2018-11-12

Novel nano-encapsulation of probucol in microgels: scanning electron micrograph characterizations, buoyancy profiling, and antioxidant assay analyses

Armin Mooranian, Nassim Zamani, Momir Mikov, Svetlana Goločorbin-Kon, Goran Stojanović, Frank Arfuso, Hani Al-Salami

Artificial Cells, Nanomedicine, and Biotechnology
An International Journal

ISSN: 2169-1401 (Print) 2169-141X (Online) Journal homepage: http://www.tandfonline.com/loi/ianb20

Novel nano-encapsulation of probucol in microgels: scanning electron micrograph characterizations, buoyancy profiling, and antioxidant assay analyses

Armin Mooranian, Nassim Zamani, Momir Mikov, Svetlana Goločorbin-Kon, Goran Stojanovic, Frank Arfuso & Hani Al-Salami

To cite this article: Armin Mooranian, Nassim Zamani, Momir Mikov, Svetlana Goločorbin-Kon, Goran Stojanovic, Frank Arfuso & Hani Al-Salami (2018): Novel nano-encapsulation of probucol in microgels: scanning electron micrograph characterizations, buoyancy profiling, and antioxidant assay analyses, Artificial Cells, Nanomedicine, and Biotechnology, DOI: 10.1080/21691401.2018.1511571

To link to this article: https://doi.org/10.1080/21691401.2018.1511571

Published online: 27 Sep 2018.

ARTIFICIAL CELLS, NANOMEDICINE, AND BIOTECHNOLOGY
https://doi.org/10.1080/21691401.2018.1511571

Novel nano-encapsulation of probucol in microgels: scanning electron micrograph characterizations, buoyancy profiling, and antioxidant assay analyses

Armin Mooranian[a], Nassim Zamani[a], Momir Mikov[b], Svetlana Goločorbin-Kon[c], Goran Stojanovic[d], Frank Arfuso[e] and Hani Al-Salami[a]

[a]Biotechnology and Drug Development Research Laboratory, School of Pharmacy and Biomedical Sciences, Curtin Health Innovation Research Institute, Curtin University, Perth, Australia; [b]Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia; [c]Department of Pharmacy, University of Novi Sad, Novi Sad, Serbia; [d]Faculty of Technical Sciences, University of Novi Sad, Novi Sad, Serbia; [e]Stem Cell and Cancer Biology Laboratory, School of Pharmacy and Biomedical Sciences, Curtin Health Innovation Research Institute, Curtin University, Perth, Australia

ABSTRACT

Smart polymers such as Eudragit (ED) have shown potential applications in oral drug delivery and targeted release. Probucol (PB) is a lipophilic drug used for hypercholesterolemia and possesses desirable antidiabetic effects such as antioxidant and cell protective effects. PB is highly hydrophobic and has poor bioavailability with significant inter- and intra-patient absorption, limiting its clinical applications in diabetes. This study aimed to design and analyse new PB-ED formulations with or without the absorption-enhancer chenodeoxycholic acid (CDCA). Sodium alginate-based microcapsules containing three different ED polymers (NM30D, RL30D and RS30D) were investigated with or without CDCA via scanning electron microscopy, energy dispersive X-ray spectroscopy (EDXR), confocal microscopy, osmotic stability, mechanical properties, buoyancy, release profiles (pH: 7.4), thermal stability and antioxidant effects. The effects of microcapsules on pancreatic β-cell survival, function, inflammatory profile and PB cellular uptake were analysed. All microcapsules showed uniform morphology and surface topography with CDCA being distributed evenly throughout the microcapsules. Osmotic stability was significantly improved in PB-NM30D and PB-RL30D microcapsules ($p < .01$ and $p < .05$, respectively), and PB-NM30D microcapsules displayed low buoyancy ($p < .01$). CDCA improved PB-NM30D effects on pancreatic β-cell function and bioenergetics, which suggests potential application of PB-NM30D-CDCA in PB delivery and diabetes treatment.

ARTICLE HISTORY
Received 22 May 2018
Revised 29 July 2018
Accepted 6 August 2018

KEYWORDS
Probucol; microencapsulation; chenodeoxycholic acid; oxidative stress; topographic analysis; diabetes

Introduction

Diabetes mellitus (DM) is a chronic disorder that develops due to either lack of adequate insulin production (Type 1; T1D) or loss of tissue sensitivity to insulin (Type 2; T2D) [1,2]. T1D develops at an early stage of life due to an inflammatory process that leads to immune attack against pancreatic cells responsible for insulin production. T2D is the most common type of DM and is a metabolic disorder with underlying pathophysiological processes including hypercholesterolemia and chronic low-grade systemic inflammation, which are exacerbated by high levels of free radicals and oxidants and subsequent oxidative stress [3,4]. Pancreatic β-cells have low endogenous antioxidant defence mechanisms and are vulnerable to oxidative stress [5]. β-cell damage is common in both types of diabetes, and all T1D patients and more than 35% of T2D require exogenous insulin injections. Accordingly, effective therapy for DM needs to not only target diabetic hyperglycaemia and loss of tissue sensitivity but also target inflammation and oxidative stress, and protect β-cells from damage [6,7].

Probucol (PB) is a medication prescribed for hypercholesterolemia. It possesses desirable antidiabetic effects such as antioxidant and β-cell protective effects [7]. PB is highly hydrophobic and has poor bioavailability and significant inter- and intra-patient absorption, which limits its potential clinical applications in DM. Recent studies in our laboratory have shown potential use of PB and various bile acids in diabetes; however, conventional PB release matrices remain inadequate and its release remains variable after oral administration [7–10]. To optimize its targeted release properties and enhance its gut absorption, PB needs to be encapsulated with a new polymer (with controlled release properties), and with an absorption enhancer. Eudragit® (ED) polymers have been recently examined in drug delivery and have shown to optimize the physical properties of many drug-delivery systems and matrices [11], whereas the bile acid chenodeoxycholic acid (CDCA) has been shown to possess absorption-enhancing properties [12]. ED polymers are composed of poly (methyl) acrylate polymer systems with unique physicochemical properties that enable them to have high flexibility, low permeability and pH-dependant swelling properties allowing for their wide range applications in pharmaceutical formulations. Both ED and CDCA need to be encapsulated and formulated with PB to form a single delivery matrix

CONTACT Hani Al-Salami hani.al-salami@curtin.edu.au Biotechnology and Drug Development Research Laboratory, School of Pharmacy and Biomedical Sciences, Curtin Health Innovation Research Institute (CHIRI), Curtin University, Perth, WA, Australia.

capable of targeted-oral delivery of PB into the lower part of the gut, where it is maximally absorbed.

Although several studies in our laboratory have examined formulations of PB alone or in combination with multiple excipients [1,2,6–10], none have shown adequate targeted-release properties or pH selective release to enable better bioavailability and clinical efficacy. However, significant published data on the use of biomaterials in diabetes suggest potential for micro- and nanocapsule devices [13–16]. Accordingly, this study aimed to design and test, *in vitro*, new formulations of PB using three ED polymers (NM30D, RL30D and RS30D) with or without the permeation-enhancing bile acid CDCA. All groups were formed via microencapsulation in alginate-based microcapsules using our well-established microencapsulation system [1,17]. The microcapsules were examined for their morphological properties (using scanning electron microscopy), elemental composition (using energy dispersive X spectroscopy), multilayered imaging (using confocal microscopy), osmotic, mechanical and thermal stability profiles (using water-loss index, intactness index and glass transition-thermal index), and PB release properties (using in-house built system, PerkinElmer Lambda 25 Spectrophotometer). Using pancreatic β-cells, the microcapsules were examined *in vitro* for their biological effects on cell viability (using MTT assays), cellular respiration and bioenergetics (using in-house built Seahorse® XF technology), glucose-stimulated insulin secretion at normoglycaemia and hyperglycaemia (using immunofluorescence, insulin ELISA kits), and the inflammatory profile (using flow-cytometric analysis).

Materials and methods

Materials

PB, alginate, CDCA, 3–(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and fluorescein isothiocyanate were purchased from Sigma-Aldrich Corporation (New South Wales, Australia). ED polymers, NM30D, RL30D and RS30D, were obtained from Evonik Industries (Victoria, Australia), and sterile phosphate-buffered saline was purchased from GE Healthcare (New South Wales, Australia). 2′,7′-Dichlorofluorescin diacetate (DCFH-DA) was purchased from Sigma-Aldrich Corporation (New South Wales, Australia). 2,2′-Azobis-2-methyl-propanimidamide, dihydrochloride purchased from Sapphire Bioscience (Australia).

Low passage pancreatic NIT-1 β-cells were maintained using an optimized culture medium consisting of Dulbecco's modified eagle's medium supplemented with 5.8 mM glucose (aliquoted from a 45% w/v solution of D-(+)-glucose solution; Sigma Aldrich Corporation, Australia), 2% penicillin–streptomycin, 11.5% foetal bovine serum, and various amino acids [18,19].

Microencapsulating formulations and capsule formation

Six formulations were prepared, three control without, and three test with CDCA. All formulations contained (per 100 ml mixture): PB (0.25 g), sodium alginate (1.8 g), ultrasonic gel (5 g) and ED polymers NM30D, RL30D and RS30D (25 ml). Formulation-1 (F1) comprised alginate-NM30D-PB mixture, formulation-2 (F2) comprised F1 and CDCA, formulation-3 (F3) comprised alginate-RL30D-PB mixture, formulation-4 (F4) comprised F3 and CDCA, formulation-5 (F5) comprised alginate-RS30D-PB mixture and formulation-6 (F6) comprised F5 and CDCA.

Microencapsulation was done using the Büchi B-390 encapsulator (Büchi, Switzerland), which consisted of a vibrational nozzle system used to generate droplets of PB suspended within the polymer matrix, with the final microcapsules forming in a 2.5% w/v calcium chloride ionic gelation hardening bath using our established methods [9,10,20].

Topography, imaging, buoyancy, osmotic, mechanical and thermal stability, and drug release measurements

Microcapsule morphology and surface topography were determined using a Zeiss Neon 40EsB FIBSEM (USA) with 0.8 nm calibrated resolution, and atomic composition was evaluated via energy dispersive X-ray spectroscopy (EDXR) using Aztec-Energy EDS Analysis Software (Oxford Instruments, UK), as previously described [9,21]. Confocal micrographs were taken using a Nikon A1 (Nikon Corporation, Japan).

Microcapsule buoyancy measurements were carried out by measuring the percentage of microcapsules floating in 100 ml of simulated intestinal fluids (enzymatic phosphate buffered at pH 7.8) [2].

The osmotic stability of the microcapsules was determined by placing 200 microcapsules in phosphate-buffered saline (pH: 7.4) for 14 days at 37 °C and calculating osmotic capacity via weight change as the percentage of initial weight [1,6,7]. Mechanical and thermal stability were measured by (1) exposing 100 microcapsules to mechanical vibrational movements using a series of oscillations and measuring resilience and resistance to fracture for 14 days and (2) differential scanning calorimetry using 5 mg weight of microcapsules and measuring changes in melting points compared with a reference [22,23]. Drug (PB) release from microcapsules at pH 7.4 was measured using an UV–Vis dissolution machine (PerkinElmer Lambda 25; PerkinElmer, USA) coupled to a closed-loop system linked to a Masterflex C/L Variable-Speed Tubing peristaltic pump (Masterflex, USA). Negative values were considered negligible [8,22].

Cell viability, antioxidant assay and biological function measurements

3–(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide viability assays were performed at two glucose concentrations (5.8 mM and 35.8 mM) over a 48-h period as described elsewhere [2,7] while oxidative stress testing was undertaken by culturing NIT-1 cells in glucose 5.8 or 35.8 mM for 48 h and incubating them with the microcapsules in two solutions for 48 h. The two solutions were DCFH-DA (4.6 mM) and 2,2′-azobis-2-methyl-propanimidamide, dihydrochloride (AAPH; 18.44 mM) and were freshly prepared prior to the testing. An Enspire Multimode Plate Reader (PerkinElmer, USA) was used for measuring absorption intensity; thus, the lower the fluorescence reading, the greater the antioxidant activity conferred

[24]. To measure glucose-stimulated insulin secretion (glucose concentrations of 15.5 mM), cellular insulin release at normoglycaemic and hyperglycaemic states were measured. Proinflammatory interferon-γ levels, and ATP production, in a hyperglycaemic state were measured from β-cells exposed to the microcapsules for 48 h. Insulin was measured using Ultrasensitive Mouse Insulin ELISA Kit (Mercodia Cooperation, Uppsala, Sweden), interferon-γ was measured using the Attune Acoustic Flow Cytometer via Cytokine Bead Array Technology (BD Bioscience, USA) while ATP production was measured using the Seahorse XF Analyser (Seahorse Bioscience, USA) as per our established methods [18,25–27].

PB cellular uptake measurements

PB uptake by cells from different microcapsules after 48 h incubation was examined at normoglycaemic and hyperglycaemic states. Cells were sonicated and contents were analysed via our well-established high pressure liquid chromatographic analysis using an isocratic Shimadzu Prominence HPLC system consisting of a Shimadzu DGU-20A5 degasser, LC-20AT liquid chromatographer, SIL-20A autosampler and SPD-20A UV/Vis detector (Shimadzu Corporation, Kyoto, Japan). The standard curve was linear with R^2=0.99. Intra- and inter-day coefficient of variations were 0.49 and 3.1%, respectively. Data were standardized as pg of PB per 1.0×10^5 cells [28].

For cell-based measurements, data were normalized for viable cell count.

Statistical analysis

Statistical analysis was performed using parametric/non-parametric or one-way analysis of variance (ANOVA) followed by Tukey posthoc as appropriate, using GraphPad Prism Version 7.04 (GraphPad, USA). Values are expressed as means ± standard error of the mean. A statistical difference was reported when the p values $<.05$.

Results and discussion

Topography and imaging of microcapsules

Topographic and imaging analyses (Figure 1) of control (F1, F3 and F5) and corresponding CDCA containing microcapsules (F2, F4 and F6) showed similar size, structural morphology, surface topographic features and CDCA distribution within the microcapsules. This suggests that neither changes in constituents between the ED formulations nor the presence of CDCA caused significant changes in the topographic and structural properties of the microcapsules. This also suggests that the microencapsulation method deployed is robust and produces consistent microcapsules regardless of variation in the microencapsulated ingredients. The findings are consistent with our published studies where our microencapsulating method produced equal size microcapsules containing drugs or cells regardless of the constituents of the microencapsulating formulation [2,29,30]. Despite the six formulations having similar topographic features, the microcapsules' stability, buoyancy and PB release profile may exhibit different characteristics due to different composition of the ED polymers or CDCA incorporation.

Buoyancy, osmotic, mechanical and thermal stability, and drug release profiles

Figure 2 shows the microcapsules' osmotic stability (A), mechanical stability (B), buoyancy (C), PB release (D) and thermal stability (E).

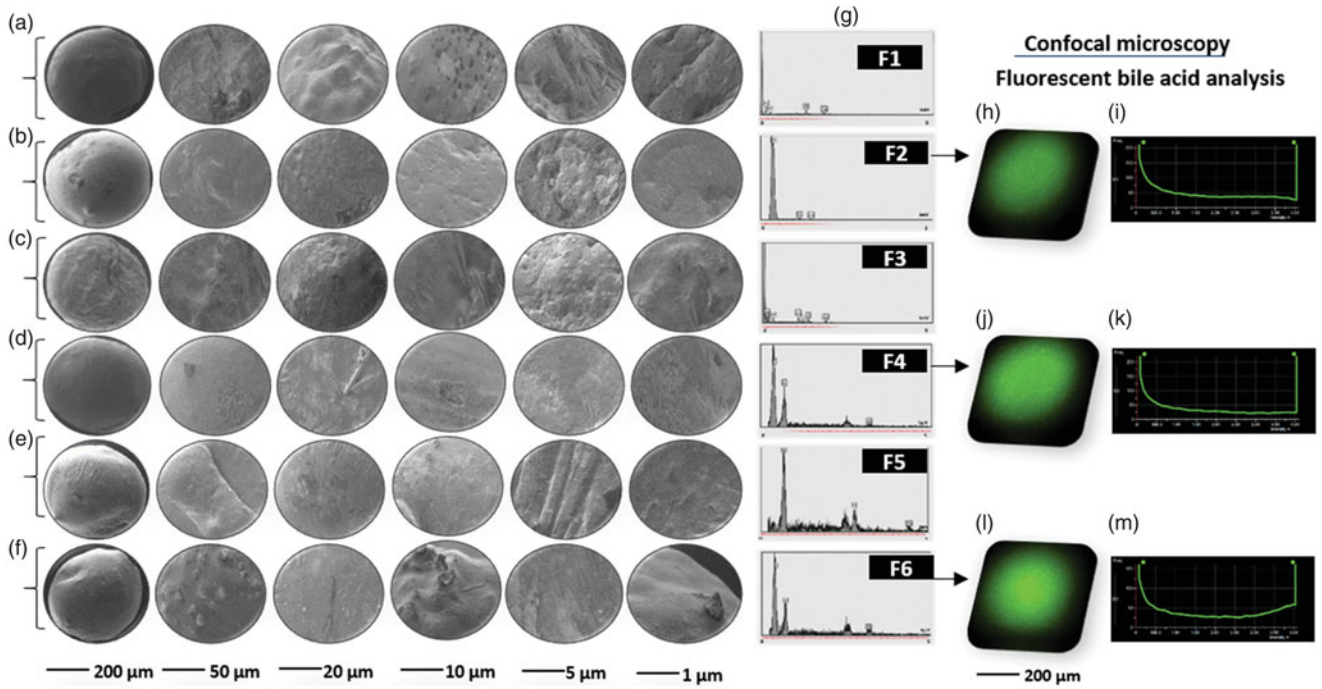

Figure 1. Scanning electron micrographs of microcapsules of F1–F6 (a–f; with F2, F4 and F6 being treatments) and corresponding energy dispersive X-ray analyses (g), and confocal imaging of stained CDCA with corresponding analytical laser depth (F2: h and i, F4: j and k and F6: l and m).

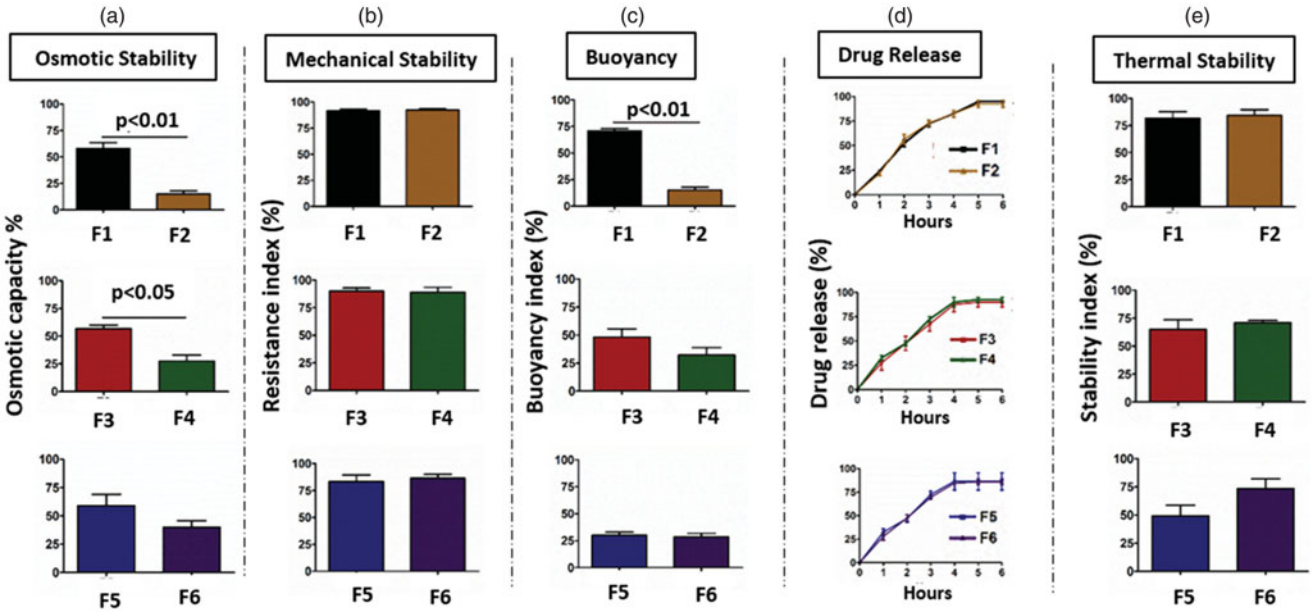

Figure 2. Osmotic stability (a), mechanical stability (b), buoyancy (c), drug release (d) and thermal stability (e) of F1–F6 microcapsules. Data are mean ± SEM, $n = 3$.

Incorporation of CDCA into F1 and F3 microcapsules reduced the osmatic capacity, thereby enhancing osmotic stability and reducing moisture loss via the microcapsules' membrane. The osmatic stabilizing effect of CDCA was not significant on F5 microcapsules, suggesting that the stabilizing effect is polymer-dependent. Mechanical stability and PB release profiles of microcapsules were maintained regardless of formulation composition or CDCA incorporation, suggesting that all formulations exhibited similar physical properties and disintegration rate at pH 7.4. PB nanoentrapment using nanoliposomes has demonstrated promise in enhancing oral absorption [31]. Thus, PB targeted release at 7.4 pH is highly desirable as this correlates to its release and potential absorption through lower part of ileal mucosa [32]. CDCA incorporation resulted in reduced buoyancy only in F2 while other formulations (F1, F3–F6) exhibited similar buoyancy, suggesting that F2 possesses higher density than gut contents and is likely to come in contact with the ileal mucosa, and potentially have better absorption at site of release. Hence, osmotic stability (Figure 2(a)) and buoyancy (Figure 2(c)) showed best results with F2 microcapsules while F4 showed improved osmotic stability (Figure 2(a)).

Clearly, to improve PB's low bioavailability and optimize its efficacy after oral delivery, a new targeted-delivery formulation is needed. Recently, we examined incorporating PB with the bile acid, deoxycholic acid. The results showed improved formulation stability, but the pattern of PB release had limitations including a multiphasic profile [9]. In other studies, alginate-CDCA showed improved release properties of PB, but total dissolution did not reach maximum concentrations within the 6-h experiment [6]. Other studies have shown that nanoparticulation of PB improves its dissolution profile. Tanka et al. investigated the potential applications of a novel wet-milling process, using the ULTRA APEX MILL, on the dissolution profile of PB. The authors found that using novel wet-milling processes and dispersing agents such as Gelucire 44/14 can significantly improve the dissolution rate and potentially increase oral absorption of PB [33]. Accordingly, our experiments were the first to investigate the applications of ED and CDCA in PB oral-targeted delivery, and our resulted showed significant and highly optimized targeted release profile, when using NM30D and CDCA in microcapsules containing PB.

Cell viability, antioxidant assay and biological function studies

Exposure of pancreatic β-cells to different microcapsules at normoglycaemic (5.8 mM glucose) and hyperglycaemic (35.8 mM glucose) states did not affect cell viability or survival over the period of the experiment, suggesting that cell viability and mass were neither directly correlated to the internal composition of the microcapsules nor affected by presence of CDCA at different glucose concentrations (Figure 3(a)). Cellular antioxidant activities of formulations, and insulin secretion at the hyperglycaemic state, were improved only by CDCA incorporation in F2 (Figure 3(b) and (c)). This suggests that antioxidant and insulin secretion effects are formulation-dependent, and that optimized PB antioxidant and cell function effects were brought about by microencapsulation with alginate-NM30D in the presence of CDCA. The improved antioxidant and insulin secretion effects of PB-CDCA in alginate-NM30D microcapsules do not seem to affect cell viability, suggesting a lack of direct relationship between cell survival and level of oxidants or insulin present in the medium at the normoglycaemic or hyperglycaemic state. In a previous study, PB microencapsulation in alginate with the bile acid cholic acid reduced cell apoptosis at the hyperglycaemic state, which suggests such effects of PB microcapsules are influenced by excipient, type of ED polymer and presence of CDCA [2]. The positive effects of PB-CDCA when microcapsulated in alginate-NM30D formulation do not seem to be directly correlated with effects on inflammatory profile since levels of the proinflammatory cytokine

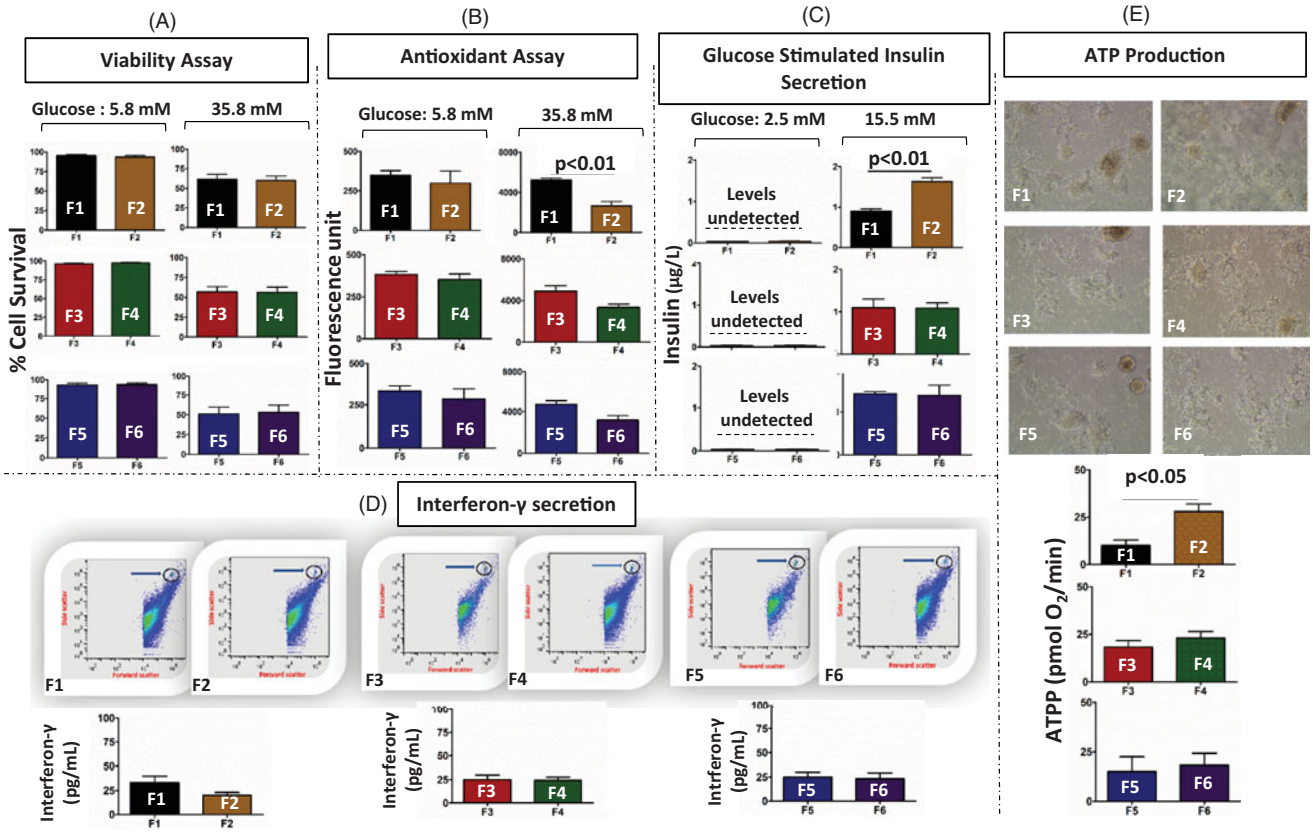

Figure 3. Cell viability (a), cellular antioxidant assay (b), insulin secretion (c), interferon-γ secretion (d) and ATP production (e) by the formulations (F1–F6). Data are mean± SEM, $n = 3$.

interferon-γ remain similar among all formulations and regardless of the presence of CDCA (Figure 3(d)). However, the positive effects of F2 microcapsules seem to be directly correlated with effects on cell bioenergetics since ATP production was elevated as a result of cell exposure to F2 microcapsules (Figure 3(e)). Accordingly, F2 seems to be the only formulation with consistent positive effects maintaining cell viability, reducing oxidants levels and enhancing insulin secretion and energy production.

Figure 3 also shows that on average, cell viability at the hyperglycaemic state was lower than the normoglycaemic state, suggesting glucotoxicity and induction of β-cell apoptosis as the result of high glucose concentrations. This is consistent with the literature. In one study, Zhang et al. investigated whether changes in glucose concentrations affect β-cell viability and function. The authors found that intermittent and constant hyperglycaemia induced apoptosis and reduced mass, with intermittent hypoglycaemia producing most detrimental effects. They authors suggested that these detrimental effects were mainly through inducing oxidant species and blocking cell cycle signalling processes at the molecular level [34]. The absence of significant effects of the microcapsules on inflammation profile suggests that either cell mass, functions and bioenergetics are not directly correlated with level of inflammation, or modulation of interferon-γ secretion requires longer exposure of cells to hyperglycaemia and high levels of free oxidants. The effects of F2 on cell biological activities may be due to PB uptake by the cell, and thus, Figure 4 shows the PB uptake by β-cells at normoglycaemic and hyperglycaemic states.

PB cellular uptake studies by NIT-1 cells

Drug uptake studies were performed to determine whether the anti-oxidant and cell-protective properties of PB were as a direct result of enhanced penetration through the phospholipid bilayer resulting in greater intracellular PB levels. Importantly, it is critical to determine whether intracellular PB concentrations vary under euglyceamic and hyperglycaemic conditions (Figure 4).

PB uptake was similar among all groups, and CDCA incorporation did not cause any significant alterations of PB cell permeation at both normoglycaemic and hyperglycaemic states. This suggests that pancreatic β-cells absorb PB intracellularly, and the level of absorption remains constant and independent of formulation excipients or presence of CDCA. This also suggests that stimulation of β-cell biological activities, and reduction of oxidant levels were not a result of more PB uptake by β-cells, but possibly due to the presence of CDCA although it did not enhance PB permeation into the cells. The potential biological effects of CDCA on insulin secretion and bioenergetics are in line with a study that showed protective and positive effects of CDCA when encapsulated with pancreatic β-cell [35]. However, not all formulations with CDCA showed biological improvements, suggesting that such effects are formulation-dependent.

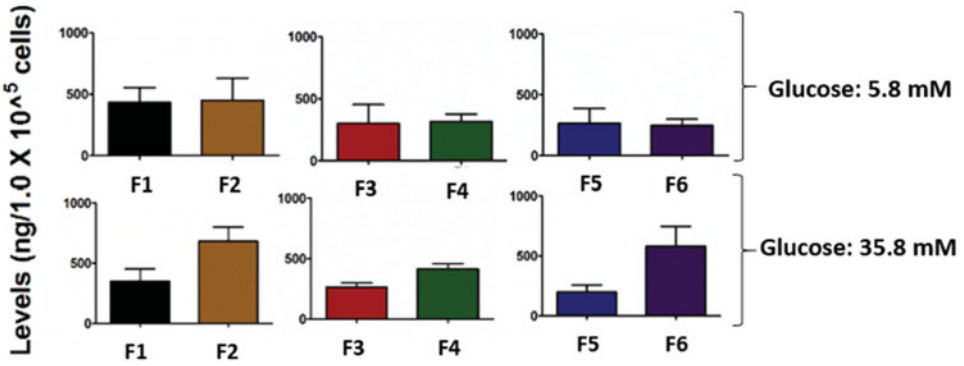

Figure 4. PB uptake by pancreatic NIT-1 cells at glucose levels of 5.8 and 35.8 mM. Data are mean±SEM, $n = 3$.

Future studies need to investigate intracellular mechanisms where PB and/or CDCA can influence β-cell viability, function and long-term performance.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

H. Al-Salami has been and is currently receiving funding from Beijing Nat-Med Biotechnology Co., Ltd. The authors would like to acknowledge the Australian Postgraduate Award (APA) and Curtin Research Scholarship (CRS). The authors also acknowledge the Curtin-seeding grant for the support and also acknowledge the use of laboratory equipment, scientific and technical assistance of Microscopy and Microanalysis Facility at Curtin University which has been partially funded by the University, State and Commonwealth Governments. The work is partially supported by the European Union Horizon 2020 MEDLEM research project and innovation program under the Marie Skłodowska-Curie Grant Agreement No 690876H.The NIT-1 cells were a generous donation from Professor Grant Morahan at the University of Western Australia.

References

[1] Negrulj R, Mooranian A, Chen-Tan N, et al. Swelling, mechanical strength, and release properties of probucol microcapsules with and without a bile acid, and their potential oral delivery in diabetes. Artif Cells Nanomed Biotechnol. 2015;44:1–7.

[2] Mooranian A, Negrulj R, Arfuso F, et al. Multicompartmental, multilayered probucol microcapsules for diabetes mellitus: formulation characterization and effects on production of insulin and inflammation in a pancreatic beta-cell line. Artif Cells Nanomed Biotechnol. 2016;44:1642–1653.

[3] Schrauwen P, Hesselink MK. Oxidative capacity, lipotoxicity, and mitochondrial damage in type 2 diabetes. Diabetes. 2004;53:1412–1417.

[4] Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res. 2010;107:1058–1070.

[5] Gorogawa S, Kajimoto Y, Umayahara Y, et al. Probucol preserves pancreatic beta-cell function through reduction of oxidative stress in type 2 diabetes. Diabetes Res Clin Pract. 2002;57:1–10.

[6] Mooranian A, Negrulj R, Mikov M, et al. Novel chenodeoxycholic acid-sodium alginate matrix in the microencapsulation of the potential antidiabetic drug, probucol. An in vitro study. J Microencapsul. 2015;32:589–597.

[7] Mooranian A, Negrulj R, Arfuso F, et al. The effect of a tertiary bile acid, taurocholic acid, on the morphology and physical characteristics of microencapsulated probucol: potential applications in diabetes: a characterization study. Drug Deliv and Transl Res. 2015;5:511–522.

[8] Mooranian A, Negrulj R, Al-Sallami HS, et al. Probucol release from novel multicompartmental microcapsules for the oral targeted delivery in type 2 diabetes. AAPS PharmSciTech. 2015;16:45–52.

[9] Mooranian A, Negrulj R, Chen-Tan N, et al. An optimized probucol microencapsulated formulation integrating a secondary bile acid (deoxycholic acid) as a permeation enhancer. Drug Des Devel Ther. 2014;8:1673–1683.

[10] Mooranian A, Negrulj R, Chen-Tan N, et al. Microencapsulation as a novel delivery method for the potential antidiabetic drug, Probucol. Drug Des Devel Ther. 2014;8:1221–1230.

[11] Barakat NS, Shazly GA, Almedany AH. Influence of polymer blends on the characterization of gliclazide-encapsulated into poly (epsilon-caprolactone) microparticles. Drug Dev Ind Pharm. 2013;39:352–362.

[12] Stojančević M, Pavlović N, Goločorbin-Kon S, et al. Application of bile acids in drug formulation and delivery. Front Life Sci. 2013;7:112–122.

[13] Di J, Yu JC, Wang Q, et al. Ultrasound-triggered noninvasive regulation of blood glucose levels using microgels integrated with insulin nanocapsules. Nano Res. 2017;10:1393–1402.

[14] Nguyen TX, Huang L, Gauthier M, et al. Recent advances in liposome surface modification for oral drug delivery. Nanomedicine (Lond). 2016;11:1169–1185.

[15] Gu Z, Aimetti AA, Wang Q, et al. Injectable nano-network for glucose-mediated insulin delivery. ACS Nano. 2013;7:4194–4201.

[16] Williams SJ, Wang Q, MacGregor RR, et al. Adhesion of pancreatic beta cells to biopolymer films. Biopolymers. 2009;91:676–685.

[17] Mooranian A, Negrulj R, Mathavan S, et al. An advanced microencapsulated system: a platform for optimized oral delivery of antidiabetic drug-bile acid formulations. Pharm Dev Technol. 2015;20:702–709.

[18] Mooranian A, Negrulj R, Al-Salami H. The effects of ionic gelation-vibrational jet flow technique in fabrication of microcapsules incorporating β-cell: applications in diabetes. CDR. 2017;13:91–96.

[19] Mooranian A, Negrulj R, Takechi R, et al. Influence of biotechnological processes, speed of formulation flow and cellular concurrent stream-integration on insulin production from β-cells as a result of co-encapsulation with a highly lipophilic bile acid. Cel Mol Bioeng. 2018;11(1):65–75.

[20] Mooranian A, Negrulj R, Chen-Tan N, et al. Novel artificial cell microencapsulation of a complex gliclazide-deoxycholic bile acid formulation: a characterization study. Drug Des Devel Ther 2014;8:1003–1012.

[21] Mooranian A, Negrulj R, Mathavan S, et al. An advanced microencapsulated system: a platform for optimized oral delivery of antidiabetic drug-bile acid formulations. Pharm Develop Technol. 2015;20(6):702–709. doi: 10.3109/10837450.2014.915570

[22] Mooranian A, Negrulj R, Al-Sallami HS, et al. Release and swelling studies of an innovative antidiabetic-bile acid microencapsulated formulation, as a novel targeted therapy for diabetes treatment. J Microencapsul. 2015;32:151–156.

[23] Mooranian A, Negrulj R, Mathavan S, et al. Stability and release kinetics of an advanced gliclazide-cholic acid formulation: the use

of artificial-cell microencapsulation in slow release targeted oral delivery of antidiabetics. J Pharm Innov. 2014;9:150–157.

[24] Wolfe KL, Liu RH. Cellular antioxidant activity (CAA) assay for assessing antioxidants, foods, and dietary supplements. J Agric Food Chem. 2007;55:8896–8907.

[25] Mooranian A, Negrulj R, Al-Salami H, et al. Designing anti-diabetic beta-cells microcapsules using polystyrenic sulfonate, polyallylamine, and a tertiary bile acid: Morphology, bioenergetics, and cytokine analysis. Biotechnol Progress. 2016;32:501–509.

[26] Mooranian A, Negrulj R, Al-Salami H. The influence of stabilized deconjugated ursodeoxycholic acid on polymer-hydrogel system of transplantable NIT-1 cells. Pharm Res. 2016;33:1182–1190.

[27] Mooranian A, Negrulj R, Al-Salami H. The incorporation of water-soluble gel matrix into bile acid-based microcapsules for the delivery of viable beta-cells of the pancreas, in diabetes treatment: biocompatibility and functionality studies. Drug Deliv Transl Res. 2016;6:17–23.

[28] Mooranian A, Negrulj R, Takechi R, et al. The biological effects of the hypolipidaemic drug probucol incorporated into bile acid-microcapsules and fed daily for 4-weeks, to an insulin-resistant mouse model: potential hypoglycaemic and anti-inflammatory effects. Drug Deliv Transl Res. 2018;8:543.

[29] Mooranian A, Tackechi R, Jamieson E, et al. Innovative microcapsules for pancreatic beta-cells harvested from mature double-transgenic mice: cell imaging, viability, induced glucose-stimulated insulin measurements and proinflammatory cytokines analysis. Pharm Res. 2017;34:1217–1223.

[30] Mooranian A, Negrulj R, Takechi R, et al. Electrokinetic potential-stabilization by bile acid-microencapsulating formulation of pancreatic beta-cells cultured in high ratio poly-L-ornithine-gel hydrogel colloidal dispersion: applications in cell-biomaterials, tissue engineering and biotechnological applications. Artif Cells Nanomed Biotechnol. 2018;46(6):1156–1162.

[31] Ma Q, Han Y, Chen C, et al. Oral absorption enhancement of probucol by PEGylated G5 PAMAM dendrimer modified nanoliposomes. Mol Pharm. 2015;12:665–674.

[32] Palin KJ, Wilson CG. The effect of different oils on the absorption of probucol in the rat. J Pharm Pharmacol. 1984;36:641–643.

[33] Tanaka Y, Inkyo M, Yumoto R, et al. Nanoparticulation of probucol, a poorly water-soluble drug, using a novel wet-milling process to improve in vitro dissolution and in vivo oral absorption. Drug Dev Ind Pharm. 2012;38:1015–1023.

[34] Zhang Z, Li J, Yang L, et al. The cytotoxic role of intermittent high glucose on apoptosis and cell viability in pancreatic beta cells. J Diabetes Res. 2014;2014:712781

[35] Mooranian A, Negrulj R, Al-Salami H. Primary bile acid chenodeoxycholic acid-based microcapsules to examine beta-cell survival and the inflammatory response. Bionanosci. 2016;6:103–109.