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Effect of surface chemistry of porous silicon microparticles on glucagon-like peptide-1 (GLP-1) loading, release and biological activity

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ABSTRACT

Recently, mesoporous silicon (PSi) microparticles have been shown to extend the duration of action of peptides, reducing the need for frequent injections. Glucagon-like peptide 1 (GLP-1) is a potential novel treatment for type 2 diabetes.

The aim of this study was to evaluate whether GLP-1 loading into PSi microparticles reduce blood glucose levels over an extended period. GLP-1 (p*I* 5.4) was loaded and released from the negatively charged thermally oxidized (TOPSi, p*I* 1.8) and thermally carbonized (TCPSi, p*I* 2.6) PSi microparticles and from the novel positively charged amine modified microparticles, designated as TOPSi-NH_2-D (p*I* 8.8) and TCPSi-NH_2-D (p*I* 8.8), respectively.

The adsorption of GLP-1 onto the PSi microparticles could be increased 3–4-fold by changing the PSi surface charge from negative to positive, indicating that the positive surface charge of PSi promoted an electrostatic interaction between the negatively charged peptide. All the GLP-1 loaded PSi microparticles lowered the blood glucose levels after a single s.c. injection but surprisingly, TOPSi-NH_2-D and TCPSi-NH_2-D were not able to prolong the effect when compared to TOPSi, TCPSi or GLP-1 solution. However, TOPSi-NH_2-D and TCPSi-NH_2-D microparticles were able to carry improved payloads of active GLP-1 encouraging continuing further attempts to achieve sustained release.

1. Introduction

Currently porous silicon (PSi) is being intensively studied for drug delivery purposes. Much effort has focused on oral delivery of small molecules since these microparticles may represent a way to improve dissolution of poorly soluble drugs due to amorphization of the drug molecules inside the PSi pores after their confinement (Xu et al., 2012a; Jarvis et al., 2012; Salonen et al., 2005). Recently, PSi has been claimed to improve macromolecule delivery (Jarvis et al., 2010; Kilpeläinen et al., 2009; Kovalainen et al., 2012) and they may have applications in multi-stage delivery systems. In this latter case, PSi microparticles can be loaded with different nanovectors such as drug- or siRNA loaded liposomes (Mann et al., 2011; Tanaka et al., 2010), iron oxide nanoparticles (Serda et al., 2010) or quantum dots (Tasciotti et al., 2008).

The surface of native PSi is reactive due to its hydride terminated surface and this has two disadvantages, a poor stability and undesirable chemical reactions with the loaded drug (Jarvis et al., 2012; Salonen et al., 2008). Therefore, one can resort to surface modification typically by oxidation, hydrosilylation or thermal carbonization in order to stabilize the PSi surface and furthermore, to optimize surface properties for drug delivery. The surface chemistry of PSi determines the extent of drug loading and release from PSi by controlling the interactions between the drug and the PSi (Jarvis et al., 2012). The loading of drugs is typically carried out by a simple immersion of the PSi into the drug solution (Salonen et al., 2008). Drug adsorption onto PSi may be governed by electrostatic or non-electrostatic interactions. For example, adsorption of methylene blue onto the surface of oxidized PSi was controlled by electrostatic interactions whereas the adsorption mechanism of

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ethyl violet and orange G was chemisorption. Notably, the protein adsorption onto unoxidized PSi surface was attributed to be due to hydrophobic interactions which resulted in structural changes and the loss of biological activity (Jarvis et al., 2010). Furthermore, the adsorption mechanism for thermally oxidized PSi was an electrostatic interaction for positively charged proteins, retaining the biological activity, and structural rearrangement for negatively charged proteins, which can lead to irreversible loss of the native protein structure (Jarvis et al., 2010). Recently zeta-potential measurements demonstrated that the negatively charged peptide is predominantly adsorbed onto the positively charges PSi nanoparticles and *vice versa* (Kaasalainen et al., 2012). Thus, both surface chemistry and pore size can affect the drug release from PSi because a loaded drug can be released by PSi dissolution and/or pore diffusion (Jarvis et al., 2012; Limnell et al., 2007). In addition, it is known that competitive adsorption plays a role in the release of peptides from PSi (Kovalainen et al., 2013).

Peptides are becoming more popular as drug compounds due to their target specificity and tolerability. However, successful delivery of peptides is challenging because of their short half-life and poor oral bioavailability. Over the years, several techniques have been developed in attempts to improve peptide delivery, but still today, the majority of clinical peptides are administered parenterally often with frequent injections (Antosova et al., 2009). We have previously demonstrated the ability of a single subcutaneous (s.c.) administration of PSi microparticles to prolong the releases of biologically active food intake regulating peptides, *i.e.* ghrelin antagonist, melanotan II and peptide YY3-36 (PYY3-36) (Kilpeläinen et al., 2009, 2011; Kovalainen et al., 2012). Glucagon like-peptide 1 (GLP-1) is a gut hormone which is involved in glucose homeostasis and appetite regulation (Sam et al., 2012). The elimination half-life of GLP-1 is very short after its systemic administration as a solution (1 min) (Cao et al., 2012) and thus, drug delivery systems which could prolong its duration of action are clearly desirable. Recently protease-operated depots of GLP-1 were shown to achieve reductions in blood glucose levels over an extended period after a single s.c. injection (Amiram et al., 2013). The aim of the present study was to evaluate if the GLP-1 delivery could be adapted by modifying the electrostatic interactions between the peptide and PSi carrier. Therefore, the negatively charged GLP-1 (isoelectric point, p*I* 5.4) was loaded and released from the negatively charged thermally oxidized (TOPSi) and thermally carbonized (TCPSi) porous silicon microparticles as well as from the novel positively charged amine modified microparticles, designated as TOPSi-NH_2-D and TCPSi-NH_2-D, corresponding to the amine modification on TOPSi and TCPSi, respectively. The biological activity of the released GLP-1 was confirmed *in vivo* by monitoring the blood glucose levels after a single s.c. injection of GLP-1 loaded microparticles.

2. Materials and methods

2.1. Materials

GLP-1 acetate (7–37) (Mw 3355.7 g/mol) was purchased from BCN Peptides (Barcelona, Spain).

The silicon wafers, used in the production of the PSi microparticles, were purchased from Cemat Silicon S.A. (Warsaw, Poland). Ethanol (99.5%) was bought from Altia (Helsinki, Finland). Hydrofluoric acid (HF) (38%) was purchased from Merck KGaA (Darmstadt, Germany). The nitrogen (99.999%) and the acetylene (99.6%) gases were from AGA (Espoo, Finland). 3-(2-Aminoethylamino)propyldimethoxymethylsilane was bought from Sigma–Aldrich (≥95.0%).

Saline solution (sodium chloride 9 mg/ml) for injections was obtained from B. Braun Melsungen AG (Melsungen, Germany). Phosphate buffered saline (PBS, pH 7.4) contained sodium chloride (JT Baker Deventer), potassium chloride (Merck KGaA, Darmstadt, Germany), disodium hydrogen phosphate (Merck KGaA, Darmstadt, Germany) and potassium dihydrogen phosphate (Merck KGaA, Darmstadt, Germany). Bovine serum albumin (BSA) was from Sigma–Aldrich (St. Louis, MO, USA).

High-performance liquid chromatography (HPLC) reagents were acetonitrile (HPLC grade, JT Baker, Deventer, The Netherlands) and trifluoroacetic acid (Sigma–Aldrich, St. Louis, MO, USA).

2.2. Microparticle preparation

PSi films were fabricated by anodizing p^+-type silicon wafers in an HF (38%)–ethanol mixture (HF:EtOH, 1:1). After drying at 65 °C for 1 h, the PSi films were ball-milled and sieved through a 400 mesh sieve to obtain the desired microparticles of sizes between 38 and 53 μm.

The PSi microparticles were stabilized with thermal oxidation (TOPSi) or carbonization (TCPSi) treatment as described earlier (Limnell et al., 2007). Surface modification of TOPSi and TCPSi was carried out by post-grafting the aminosilane of 3-(2-aminoethylamino) propyldimethoxymethylsilane in anhydrous toluene, as recently described in detail (Xu et al., 2012b). The amine modified samples were designated as TOPSi-NH_2-D and TCPSi-NH_2-D, corresponding to the amine modification on TOPSi and TCPSi, respectively.

The films of TOPSi-NH_2-D and TCPSi-NH_2-D were also prepared in order to measure the contact angle. The amine modification procedures of film samples were identical with those of microparticles.

2.3. Microparticle characterization

The samples were characterized with N_2 ad/desorption (Tristar II 3020, Micromeritics) to measure specific surface area, pore volume and pore diameter. The specific surface area was calculated using the multiple-point Brunauer–Emmett–Teller (BET) method (Brunauer et al., 1938). The pore size distribution was calculated from the desorption branch according to the Barrett–Joyner–Halenda (BJH) theory (Barrett et al., 1951). The amount of amino groups on the surface of PSi was verified by elemental analysis (CHNS analyzer, Vario MICRO cube). The properties of microparticles before the GLP-1 loading are summarized in Table 1.

To evaluate the hydrophilicity/hydrophobicity of PSi materials, contact angles were measured by placing a drop of deionized water onto the surface of the film samples. The images of the water drop on the film substrate were captured by a digital camera (Sony DSC-H20).

PSi microparticles with and without GLP-1 loading were studied with FT-IR spectroscopy (Thermo Scientific Nicolet 8700). The powders were mixed with KBr in a 1% (w/w) mixture, which were pressed into tablets with a pressure of 5 ton for 5 min.

2.4. Peptide loading

GLP-1 peptide was dissolved in deionized water (100 mg/ml) and the PSi microparticles (100 mg) were immersed in the peptide solution (1.0 ml) for 1.5 h at room temperature. The loading solution was treated with ultrasound 3 times during the loading to guarantee homogeneity. In order to confirm the effect of electrostatic interactions between TOPSi-NH_2-D (p*I* 8.8) and GLP-1 (p*I* 5.4) on the loading degree, the peptide loading was also carried out in phosphate buffer solution (PBS, pH 9.0), using the same protocol as described above. Finally, the microparticles were filtered from the solution and dried for 3 h at room temperature and then to remove residual water, for an additional 30 min in a vacuum at