Microfabricated Porous Silicon Particles Enhance Paracellular Delivery of Insulin across Intestinal Caco-2 Cell Monolayers

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Purpose. Novel porous silicon microparticles were fabricated and loaded with fluorescein isothiocyanate (FITC)-insulin, a model hydrophilic pharmacologically active protein, along with varied doses of sodium laurate (C12), a well-known permeation enhancer.

Methods. Particle and liquid formulations were compared as a function of apical to basolateral flux of FITC-insulin across differentiated human intestinal Caco-2 cell monolayers grown on Transwell® inserts.

Results. The flux of FITC-insulin from silicon particles across cell monolayers was nearly 10-fold higher compared with liquid formulations with permeation enhancer and approximately 50-fold compared with liquid formulations without enhancer. By increasing C12 dose per particle with a concomitant decrease in total particles added per monolayer, the percent of FITC-insulin transport resulted in a linear increase up to 25% monolayer coverage.

Conclusions. Although maintaining monolayer integrity and transepithelial electrical resistance, maximum drug transport (20%/h) was achieved with 0.337 μg C12 dose per particle, and total particle loading at 25% monolayer coverage.

KEY WORDS: paracellular transport; tight junction; permeation enhancer; sodium laurate; sodium caprate; insulin; nanoparticles.

INTRODUCTION

Paracellular transport along the epithelial lumen of the small intestine is an attractive route of oral absorption for hydrophilic drug molecules. However, tight junctions between adjacent enterocytes form a highly impenetrable barrier. In an effort to increase intestinal absorption of water-soluble drugs, various agents, collectively referred to as permeation enhancers, have been found to reversibly open epithelial tight junctions. To date, several studies have focused on the enhancing effects of the sodium salts of medium chain fatty acids, such as sodium caprate (C10) and sodium laurate (C12). C10 significantly (up to 10-fold) increases paracellular transport of various hydrophilic compounds, such as mannitol, FD-4000 (fluorescein isothiocyanate [FITC]-labeled dextran), and inulin, (1–3). Furthermore, *in vitro* and *in vivo* studies suggest that C10 affects the phosphatidylinositol pathway that ultimately results in the contraction of actin filaments at the tight junctions (4–7). There is evidence that the physiologic effect of C10 is reversible and noncytotoxic (8–10). A major disadvantage of permeation enhancers is their lack of specificity, opening the possibility that food-borne pathogens and toxins migrate along with therapeutic compounds. To minimize this drawback, we have developed OralMEDDS (Oral Micro-Engineered Delivery Devices), novel porous silicon particles that can be used as oral drug-delivery vehicles. Once prepared, the particles can be loaded in a liquid drug formulation through simple capillary action. Interstitial air is removed by vacuum aspiration, and the formulation is dried completely using vacuum drying or freeze-drying. OralMEDDS particles are designed to target intestinal epithelial cells, adhere to the apical cell surface, and deliver a drug formulation containing a permeation enhancer that will open the local tight junctions of the paracellular transport pathway. The absorption of macromolecules and hydrophilic drugs, which are unable to undergo transcellular transport across lipid membranes, is largely restricted to this paracellular route. Therefore, the intestinal absorption of orally administered water-soluble drugs can be greatly enhanced through the utilization of OralMEDDs particles.

It was the aim of this study to characterize OralMEDDs with respect to formulation and drug loading and to select the most suitable permeation enhancer. Of these enhancers, sodium laurate (C12) and sodium caprate (C10) were initially analyzed and compared for enhancing paracellular absorption as a function of transport of a model hydrophilic drug, FITC-insulin. The theoretical advantage to using porous particles loaded with a combination of drug and permeation enhancer as opposed to administering both in a liquid formulation is based on the hypothesis that the particle will localize drug and enhancer along the apical side of the epithelial cells lining the intestinal lumen. As a possible result of this localization, drug and enhancer are predicted to dissolve out of the pores of the particle and become concentrated along the apical side of the epithelial cells, which includes the paracellular tight junctions.

To test drug transport from OralMEDDs across the intestinal lining, we used a Caco-2 cell culture system. When grown to confluence on a synthetic membrane support (Transwell®), these cells effectively mimic the small intestinal epithelium (11). Although not an exact representation of the gut, the cells differentiate completely with tight junctions and thus represent an impervious barrier to simple diffusion.

MATERIALS AND METHODS

Materials

Porous silicon particles were fabricated at Case Western Reserve University, Microfabrication Facility, using single side polished, p+ type, silicon wafers. Silicon of p+ type with a resistivity of 0.005–0.02 Ωcm^2 was chosen for all porous particle fabrications because it yields a pore distribution of 20–100 nm. Anodization and electropolishing were performed using an custom-built, double-sided anodization tank modified from Shih and colleagues (12). The particles were prepared and filled using liquid capillary action with sodium laurate, or sodium caprate, cornstarch, and FITC-labeled porcine insulin (Sigma Chemical Co., St. Louis, MO, USA). All tissue culture media, transport assay reagents, and additives were obtained from Gibco BRL® Life Technologies® (Rock-

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ville, MD, USA). Caco-2 cells originating from human colorectal carcinoma were obtained from the American Type Culture Collection (Rockville, MD, USA). Six- and 12-well, polycarbonate membrane Transwells® for all transport experiments were obtained from the Corning Costar® Corporation (Cambridge, MA, USA).

Fabrication of Porous Silicon Particles

Thin film deposition, photolithography, and selective etching were performed on p+-type silicon wafers to generate porous particles. Briefly, ~2500Å of silicon nitride was grown on the silicon wafers using low-pressure chemical vapor deposition. The wafers were then lithographically patterned, and the exposed areas of the photoresist were etched with SF_6 plasma. The remaining photoresist was removed with piranha (3:1 mixture of H_2SO_4 and H_2O_2 by volume). Electrochemical anodization of the silicon in HF/ethanol solution was performed using a double-sided, Teflon, anodization tank with platinum electrodes (Fig. 1). The anodization tank was filled with 500 mL of HF/ethanol solution (1:1 by volume) and current was applied upon connecting the electrodes to a power supply. Porosification exclusively took place along the anodic side of the silicon wafers. Anodization was performed with a current density of 2.19 mA/cm^2 and is described by the following series of reactions:

$$Si + 2HF + \lambda h^+ \rightarrow SiF_2 + 2H^+ + (2 - \lambda)e^- \quad (1)$$

$$SiF_2 + 2HF \rightarrow SiF_4 + H_2 \quad (2)$$

$$SiH_4 + 2HF \rightarrow H_2SiF_6 \quad (3)$$

where h^+ and e^- are the exchanged hole and electron, respectively, and λ represents the number of charges exchanged during the initial reaction (13). The wafers were next electropolished (dilute HF/ethanol; 9–12 mA/cm^2) and an adhesive tape, that is water soluble above 50°C, was used to remove the particles from the wafers. A majority of the adhesive was removed by exposing the particles to boiling water for 10 min, followed by centrifugation to remove water. To ensure complete removal of adhesive tape, this boiling/centrifugation step was repeated several times. Particles were then rinsed several times with methanol, followed by pentane, and then filtered and dried in an oven at 50°C.

Particle Loading and Liquid Formulations

Polymeric cornstarch was added as an excipient in all particle formulations to regulate enhancer and FITC-insulin dissolution. Experiments comparing particle formulations to liquid formulations included the same amount of starch excipient in the liquid treatments. Exactly 2.0 mg of porous silicon particles were weighed in 1.0 mL of high-walled glass autosampler vials. Homogenous liquid formulations containing FITC-insulin, sodium laurate, and 1:3 (w/w) ratio of corn starch to sodium laurate were prepared and slowly loaded onto the porous silicon particles using a micropipettor. This solution was taken into the particles through capillary action. C12-containing liquid formulations were kept at >37°C to prevent solidification before loading particles. To prevent air drying of loaded particles before vacuum drying, vials were immediately capped after loading. All particles were initially frozen at 0°C for 1 h before vacuum drying. The lyophilization flask was equilibrated in a dry ice/ethanol bath before using in vacuum drying. Uncapped vials were placed into a 50-mL conical tube and positioned in the lyophilization flask. The flask was connected to Precision Scientific DD-90 vacuum pump. Lyophilization was performed overnight at room temperature at <90 mTorr. Lyophilized/loaded particles were stored in a room temperature desiccator protected from light.

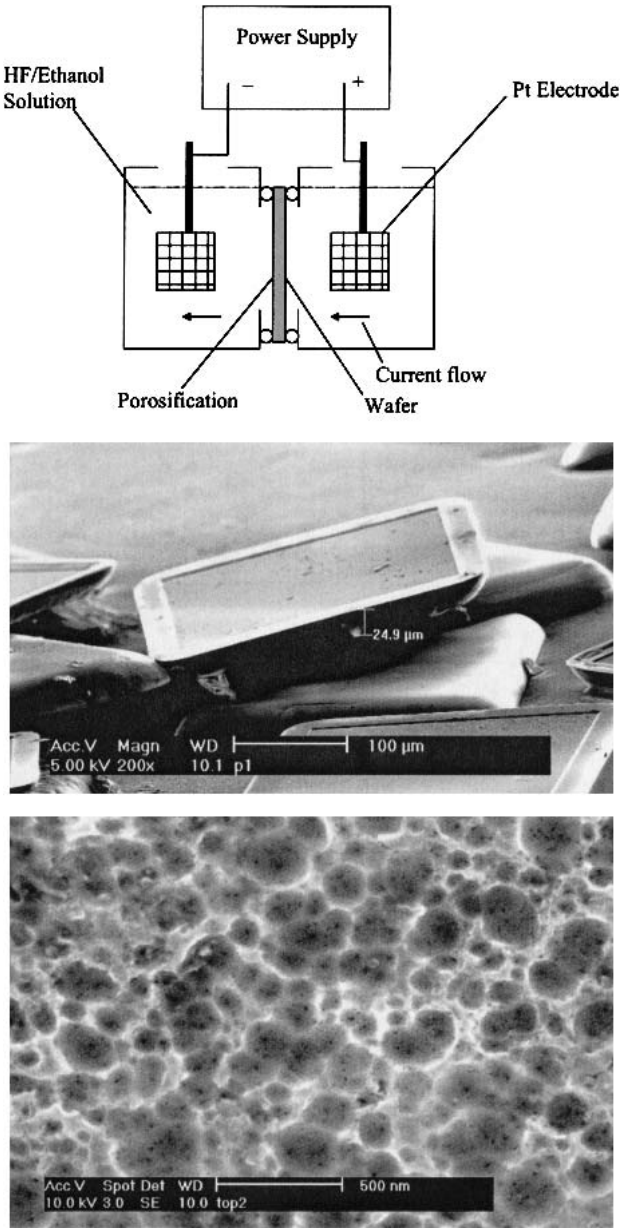


Fig. 1. Top, schematic diagram of the double-sided Teflon anodization tank. Middle, scanning electron microscopy image of a porous silicon particle demonstrating the thickness. Bottom, scanning electron microscopy image of a porous silicon particle demonstrating the pore size distribution of ~20–100 nm.

Cell Culture Conditions

Caco-2 cell cultures were grown and expanded in Dulbecco's modified eagles medium supplemented with 10% (v/v) fetal bovine serum, 25 mM *N*-hydroxyethylpiperazine-

N′-2-ethanesulfonate, 1% (v/v) nonessential amino acids, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine. Cultures were maintained in an incubator set at 37°C with a constant atmosphere of 95% relative humidity and 5% CO_2. Cells grown to 70–80% confluence were split using phosphate-buffered saline without Ca^{2+} or Mg^{2+} and containing 0.02% (w/v) ethylenediamine tetraacetic acid, which was used as a pretreatment to trypsinization. Cells were seeded onto Costar® polycarbonate membrane Transwell® inserts (0.4-μm pore size and 1.0 or 4.7 cm^2 surface area) at a cell density of 6.6×10^4 cells/cm^2. Before seeding cells onto these inserts, the polycarbonate membranes were coated with a filter-sterilized 33% (v/v) solution of rat-tail collagen (Becton-Dickinson Laboratories, Bedford, MA) in 60% ethanol. Coated inserts were left to dry in a cell culture hood before seeding. From the day of initial seeding up to the day of each transport experiment, cultures were maintained in supplemented Dulbecco's modified eagles medium that was applied above and below growing cell monolayers. Seeded cells were fed fresh media every other day for the first 7 days, and then every day there after for the next 2 weeks. Caco-2 cell monolayers grown between 21–30 days were used in all transport experiments. Cell monolayers exhibiting transepithelial electrical resistance (TEER) values of at least 220 Ωcm^2 were used in transport studies and included cell passages ranging from 33 to 45.

Transport Studies

The protocol implemented in transport assays was modified from that detailed by Lindmark and colleagues (5). Experiments were performed using two transport media. Apical bathing solution (APBS) was applied to the apical side of monolayers and served as the donor solvent. Basolateral bathing solution (BLBS) was applied to the basolateral side of monolayers and served as the acceptor medium. APBS consists of 1× HBSS without Ca^{2+} and Mg^{2+}, 25 mM D-glucose, and 10 mM *N*-hydroxyethylpiperazine-*N′*-2-ethanesulfonate, pH 7.4. BLBS is the same as APBS except that the cations, Ca^{2+} and Mg^{2+}, are included in Hank's balanced salt solution to aid in maintaining cell monolayer integrity. Cations were omitted from APBS to prevent precipitation of both medium chain fatty acids, sodium caprate (C10) and sodium laurate (C12). Before initiating transport experiments with permeation enhancers, cell monolayers were washed three times with prewarmed 1× Dulbecco's phosphate-buffered saline, pH 7.4, and subsequently equilibrated in the above-mentioned bathing solutions at 37°C with 95% relative humidity for 30 min. Caco-2 cell monolayer integrity was determined through apical to basolateral transport of [^{14}C]-mannitol, and through TEER measurements, as were recorded using an EVOM epithelial volt-ohmmeter (World Precision Instruments, Inc., Sarasota, FL, USA). TEER values were compared to readings for naked (without cells) polycarbonate membrane inserts submerged in the same bathing solutions as experimentals. Resistance measurements were also recorded at 15, 30, 45, 60, 90, 120, 150, and 180 min post-drug/particle treatment to determine the complete drug dissolution time from porous silicon particles. For all transport experiments, 10-μL donor (apical) samples were taken at 0 and 180 min post-drug administration. A total of 200 μL acceptor (basolateral) samples were taken at 0, 15, 30, 45, 60, 90, 120, 150, and 180 min post-drug administration. For each acceptor sample taken, 200 μL of fresh BLBS was added back to the basolateral side of monolayers to maintain sink conditions.

All liquid formulations were prepared the day of the experiment in the donor solution (APBS) and were added directly to cell monolayers grown on Transwell® inserts. Where liquid drug formulations were replaced with porous silicon particles, the particles were added to Caco-2 monolayers by sprinkling them from siliconized-low retention microcentrifuge tubes through a 3-mm diameter filter paper cone positioned directly over the monolayer to prevent inadvertently transferring particles to the basolateral side of the Transwell® insert. Those particles that did not immediately sink were forced down to the monolayer by gently pipetting 0.2 mL of APBS onto the particles in as drop-wise fashion to break surface tension. There was no stirring of the apical-side solution during the test to ensure that the particles remained in contact with the monolayer. The cells were maintained at 37°C with 95% relative humidity, between sampling times. A maximum number of 24 wells could be run at one time, affording eight test conditions with at least three replicates each.

Quantification of FITC-Insulin Transport across Caco-2 Cell Monolayers

Apical and basolateral side samples were transferred to a 96-well titer plate containing a series of pre-made FITC-insulin standards. FITC-insulin was detected using a Packard Fluorocount® Microplate Fluorometer (Packard Instrument Company, Meriden, CT, USA) with an excitation filter of 485 nm and an emission filter of 530 nm. Raw data were corrected for background as well as basolateral side dilution due to maintaining sink conditions.

FITC-Insulin Stability and Gel Electrophoresis

A fresh working solution of 24.5 μM FITC-insulin was prepared in BLBS (prewarmed in a 37°C water bath) the first day of the experiment. Confluent and differentiated Caco-2 cell monolayers, maintained in 25-cm^2 culture-treated Falcon flasks, were initially washed twice with warm Dulbecco's phosphate-buffered saline (37°C), pH 7.4. All monolayers were pre-equilibrated in warm BLBS in an incubator set at 95% relative humidity and 37°C, for 30 min. The bathing solution was aspirated off and replaced with 5 mL homogenous stock solution of 24.5 μM FITC-insulin in BLBS. At time points 0, 2, 4, 12, and 24 h post-peptide exposure, 200-μL samples were obtained from each of three replicate cultures. In between sampling, cells were maintained in the dark at 37°C and 95% humidity. Samples were concentrated using a refrigerated SpeedVac lyophilization system (Savant Instruments, Inc., Holbrook, NY, USA). Samples were then quantitated using the BCA protein assay (Sigma Chemical Co., St. Louis, MO). A 10–20% gradient tricine sodium dodecylsulphate (SDS)-polyacrylamide gel (Bio-Rad, Hercules, CA, USA) was loaded with 20 μg of each sample along with 20 μg of FITC-insulin that was not exposed to Caco-2 cells, and served as a negative control. A low range molecular weight protein ladder from GibcoBRL Life Technologies was also loaded. The gel was run for 45 min at 130 V and then stained

according to traditional procedures with BLUPRINT™ Fast-PAGE stain (GIBCO-BRL, Grand Island, NY, USA).

Statistics

All data are expressed as means ± SD of at least three separate experiments. For liquid and particle formulation comparisons, statistical analysis was performed by Analysis of Variance using the program SYSTAT (v 6.2.1, Systat Software, Inc. Richmond, CA, USA).

RESULTS

Particle Fabrication

OralMEDDs particles were fabricated in a custom-built anodization tank (Fig. 1 Top). After particle release from wafer support, scanning electron microscopy was performed to characterize the particle dimensions. Three separate experimental batches were generated and their dimensions ranged from 150 × 150 × 25 μm to 240 × 240 × 25 μm (Fig. 1 Middle, Table I) with a pore distribution of 20–100 nm (Fig. 1 Bottom). The particle dimensions reflect an average surface area of approximately 23–57 μm^2 (Table I). The apical surface area of a Caco-2 cell is roughly 15 μm^2; thus, it can be envisioned that a single particle would span approximately 10 to 20 cells in each direction, potentially covering 100–400 cells per particle (Fig. 2). The variations in particle size dimensions is ultimately determined by the accuracy of the photolithographic process, and variations from batch to batch were taken into account when calculating the number of particles required for total cell monolayer coverage (Table I).

Protein Stability

To determine whether endogenous proteases secreted by differentiated Caco-2 cells caused FITC-insulin degradation during the course of a transport experiment, an additional study was carried out under conditions identical to those during a transport experiment (37°C, identical bathing solutions). Incubation of FITC-insulin with Caco-2 cell monolayers reveals that minimal degradation occurs over the typical 3-h time period of our *in vitro* experiments (Fig. 3), approximately 5–10%. Longer incubation up to 24 h reveals significant peptide degradation. This suggests a favorable window exists for insulin delivery within 3–6 h after administration to minimize degradation and, hence, optimize potential delivery.

Effect of Permeation Enhancers on FITC-Insulin Transport

Confluent Caco-2 cell monolayers were treated with isotropic liquid formulations of FITC-insulin in the presence or absence of either C10 or C12. These studies were performed to select the most suitable candidate enhancer and optimize enhancer concentrations for maximal paracellular drug transport. The effect of either C10 (5 mM and 10 mM) or C12 (0.375 mM and 0.75 mM) on paracellular drug transport enhancement was determined as a function of FITC-insulin translocation from the apical (donor) side to the basolateral (acceptor) side. Compared to control (i.e., without permeation enhancer), the inclusion of 0.75 mM C12 increases the flux of FITC-insulin approximately 8-fold (Fig. 4B). At half this concentration a 2-fold increase in FITC-insulin transport was observed. In contrast, C10 did not significantly enhance drug absorption at either concentration (Fig. 4A). To validate that C12 permeation enhancement was not the effect of monolayer disruption, 1.89 mM (0.2 μCi/mL) [^{14}C]-mannitol was included in the donor solutions. [^{14}C]-mannitol, a model hydrophilic compound that traverses the intestinal epithelium via paracellular diffusion, serves as a monolayer integrity marker. At 0.75 mM C12, the transepithelial [^{14}C]-mannitol flux amounted 1.50 ± 0.13%/h·cm^2 (Fig 4C). This value is about an 8-fold increase over control, revealing a similar permeation enhancement to that observed for FITC-insulin. The same trend was observed with all other enhancer formulations. The permeation enhancement effect at 0.75 mM C12 are in good agreement with those observed by Lindmark and co-workers (5). Based on these results, C12 was further used in the porous silicon formulations. To assess the effect of particles on monolayer integrity, mannitol transport was determined in presence of particles containing only starch and FITC-Insulin (without enhancer). Overall FITC-insulin transport and mannitol permeation was not significantly different from liquid formulations (data not shown).

Effect of Sodium Laurate as a Function of Delivery Vehicle

To choose the optimal dose of C12 per particle, the amount of C12 per OralMEDD was varied with a constant FITC-insulin concentration. Control experiments were carried out with liquid formulations containing identical doses of FITC-insulin and permeation enhancer (Fig. 5). The FITC-

Table I. Experimental Particle Batch Dimensions: Relationship to Cell Monolayer Coverage

Particle dimensions (mm)	Surface area (mm^2)	Particle volume (mm^3)	Particles/monolayer[a]	Volume/monolayer (cm^3)[b]	g/monolayers (g)[c]
0.239 × 0.239 × 0.025	5.71E-02	1.43E-03	1978	2.83E-03	1.97E-03
0.187 × 0.187 × 0.025	3.50E-02	8.74E-04	3231	2.83E-03	1.97E-03
0.153 × 0.153 × 0.025	2.34E-02	5.85E-04	4827	2.83E-03	1.97E-03

[a] Total number of particles required for 100% monolayer coverage as calculated by Transwell™ insert area (113 mm^2) divided by particle surface area.

[b] Total volume occupied by the number of particles making up 100% monolayer coverage. Calculations involved (particle volume × no./monolayers)/1000. By further multiplying this value by the porosity of the particle (70% for all particles), the loading capacity of each particle is determined (1.98 μL).

[c] Mass in grams of particles required for 100% monolayer coverage, as determined by the product of the volume/monolayers and the density of pure nonanodized silicon ($2.83 \cdot 10^{-03}$ cc × 2.33 g/cc = 6.59 mg). To correct for silicon postanodization, particle porosity was taken into account (constant 70% porosity for all particles). Thus, the final amount of particles required for 100% monolayer coverage is ~2.0 mg.

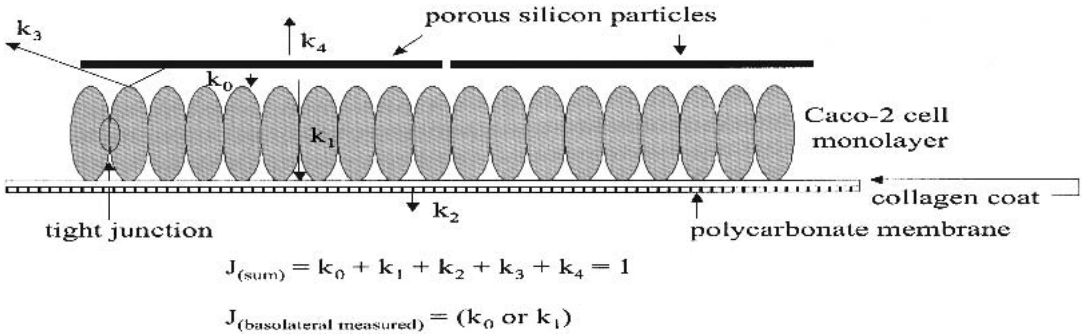


Fig. 2. Microenvironment of a porous silicon particle at the surface of confluent and differentiated Caco-2 cell monolayer grown on a Costar Transwell® membrane insert, including modeled kinetic constants (not to scale).

insulin flux is greatly enhanced (up to 10-fold at 2.25 mM C12) when delivered to the Caco-2 cell monolayer in porous silicon particles as compared to the same dose delivered in liquid form.

TEER measurements and [^{14}C]-mannitol flux in liquid formulations followed the same trend in permeation enhancement as compared to FITC-insulin; in contrast, TEER values and mannitol flux for particle formulations followed the trend for liquid formulations, suggesting that the cell monolayer was not compromised and that overall FITC-insulin permeation enhancement could be attributed solely to the OralMEDDs system. It is interesting to note that permeation enhancement of up to 10%/h·cm^2 is comparable to the transepithelial flux observed with compounds with very high membrane permeability, e.g., actively transported compounds such as taurocholic acid (14).

Effect of Varying Enhancer Dose per Particle and Particle Concentration per Monolayer

These studies were performed with the total amount of FITC-insulin presented to the cells constant. As the dose per particle increases (and the particle concentration, i.e., the total number of particles, decreases), the percent of FITC-insulin that crosses the Caco-2 cell monolayer increases in a linear fashion until a quarter of the available cell monolayer is covered (Fig. 6). When a large dose is loaded into a particle concentration that is the equivalent to an eighth of monolayer, the amount of FITC-insulin transported per hour decreases. Thus, the greatest drug transport (20%) occurs at a laurate concentration of 0.337 μg per particle using a particle concentration that will cover a fourth of the surface area of the Caco-2 monolayer (Fig. 6).

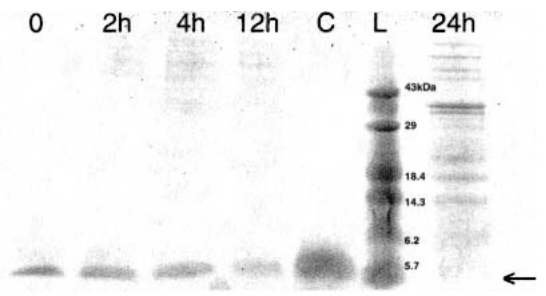


Fig 3. SDS-polyacrylamide gel electrophoresis analysis of FITC-insulin stability upon incubation with differentiated Caco-2 cell monolayers. Samples were analyzed at $t = 0$, 2, 4, 12 and 24 h post-incubation. The arrow indicates insulin α- and β-chains, 5.7 kDa (C = control insulin; L = molecular weight ladder).

Effects of Increasing the Number of Particles with a Fixed C12 Dose/Particle

The rationale for this experiment is to determine the relationship between effective permeation enhancement and Caco-2 cell surface coverage with particles. For all data represented in Fig. 7, doses for C12 and FITC-insulin were kept constant (0.052 μg and 0.003 μg, respectively) in all porous silicon particle formulations. Liquid formulations represent equivalent C12 and FITC-insulin doses that were directly administered to Caco-2 cell monolayers. At doses below 25% particle monolayer concentrations, the transport rate decreases to below detectable limits.

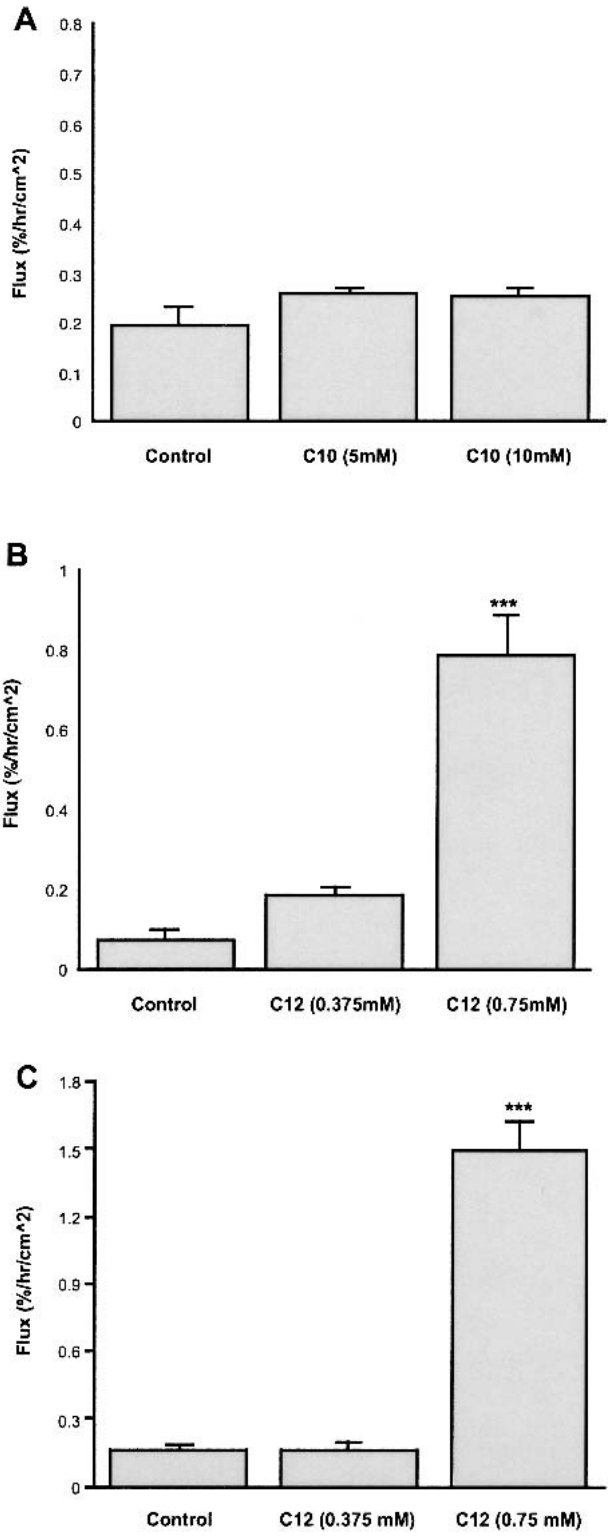
DISCUSSION

This study was conducted to determine whether formulated porous silicon particles could efficiently deliver a model peptide drug dose through a representative intestinal epithelial cellular system in a manner that surpasses traditional liquid drug formulations. Not only would this drug delivery system enhance bioavailability through an oral route by administering high local concentrations of drug locally at the site of absorption but also be used as a protective matrix for peptide drugs that can be easily degraded by intestinal enzymes. The results of this study strongly suggest that peptide drug delivery via porous silicon particle is much more efficient than liquid doses.

In the current study we selected sodium laurate as a suitable permeation enhancer to enhance the epithelial permeability of a model hydrophilic macromolecule, insulin (Fig. 4b). We observed a 8-fold increase in FITC-insulin permeability in the presence of this permeation enhancer, which is in good agreement with the data by Lindmark and co-workers (5). After incorporation of sodium laurate into porous silicon particles, we show that FITC-insulin is transported at a rate nearly 10 times faster when compared with a dose in free solution (Fig. 5). This effect is mainly caused by the very high local concentration of both the permeation enhancer (sodium laurate) and FITC-insulin directly at tight junctions, thus dramatically augmenting drug flux across the cell monolayer. The combined data of mannitol flux and TEER measurements indicate that monolayer integrity is not compromised, strongly suggesting that permeation enhancement is facilitated by the silicon delivery devices.

It is important to note that the liquid formulations used in this experiment already represent a significant increase (2 to 10-fold) over aqueous insulin formulations without permeation enhancer (control); thus, the transepithelial FITC-insulin flux from OralMEDDs particles is approximately 25- to 100-fold enhanced over control.

This study contains two important variables: 1) the particle density per monolayer, that is, the number of particles delivered to a Caco-2 cell monolayer; 2) the ratio of drug and enhancer amount per particle. To optimize these variables, we further examined the influence of drug and enhancer dose as a function of particle dosing per monolayer (Fig. 6). The most efficient concentration of enhancer and particle coverage is 0.337 μg sodium laurate per particle and 25% cell monolayer coverage, respectively. From a physiologic perspective, this is a desirable situation in that the total surface area required to significantly enhance drug transport can be compensated for by increasing the permeation enhancer dos-



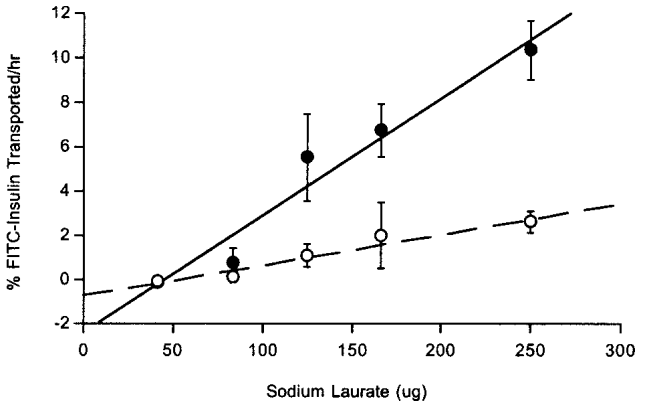


Fig. 5. Effect of C12 dose/particle (0.021–0.126 μg) on FITC-insulin transport. Particle formulations (closed circles) were compared to isotropic liquid formulations (open circles) with similar FITC-insulin and C12 concentrations. FITC-insulin concentration was maintained at 29 μg/mL (5.0 μM) in the liquid formulations. A total of 14.5 μg of FITC-insulin (2.5 nmol) represented total dose/monolayer after adding formulated particles. Upon complete dissolution of FITC-insulin from particles, an equivalent concentration is obtained (5.0 μM). Liquid formulations also contained varying concentrations of C12 (i.e., 0.375, 0.75, 1.125, 1.5, and 2.25 mM). Equivalent amounts of C12 were represented in particle formulations dosed/monolayer, which included doses 41.7, 83.3, 125, 166.6, and 250 μg. Upon complete dissolution from the particles into APBS donor solution, molar concentrations of C12 correspond to 0.375, 0.75, 1.125, 1.5, and 2.25 mM, respectively. The total C12 dose/monolayer range is 41–250 μg. A constant dose of FITC-insulin per particle, or in solution, is maintained for all experiments (1.2 ng). The total number of particles used per monolayer was kept constant (i.e., % monolayer coverage = 100).

Fig. 4. Effects of sodium caprate (C10) (A) and sodium laurate (C12) (B) on the paracellular transport of FITC-insulin (A, B) and [^{14}C]-mannitol (C) across confluent Caco-2 monolayers. FITC-insulin stock solution (1 mg/mL; 160 μM) was dissolved in 1× Dulbecco's phosphate-buffered saline, pH 7.4. On the day of the experiment, donor (apical) solutions were prepared by diluting an aliquot of this stock solution into APBS to make a final concentration of 0.5 μM (3.13 μg/mL) FITC-insulin. Stock solutions of C10 and C12 were prepared in ddH2O (97.1 mg/mL and 11.12 mg/mL, respectively). Aliquots were diluted in APBS donor solutions with 0.5 μM FITC-insulin. C10 formulations contained 0.97 mg/mL (5 mM) or 1.94 mg/mL (10 mM), and C12 formulations contained 0.083 mg/mL (0.375 mM) or 0.167 mg/mL (0.75 mM). A volume of 1 mL or 0.5 mL (for 6-well and 12-well Transwell® inserts, respectively) of these donor solutions were added directly to differentiated Caco-2 cell monolayers. Values are the mean ± SD of n = 3 for both C10 and C12 treatments, and n = 6 for control treatments. ***$p < 0.001$.

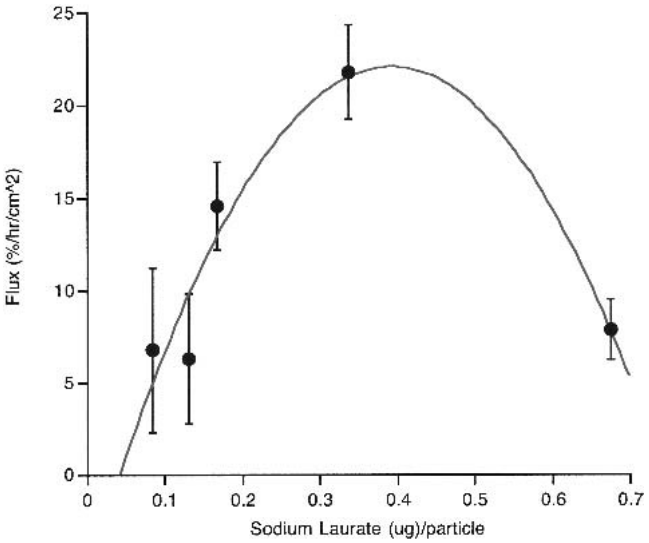


Fig. 6. Varying C12 dose/particle (0.084–0.674 μg) as a function of adjusted monolayer coverage. Total FITC-insulin dose was kept at 14.5 μg (upon complete dissolution from particles, equals 29 μg/mL or 5.0 μM). C12 dose/particle varied from 0.084–0.674 μg. The amount of particles loaded into the APBS donor solution were calculated based on mass to determine the % monolayer coverage (i.e., 2.5 mg = 100%, 1.25 mg = 50%, 0.625 mg = 25%, 0.3125 mg = 12.5%, 0.15625 mg = 6.25%). Total C12 dose/monolayer was maintained at 166.6 μg (upon complete dissolution from the particles equals 1.5 mM).

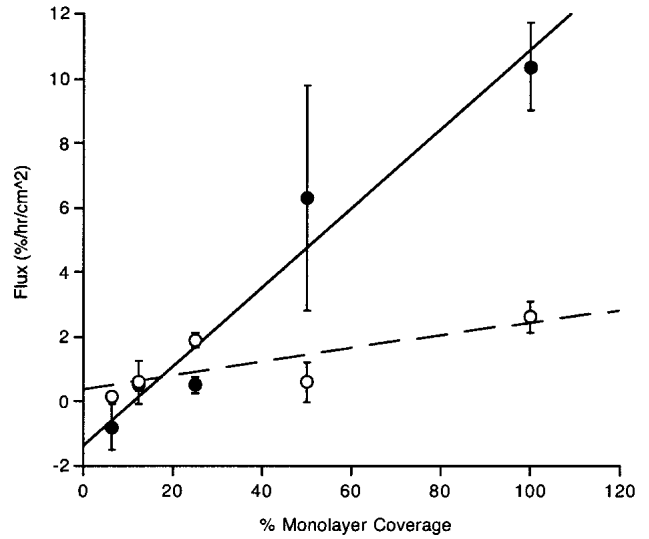


Fig. 7. The effect of varying the amount of porous silicon particles added per monolayer as a function of FITC-insulin transport (closed circles) as compared to liquid formulations (open circles). In addition to what is detailed in the manuscript for this figure, total C12 dose/monolayer in particle formulations was 166.6 μg (upon complete dissolution from porous particles equals 1.5 mM). Total FITC-insulin dose/monolayer in particle formulation was maintained at 14.5 μg, which upon complete dissolution from the particles is equivalent to 5.0 μM. Equivalent concentrations for C12 (1.5 mM or 333.2 μg/mL) and FITC-insulin (5.0 μM or 29 μg/mL) was represented in all liquid formulations. Data points represent 6.25-100% monolayer coverage. C12 dose/particle was kept constant at 0.052 μg, and FITC-insulin dose/particle was maintained at 0.003 μg. Total C12 dose was fixed at 166 μg.

age per particle. The current study uses insulin as a model peptide drug for measuring transport using porous silicon particles; however, it will be necessary to optimize drug/enhancer ratios for individual drugs that would lead to the most efficient transport enhancement. To translate the current study to the *in vivo* situation, it will be necessary to functionalize the surface of silicon particles for the attachment of groups that target specifically to the epithelial surface of the gastrointestinal tract. This can be achieved by attaching common mucoadhesive moieties, such as tomato and other lectins. Future studies are aimed at exploring the possibility of silicon surface modifications.

In summary, this study shows that drug permeation rates across Caco-2 cell monolayers can be enhanced significantly using porous silicon particles as delivery vehicles. Of particular note, the drug transport efficiency can be augmented at least 10-fold when drug formulations are delivered in porous silicon particles when compared to liquid formulations, and up to 100-fold when compared to formulations without permeation enhancers.

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