Nanoscale analysis of insulin-biopolymeric nanoparticles using atomic force microscopy

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INTRODUCTION

The treatment of several metabolic diseases such as diabetes mellitus has evolved through knowledge gained in nanotechnology applied to medicine. Type I diabetes mellitus is a disorder of glucose metabolism generally attributed to the absence of insulin secretion (Reis, C.P. et al. 2007). Daily injections of insulin are thus indespensable for glucose homeostasis. The nanoencapsulation and orally dosed insulin have presented an approach to mimic the physiological fate of insulin, providing more adequate glucose homeostasis than subcutaneous injections (Carino, G.P. et al. 1999; Owens, D.R. et al. 2003). Orally dosed insulin requires the design of nanoparticles that transport and protect insulin from the deleterious environment of the gastrointestinal tract (GIT), and increase insulin residence time and permeability at the absorption site in the intestinal mucosa (Carino, G.P. et al. 1999; Woitiski, C.B. et al. 2008). The aim of the study was to characterize insulin-loaded nanoparticles in terms of particle size and morphology that governs the functional behaviour of nanoparticles *in vivo*.

Particle size has been controlled through experimental parameters of the preparation method, or by physical properties of polymers that constitute the nanoparticle (Champion, J.A. et al. 2007). Particles less than 100 nm are taken up by Peyer's patches and via enterocytes to a higher extent than particles greater than 300 nm (Carino, G.P. et al. 2000). Indeed morphology, along with size and surface chemistry, is a critical parameter for particle carrier function. The most basic function depends on the morphology, which comprises particle uptake and also insulin release from the nanoparticle. Generally, nanoparticles have increased insulin uptake and translocation through the GIT (Sarmento, B. et al. 2007; Reis, C.P. et al. 2008), and have lead to a fast insulin release due to larger surface area (Florence, A.T. 2005). Spherical nanoparticles are taken up from any site of attachment at the intestinal mucosa due to the particle symmetry, and the insulin release from nanoparticles has demonstrated the importance of the surface area (Dunne, M. et al. 2000; Champion, J.A. et al. 2007).

MATERIALS AND METHODS

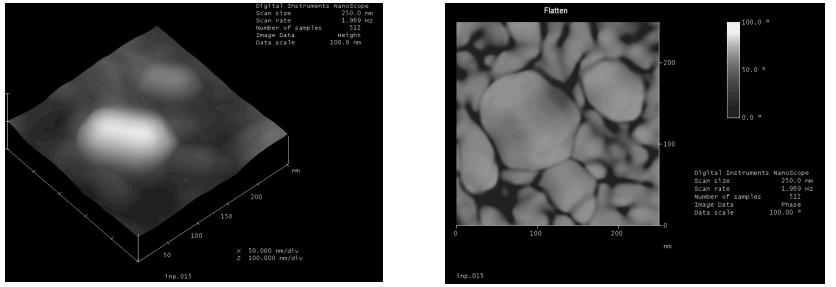
Size, morphology and surface topography of insulin-loaded nanoparticles were determined by atomic force microscopy (AFM) using Nanoscope IIIa controller and multimode AFM head (Digital Instruments Inc., USA) set to tapping mode. Particles were scanned at 20 ± 1°C and 5 ± 0.5% humidity with a noncontact single crystal silicon tip with AU reflective coating (NSG series, NT-MDT Co., Russia) with 44.01 N/mm spring constant and 315.62 Hz resonance frequency. The scanning speed was optimized between 0.5 and 2.0 Hz depending on the scan size with maximum range of 2.5 µm x 2.5 µm X 500 nm. All images were recorded and analyzed by both height and phase data types. For the size and morphology characterization, the insulin-loaded nanoparticle suspension was adsorbed onto freshly cleaved mica and then dried at controlled temperature and humidity.

RESULTS AND DISCUSSION

Nanoparticles described as multilayer biopolymer complex retaining insulin within the nanoparticle nucleus were prepared by ionotropic gelation followed by polyelectrolyte complexation as previously described (Woitiski, C.B. et al. 2009). Nanoparticles consisting of calcium cross-linked alginate, dextran sulfate and insulin coated with chitosan and poloxamer were analyzed by AFM for characterization of particle size, morphology and surface topography. Nanoparticles were coated with albumin as a sacrificial enzymatic degradation target for proteases in the stomach (Reis, C.P. et al. 2008).

Uncoated particles with 100 nm and particles with 320 nm coated with albumin with size determined by photon correlation spectroscopy (PCS) were examined by AFM imaging. The height data obtained from the amplitude scan of the tapping mode may provide a correlation with particle size (Ramirez-Aguilar, K.A. et al. 1998), depending upon the tip shape (Howald, L. et al. 1994). As seen in Figures 1 and 2, uncoated particles with weight less than 100 nm and albumin coated nanoparticles less than 200 nm were measued by AFM. The spherical nanoparticle shape is observed in figure 1-b and 2 with phase data type image, presenting rough surface topography expected by formation of multilayer nanoparticles. Particles coated with albumin presented a slightly rough surface than uncoated nanoparticles.

The accurate evaluation of size and morphology is dependent on the tip shape and other image factors such as piezoelectric scanner hysteresis and drift (Ramirez-Aguilar, K.A. et al. 1998). Therefore, the measurements of nanoparticles size and morphology are estimated. A representative tip shape is shown on the cross-section measurement of a spherical nanoparticle in Fig. 3. The tip used for AFM imaging was asymmetrical, tetrahedral-shape silicon with cylindrical apex, and front and back plane angles of 10°± 2° and 30°± 2°, respectively. Assuming that a 0° scan angle is used with trace capture, the 10° slope first comes across to the particle surface causing asymmetry in the AFM image (Ramirez-Aguilar, K.A. et al. 1998). The cross-sectional analyses of insulin-loaded nanoparticles are seen in Fig. 4. In addition, nanoparticles were dried at room temperature, which may result in different particle size than that nanoparticles in suspension determined by PCS.



(a) **(b)**

Fig. 1. AFM tapping mode images of uncoated insulin nanoparticles, adsorbed onto mica and analyzed by height (a, b, c,) and phase (d) data type. Scale: (a) 2.5 µm x 2.5 µm x 500 nm, (b) 1.0 µm x 1.0 µm x 250 nm, (c) 250 nm x 250 nm x 100 nm, (d) 250 nm x 250 nm.

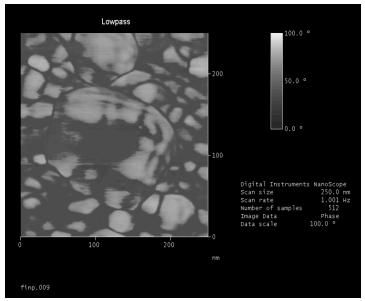


Fig. 2. AFM tapping mode images of insulin nanoparticle coated with albumin analyzed by phase data type. Scale: (a) 250 nm x 250 nm x 100 nm, (b) 250 nm x 250 nm.

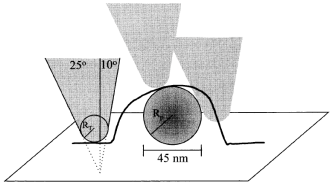
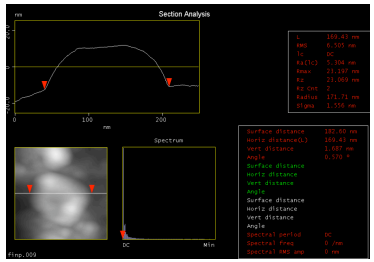
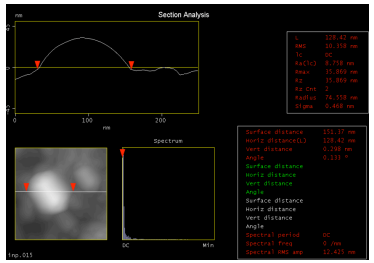


Fig. 3. Spherical nanoparticle and model tip for cross-sectional analysis. The tip apex is cylindrical with a radius of curvature *R*T, and front and back plane angles of 10° and 25°, respectively (Ramirez-Aguilar, K.A. et al. 1998).



(c)



(d)

Fig. 4. Cross-section analyses of insulin nanoparticles coated (a) and not coated with albumin (b). The tip apex is cylindrical with radius of curvature *R*T of 10 nm, and front and back plane angles of 10°± 2° and 30°± 2°, respectively

CONCLUSION

Nanoparticle size and morphology represent critical parameters for orally dosed insulin, determining insulin and nanoparticle uptake and translocation through the GIT and insulin release from nanoparticles. AFM images were used to estimate particle size and to examine the particle morphology, and the technique presented to be appropriate for the analysis of surface topography.

These results can display alternatives for drug delivery design, however additional particle characterization by alternative methods of analysis and imaging are required to determine the nanoparticle performance *in vivo*.

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