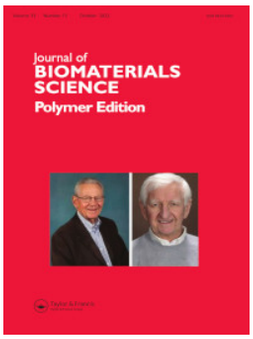

Journal of Biomaterials Science, Polymer Edition

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/tbsp20

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To cite this article: Lara L. Reys, Simone S. Silva, Diana Soares da Costa, Rui L. Reis & Tiago H. Silva (2022) Fucoidan-based hydrogels particles as versatile carriers for diabetes treatment strategies, Journal of Biomaterials Science, Polymer Edition, 33:15, 1939-1954, DOI: 10.1080/09205063.2022.2088533

To link to this article: https://doi.org/10.1080/09205063.2022.2088533

Published online: 20 Jun 2022.

JOURNAL OF BIOMATERIALS SCIENCE, POLYMER EDITION
2022, VOL. 33, NO. 15, 1939–1954
https://doi.org/10.1080/09205063.2022.2088533

Fucoidan-based hydrogels particles as versatile carriers for diabetes treatment strategies

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ABSTRACT

There is a current lack of fully efficient therapies for diabetes mellitus, a chronic disease where the metabolism of blood glucose is severely hindered by a deficit in insulin or cell resistance to this hormone. Therefore, it is crucial to develop new therapeutic strategies to treat this disease, including devices for the controlled delivery of insulin or encapsulation of insulin-producing cells. In this work, fucoidan (Fu) – a marine sulfated polysaccharide exhibiting relevant properties on reducing blood glucose and antioxidant and anti-inflammatory effects – was used for the development of versatile carriers envisaging diabetes advanced therapies. Fu was functionalized by methacrylation (MFu) using 8% and 12% (v/v) of methacrylic anhydride and further photocrosslinked using visible light in the presence of triethanolamine and eosin-y to produce hydrogel particles. Degree of methacrylation varied between 2.78 and 6.50, as determined by ^{1}HNMR, and the produced particles have an average diameter ranging from 0.63 to 1.3 mm (dry state). Insulin (5%) was added to MFu solution to produce drug-loaded particles and the release profile was assessed in phosphate buffer solution (PBS) and simulated intestinal fluid (SIF) for 24 h. Insulin was released in a sustained manner during the initial 8 h, reaching then a plateau, higher in PBS than in SIF, indicating that lower pH favors drug liberation. Moreover, the ability of MFu particles to serve as templates for the culture of human pancreatic cells was assessed using 1.1B4 cell line during up to 7 days. During the culture period studied, pancreatic beta cells were proliferating, with a global viability over 80% and tend to form pseudo-islets, thus suggesting that the proposed biomaterial could be a good candidate as versatile carrier for diabetes treatment as they sustain the release of insulin and support pancreatic beta cells viability.

ARTICLE HISTORY
Received 28 June 2021
Accepted 5 May 2022

KEYWORDS
Fucoidan; diabetes mellitus; marine biomaterials; photocrosslinking; insulin; pancreatic cells

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1. Introduction

Diabetes mellitus is a life-threating disease that affects 450 million of people in the world, being the seventh most common cause of death. Diabetes is a metabolic disease characterized by abnormally high levels of glucose in the blood caused by insufficient production of insulin, or resistance to this hormone, which is secreted by β cells within the pancreatic islets [1, 2]. Etiologically, diabetes can be subdivided into different types, namely type I, type II, and gestational diabetes [3, 4], although recently some researchers suggested a more complex organization related with disease outcomes and associated complications [5]. Type I diabetes (T1D) is characterized by an absolute deficiency in the production of insulin by the pancreas, as a result of autoimmune destruction of the insulin-producing β cells. As insulin is the hormone promoting the uptake of glucose by cells, this sugar is not metabolized for the production of energy and hyperglycemia occurs, resulting in severe complications [6]. Without insulin treatment, T1D patients are exposed to the risk of death from acute ketoacidosis [7]. By its turn, type II diabetes (T2D) is characterized by the development of resistance to insulin by cells and, over time, insulin production by β-cells becomes insufficient to sustain a proper glucose metabolism [3, 8]. The pathogenesis of T2D is mainly associated with increasing age, unhealthy diets, obesity, and a sedentary lifestyle, which explains why the mainstay of both treatment and prevention of T2D is dieting and physical activity, although some anti-diabetic drugs can be also prescribed, as insulin sensitizers (as thiazolidinediones) or secretagogues (as sulphonyreas) [1, 9].

Despite being much less abundant than T2D, the higher morbidity, severity of the symptoms and drastic impact on patients' quality of life associated with T1D pushed the development of new therapeutic strategies for this disease, as well as models to better understand it. In this perspective, insulin sustained delivery systems have been proposed as a therapeutic approach [10–15], as well as the development of devices for the encapsulation of insulin-producing pancreatic cells and islets of Langerhans, mainly based in hydrogels [16]. Different materials and processing methodologies are being explored, as carboxymethyl cellulose cryogels [17], hydrophilic polyurethanes processed as porous tough hydrogels [18], poly(ethylene glycol) hydrogel membrane functionalized with glucagon-like peptide [19], and more frequently alginate-based systems [20],including alginate-poly-L-lysine-alginate microcapsules[21], 3D printed porous alginate hydrogels [22], and even alginate coatings into nylon threads [23]. The resulting constructs are generally envisaged for further implantation near the patient liver [24, 25], but other implantation sites are also being studied, such as kidney capsule, spleen, intraperitoneal transplantation and omentum pouch, gastrointestinal wall intramuscular and subcutaneous site. In all the rational is the same, with the device being designed to support cell viability and reestablish the physiological secretion of insulin, while protecting the encapsulated cells from the host immune system [24, 26], although currently several bottlenecks are still to be surpassed [20, 27]. One of these is the foreign-body response that encapsulation materials may elicit, recognized for instance in alginate hydrogels, to which the combination with immunomodulating compounds may be the solution, as recently proposed with a chemically-modified alginate Z1-Y15 encapsulating viable pancreatic islets being implanted

in a non-human primate model during 4 months without the need of immunosuppression drugs [28]. Other strategies also involve the use of bioactive compounds from marine resources as potential anti-diabetic drugs [29], such as fucoidan (Fu) a marine polysaccharide which has been reported as capable to regulate blood glucose homeostasis [30], besides several other biological activities, namely regarding antioxidant and immunomodulation effects [31]. Moreover, being a polysaccharide found in the cell wall of brown macroalgae, its role as a structural component for the development of biomaterials is being also addressed [32]. Thus, we hypothesized that Fu-based hydrogels may be attractive for the development of multifunctional carriers, as besides acting as support material for cells and drugs, may also enable the improvement of encapsulated cells viability by reducing oxidative stress [33] or modulate the immune response upon implantation. In this regard, the work reported herein aimed the establishment of Fu hydrogels without the use of additional polymers and evaluate their capacity to support drug loading and sustained release, as well as *in vitro* culture of pancreatic cells, as first assessment of carrier functionality.

Fu is a heterogeneous and anionic polysaccharide that contains L-fucose and sulfate groups, together with other sugars, as xylose and glucuronic acid [34, 35]. The diversity of biological activities being reported in the literature may vary depending on the composition and structural traits, namely the content (charge density), distribution and bonding of the sulfate substitutions and the purity of the extract. These characteristics are a direct result of the seaweeds used as raw-material and the extraction methodologies employed, among others [35]. Fu has a high solubility in water, which is interesting for its delivery as a bioactive compound; however it could be a problem for the production of stable structures in aqueous media using Fu alone, as this kind of structures are not able to withstand aqueous media without following quick solubilization [36, 37]. In fact, most of the reported studies involve Fu composites with natural or synthetic macromolecules, namely Fu with collagen [38], chitosan [39–41], chondroitin sulphate [42], poly (caprolactone) (PCL) [38, 43], and hydroxyapatite [44, 45], have been reported as 2D (films) [38, 39], or 3D structures (hydrogels [39, 46], microspheres [47], and fibers [37]). However, there is an absence of studies using pure Fu matrices. Nevertheless, our group has recently shown a process to obtain structures of Fu alone using functionalization by methacrylation and further photocrosslinking with visible light [25, 36].

Herein in this new work the established methodology was used to produce Fu-based hydrogel particles and their potential as biomaterial envisaging new diabetes therapies was explored, from the materials perspective: from one side, as insulin delivery system by studying drug encapsulation and further release in phosphate buffer solution (PBS), simulated gastric fluid (SGF) and simulated intestinal fluid (SIF); on the other side, as pancreatic cells encapsulation device by assessing the ability of the developed materials to support adhesion and proliferation of human pancreatic cells (1.1B4).

2. Materials and methods

2.1. Materials

Fu in powder from brown algae *Fucus vesiculosus* (Maritech Fucoidan, Marinova, Australia, batch # FVF2011527), methacrylic anhydride (MA, Sigma, Germany, cat

no. 276685), triethanol amine (TEOA, Sigma, USA, cat no. T58300), eosin-y (Sigma, USA, cat no. E4009), N-vinylpyrrolidone (NVP, JMGS, Portugal, cat no. 140925000), tetraethyl orthosilicate (TEOS, VWR,USA, cat no. 8.00658.1000), and 1H, 1H, 2H, 2H-perfluorodecyl-trichlorosilane (PFDTS, Sigma, USA, cat no. 658758), of analytic grade (Sigma-Aldrich), were used as received.

2.2. Functionalization of Fu

Details of the Fu modification are given elsewhere [36], but briefly, fucoidan was modified (MFu) by methacrylation reaction: 4% (w/v) of Fu aqueous solution was mixed with methacrylate anhydride (MA) in two concentrations, 8% (to produce MFu1) or 12% (to produce MFu2), at 50 °C, allowing to react for 6 h. After, the resulting MFu materials were dialyzed against water using 12–14 kDa cutoff dialysis tubing for 4 days at 4 °C to remove the unreacted material. MFu materials were further purified with pure acetone, lyophilized and stored at 4 °C, protected from light, until further use.

2.3. Characterization of Fu and MFu

2.3.1. Fourier transform infrared spectroscopy

The Fourier transform infrared spectroscopy (FTIR) was performed on Shimadzu IR Prestige 21 Spectrometer (Shimadzu, Japan, A21004200704). The samples were powdered, mixed with potassium bromide (KBr) and processed into pellets and scanned by infrared radiation in the range 4000–400 cm^{-1}, with a resolution of 16 cm^{-1}. Each spectrum was recorded as an average of 32 scans.

2.3.2. Nuclear magnetic resonance (^{1}HNMR)

The methacrylation of Fu was quantified by ^{1}HNRM spectroscopy. The ^{1}HNRM spectra of Fu and MFu were collected in deuterated water (D_2O) at 5 mg/ml and 50 °C, being recorded on Bruker Avance III spectral conditions: 300 Hz spectra with 90° impulses and 4 s acquisition time.

2.3.3. Zeta potential

The zeta potentials of Fu and modified fucoidan (MFu1, MFu2) samples in powder were measured by laser Doppler anemometry using a Malvern Zeta sizer 3000 HS (Malvern Instruments, UK). The samples powders were prepared by dispersing 3 mg in 5 ml of filtrated ultrapure water. Each analysis was made at 25 °C for 60 s. Independent triplicate experiments were performed for obtaining statistical significance.

2.4. Preparation of Fu-based hydrogels particles (MFu hydrogel particles)

The methodology used for the preparation of MFu hydrogel particles is shown schematically in Figure 1. MFu solutions were prepared by adding MFu1 or MFu2 (5%, w/v) to a photoinitiator (PI) solution consisting of 0.3% (w/v) eosin-y (photoinitiator) in N-vinylpyrrolidone (comonomer) and 5 M triethanolamine (TEOA) (co-initiator)

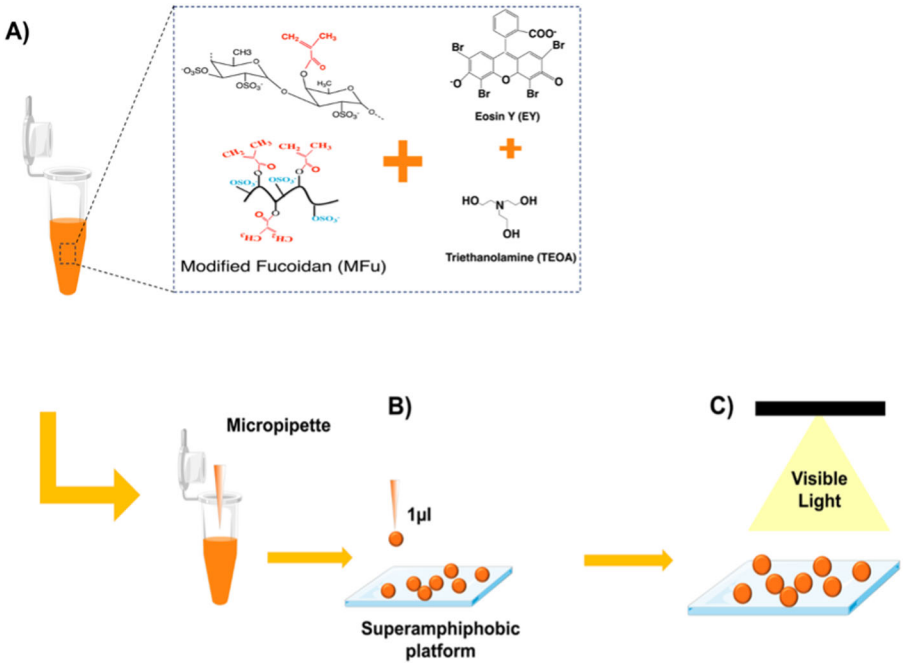

Figure 1. Scheme to illustrate the formation of the fucoidan-based hydrogels particles using visible light and superamphiphobic surface: (A) solution of modified fucoidan (MFu) and photoinitiators (B) pipetting volumes between 1 and 5 µL of the solution to superamphiphobic surface and (C) exposure of the solution droplets to visible light to promote photocrosslinking and thus produce the MFu hydrogel particles.

in water. MFu hydrogel particles were produced by pipetting volumes between 1 and 5 µL of the respective MFu solution onto a superamphiphobic surface, which high repellence hindered the solution to spread, thus keeping the integrity of the liquid droplets, resulting in spheroidal particles upon photocrosslinking with visible light [48–50]. MFu hydrogel particles loaded with insulin were produced in the same way but using MFu solutions with 5 mg/ml of insulin. This procedure assures that all the drug is encapsulated in particles [48].

2.5. Morphology of MFu hydrogel particles

The MFu hydrogel particles morphology was observed using a NanoSEM-194 FEI Nova 200 (FEG/SEM) scanning electron microscope. Before SEM analysis, samples were frozen at −80 °C and lyophilized, to obtain dehydrated materials, and further gold-sputtered by using a Quorum/Polaron model E 6700 equipment and the analysis was performed with an acceleration voltage of 15.00 kV and magnification from 30× to 60×.

2.6. Assessment of in vitro insulin release

To quantify the insulin release and predict the bioavailability of insulin encapsulation in MFu hydrogel particles, we incubated these drug-charged particles in a simulated

gastric fluid (SGF) and in a simulated intestinal fluid (SIF). The SGF solution was prepared in aqueous solution, for 1 L of SGF we use 2 g of sodium chloride (NaCl), 3.2 g of pepsin derived from stomach mucosa and 7 ml of concentrated hydrochloric acid (HCl), while SIF was prepared in aqueous solution, for 1 L of this buffer we use 6.8 g of monobasic potassium phosphate, 77 ml of 0.2 N sodium hydroxide (NaOH) and 1 g of pancreatin. Ten MFu hydrogel particles loaded with insulin were placed in 20 ml of release PBS and incubated at 37 °C under stirring. The release media was SIF (pH 6.5) for 4 h [51]. At appropriate time intervals (0.5–6 h), 1 ml of the solution was removed for analysis and replaced by fresh solution. The absorbance at 272 nm was measured on a multiwell microplate reader (SynergyHT, Biotek Instruments, microplate reader-Gen 5 2.01) and the amount of insulin released from the particles was determined from a previously obtained calibration curve (0–6 mg/ml) [51]. All the samples were analyzed in triplicate.

2.7. Biological characterization by culturing human pancreatic cells

The ability of the developed materials to sustain the viability of human pancreatic cells was assessed by direct contact of 1.1B4 HPC cell line (Sigma) in MFu2 hydrogel particles for 1, 5 and 7 days. The hybrid cell line 1.1B4 was formed by the electrofusion of a primary culture of human pancreatic islets with PANC-1, a human pancreatic ductal carcinoma cell line (ECACC catalogue number 87092802). 1.1B4 cells have been shown to express insulin, glucokinase, IAPP and GLUT1 glucose transporter, thus being considered as an appropriate model for this study.

2.7.1. Cell culture

For these experiments, 1.1B4 HPC was cultured in Roswell Park Memorial Institute (RPMI 1640) medium (Invitrogen, USA) supplemented with 10% fetal bovine serum (FBS) (Invitrogen, USA) and 1% antibiotic/antimycotic (A/B) (Invitrogen, USA) at a temperature of 37 °C and 5% of CO_2 until achieving 90% confluence. The medium was changed every 2–3 days. Each experimental condition was tested in triplicate and three independent assays were performed.

2.7.2. Direct contact

These cells were seeded on the surface of the materials (10 MFu2 hydrogel particles/well) and TCPs at density of 3.5×10^5 cells/ml using 500 µL/well and cultured for 1, 5 and 7 days. The samples were incubated at 37 °C in humidified 95% air and 5% CO_2 atmosphere. After each time point, the samples were washed with PBS and metabolic activity (MTS assay), proliferation (DNA quantification), viability and cell morphology by confocal laser scanning microscopy (CLSM) was performed.

2.7.3. Metabolic activity assay

Metabolic activity was evaluated by MTS (3-(4,5-dimethythiazol-2y)-5-(3-carboxymethoxyphenyl) assay. The metabolic activity of 1.1B4 cells in MFu2 hydrogel particles was determined using MTS assay. Samples were incubated with an MTS solution prepared using a 1:5 ratio of MTS reagent and DMEM without phenol red and 1% ATB

solution for 3 h at 37 °C. The optical density (O.D.) was read at 490 nm on a multi-well microplate reader (Bio-Tek Instruments, US).

2.7.4. dsDNA quantification

Cell proliferation was evaluated using fluorimetric Picogreen double stranded DNA assay according to the manufacturer's instructions (Quanti-it, pico green dsDNA kit P7589, Invitrogen). Briefly, samples were collected at 1, 5 and 7 days washed with PBS and transferred into 1.5 ml tubes containing 1 ml of ultra-pure water and stored at −80 °C until testing. Before the dsDNA quantification, the structures were thawed and sonicated for 20 min. The quantification was performed in triplicate according to supplier instructions. All quantitative experiments were run in triplicate, and the results are expressed as a mean ± standard deviation.

2.7.5. Live/dead assay

The cellular viability was assessed using the live/dead assay (calcein AM/propidium iodide (PI) staining). Briefly, the cell-laden MFu2 hydrogel particles were incubated for 15 min with 2 μL calcein-AM (1 mg/mL, Molecular Probes, Invitrogen, USA) and 1 μL PI (1 mg/mL, Molecular Probes, Invitrogen, USA) in 1 mL of Dulbecco's phosphate buffered saline (DPBS) protected from light. After incubation the staining solution was removed and DPBS was added to remove residual staining. This washing step was repeated twice before image acquisition by confocal laser scanning microscopy (CLSM, TCS SP8, Leica, Germany).

2.7.6. Cell morphology

Adhesion, morphology, and distribution of 1.1B4 cells on MFu2 particles – and within, using particles cutted in half and addressing the cross-section – were analyzed by CLSM. To evaluate cell morphology, cells nuclei were stained with DAPI (blue) and F-actin filaments with phalloidin (red). The particles were fixated with 10% formalin for 30 min and then blocked with 3% bovine serum albumin (BSA) for 30 min. The structures were permeabilized with 0.1% Triton X-100 for 5 min, incubated with phalloidin-TRITC for 20 min at room temperature, followed by washing with PBS and staining with 5 μg/mL DAPI (2-(4-amidinophenyl)-1H-indole-6-carboxamidine) for 30 min. Fluorescence images from the stained constructs were obtained by CLSM (Confocal Laser Scanning Microscopy) with incubation (TCS SP8, Leica).

2.8. Statistical analysis

Statistical analysis of the data was performed using nonparametric Kruskal–Wallis test and the post hoc Tukey's multiple comparison tests, by Graph Pad Prism 5.0. Differences between the groups with $p < 0.05$ were considered statistically significant.

Table 1. Methacrylation degree (MD) and zeta potential of bare and modified fucoidan, as determined by ^{1}HNMR and laser Doppler anemometry, respectively.

Methods	Fu[†]	MFu1[‡]	MFu2[‡]
Ma[§] concentration [v/v]	—	8 %	12 %
MD[¶] [%]	—	2.78	6.50
Zeta potential (mv)	−30.40	−32.98	−62.10

Abbreviations: Fu: fucoidan; Ma: methacrylate anhydride; MD: methacrylation degree; MFu: modified fucoidan.

3. Results and discussion

3.1. Characterization of Fu and MFu

Fu is an underexploited sulfated polysaccharide extracted from brown algae, which has interesting chemical and biological properties. The major obstacle for using Fu on polymeric devices for biomedical applications is the high solubility in water. In order to control the solubility of Fu in water and increase its processability, while avoiding the blending with other polymers, a chemical modification was studied, a methacrylation reaction, enabling its further gelation by photocrosslinking. The chemical modification of Fu by methacrylation was assessed by FTIR, ^{1}H NMR and Zeta potential measurements and the results are shown in Table 1. In the FTIR (Figure 2(A)), the two spectra show an absorbance peak at 1000–1270 cm^{-1} corresponding to the stretching vibration of the sulfur-oxygen double bond (S = O) of sulfate group with different intensity [52]. This means that some acidic hydrolysis could occur during the methacrylation reaction removing some sulphate groups. The spectra of MFu revealed the appearance of carbon–carbon double bond characteristic peak (C = C) at 1400–1550 cm^{-1}, accompanied by the occurrence of characteristic ester peak (C = O) at 1680–1750 cm^{-1}, existent in MFu but not in Fu, thus confirming the methacrylation of Fu [36, 53, 54]. The functionalization of Fu with methacrylic groups was confirmed by ^{1}HNMR with the presence two peaks referent to methylene (=CH_2) characteristic signal, at $\delta = 5.5$–6 ppm (Figure 2(E,F)) and the other at $\delta = 1.9$–2 ppm peak corresponds to methyl (CH_3) (Figure 2(E–G)) both from incorporated methacrylated groups, in the NMR spectra of MFu (Figure 2(E)). Different methacrylation degrees (MD) were obtained, which depended on the concentration of methacrylated anhydride (MA) used for functionalization. In addition, the zeta potential measurement confirmed the anionic potential of both Fu, MFu1 and MFu2, with values varying between −30 and −62 mV.

3.2. Characterization of MFu hydrogel particles

3.2.1. Scanning electron microscopy

The morphology of MFu hydrogel particles, prepared by photocrosslinking of MFu solution drops deposited on superamphiphobic surfaces, was evaluated in dry state using scanning electron microscopy (SEM), with illustrative images being depicted in Figure 3. The SEM images show that the produced Fu particles have a spherical shape, as expected according to the production methodology used, with minimal roughness and varying size, with diameters ranging from 0.63 to 1.30 mm (values for particles in dry state), depending on the volume of the solution drop, which compare

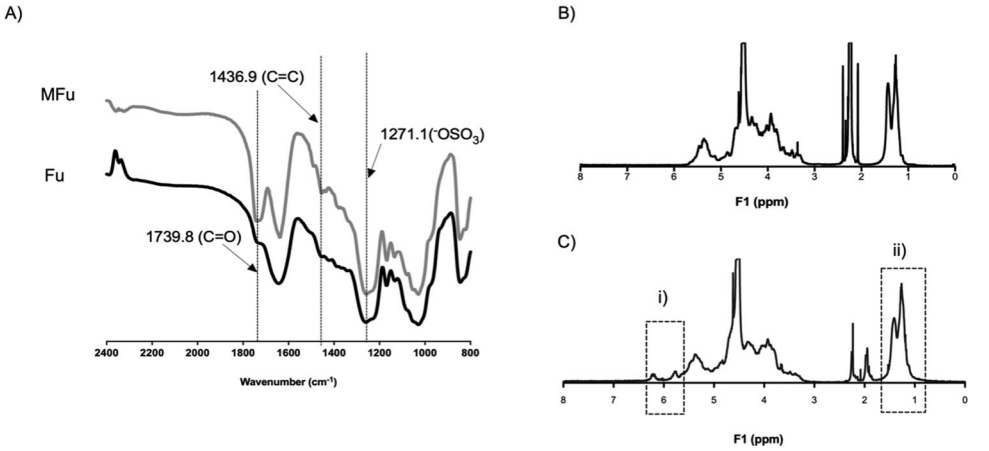

Figure 2. (A) FTIR spectra fucoidan (Fu) and modified fucoidan (MFu). ^{1}HNMR spectra of (B) fucoidan (Fu) and (C) modified fucoidan (MFu), (i) methylene groups ($=CH_2$) and (ii) methyl group (CH_3).

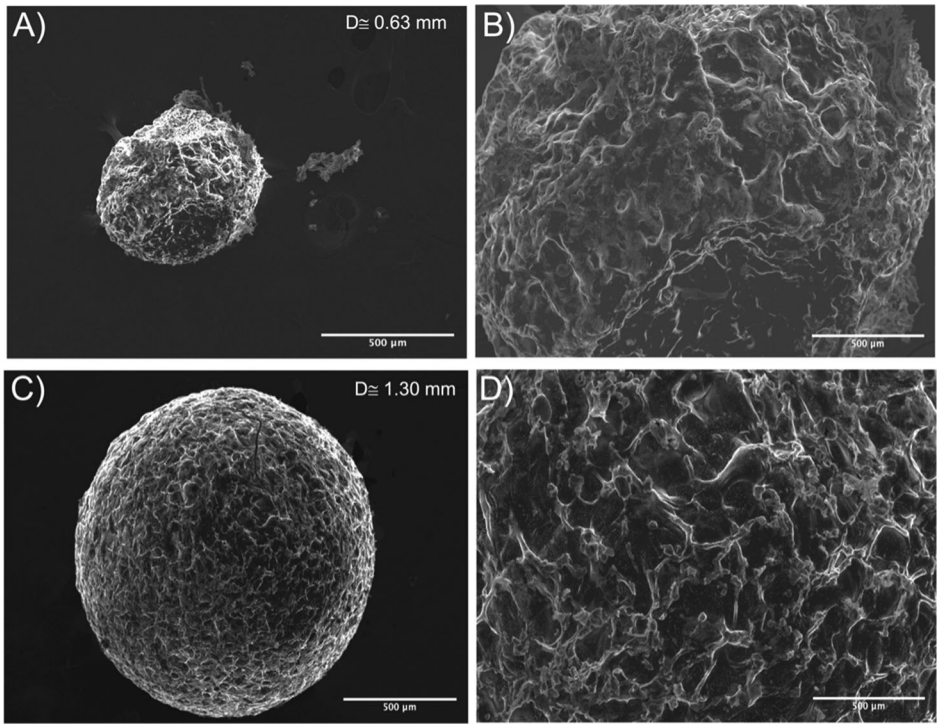

Figure 3. SEM images of MFu hydrogel particles: (A) MFu1, (B) higher magnification of the previous image, (C) MFu2, (D) higher magnification of the previous image; scale bar: 500 µm in (A) and (C), 50 µm in (B) and (D).

with diameters of 1.2–2.1 mm expected for wet particles produced from 1 to 5 µL solution droplets. This same photochemical methodology could be used to obtain hydrogels with different shapes, as discs, cylinders or cubes, if adequate molds were employed.

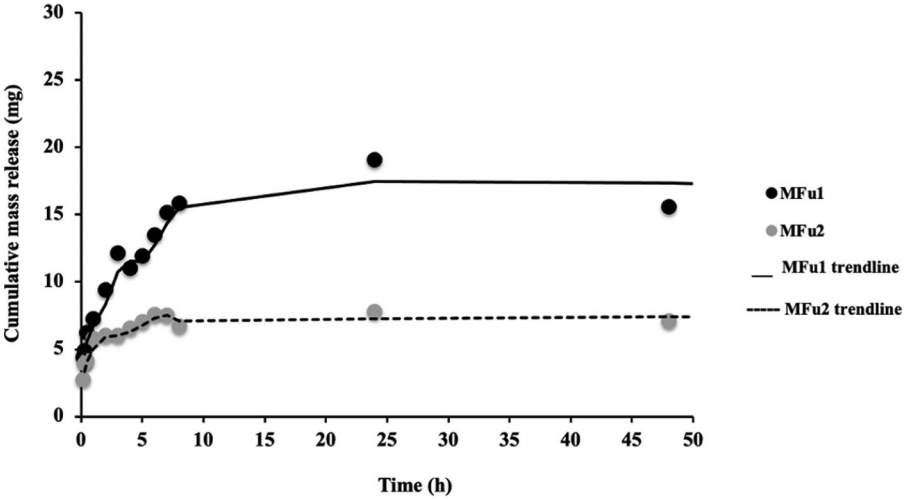

Figure 4. Insulin release from MFu1 and MFu2 hydrogel particles in PBS, assessing the effect of fucoidan with different methacrylation degree. The shown release curves are trend lines, determined from the experimental points, as guides to the eye.

3.2.2. Release of insulin in vitro

Hydrogel particles encapsulating insulin may protect the drug against the proteolytic attack and further release it at or near the cellular membrane to optimize the driving force for passive permeation [55, 56]. As first assessment of the release profile of insulin, we incubated MFu hydrogel particles loaded insulin in phosphate buffer saline (PBS), using as well MFu hydrogel particles without insulin in the assay as a control group (CTRL), and quantifying the released insulin over time. MFu 1 particles (lower methacrylation degree) allow a higher release of insulin in PBS, when compared with the release from MFu2 (higher methacrylation degree), as shown in Figure 4. We hypothesized that the mesh size in MFu2 could be smaller than MFu1, probably due to the higher methacrylation degree and consequently a higher cross-linking degree, which implies hampering the diffusion of the encapsulated insulin. The behavior of insulin release in PBS is in agreement with previous studies with other polysaccharide systems [56, 57].

The oral route is considered an acceptable and convenient route of insulin administration for treatment of diabetes. One major problem associated with the oral delivery of insulin is its low bioavailability (due to the proteolytic activity of the acidic juice in the stomach and susceptibility to enzymatic attack) and physical instability [55, 56]. In this regard, MFu2 hydrogel particles loaded with insulin were incubated in simulated gastric fluid (SGF) and in simulated intestinal fluid (SIF), not in sequence, and the insulin release quantified thus assessing the effect of pH on insulin release. It was observed a higher release of insulin from the hydrogel particles in SGF than in PBS, and in the latter higher than in SIF, which seems to indicate that lower pH facilitates insulin diffusion to the media, probably due to an effect on polymeric matrix by increment of mesh or stiffness decrease. Nevertheless, considering a gastrointestinal time in stomach of 1 h [58], only part of the encapsulated insulin would be released from MFu2 hydrogel particles in SGF during this time, from which a

significant amount of insulin would still be loaded in the MFu2 hydrogel particles for later release in intestine. In this regard, the proposed system might have a potential to be used in oral administration vehicles, being now needed to evaluate the functionality of the released insulin, as well as the release profiles in sequential media in response to low and high glucose levels.

3.3. Biological characterization

The potential of the developed MFu2 hydrogel particles to support the culture of pancreatic beta cells (ultimately aiming to mimic the pancreatic islets microenvironment) was addressed by evaluation of cytocompatibility upon culturing 1.1B4 human pancreatic beta cells (1.1B4-HPC). The direct contact assay on MFu2 hydrogel particles demonstrated that 1.1B4 cells were effectively able to proliferate from the initial time point up to 7 days in culture, as shown by the results of DNA quantification (Figure 5(A,B)).

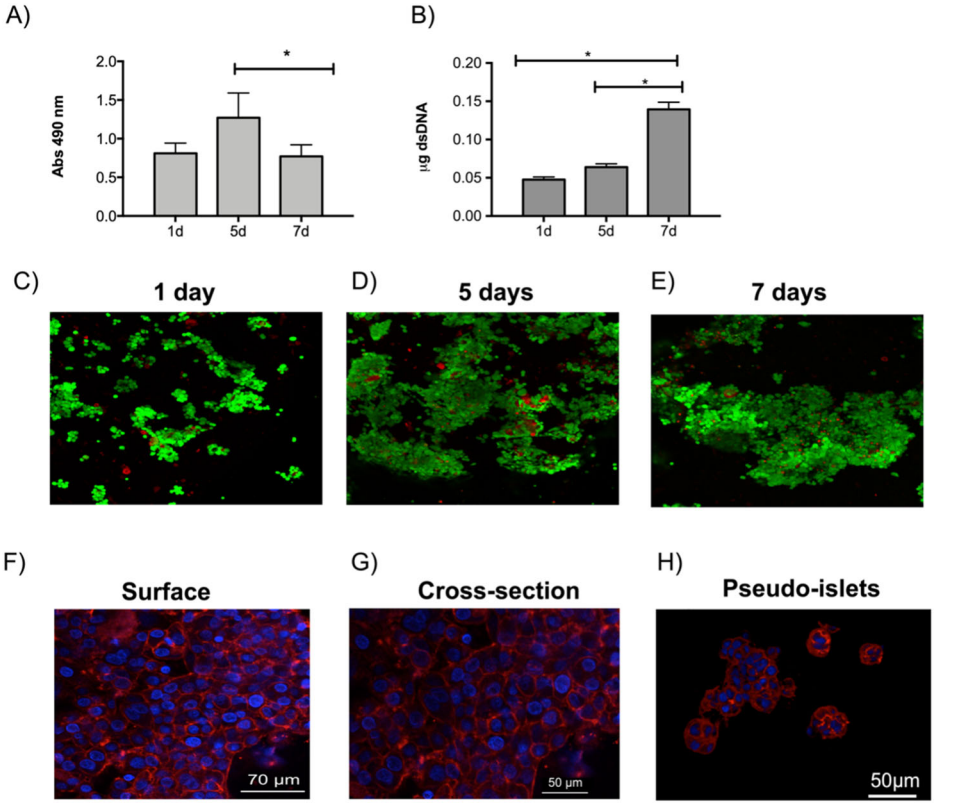

Figure 5. Biological performance of 1.1B4 cells in MFu2 hydrogel particles. (A) MTS assay and (B) dsDNA content of 1.1B4 cells, seeded and cultured up to 7 days on MFu2 hydrogel particles. Data means ± SD (* $p < 0.05$, nonparametric Kruskal–Wallis). (C–E) cell viability demonstrated by live (green)/dead (red) staining for 1.1B4 cells after (C) 1, (D) 5 and (E) 7 days in direct contact with MFu2 hydrogel particles, scale bar: 100 µm. (F–H) confocal laser micrographs of 1.1B4 cells cultured on MFu2 hydrogel particles during 7 days: (F) surface of the particles, (G) cross section and (H) detail of isolated pseudo-islets; the cells were stained with phalloidin-TRITC for actin filaments (red) and Hoechst 33342 for nuclei (blue); scale bars: 70 µm for (F) and 50 µm for (G) and (H).

Moreover, cell adhesion on MFu hydrogel particles, as well as cell viability, morphology at the particles' surface and morphology inside the particles (using particles cutted in half and addressing the cross-section) were evaluated by CLSM. The MFu2 hydrogel particles shown a good cytocompatibility, with viability above 80% and almost no dead cells observed after 7 days of culture. (Figure 5(C–E)). This is compatible with the results obtained by Velasco-Mallorquí et al. [17] who also observed a reduction in cell metabolic activity with time in respect to controls, although mostly associated to a reduction in cell proliferation. By their turn, Acarregui et al. [21] were also able to encapsulate 1.1B4 cells, but in alginate-derived materials, with most cells remaining viable upon 7 days of culture. In which regards cell morphology, cells shown round-like shape (Figure 5(F,G)) and tended to agglomerate after 7 days in culture to form pseudo-islets (Figure 5(H)), characteristic of pancreatic cells during the culture period of study. This is compatible with the original assessment of formation of pseudo-islets using this cell line, observed to be formed readily over 3–7 days of culture in low-adhering plastic [59], but also when cells were encapsulated in carboxymethyl cellulose cryogels [17]. Interestingly, images acquired from cross-sections of MFu2 hydrogel particles cutted in half (Figure 5(H)) show that 1.1B4 cells were observed not only at the surface of the particles, but were also able to migrate into particle core and proliferate within the developed structures, suggesting the cytocompatibility of the proposed MFu2 hydrogel particles, considering the reported limited success of encapsulation of pancreatic cells in hydrogels attributed to limited diffusion of oxygen and nutrients [60, 61]. Nevertheless, despite the cellular structural organization favorable to adequate function [59] has apparently been achieved, the functionality of the pseudo-islets needs to be confirmed by assessing insulin secretion in response to stimulation with glucose, as well as evaluating the expression of other markers, as genes involved in cell-to-cell communication, which being outside the scope of this work, should be the subject of a future study.

4. Conclusions

A methodology for the production of Fu hydrogel particles loaded with insulin was established, based in methacrylation of Fu followed by photocrosslinking in which droplets of functionalized Fu solution comprising insulin gave rise to spheroidal drug-loaded hydrogel particles. The MFu hydrogel particles revealed to be able to release insulin in a sustained fashion in both PBS and SIF, depending on methacrylation degree and, consequently, on hydrogel mesh. Moreover, these particles were also able to support the culture and encapsulation of human pancreatic cells (1.1B4), which were able to proliferate and migrate into the particles core and further agglomerate into pseudo-islets. The results suggested that MFu2 hydrogel particles may be a good candidate for drug delivery vehicle or immuno-protectant devices for insulin-producing pancreatic cells, opening the doors for future assessment of functionality, namely regarding response to glucose high and low levels, as well as to host immune system, envisaging advanced therapeutic solutions for diabetes mellitus treatment.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

We acknowledge ERDF for the financial support through POCTEP Project 0687_NOVOMAR_1_P, under the scope of INTERREG 2007-2013, and project 0302_CVMAR_I_1_P, under the scope of INTERREG España-Portugal 2014-2020, and Structured Projects NORTE-01-0145-FEDER-000021, NORTE-01-0145-FEDER-000023 and ATLANTIDA (ref. NORTE-01–0145-FEDER-000040), under the scope of Programa Operacional Regional do Norte (Norte 2020). Funding from the Portuguese Foundation for Science and Technology for doctoral grant (SFRH/BD/112139/2015) and post-doctoral grant (SFRH/BPD/85790/2012) is also acknowledged.

References

[1] Association AD. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2010; 33(Suppl 1):S62–S69.
[2] Amer LD, Mahoney MJ, Bryant SJ. Tissue engineering approaches to cell-based type 1 diabetes therapy. Tissue Eng Part B Rev. 2014;20(5):455–467.
[3] Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med. 1998;15(7):539–553.
[4] Crowther CA, Hiller JE, Moss JR, et al. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. N Engl J Med. 2005;352(24):2477–2486.
[5] Ahlqvist E, Storm P, Käräjämäki A, et al. Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. Lancet Diabetes Endocrinol. 2018;6(5):361–369.
[6] Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. Lancet. 2014;383(9911): 69–82.
[7] de Groot M. Immunoprotection of pancreatic islets: in vitro studies into causes of microencapsulated graft failure [doctoral thesis]. Groningen: University of Groningen; 2004.
[8] Roep BO, Peakman M. Antigen targets of type 1 diabetes autoimmunity. Cold Spring Harb Perspect Med. 2012;2(4):a007781.
[9] He Z-X, Zhou Z-W, Yang Y, et al. Overview of clinically approved oral antidiabetic agents for the treatment of type 2 diabetes mellitus. Clin Exp Pharmacol Physiol. 2015; 42(2):125–138.
[10] Xie J, Li A, Li J. Advances in pH-sensitive polymers for smart insulin delivery. Macromol Rapid Commun. 2017;38(23):1700413.
[11] Elshaarani T, Yu H, Wang L, et al. Dextran-crosslinked glucose responsive nanogels with a self-regulated insulin release at physiological conditions. Eur Polym J. 2020;125: 109505.
[12] Cubayachi C, Lemos CN, Pereira F, et al. Silk fibroin films stabilizes and releases bioactive insulin for the treatment of corneal wounds. Eur Polym J. 2019;118:502–513.
[13] Chen BZ, Ashfaq M, Zhu DD, et al. Controlled delivery of insulin using rapidly separating microneedles fabricated from genipin-crosslinked gelatin. Macromol Rapid Commun. 2018;39(20):1800075.
[14] Bhattacharyya A, Mukherjee D, Mishra R, et al. Preparation of polyurethane–alginate/ chitosan core shell nanoparticles for the purpose of oral insulin delivery. Eur Polym J. 2017;92:294–313.
[15] Reis CP, Ribeiro AJ, Neufeld RJ, et al. Alginate microparticles as novel carrier for oral insulin delivery. Biotechnol Bioeng. 2007;96(5):977–989.
[16] Fuchs S, Ernst AU, Wang L-H, et al. Hydrogels in emerging technologies for type 1 diabetes. Chem Rev. 2021;121(18):11458–11526.

[17] Velasco-Mallorquí F, Rodríguez-Comas J, Ramón-Azcón J. Cellulose-based scaffolds enhance pseudoislets formation and functionality. Biofabrication. 2021;13(3):035044.
[18] Naficy S, Dehghani F, Chew YV, et al. Engineering a porous hydrogel-based device for cell transplantation. ACS Appl Bio Mater. 2020;3(4):1986–1994.
[19] Lin C-C, Anseth KS. Glucagon-like peptide-1 functionalized PEG hydrogels promote survival and function of encapsulated pancreatic beta-cells. Biomacromolecules. 2009; 10(9):2460–2467.
[20] Strand BL, Coron AE, Skjak-Braek G. Current and future perspectives on alginate encapsulated pancreatic islet. Stem Cells Transl Med. 2017;6(4):1053–1058.
[21] Acarregui A, Ciriza J, Saenz del Burgo L, et al. Characterization of an encapsulated insulin secreting human pancreatic beta cell line in a modular microfluidic device. J Drug Target. 2018;26(1):36–44.
[22] Marchioli G, van Gurp L, van Krieken PP, et al. Fabrication of three-dimensional bioplotted hydrogel scaffolds for islets of langerhans transplantation. Biofabrication. 2015; 7(2):025009.
[23] An D, Chiu A, Flanders JA, et al. Designing a retrievable and scalable cell encapsulation device for potential treatment of type 1 diabetes. Proc Natl Acad Sci USA. 2018;115: E263–E272.
[24] Merani S, Toso C, Emamaullee J, et al. Optimal implantation site for pancreatic islet transplantation. Br J Surg. 2008;95(12):1449–1461.
[25] Shih Lin CCH, Shih H, Lin CC, et al. Visible-light-mediated thiol-ene hydrogelation using eosin-Y as the only photoinitiator. Macromol Rapid Commun. 2013;34(3): 269–273.
[26] Fotino N, Fotino C, Pileggi A. Re-engineering islet cell transplantation. Pharmacol Res. 2015;98:76–85.
[27] Korsgren O. Islet encapsulation: physiological possibilities and limitations. Diabetes. 2017;66(7):1748–1754.
[28] Bochenek MA, Veiseh O, Vegas AJ, et al. Alginate encapsulation as long-term immune protection of allogeneic pancreatic islet cells transplanted into the omental bursa of macaques. Nat Biomed Eng. 2018;2(11):810–821.
[29] Lauritano C, Ianora A. Marine organisms with anti-diabetes properties. Mar Drugs. 2016;14(12):220.
[30] Kim KJ, Yoon KY, Lee BY. Fucoidan regulate blood glucose homeostasis in C57BL/KSJ m+/+db and C57BL/KSJ db/db mice. Fitoterapia. 2012;83(6):1105–1109.
[31] Li B, Lu F, Wei X, et al. Fucoidan: structure and bioactivity. Molecules. 2008;13(8): 1671–1695.
[32] Silva TH, Alves A, Popa EG, et al. Marine algae sulfated polysaccharides for tissue engineering and drug delivery approaches. Biomatter. 2012;2(4):278–289.
[33] Reys LL, Vaithilingam V, Sthijns M, et al. Fucoidan hydrogels significantly alleviate oxidative stress and enhance the endocrine function of encapsulated beta cells. Adv Funct Mater. 2021;31(35):2011205.
[34] Sezer A, Cevher E. Fucoidan: a versatile biopolymer for biomedical applications. In: Zilberman M, editor. Act implant scaffolds tissue regen. Berlin, Heidelberg: Springer; 2011. p. 377.
[35] Ale MT, Mikkelsen JD, Meyer AS. Important determinants for fucoidan bioactivity: a critical review of structure-function relations and extraction methods for fucose-containing sulfated polysaccharides from brown seaweeds. Mar Drugs. 2011;9(10): 2106–2130.
[36] Reys LL, Silva SS, Soares da Costa D, et al. Fucoidan hydrogels photo-cross-linked with visible radiation as matrices for cell culture. ACS Biomater Sci Eng. 2016;2(7): 1151–1161.
[37] Lee JS, Jin GH, Yeo MG, et al. Fabrication of electrospun biocomposites comprising polycaprolactone/fucoidan for tissue regeneration. Carbohydr Polym. 2012;90(1): 181–188.

[38] Perumal RK, Perumal S, Thangam R, et al. Collagen-fucoidan blend film with the potential to induce fibroblast proliferation for regenerative applications. Int J Biol Macromol. 2018;106:1032–1040.
[39] Sezer AD, Cevher E, Hatipoğlu F, et al. Preparation of fucoidan-chitosan hydrogel and its application as burn healing accelerator on rabbits. Biol Pharm Bull. 2008;31(12): 2326–2333.
[40] Sezer AD, Cevher E, Hatipoğlu F, et al. The use of fucosphere in the treatment of dermal burns in rabbits. Eur J Pharm Biopharm. 2008;69(1):189–198.
[41] Murakami K, Ishihara M, Aoki H, et al. Enhanced healing of mitomycin C-treated healing-impaired wounds in rats with hydrosheets composed of chitin/chitosan, fucoidan, and alginate as wound dressings. Wound Repair Regen. 2010;18(5):478–485.
[42] Ferreira VRA, Azenha MA, Bustamante AG, et al. Metal cation sorption ability of immobilized and reticulated chondroitin sulfate or fucoidan through a sol-gel crosslinking scheme. Mater Today Commun. 2016;8:172–182.
[43] Jin G, Kim GH. Rapid-prototyped PCL/fucoidan composite scaffolds for bone tissue regeneration: design, fabrication, and physical/biological properties. J Mater Chem. 2011;21(44):17710–17718.
[44] Jeong HS, Venkatesan J, Kim S-K. Hydroxyapatite-fucoidan nanocomposites for bone tissue engineering. Int J Biol Macromol. 2013;57:138–141.
[45] Lowe B, Venkatesan J, Anil S, et al. Preparation and characterization of chitosan-natural nano hydroxyapatite-fucoidan nanocomposites for bone tissue engineering. Int J Biol Macromol. 2016;93(Pt B):1479–1487.
[46] Murakami K, Aoki H, Nakamura S, et al. Hydrogel blends of chitin/chitosan, fucoidan and alginate as healing-impaired wound dressings. Biomaterials. 2010;31(1):83–90.
[47] Sezer AD, Akbuğa J. Fucosphere—new microsphere carriers for peptide and protein delivery: preparation and in vitro characterization. J Microencapsul. 2006;23:513–522.
[48] Rial-Hermida MI, Oliveira NM, Concheiro A, et al. Bioinspired superamphiphobic surfaces as a tool for polymer- and solvent-independent preparation of drug-loaded spherical particles. Acta Biomater. 2014;10(10):4314–4322.
[49] Deng X, Mammen L, Butt H-J, et al. Candle soot as a template for a transparent robust superamphiphobic coating. Science. 2012;335(6064):67–70.
[50] Shih H, Mirmira RG, Lin CC. Visible light-initiated interfacial thiol-norbornene photopolymerization for forming islet surface conformal coating. J Mater Chem B. 2015;3: 170–175.
[51] Tahtat D, Mahlous M, Benamer S, et al. Oral delivery of insulin from alginate/chitosan crosslinked by glutaraldehyde. Int J Biol Macromol. 2013;58:160–168.
[52] Sabourel P, Chaubet F, Rouzet F, et al. Purification of a low molecular weight fucoidan for SPECT molecular imaging of myocardial infarction. Mar Drugs. 2014;12(9): 4851–4867.
[53] Coutinho DF, Sant SV, Shin H, et al. Modified gellan gum hydrogels with tunable physical and mechanical properties. Biomaterials. 2010;31(29):7494–7502.
[54] Morelli A, Chiellini F, Morelli FA. Ulvan as a new type of biomaterial from renewable resources: functionalization and hydrogel preparation. Macromol Chem Phys. 2010; 211(7):821–832.
[55] Sood A, Panchagnula R. Peroral route: an opportunity for protein and peptide drug delivery. Chem Rev. 2001;101(11):3275–3303.
[56] Silva CM, Ribeiro AJ, Ferreira D, et al. Insulin encapsulation in reinforced alginate microspheres prepared by internal gelation. Eur J Pharm Sci. 2006;29(2):148–159.
[57] Mukhopadhyay P, Sarkar K, Chakraborty M, et al. Oral insulin delivery by self-assembled chitosan nanoparticles: in vitro and in vivo studies in diabetic animal model. Mater Sci Eng C Mater Biol Appl. 2013;33(1):376–382.
[58] Shahbazi M-A, Santos HA. Improving oral absorption via drug-loaded nanocarriers: absorption mechanisms, intestinal models and rational fabrication. Curr Drug Metab. 2013;14(1):28–56.

[59] Guo-Parke H, McCluskey JT, Kelly C, et al. Configuration of electrofusion-derived human insulin-secreting cell line as pseudoislets enhances functionality and therapeutic utility. J Endocrinol. 2012;214(3):257–265.
[60] Johnson AS, Fisher RJ, Weir GC, et al. Oxygen consumption and diffusion in assemblages of respiring spheres: performance enhancement of a bioartificial pancreas. Chem Eng Sci. 2009;64(22):4470–4487.
[61] Li W, Lee S, Ma M, et al. Microbead-based biomimetic synthetic neighbors enhance survival and function of rat pancreatic β-cells. Sci Rep. 2013;3:2863.