperature of methyl cellulose solution bellow physiological range and improved the rheological features of methyl cellulose *in situ* gel, which led to a suitably viscous formulation for the dysphagic patients to swallow. Sufficient gel rigidity allows the gel formulation to persist in the rat stomach over 3 h long time period and as a result a prolonged release of drug was accomplished over this time interval from the methyl cellulose/pectin formulations. Shimoyama *et al*. [47] developed an oral *in situ* gelling methyl cellulose/alginate formulation for prolonged delivery of drug to dysphagic patients. Liquid formulation containing proper composition of methyl cellulose and alginate were properly viscous for instillation to patients with dysphagia and it constructed gels in physiological environment with features of sustained release. Itoh *et al*. [23] also developed a methyl cellulose based *in situ* gel oral drug delivery formulation in combination with sorbitol. A 25 and 30% of D-sorbitol was capable to reduce gelation temperature of 1.0 to 2.0% (w/v) methylcellulose solutions near to physiological range and improve the rigidity of the gel. The gel formulation consisting of methyl cellulose of 2.0% (w/v) and D-sorbitol of 25% (w/v) was able to diffuse acetaminophene *in vitro* over a period of 6 h and persist gel integrity in the pH between 1.2-6.8, which is good indication of potential applicability of the *in situ* gelling vehicles for the delivery of drugs in sustained way. Sharma *et al*. [48] fabricated a methyl cellulose based oral sustained *in situ* gel drug delivery vehicle for the same purpose. The formulation containing methyl cellulose of 1.5 wt.%, sodium alginate of 1.5 wt.%, $CaCO_3$ of 3 wt.%, NaCl of 2% and polyethylene glycol of 0.05% undergo gelation in rabbit stomach and diffusion of paracetamol from the gel takes place in more sustained way in comparison to the market accessible calpol® suspension having similar paracetamol dose. Addition of methyl cellulose, sodium chloride and polyethylene glycol improved the drug retention efficacy of gel. Methyl cellulose based oral *in situ* gel solution have additionally been used in the treatment of periodontitis. With the aim to overcome poor drug retention property and prevent pain during implantation of inserts, temperature responsive *in situ* gelling drug delivery vehicle have been offered as a suitable alternative for local intra-pocket delivery of drug [49]. For the treatment of periodontitis, Boonrat *et al*. [50] examined the use of mucoadhesive *in situ* gel by combining various proportion of pluronic F127 and methyl cellulose and proved that their designed gel formulation might have benificial effect for the recovery of periodontitis by controlling the release rate. Methyl cellulose improved the gelation property of pluronic gel by its mucoadhesive and viscosity enhancing property and as a result drug release was slowed down for prolonged period.

Subcutaneous drug delivery: Drug like insulin is generally administered in human body through subcutaneous (s.c) path-

way. Using long-acting insulin dosage through the s.c. route, the basal insulin level was maintained. To improve poor patient compliance in parenteral routes, the drug dose frequency must be reduced and this can be accomplished by regulating insulin release from the delivery formulation. In case of normal insulin, it starts acting after 30-60 min and its effect last for 5-8 h. *In situ* gel polymers have been proved to be a promising candidate for the delivery of insulin. Nasir *et al*. [51] combined pluronic with methyl cellulose and hydroxypropyl methyl cellulose (HPMC) to fabricate a vehicle that can be easily administered into the body as free flowing liquid but readily transform into a stiff gel inside the body to provide sustained insulin release over an extended period of time while preserving the therapeutic plasma level. From the *in vitro* as well as *in vivo* study, it was found that 15 wt.% pluronic F-127 and 3 wt.% methyl cellulose containing formulation was sufficiently reliable and effective for prolonging the release of insulin and retaining the normal level of basal plasma insulin for 10 days. Another thermoreversible subcutaneous drug delivery system for diclofenac sodium was developed by Nasir *et al*. [52]. They used virgin poloxamer 407, methyl cellulose, HPMC and PEG as well as combination of these polymers in various proportion to construct the thermoresponsive delivery vehicle. According to their drug delivery data, the researched polymers poloxamer, methyl cellulose and PEG are promising candidates to prolong the drug release due to special thermoreversible gelation feature. The release rate was prolonged by 72 h, when 3 wt.% of methyl cellulose was added to 15 wt.% of virgin poloxamer solution.

Injectable thermoresponsive gels for local delivery: Localized drug delivery has drawn a lot of focus recently in an effort to improve therapeutic effect and reduce undesirable effects *via* intravenous, subcutaneous and intramuscular administration routes. Rangabhatla *et al*. [53] fabricated an *in situ* gel-based formulation of methyl cellulose for targeted etidronate delivery for osteogenesis disease in regulated manner. Methyl cellulose (4 wt.%) was blended with various concentrations of pluronic F127 for the preparation of novel hydrogels based injectable drug delivery solution. The developed formulation was confirmed to be a viable option for prolonging the *in vitro* release of drug for a period longer than 28 days. Here, methyl cellulose was found to improve the mechanical strength of pluronic gel extensively and decrease the rate of gel degradation rendering the gel formulation more useful for functioning long term osteogenesis drug delivery. Kim *et al*. [54] developed an injectable thermoresponsive gel/micelle combined formulation for sustained release of docetaxel locally by blending low molecular weight methyl cellulose gel with pluronic F127 micelles and docetaxel drug. Gel formation of methyl cellulose occurred at 15 to 40 ºC in presence of ammonium sulfate salt and pluronic F127 and gel forming temperature of methyl cellulose dropped as salt concentration increased. From the combined formulation, a biphasic release of docetaxel was observed for more than 35 days and stability of docetaxel was preserved throughout the release period. Due to the sustained docetaxel release in the tumor area, the combined formulation resulted complete eradication of tumor at high injectable dose of docetaxel with no adverse effects and minimum lethality caused by toxic effect of docetaxel in compared to free docetaxel formulation. Gupta *et al*. [33] blended methyl cellulose with hyaluronan to get fast gelling non-cell adhesive, degradable and biocompatible as an injectable intrathecal drug delivery system for localized release of growth factor. They reported successively that HAMC did not have an adverse effect on the spinal cord and improved cell survival of retinal stem cell-derived rods and neural stem cells.

Methyl cellulose based *in situ* hydrogel for tissue engineering: The scientific technique of substituting or repairing the injured tissue or organs that are not performing properly is the most enthralling invention in biological science. Transplanting organs is a technology that has been extensively studied, but its usefulness is limited in practical situation due to lack of organ, issues regarding immune system and high probability of rejection [55,56]. Tissue engineering is the most recently developed technique that may be a successful way out to support the native cells in healing the lost or damaged organs. For this technique, a highly biomimetic niche is created by providing an extracellular matrix (ECM), which is designed artificially. The artificially designed ECM also known as "scaffold" that can carry cells and growth factors and help the regeneration of native cells in the damaged area [57]. The scaffold materials [58] can be attached with living cells and can be inserted into various tissues in accordance with particular alternative tissues [59]. In order to facilitate the proliferation and differentiation of seed cells, it is essential to provide a cell scaffold composed of biomaterials that closely resemble a constructed extracellular matrix [60]. Because of this, the selection of scaffold materials is crucial for their biocompatibility and ability to adhere to cell [61]. Consequently, natural polymers are being used extensively as scaffolds material for tissue engineering. Development of injectable scaffold has gained recognition since they are simple to administer without surgery into the wounded area by pushing syringe [62]. In order to create injectable scaffolds for tissue engineering, smart hydrogel, also known as *in situ* hydrogel, has received significant scientific attention. Thermosensitive *in situ* injectable hydrogel systems possess some intrinsic benefits: (i) they can be inserted through less invasive technique; (ii) incorporation of drugs and/or cells can be done by simply blending them prior to injection; (iii) cavities with irregular shape may be filled, without fabricating custom-shaped architecture, reducing host tissue scaffold gap and maintaining continuation of the mechanical forces at the tissue-hydrogel contact [63,64]; and (iv) mechanical characteristics are comparable to those of soft tissue [65].

Methyl cellulose is proved to be biocompatible and extensive research has been performed on its gelling behaviour [9,14,16, 32,33]. It is injectable *in vivo* using a regular syringe at ambient temperature [66,67]. The proper crosslinking kinetics plays an important role when any hydrogel materials are being evaluated for tissue engineering. From one side, gelation should occur quite slowly to prevent gel formation before a full injection is pushed to completely fill the injure side, yet, it should nonetheless move quickly enough to avoid it from straying from the intended place. Analyzing the crosslinking kinetics, the rheological and biological properties as well as cytotoxicity,

it was proved that methyl cellulose hydrogel is much relevant as tissue engineering scaffold [8]. Methyl cellulose based hydrogels are extensively studied as a scaffolding substance for skin, muscle, bone, cartilage, blood vessels and neural system tissue engineering. Methyl cellulose has also been proved to support regeneration of nerve in peripheral nerve conduits [68]. Tate *et al*. [32] examined the application of methyl cellulose as a scaffolding material for tissue engineering in the defected brain. Suitable thermogelation properties of methyl cellulose were attained by manipulating methyl cellulose concentration and solvent pattern. Methyl cellulose gel was shown to be degraded very slowly in primary state but it was quite stable up to two week times period, which proved that *in situ* gel-based scaffold of methyl cellulose can provide long lasting support for cell regeneration. Concentrations of methyl cellulose up to 8% were still safe and did not cause cell death in primary rat astrocytes or neurons at 1 or 7 days. Examining injury and methyl cellulose grafting it was proved that the presence of methyl cellulose did not enhance damage response and alter the pattern of gliosis. All of their findings suggested that methyl cellulose is a suitable nontoxic injectable scaffold for the reconstruction of brain injuries. Stabenfeldt *et al*. [69] concentrated on developing a thermoresponsive methyl cellulose hydrogel that has been tethered with laminin-1 (LN) for delivering cells and therapeutics to damaged central nervous system (CNS). It has been demonstrated that the LN tethered methyl cellulose hydrogel scaffold promotes primary neuronal cell adhesion and survival. Therefore, the designed scaffold may offer a reliable delivery method to wonded tissue of CNS for transplantation techniques of neural cell. Martin *et al*. [67] designed a thermoreversible hydrogel blend system by combining methyl cellulose with agarose that solidified rapidly under physiological condition and might be utilized to support scaffolding and deliver therapeutic agents for directed neuronal regrowth *via* a damaged location. For improved mechanical property and prolonged stability, Stalling *et al.* [70] synthesized photopolymerized methacrylated methylcellulose (MA-MC) hydrogels by chemically modified methyl cellulose with functional methacrylate groups and photocrosslinked for reconstructive application of soft tissue. Their findings demonstrated that the fabricated hydrogel formulations containing MA-MC of higher weight percentages had better compressive moduli than hydrogels with less weight percentages. Therefore, the hydrogels with higher concentration of MA-MC were able to retain their shape with minimal degradation and swelling. The substances exhibited no cytotoxicity in any of the evaluated formulations and relatively small inflammation. All of these traits suggested that the hydrogel may be used for soft tissue restoration. Tang *et al.* [71] researched the characteristics of a thermoreversible injectable hydrogel consisting of chitosan (CS), methyl cellulose (MC) and salts mixture for employing as a synthetic three-dimensional tissue engineering matrix. The application of the CS/MC/Na_3PO_4 hydrogel as a scaffold for cartilage cells led to excellent cell survival as well as proliferation. For the purpose of augmentation and reconstruction of soft tissue, Gold *et al.* [72] also synthesized methacrylate functional groups modified methyl cellulose and used redox-initiation scheme of polymerization to fabricate hydrogels having adjustable properties comparable to soft tissue. The fabricated hydrogel provides appropriate gelation which make it simple to inject, complete degradation through enzymes and cytocompatible character which are relevant for clinical research. In order to deliver stem cells to skin wounds *via* injection, Kim *et al.* [73] presented a thermoresponsive hydrogel made of soluble extracellular matrix (sECM) and methyl cellulose. The sECM-MC formulation showed a rapid hydrogel formation at physiological temperature. According to their findings, sECM-MC hydrogels not only offered a cell niche which maintained cell shape and improved viability and engraftment of stem cell but also provided biological hint to accelerate regeneration of injured tissue without creating any significant cytotoxic effect. Zhuo *et al.* [65] developed an injectable hyaluronan-methyl cellulose (HAMC) composite hydrogel that was crosslinked with PEG for the goal of creating a scaffold for neural tissue reconstruction. The HAMC was in a sol state at room temperature, making it simple to inject, cytocompatable, biodegradable and capable of quickly forming *in situ* gels to take on the shape needed for injured tissue. PEG additionally improved the rheological characteristics as well as stability of the hydrogel composite through its impact of cross-linking to methyl cellulose that may effectively extend the residence duration of this hydrogel. Kim *et al.* [74] introduced calcium phosphate nanoparticles (CaP NPs) containing methyl cellulose composite hydrogel (MC-CaP NPs) as injectable scaffold for bone tissue engineering. The methyl cellulose gels at physiological temperature due to the salt-out action caused by the precursor salts. The proposed hydrogel was proved to be appropriate in terms of injectability, biocompatibility and capacity to speed up the regeneration of new and mature bone. A thermosensitive injectable scaffold made of nanotailored hyaluronic acid modified methyl cellulose (nHAMC) has recently been developed by Das *et al.* [75] to encourage regeneration of cells by permitting fast cellular activity. Incorporating nanotailored hyaluronic acid reduced the demand for high salt content in order to lower the gelling temperature and effectively boosted the gel strength and decreased the gel dissolution rate of nHAMC compared to methyl cellulose blend containing methyl cellulose and hyaluronic acid. In the presence of nanotailored hyaluronic acid, cytoskeletal actin polymerization and wound healing rate enhanced in comparison to methyl cellulose alone exhibit the slowest healing rate that is shown by least number of protrusions and smoother cell surface. Increasing the nanotailored hyaluronic acid concentration and lowering the NaCl amount gives the desired hydrogel scaffold cyto-compatibility and hemo-compatibility [75].

Fattahpour *et al.* [76] developed the hydrogels of carboxymethyl chitosan (CMC), methylcellulose (MC), pluronic (P) and zinc chloride. Meloxicam loaded into nanoparticles was encapsulated into the hydrogel. Methyl cellulose was used to reinforce the mechanical property of CMC based scaffold and pluronic for dispersion of nanoparticles. Zinc ions were chosen for their ability to crosslink hydrogels, promote proliferation of chondrocyte and have antibacterial properties. This nanoparticle encapsulated hydrogel may be appropriate as a novel scaffold biomaterial for the regeneration of cartilage tissue

due to its good biocompatible, bioadhesion nature as well as the capacity to support proliferation and expansion of cell. Sa-Limaha *et al*. [77] proved that poly(*N*-isopropylacrylamide)-*g*-methylcellulose (PNIPAAm-*g*-MC) thermoreversible hydrogel can successfully act as an injectable 3D support for repairing the articular cartilage tissue. There results proved that the encapsulated chondrogenic cell within the hydrogel scaffold retained their viability and preserved chondrocytes behaviour as well as phenotype keeping round shaped like morphology. Sajadi-Javan *et al*. [78] fabricated a novel thermosensitive injectable hydrogel scaffold for bone tissue engineering by combining methyl cellulose and Persian gum (PG) and improved the gel quality by adding halloysite nanotubes (HNTs) loading with taxifolin (TAX). The hydrogel's mechanical and biological properties were improved by the addition of PG and HNTs and when 1% PG and 3% HNTs were combined with methyl cellulose, the resultant hydrogel was simple to inject, gelled instantly after injection into body and provided the maximum mechanical strength. Higher cell adhesion, cell growth and gene expression were evident in the resulting hydrogel. The presence of HNTs-TAX facilitated osteogenic differentiation.

Cell sheet engineering (CSE) has been established as an alternate strategy for tissue engineering in order to address some of the issues with conventional scaffold-based tissue engineering. The use of cell sheet engineering offers the benefit of avoiding the use of biodegradable scaffolds that may have issues with their *in vivo* degradation, such as a rapid rate of breakdown, the production of toxic chemicals or an unwanted inflammatory reaction [79-81]. Cell-sheet engineering is a new approach that is scaffold free and suitable to regenerate cell-dense tissues like corneal [82], cardiac [83,84] and 3D tissues [85]. Cell sheet engineering (CSE) also develop smart surfaces of cell culture that permit the culture of cell *in vitro* and the intact cell sheets are detached by particular stimuli (such as temperature fluctuation), retaining the gap junctions between cells and the extracellular matrix (ECM) proteins [86]. One approach of CSE is to create a surface that responds to temperature, allowing cultured cells to be harvested as intact sheets with their implanted extracellular matrix for use in tissue engineering. This method avoids any enzymatic treatment for detachment of cell sheet, which can cause cell injury and the loss of differentiated phenotypes when used during harvesting of cultured cells [87,88]. The ECM is still present on the basal surface of the cell sheets, which allows for their immediate transplantation into tissue beds or even their overlapping to produce 3D structures, which resembles tissue [89]. Temperature responsive hydrogels are used to develop the thermoresponsive culture surfaces for the production of cell sheets. Okano's team [90-92] reported a unique method of CSE for reconstructions of tissue by chemically grafting a thermoresponsive polymer, poly(N-isopropylacrylamide) (PNIPAAm) over tissue culture polystyrene (TCPS) plates. The cultured cells can be harvested from PNIPAAm hydrogel-based culture surface as a continuous cell sheet by simply reducing the incubation temperature to 20 ºC as PNIPAAm hydrogel surface exhibit hydrophilic characteristics at 20 ºC and hydrophobic characteristics at 37 ºC. The harvested cell sheets have been utilized for varieties of tissue reconstructions, such as ocular surfaces, periodontal ligaments, cardiac patches and bladder augmentations. The entire grafting procedure requires a lot of time and complexity. Chen *et al*. [93] developed a unique but simple and affordable living cell sheet harvesting technique utilizing thermoreversible hydrogels made of methyl cellulose. This approach involved pouring aqueous methyl cellulose solutions in combination with phosphate buffered saline (PBS) onto TCPS dishes at ambient temperature, which eventually transform to gel at 37 ºC. The gel coated TCPS dish was further uniformly spread with an aqueous neutral solution of collagen at 4 ºC for improving cell adhesion which over time rebuilt slowly and create a thin coating of collagen (MC/PBS/collagen hydrogel). When cells attained confluency, a continuous single layer of cell sheet is developed on the hydrogel surface of MC/PBS/collagen, which can be detached from the hydrogel surface by incubation at 20 °C and liquefying the hydrogel. Results from the MTT experiment showed that cells grown on the hydrogel-coated surface were more active than cells grown on an untreated TCPS plate. Once the detached cell sheet has been harvested, the residual viscous hydrogel system can be reused. Furthermore, a cell sheet of multilayer may be cultured using the established hydrogel system. One can employ the collected living cell sheets to reconstruct tissue.

Thirumala *et al*. [94] established an improved collagen layered methyl cellulose hydrogel approach for developing the cell sheets, basically modified for working with adipose tissue derived stromal/stem cells (ASCs) and for creating cell of multi-dimension for the purpose of tissue engineering. The uniformly spread bovine collagen above the methyl cellulose hydrogel layered surface at 37 ºC extensively improve the adhesion and growth of cell on the methyl cellulose hydrogel system. This harmless technique of cell extraction utilizing methyl cellulose layered TCPS dishes enables construction of cell sheet of single or multilayere while retaining cell–cell and cell–extracellular matrices. Methyl cellulose based thermosensitive hydrogels was also used by Altomare *et al*. [95] as temporary substrate for biofabricating cell sheet. In this case, different salts like sodium sulphate, sodium phosphate, calcium chloride or phosphate buffered saline was combined with methyl cellulose powder of 2-12 wt.% for improving the preparation procedure, for better regulation of the gelation temperature and improvement of gel stability. No harmful release was observed from any of the investigated hydrogels in *in vitro* cytotoxicity experiments. Among all other tested compositions, the hydrogel formulation made of 8 wt.% methyl cellulose and 0.05 M Na_2SO_4 was found to spontaneously detach single layer of cells that had been seeded previously on the hydrogel surface as it undergo phase transition around 37 ºC. By using the thermoresponsive properties of methyl cellulose hydrogel, they were able to generate a cell sheet effectively and then successfully separate it from the hydrogel surface without using enzymes. For the purpose of cell sheets engineering, Contessi *et al*. [96] also investigated the thermoresponsive characteristics of methyl cellulose hydrogels. They proved that 8 wt.% methyl cellulose hydrogels with 0.1 M Na_2SO_4 or PBS 20 g/L additives

were specially promising for the application in cell sheet engineering, showing sol-gel phase transition around 37 ºC.

3D Bioprinting for fabricating tissue engineering scaffold: Bioprinting is a computer-based processes by which tissue like structures or organs are fabricated from living cell *in vitro* in accordance with preconfigured design [97]. In this process, cell or biomaterials are deposited layer by layer to create bio-inks. 3D printing may be laser-based, inkjet-based, valve based or extrusion based. The last one has drawn greater interest for fabricating solid tissue engineering scaffold due to its capability to print highly viscous bio-ink having high density of cell [98-100]. The bio-fabrication process improving parameters may create negative impact on biological condition and therefore limited the clinical usages of 3D printed product. Biocompatibility, variable gelation, viscoelastic and shear-thinning properties are the fundamental needs for 3D bioprinting [101]. Hydrogels are considered as appropriate material for bio-inks for 3D bioprinting due to their capacity to mimic the tissue environment and control cell fate. From the aspect of printable capacity, a hydrogel must possess the following characteristics: (i) Sufficient shear-thinning property that maintain potentiality of cell by protecting them against excessive shear stress during extrusion, which assist bioprinting [102]; (ii) excellent recoverability, allowing bioink to regain its original viscosity after printing [103]; (iii) adequate mechanical strength to support the weight of printed structure; (iv) hydrogels and the degraded byproducts must be biocompatible and non-toxic; (v) rapid post cross-linking ability for providing stability in biological media; and (vi) good printability (flowability) during printing and achieve good resolution for printed construct

It is apparent that hydrogels of one component cannot simultaneously meet all of the aforementioned needs due to the fact that most suffer from inadequate mechanical strength and an inappropriate disintegration rate in comparison to local tissues [104]. In light of this, hydrogel composites appear to be an effective way for imposing many capabilities to a bioink. The use of thermoreversible hydrogel is being investigated for use in bioprinting since (i) the sol-gel state can be easily tuned by altering temperature; (ii) it may be quickly printed or extruded with excellent shape fidelity; and (iii) fast gelation allows for outstanding resolution [104]. Furthermore, a number of hydrogels also offer favourable cell compatibility. Recently, hydrogels based on methyl cellulose have been used in 3D bioprinting due to methyl cellulose's special ability to gel at a temperature between 40 and 50 ºC, which is close to physiological temperature [105]. It could be used as support materials during bioprinting. Moreover, integrating other hydrogels with methyl cellulose for tissue engineering applications are also possible [106,107]. Although methyl cellulose is non-toxic, biocompatible and thixotropic in nature but its application in 3D bioprinting has been constrained by its week mechanical characteristics and absence of post cross-linking capability. Methyl cellulose-based hydrogel is applied for 3D bioprinting only in a few research [108-110]. In these investigations, the hydrogels based on methyl cellulose were productively printed into 3D constructions having regulated size and shape by adjusting the parameters of 3D printing. Even though the printed hydrogel of methyl cellulose confirmed its cytocompatibility however, it only showed transient stability for a period of 2-3 days after being immersed in culture medium. Due to this, methyl cellulose is being combined with other viscosity enhancing polymers in order to use it as component for printing. Methyl cellulose has been employed in combinations with alginate to create suitable inks for 3D bioprinting because of their biocompatibility as well as stability of structure. For the application of multicomponent methyl cellulose based hydrogel in 3D bioprinting, a bioink of methyl cellulose/alginate combined system has been presented by Schütz *et al*. [111], where alginate was functioned for post cross-linking in Ca^{2+} environment and the role of methyl cellulose was to enhance viscosity. The resultant constructs were highly elastic and stable with increased microporosity due to temporary occurrence of methyl cellulose. The added methyl cellulose to the alginate solution of low concentration significantly enhanced the hydrogel material's printability, which allows for plotting 3D constructs with dimension of centimeter range with modified structural design and of great shape fidelity. The combined methyl cellulose based hydrogel material was also appropriate of producing a cell-laden matrix with high cell viability which demonstrated that methyl cellulose had no harmful effects. It has also been proved that embedded cells retain their differentiation capacity, which is a crucial condition for the development of useful tissue engineering structures.

Li *et al*. [112] treated trisodium citrate, a chelating agent, with alginate/methyl cellulose mixed hydrogel for applying it as an effective component for 3D bioprinting. The added chelating agent considerably increased the interfacial bonding between the layers, enhancing the attraction between the printed layers. The resulting hydrogel showed excellent extrudability, stackability and extremely thixotropic properties that proved it as an effective material for 3D bioprinting. Karavasili *et al*. [113] reported on the invention of 3D printable alginate/methyl cellulose hydrogel blended with bioactive ingredients for the application of wound repairing. All the hydrogel formulations were biocompatible and stimulated cell proliferation as confirmed by *in vitro* wond healing assey. The 3D printed samples showed sufficient moisture sorption ability, that are crucial to balance moisture at the location of injury, while simultaneously providing antimicrobial action against Gram-positive and Gram-negative bacteria. Rastin *et al*. [114] developed methyl cellulose/gelatin methacryloyl (MC/GelMA) bioink having excellent shape integrity and enhanced stability in biological medium. Methyl cellulose was functioned as printing material and GelMA was used for the permanent photo-cross-linking in the condition of UV irradiation. Although the printed methyl cellulose distorted and dissociated quickly within 2-3 days in biological medium due to lack of post cross-linking capability but UV cross-linked MC/GelMA bioink maintained its stability for several months. GelMA improved rheological properties of the system which caused the MC/GelMA ink to behave in a self-supporting manner after printing. Numerous 1D, 2D and 3D constructs were successfully printed as a consequence of the bioink's exceptional shape integrity, which increased resolution and printability. Furthermore, human primary osteo-

blasts grown in the MC/GelMA hydrogel exhibited more than 95% of cell viability. Hyaluronic acid (HA)/methyl cellulose (MC) hydrogels have revealed promising outcomes for the applications of 3D bioprinting. According to Law *et al*. [115], hyaluronic acid (HA) and methylcellulose (MC) containing hydrogel (HAMC) is quick-gelling and printable that also has desirable mechanical characteristics and cell viability. HAMC may become a potential hydrogel for a range of 3D bioprinting applications with further study. For 3D bioprinting, Cofiño *et al*. [116] fabricated a self-assembled peptide (SAP)/methyl cellulose based improved bioink. They used RAD16-I as one of the most useful self-assembled peptide for tissue engineering application. To increase the viscosity of the bioink and enable 3D printing of preset structures, methyl cellulose was added to the RAD16-I solution. In presence of methyl cellulose, the final constructs exhibited excellent structural integrity and stability and presented higher cell viability even after 7 days of growth of implanted cell. Additionally, the ability of the implanted rat mesenchymal stem cells (rMSCs) inside the RAD/MC 3D-bioprinted scaffolds to develop to the adipogenic linage suggested that this unique biomaterial is suitabile for applications of soft tissue engineering. To enhance the structural integrity as well as mechanical strength of methyl cellulose hydrogel used as 3D bio-ink material, Shin *et al*. [117] synthesized dual crosslinkable tyramine-modified methyl cellulose (MC-Tyr), which exhibited outstanding printability and mechanical characteristics for 3D bio-ink. Using a two-step crosslinking technique, the cell-entrapped MC-Tyr bioink was properly extruded into stable 3D hydrogel constructions with great resolution. The MC-Tyr scaffolds showed good cell viability and cell growth in addition to great fidelity and integrity. Boonlai *et al*. [118] investigated hydrogel based bioinks made of methyl cellulose and κ-carrageenan of different concentrations for extrusion-based bioprinting. Hydrogel's physical characteristic was successfully improved by using 0.1% w/w KCl which promotes ionic crosslinking with κ-carrageenan. The mechanical properties of the hydrogel were improved by incorporating cellulose nanocrystal (CNC) of different concentrations. The incorporation of CNC in the hydrogels resulted in the better thixotropic behaviour and with higher CNC content better shear thinning behaviour was seen that are crucial for extrusion-based bioprinting. Boonlai *et al*. [118] proved that pluronic F127 (PF) and methyl cellulose containing hydrogels offered favourable characteristics for a potential 3D bioprinted material. They have developed and investigated bioinks containing pluronic F127 of different concentrations and methyl cellulose of 4% w/w concentration for extrusion based bioprinting. Thixotropic, shear-thinning behaviour and printability of methyl cellulose and pluronic F127 (18 and 20%) mixture were all excellent. When pluronic F127 was combined with methyl cellulose, mechanical property increased noticeably. The 3D bioprinted constructs of 18%PF/MC and 20%PF/MC demonstrated more than 97% cell viability [119].

Conclusion

Over the last few decades, numerous studies have shown that methyl cellulose based *in situ* hydrogel, both alone and in combination with other materials may be successfully utilized for controlled drug delivery and tissue engineering application. Methyl cellulose-based *in situ* hydrogel system may perform extremely well as a vehicle for controlled drug delivery to get around many of the challenges associated with traditional drug delivery systems. Removal of methyl cellulose gel is not crucial after entire drug release due to *in vivo* swelling and dissolution. Analysis of the crosslinking kinetics, rheological and biological properties as well as cytotoxicity proved that methyl cellulose hydrogel is very much relevant as tissue engineering scaffold. Methyl cellulose based hydrogels are extensively studied as a scaffolding substance for skin, muscle, bone, cartilage, blood vessels and neural system tissue engineering and also been proved to support regeneration of nerve in peripheral nerve conduits. Methyl cellulose is also utilized to develop the thermo-responsive culture surfaces for the production of cell sheet in cell sheet engineering (CSE) which is an alternate strategy for tissue engineering in order to avoid the use of biodegradable scaffolds that may have issues with their *in vivo* degradation. Most recent research have shown that multicomponent methyl cellulose based hydrogel may be printable component in 3D bioprinting due to its special ability to gel at physiological temperature as well as its biocompatible, non-toxic and thixotropic nature. In summary, it can be asserted that while *in vitro*, *in vivo* and pre-clinical investigations have yielded encouraging results, additional clinical trials are necessary to address several noteworthy unresolved medical concerns.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. P.L. Nasatto, F. Pignon, J.L.M. Silveira, M.E.R. Duarte, M.D. Noseda and M. Rinaudo, *Polymers*, **7**, 777 (2015); https://doi.org/10.3390/polym7050777
2. J. Brady, T. Dürig, P.I. Lee and J.-X. Li, in eds.: Y. Qiu, Y. Chen, G. G.Z. Zhang, L. Yu and R.V. Mantri, Developing Solid Oral Dosage Forms, Academic Press, edn. 2, Chap. 7, p. 181 (2017); https://doi.org/10.1016/B978-0-12-802447-8.00007-8
3. D.G. Coffey, D.A. Bell and A. Henderson, in eds.: A.M. Stephen, Cellulose and Cellulose Derivatives, In: Food Polysaccharides and Their Applications, Marcel Dekker Inc.: New York, Chap. 5 p. 123 (1995).
4. E. Doelker, in eds.: R.S. Langer and N.A. Peppas, Cellulose derivatives. Biopolymers I, In: Advances in Polymer Science, Springer, Berlin, Heidelberg, vol 107. (2005); https://doi.org/10.1007/BFb0027554
5. M. Hirrien, Ph.D. Thesis, Comportement des Méthylcelluloses en Relation Avec leur Structure. Joseph Fourier University, Grenoble, France (1996) (In French).
6. W. Huang, N. Zhang, H. Hua, T. Liu, Y. Tang, L. Fu, Y. Yang, X. Ma and Y. Zhao, *Biomed. Pharmacother.*, **83**, 107 (2016); https://doi.org/10.1016/j.biopha.2016.06.024
7. Y. Tang, X. Wang, Y. Li, M. Lei, Y. Du, J.F. Kennedy and C.J. Knill, *Carbohydr. Polym.*, **82**, 833 (2010); https://doi.org/10.1016/j.carbpol.2010.06.003
8. B. Niemczyk-Soczynska, A. Gradys, D. Kolbuk, A. Krzton-Maziopa and P. Sajkiewicz, *Polymers*, **11**, 1772 (2019); https://doi.org/10.3390/polym11111772
9. L. Li, P.M. Thangamathesvaran, C.Y. Yue, K.C. Tam, X. Hu and Y.C. Lam, *Langmuir*, **17**, 8062 (2001); https://doi.org/10.1021/la010917r

10. L. Bonetti, L. De Nardo and S. Farè, *Tissue Eng. Part B Rev.*, **27**, 486 (2021); https://doi.org/10.1089/ten.teb.2020.0202
11. Y. Lin, J. Sun, G. Jiang, J. Zan and F. Ding, *Chin. J. Chem. Eng.*, **15**, 566 (2007); https://doi.org/10.1016/S1004-9541(07)60125-6
12. M. Takahashi, M. Shimazaki and J. Yamamoto, *J. Polym. Sci., B, Polym. Phys.*, **39**, 91 (2001); https://doi.org/10.1002/1099-0488(20010101)39:1<91::AID-POLB80>3.0.CO;2-C
13. Y. Xu and L. Li, *Polymer*, **46**, 7410 (2005); https://doi.org/10.1016/j.polymer.2005.05.128
14. Y. Xu, C. Wang, K.C. Tam and L. Li, *Langmuir*, **20**, 646 (2004); https://doi.org/10.1021/la0356295
15. Y. Zhang and P.S. Cremer, *Curr. Opin. Chem. Biol.*, **10**, 658 (2006); https://doi.org/10.1016/j.cbpa.2006.09.020
16. H.F. Liang, M.H. Hong, R.M. Ho, C.K. Chung, Y.H. Lin, C.H. Chen and H.W. Sung, *Biomacromolecules*, **5**, 1917 (2004); https://doi.org/10.1021/bm049813w
17. G. Levy and T.W. Schwarz, *J. Am. Pharm. Assoc.*, **47**, 44 (1958); https://doi.org/10.1002/jps.3030470113
18. M.K. Bain, M. Bhowmik, D. Maity, N.K. Bera, S. Ghosh and D. Chattopadhyay, *J. Appl. Polym. Sci.*, **118**, 631 (2010); https://doi.org/10.1002/app.32350
19. M. Bhowmik, M.K. Bain, L.K. Ghosh and D. Chattopadhyay, *Pharm. Dev. Technol.*, **16**, 385 (2011); https://doi.org/10.3109/10837451003774369
20. M. Bhowmik, S. Das, J. Sinha, S. Bag, D. Chattopadhyay and L.K. Ghosh, *Asian J. Chem.*, **22**, 2147 (2010).
21. N. Iso and D. Yamamoto, *Agric. Biol. Chem.*, **34**, 1867 (1970); https://doi.org/10.1080/00021369.1970.10859863
22. M.K. Bain, M. Bhowmik, S.N. Ghosh and D. Chattopadhyay, *J. Appl. Polym. Sci.*, **113**, 1241 (2009); https://doi.org/10.1002/app.30040
23. K. Itoh, T. Hatakeyama, T. Kimura, T. Shimoyama, S. Miyazaki, A. D'Emanuele and D. Attwood, *Chem. Pharm. Bull.*, **58**, 247 (2010); https://doi.org/10.1248/cpb.58.247
24. Q. Kuang, G. Cheng, J. Zhao and Y. Li, *J. Appl. Polym. Sci.*, **100**, 4120 (2006); https://doi.org/10.1002/app.23375
25. A.H. El-Kamel, *Int. J. Pharm.*, **241**, 47 (2002); https://doi.org/10.1016/S0378-5173(02)00234-X
26. M. Dewan, B. Bhowmick, G. Sarkar, D. Rana, M.K. Bain, M. Bhowmik and D. Chattopadhyay, *Int. J. Biol. Macromol.*, **72**, 706 (2015); https://doi.org/10.1016/j.ijbiomac.2014.09.021
27. I.P. Kaur, M. Singh and M. Kanwar, *Int. J. Pharm.*, **199**, 119 (2000); https://doi.org/10.1016/S0378-5173(00)00359-8
28. M. Bhowmik, P. Kumari, G. Sarkar, M.K. Bain, B. Bhowmick, M.M.R. Mollick, D. Mondal, D. Maity, D. Rana, D. Bhattacharjee and D. Chattopadhyay, *Int. J. Biol. Macromol.*, **62**, 117 (2013); https://doi.org/10.1016/j.ijbiomac.2013.08.024
29. M.K. Bain, B. Bhowmick, D. Maity, D. Mondal, M.M.R. Mollick, D. Rana and D. Chattopadhyay, *Int. J. Biol. Macromol.*, **51**, 831 (2012); https://doi.org/10.1016/j.ijbiomac.2012.07.028
30. M.K. Bain, D. Maity, B. Bhowmick, D. Mondal, M.M.R. Mollick, G. Sarkar, M. Bhowmik, D. Rana and D. Chattopadhyay, *Carbohydr. Polym.*, **91**, 529 (2013); https://doi.org/10.1016/j.carbpol.2012.08.040
31. H. Qi, W. Chen, C. Huang, L. Li, C. Chen, W. Li and C. Wu, *Int. J. Pharm.*, **337**, 178 (2007); https://doi.org/10.1016/j.ijpharm.2006.12.038
32. M.C. Tate, D.A. Shear, S.W. Hoffman, D.G. Stein and M.C. LaPlaca, *Biomaterials*, **22**, 1113 (2001); https://doi.org/10.1016/S0142-9612(00)00348-3
33. D. Gupta, C.H. Tator and M.S. Shoichet, *Biomaterials*, **27**, 2370 (2006); https://doi.org/10.1016/j.biomaterials.2005.11.015
34. H.M. Haddad and S.P. Loucas, US Patent 5,556,633 (1996).
35. M. Ahuja, G. Singh and D.K. Majumdar, *Sci. Pharm.*, **76**, 505 (2008); https://doi.org/10.3797/scipharm.0804-22
36. M. Takeuchi, S. Kageyama, H. Suzuki, T. Wada, Y. Notsu and F. Ishii, *Colloid Polym. Sci.*, **281**, 1178 (2003); https://doi.org/10.1007/s00396-003-0893-2
37. M. Takeuchi, S. Kageyama, H. Suzuki, T. Wada, Y. Toyoda, T. Oguma, Y. Ezure, Y. Tsuriya, T. Kato and F. Ishii, *Mater. Technol.*, **17**, 445 (1999).
38. K. Shimokawa, K. Saegusa and F. Ishii, *Colloids Surf. B Biointerfaces*, **74**, 56 (2009); https://doi.org/10.1016/j.colsurfb.2009.06.022
39. S. Kumar, B.O. Haglund and K.J. Himmelstein, *J. Ocul. Pharmacol. Ther.*, **10**, 47 (1994); https://doi.org/10.1089/jop.1994.10.47
40. W.H. Mueller and D.L. Deardorff, *J. Am. Pharm. Assoc.*, **45**, 334 (1956); https://doi.org/10.1002/jps.3030450518
41. M.K. Bain, B. Bhowmick, D. Maity, D. Mondal, M.M.R. Mollick, B.K. Paul, M. Bhowmik, D. Rana and D. Chattopadhyay, *Int. J. Biol. Macromol.*, **50**, 565 (2012); https://doi.org/10.1016/j.ijbiomac.2012.01.025
42. B. Bhowmick, G. Sarkar, D. Rana, I. Roy, N.R. Saha, M. Bhowmik, S. Ghosh and D. Chattopadhyay, *RSC Adv.*, **5**, 60386 (2015); https://doi.org/10.1039/C5RA06858D
43. N. Nagai, M. Minami, S. Deguchi, H. Otake, H. Sasaki and N. Yamamoto, *Front. Bioeng. Biotechnol.*, **8**, 764 (2020); https://doi.org/10.3389/fbioe.2020.00764
44. J. Kelly, G. D'Cruz and D. Wright, *Dysphagia*, **24**, 49 (2009); https://doi.org/10.1007/s00455-008-9170-3
45. J. Kelly, G. D'Cruz and D. Wright, *J. Adv. Nurs.*, **66**, 82 (2010); https://doi.org/10.1111/j.1365-2648.2009.05145.x
46. K. Itoh, T. Hatakeyama, T. Shimoyama, S. Miyazaki, A. D'Emanuele and D. Attwood, *Drug Dev. Ind. Pharm.*, **37**, 790 (2011); https://doi.org/10.3109/03639045.2010.541465
47. T. Shimoyama, K. Itoh, M. Kobayashi, S. Miyazaki, A. D'Emanuele and D. Attwood, *Drug Dev. Ind. Pharm.*, **38**, 952 (2012); https://doi.org/10.3109/03639045.2011.634809
48. S. Sharma, G. Sarkar, B. Srestha, D. Chattopadhyay and M. Bhowmik, *Int. J. Biol. Macromol.*, **134**, 864 (2019); https://doi.org/10.1016/j.ijbiomac.2019.05.092
49. B. Vigani, S. Rossi, G. Sandri, M.C. Bonferoni, C.M. Caramella and F. Ferrari, *Pharmaceutics*, **12**, 859 (2020); https://doi.org/10.3390/pharmaceutics12090859
50. O. Boonrat, V. Tantishaiyakul and N. Hirun, *Polym. Bull.*, **79**, 4515 (2022); https://doi.org/10.1007/s00289-021-03722-w
51. F. Nasir, Z. Iqbal, A. Khan, J.A. Khan, A. Khan, F. Khuda, S. Zakir, N. Yousaf, I. Khan, Y. Shah, M.I. Khan and N. Shahbaz, *Drug Dev. Ind. Pharm.*, **40**, 1503 (2014); https://doi.org/10.3109/03639045.2013.831441
52. F. Nasir, Z. Iqbal, J.A. Khan, A. Khan, F. Khuda, L. Ahmad, A. Khan, A. Khan, A. Dayoo and Roohullah, *Int. J. Pharm.*, **439**, 120 (2012); https://doi.org/10.1016/j.ijpharm.2012.10.009
53. A.S.L. Rangabhatla, V. Tantishaiyakul, K. Oungbho and O. Boonrat, *Int. J. Pharm.*, **499**, 110 (2016); https://doi.org/10.1016/j.ijpharm.2015.12.070
54. J.K. Kim, Y.W. Won, K.S. Lim and Y.H. Kim, *Pharm. Res.*, **29**, 525 (2012); https://doi.org/10.1007/s11095-011-0581-8
55. B. Das, P. Chattopadhyay, D. Mishra, T.K. Maiti, S. Maji, R. Narayan and N. Karak, *J. Mater. Chem. B Mater. Biol. Med.*, **1**, 4115 (2013); https://doi.org/10.1039/c3tb20693a
56. B. Das, M. Mandal, A. Upadhyay, P. Chattopadhyay and N. Karak, *Biomed. Mater.*, **8**, 035003 (2013); https://doi.org/10.1088/1748-6041/8/3/035003
57. B. Das, P. Chattopadhyay, S. Maji, A. Upadhyay, M.D. Purkayastha, C.L. Mohanta, T.K. Maity and N. Karak, *Biomed. Mater.*, **10**, 025011 (2015); https://doi.org/10.1088/1748-6041/10/2/025011
58. D.W. Hutmacher, J.T. Schantz, C.X.F. Lam, K.C. Tan and T.C. Lim, *J. Tissue Eng. Regen. Med.*, **1**, 245 (2007); https://doi.org/10.1002/term.24
59. P.X. Ma, *Mater. Today*, **7**, 30 (2004); https://doi.org/10.1016/S1369-7021(04)00233-0
60. L.A. Smith and P.X. Ma, *Colloids Surf. B Biointerfaces*, **21**, 10243 (2011); https://doi.org/10.1016/j.colsurfb.2003.12.004
61. J. Yin, Y. Fang, L. Xu and A. Ahmed, *Int. J. Biol. Macromol.*, **183**, 1210 (2021); https://doi.org/10.1016/j.ijbiomac.2021.05.026

62. H. Tan, C.R. Chu, K.A. Payne and K.G. Marra, *Biomaterials*, **30**, 2499 (2009); https://doi.org/10.1016/j.biomaterials.2008.12.080
63. E. Ruel-Gariépy and J.C. Leroux, *Eur. J. Pharm. Biopharm.*, **58**, 409 (2004); https://doi.org/10.1016/j.ejpb.2004.03.019
64. H.J. Moon, D.Y. Ko, M.H. Park, M.K. Joo and B. Jeong, *Chem. Soc. Rev.*, **41**, 4860 (2012); https://doi.org/10.1039/c2cs35078e
65. F. Zhuo, X. Liu, Q. Gao, Y. Wang, K. Hu and Q. Cai, *Mater. Sci. Eng. C*, **81**, 1 (2017); https://doi.org/10.1016/j.msec.2017.07.029
66. E.C. Tsai, P.D. Dalton, M.S. Shoichet and C.H. Tator, *Biomaterials*, **27**, 519 (2006); https://doi.org/10.1016/j.biomaterials.2005.07.025
67. B.C. Martin, E.J. Minner, S.L. Wiseman, R.L. Klank and R.J. Gilbert, *J. Neural Eng.*, **5**, 221 (2008); https://doi.org/10.1088/1741-2560/5/2/013
68. M.R. Wells, K. Kraus, D.K. Batter, D.G. Blunt, J. Weremowitz, S.E. Lynch, H.N. Antoniades and H.A. Hansson, *Exp. Neurol.*, **146**, 395 (1997); https://doi.org/10.1006/exnr.1997.6543
69. S.E. Stabenfeldt, A.J. García and M.C. LaPlaca, *J. Biomed. Mater. Res. A*, **77A**, 718 (2006); https://doi.org/10.1002/jbm.a.30638
70. S.S. Stalling, S.O. Akintoye and S.B. Nicoll, *Acta Biomater.*, **5**, 1911 (2009); https://doi.org/10.1016/j.actbio.2009.02.020
71. Y. Tang, X. Wang, Y. Li, M. Lei, Y. Du, J.F. Kennedy and C.J. Knill, *Carbohydr. Polym.*, **82**, 833 (2010); https://doi.org/10.1016/j.carbpol.2010.06.003
72. G.T. Gold, D.M. Varma, D. Harbottle, M.S. Gupta, S.S. Stalling, P.J. Taub and S.B. Nicoll, *J. Biomed. Mater. Res. A*, **102**, 4536 (2014); https://doi.org/10.1002/jbm.a.35132
73. E.J. Kim, J.S. Choi, J.S. Kim, Y.C. Choi and Y.W. Cho, *Biomacromolecules*, **17**, 4 (2016); https://doi.org/10.1021/acs.biomac.5b01566
74. M.H. Kim, B.S. Kim, H. Park, J. Lee and W.H. Park, *Int. J. Biol. Macromol.*, **109**, 57 (2018); https://doi.org/10.1016/j.ijbiomac.2017.12.068
75. B. Das, A. Basu, S. Maji, K. Dutta, M. Dewan, A. Adhikary, T.K. Maiti and D. Chattopadhyay, *Carbohydr. Polym.*, **237**, 116146 (2020); https://doi.org/10.1016/j.carbpol.2020.116146
76. S. Fattahpour, M. Shamanian, N. Tavakoli, M. Fathi, H. Sadeghi-Aliabadi, S.R. Sheykhi, M. Fesharaki and S. Fattahpour, *Int. J. Biol. Macromol.*, **151**, 220 (2020); https://doi.org/10.1016/j.ijbiomac.2020.02.002
77. H. Sá-Lima, K. Tuzlakoglu, J.F. Mano and R.L. Reis, *J. Biomed. Mater. Res. A*, **98**, 596 (2011); https://doi.org/10.1002/jbm.a.33140
78. Z.S. Sajadi-Javan, J. Varshosaz, M. Mirian, M. Manshaei and A. Aminzadeh, *Cellulose*, **29**, 2413 (2022); https://doi.org/10.1007/s10570-021-04383-8
79. M. Vert, S.M. Li, G. Spenlehauer and P. Guerin, *J. Mater. Sci. Mater. Med.*, **3**, 432 (1992); https://doi.org/10.1007/BF00701240
80. M. Vert, J. Mauduit and S. Li, *Biomaterials*, **15**, 1209 (1994); https://doi.org/10.1016/0142-9612(94)90271-2
81. S. Li, *J. Biomed. Mater. Res.*, **48**, 342 (1999); https://doi.org/10.1002/(SICI)1097-4636(1999)48:3<342::AID-JBM20>3.0.CO;2-7
82. T. Sumide, K. Nishida, M. Yamato, T. Ide, Y. Hayashida, K. Watanabe, J. Yang, C. Kohno, A. Kikuchi, N. Maeda, H. Watanabe, T. Okano and Y. Tano, *FASEB J.*, **20**, 392 (2006); https://doi.org/10.1096/fj.04-3035fje
83. Y. Haraguchi, T. Shimizu, M. Yamato and T. Okano, *Cardiol. Res. Pract.*, **2011**, 845170 (2011); https://doi.org/10.4061/2011/845170
84. Y. Haraguchi, T. Shimizu, T. Sasagawa, H. Sekine, K. Sakaguchi, T. Kikuchi, W. Sekine, S. Sekiya, M. Yamato, M. Umezu and T. Okano, *Nat. Protoc.*, **7**, 850 (2012); https://doi.org/10.1038/nprot.2012.027
85. K. Sakaguchi, T. Shimizu, S. Horaguchi, H. Sekine, M. Yamato, M. Umezu and T. Okano, *Sci. Rep.*, **3**, 1316 (2013); https://doi.org/10.1038/srep01316
86. A. Cochis, L. Bonetti, R. Sorrentino, N. Contessi Negrini, F. Grassi, M. Leigheb, L. Rimondini and S. Farè, *Materials*, **11**, 579 (2018); https://doi.org/10.3390/ma11040579
87. A. Kushida, M. Yamato, Y. Isoi, A. Kikuchi and T. Okano, *Eur. Cell. Mater.*, **10**, 23 (2005); https://doi.org/10.22203/eCM.v010a03
88. A. Kushida, M. Yamato, A. Kikuchi and T. Okano, *J. Biomed. Mater. Res.*, **54**, 37 (2001); https://doi.org/10.1002/1097-4636(200101)54:1<37::AID-JBM5>3.0.CO;2-7
89. G. Chen, Y. Qi, L. Niu, T. Di, J. Zhong, T. Fang and W. Yan, *Biomed. Rep.*, **3**, 749 (2015); https://doi.org/10.3892/br.2015.522
90. T. Okano, N. Yamada, H. Sakai and Y. Sakurai, *J. Biomed. Mater. Res.*, **27**, 1243 (1993); https://doi.org/10.1002/jbm.820271005
91. T. Okano, N. Yamada, M. Okuhara, H. Sakai and Y. Sakurai, *Biomaterials*, **16**, 297 (1995); https://doi.org/10.1016/0142-9612(95)93257-E
92. T. Shimizu, M. Yamato, A. Kikuchi and T. Okano, *Biomaterials*, **24**, 2309 (2003); https://doi.org/10.1016/S0142-9612(03)00110-8
93. C.H. Chen, C.C. Tsai, W. Chen, F.L. Mi, H.F. Liang, S.C. Chen and H.-W. Sung, *Biomacromolecules*, **7**, 736 (2006); https://doi.org/10.1021/bm0506400
94. S. Thirumala, J.M. Gimble and R.V. Devireddy, *Cells*, **2**, 460 (2013); https://doi.org/10.3390/cells2030460
95. L. Altomare, A. Cochis, A. Carletta, L. Rimondini and S. Fare, *J. Mater. Sci. Mater. Med.*, **27**, 95 (2016); https://doi.org/10.1007/s10856-016-5703-8
96. N. Contessi, L. Altomare, A. Filipponi and S. Farè, *Mater. Lett.*, **207**, 157 (2017); https://doi.org/10.1016/j.matlet.2017.07.023
97. D. Chimene, R. Kaunas and A.K. Gaharwar, *Adv. Mater.*, **32**, 1902026 (2020); https://doi.org/10.1002/adma.201902026
98. K. Hölzl, S. Lin, L. Tytgat, S. Van Vlierberghe, L. Gu and A. Ovsianikov, *Biofabrication*, **8**, 032002 (2016); https://doi.org/10.1088/1758-5090/8/3/032002
99. J.H.Y. Chung, S. Naficy, Z. Yue, R. Kapsa, A. Quigley, S.E. Moulton and G.G. Wallace, *Biomater. Sci.*, **1**, 763 (2013); https://doi.org/10.1039/c3bm00012e
100. J.M. Lee and W.Y. Yeong, *Adv. Healthc. Mater.*, **5**, 2856 (2016); https://doi.org/10.1002/adhm.201600435
101. T. Gao, G.J. Gillispie, J.S. Copus, A.K. Pr, Y.J. Seol, A. Atala, J.J. Yoo and S.J. Lee, *Biofabrication*, **10**, 034106 (2018); https://doi.org/10.1088/1758-5090/aacdc7
102. N. Paxton, W. Smolan, T. Böck, F. Melchels, J. Groll and T. Jungst, *Biofabrication*, **9**, 044107 (2017); https://doi.org/10.1088/1758-5090/aa8dd8
103. D. Chimene, K.K. Lennox, R.R. Kaunas and A.K. Gaharwar, *Ann. Biomed. Eng.*, **44**, 2090 (2016); https://doi.org/10.1007/s10439-016-1638-y
104. F.P.W. Melchels, M.A.N. Domingos, T.J. Klein, J. Malda, P.J. Bartolo and D.W. Hutmacher, *Prog. Polym. Sci.*, **37**, 1079 (2012); https://doi.org/10.1016/j.progpolymsci.2011.11.007
105. M.R. Matanovic, J. Kristl and P.A. Grabnar, *Int. J. Pharm.*, **472**, 262 (2014); https://doi.org/10.1016/j.ijpharm.2014.06.029
106. Z. Li and J. Guan, *Expert Opin. Drug Deliv.*, **8**, 991 (2011); https://doi.org/10.1517/17425247.2011.581656
107. C.M. Piard, Y. Chen and J.P. Fisher, *Clin. Rev. Bone Miner. Metab.*, **13**, 245 (2015); https://doi.org/10.1007/s12018-015-9198-5
108. A. Cochis, L. Bonetti, R. Sorrentino, N. Contessi Negrini, F. Grassi, M. Leigheb, L. Rimondini and S. Farè, *Materials*, **11**, 579 (2018); https://doi.org/10.3390/ma11040579

109. N. Contessi Negrini, L. Bonetti, L. Contili and S. Farè, *Bioprinting*, **10**, e00024 (2018); https://doi.org/10.1016/j.bprint.2018.e00024
110. R. Suntornnond, J. An and C.K. Chua, *Macromol. Mater. Eng.*, **302**, 1600266 (2017); https://doi.org/10.1002/mame.201600266
111. K. Schütz, A.M. Placht, B. Paul, S. Brüggemeier, M. Gelinsky and A. Lode, *J. Tissue Eng. Regen. Med.*, **11**, 1574 (2017); https://doi.org/10.1002/term.2058
112. H. Li, Y.J. Tan, K.F. Leong and L. Li, *ACS Appl. Mater. Interfaces*, **9**, 20086 (2017); https://doi.org/10.1021/acsami.7b04216
113. C. Karavasili, K. Tsongas, I.I. Andreadis, E.G. Andriotis, E.T. Papachristou, R.M. Papi, D. Tzetzis and D.G. Fatouros, *Carbohydr. Polym.*, **247**, 116666 (2020); https://doi.org/10.1016/j.carbpol.2020.116666
114. H. Rastin, R.T. Ormsby, G.J. Atkins and D. Losic, *ACS Appl. Bio Mater.*, **3**, 1815 (2020); https://doi.org/10.1021/acsabm.0c00169
115. N. Law, B. Doney, H. Glover, Y. Qin, Z.M. Aman, T.B. Sercombe, L.J. Liew, R.J. Dilley and B.J. Doyle, *J. Mech. Behav. Biomed. Mater.*, **77**, 389 (2018); https://doi.org/10.1016/j.jmbbm.2017.09.031
116. C. Cofiño, S. Perez Amodio, C.E. Semino, E. Engel and M.A. Mateos-Timoneda, *Macromol. Mater. Eng.*, **304**, 1900353 (2019); https://doi.org/10.1002/mame.201900353
117. J.Y. Shin, Y.H. Yeo, J.E. Jeong, S.A. Park and W.H. Park, *Carbohydr. Polym.*, **238**, 116192 (2020); https://doi.org/10.1016/j.carbpol.2020.116192
118. W. Boonlai, V. Tantishaiyakul and N. Hirun, *Polym. Int.*, **71**, 181 (2022); https://doi.org/10.1002/pi.6298
119. W. Boonlai, N. Hirun, K. Suknuntha and V. Tantishaiyakul, *Polym. Bull.*, **80**, 4555 (2023); https://doi.org/10.1007/s00289-022-04271-6