Supporting Information:

Materials and Methods

Device Fabrication

Silicon nanowires were grown conformally on controlled pore glass particles (CPG, 30-70 μm width) with 200 nm pore size (Sigma Aldrich) and 30-50 μm glass microspheres (Polysciences, Warrington, PA) using a standard vapor-liquid-solid deposition[1].

Nanowire Characterization

The surface areas of three batches were measured using a multipoint surface area analysis using Krypton gas (Micromeritics Analytical Services, Norcross, GA).

Scanning Electron Microscopy

Devices were dried onto glass slides or loaded directly onto conductive tape for SEM imaging. All images were taken with a NovelX MySEM tabletop scanning electron microscope.

Controlled Pore Glass Loading

The CPG particles were loaded by placing approximately 5-10 mg of devices in 500 μL of a loading solution and heating at 35°C for approximately 24 hours (until dry). Loaded particles were washed with PBS in a filter flask to remove residual protein crystals. Bovine immunoglobulin G (IgG, 10 mg/mL, Biomeda), trypan blue (0.4% w/v in normal saline, Mediatech), and bovine pancreatic insulin (10 mg/mL, Sigma) were used as loading solutions. Bovine serum albumin (Sigma) was mixed with phosphate-buffered saline (Fisher Scientific) to produce loading solutions at concentrations of 11.9 ± 3.6 mg/mL (s.d.).

Microsphere Loading

Two experiments were conducted to understand the loading capabilities of the three different batches of microspheres: control microspheres with no nanowires, microspheres with short nanowires, and microspheres with long nanowires attached. In the first experiment, microspheres were loaded with bovine serum albumin (BSA) at a concentration of 10 mg/ml in phosphate buffered saline (PBS). Elution was then observed over the course of six hours, with timepoints being taken at various intervals. In the second experiment, microspheres with long nanowires were loaded with concentrations of of 1 mg/ml, 10 mg/ml, 50 mg/ml and 100 mg/ml and elution was observed over the course of six hours. In both experiments, the eluted samples were measured for protein concentration using a BCA assay. All samples were made in triplicate.

The microspheres were loaded by placing approximately 70 mg of the microspheres in 500 μl of the BSA solution. The vial was then partially covered with aluminum foil and placed on a hot plate at 35˚C for 72 hours to dry. Excess BSA was removed by washing the microspheres with PBS and running them through a vacuum filter. The spheres were transferred to a new vial and weighed.

Elution and Quantification
The loaded CPG particles were suspended in 3 ml of PBS on a shaker plate for up to 10 days. However, due to instability of the drug molecules, only data points from 0 to 48 hr were considered. At each time, 150 µL of solution was removed to a 96 well plate for quantification, and 150 µL of PBS was added to replace it (with appropriate adjustments to the calculation of eluted protein concentration).

The loaded microspheres were suspended in 3 ml of PBS for six hours. At approximately 0 hr, 40 min, 1 hr, 2h, 4 hr and 6 hr, 150 µl of the solution was removed after the suspension had been stirred and the microspheres allowed to settle. The sample was placed in a 96 well plate and 150 µl of PBS was added to the suspension to replace the removed quantity.

For BSA, IgG, and insulin, the protein concentrations in the samples were analyzed using a micro and/or regular BCA assay and a spectrometer. The absorbance of the samples was read at a wavelength of 560 nm. Trypan blue was measured directly at an absorbance wavelength of 590 nm.

Adhesion
Parallel plate flow studies were conducted to quantify particle and microsphere adhesion to cells under shear. Devices suspended in 2% mucin (Porcine Gastric Mucin, Type II, Sigma) in water were added to cells immediately prior to assembly of the flow chamber (Glycotech, Gaithersburg, MD). The mucin flow rate was increased across the cells and devices to produce shears ranging from 0 to 170 dynes/cm^2 after [1]. Devices were imaged on a Nikon Eclipse TI-E motorized inverted microscope at 2x and stitched using NIS-Elements Advanced Research software. From these images, devices were counted at each shear using Adobe Photoshop CS4.

Surface Area and Geometric Calculations

Geometric modeling was used to estimate the number of nanowires per device and the increase in surface area due to nanowires. Assuming the microsphere beads are spherical, they have a surface area, SA_b, of

$$SA_b = 4\pi R_b^2$$

Where R_b = the radius of the bead (approximately 24 µm, based on measurements). Nanowires are approximated as cylinders with R_{nw} defined as the average nanowire radius (as measured by SEM and TEM), and l_{nw} defined as the average nanowire length. Thus, the surface area per nanowire, SA_{nw}, is

$$SA_{1NW} = 2\pi R_{NW}\ell$$

The number of nanowires per bead can be calculated based on an assumed percent of surface area covered by nanowires, $SA_{assumed}$ (variable by bead batch). This gives the number of nanowires, N_{nw}, as

$$N_{NW} = \frac{SA_{assumed} SA_b}{\pi R_{NW}^{\ 2}} = SA_{assumed} \frac{4R_b^{\ 2}}{R_{NW}^{\ 2}}$$

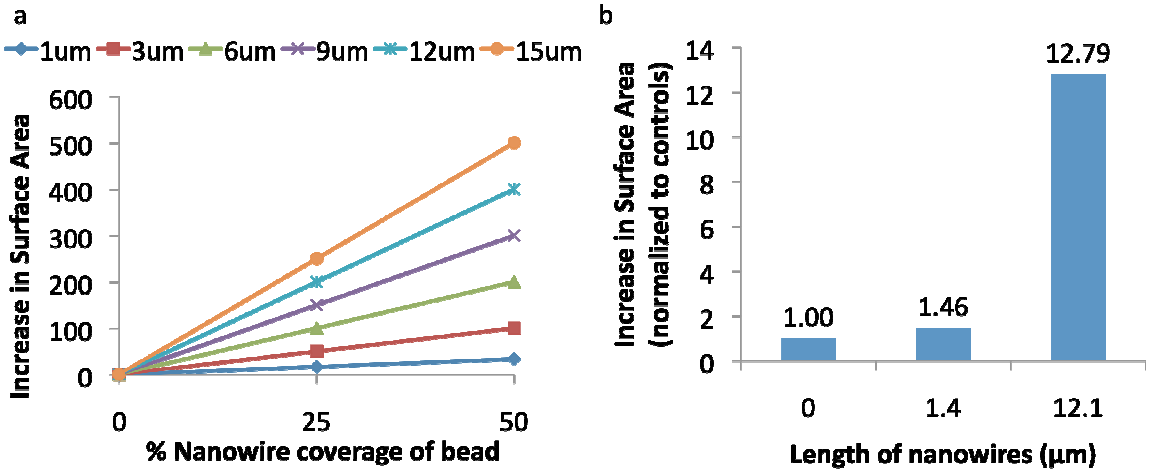
.

Thus, the increase in surface area per device due to the nanowires is

$$\frac{SA_{NW} + SA_b}{SA_b} = \frac{N_{NW} SA_{1NW} + SA_b}{SA_b} = \frac{2SA_{assumed}\ell}{R_{NW}} + 1$$

,

assuming that the surface area of the tips of the nanowires is the same as that taken up by their bases. Using data from SEM and TEM measurements, the increase in surface area can be calculated as a function of the assumed surface area covered by nanowires (% Nanowire coverage of bead) and the nanowire length, given a nanowire radius (assumed 30 nm, in Supporting Figure 1).



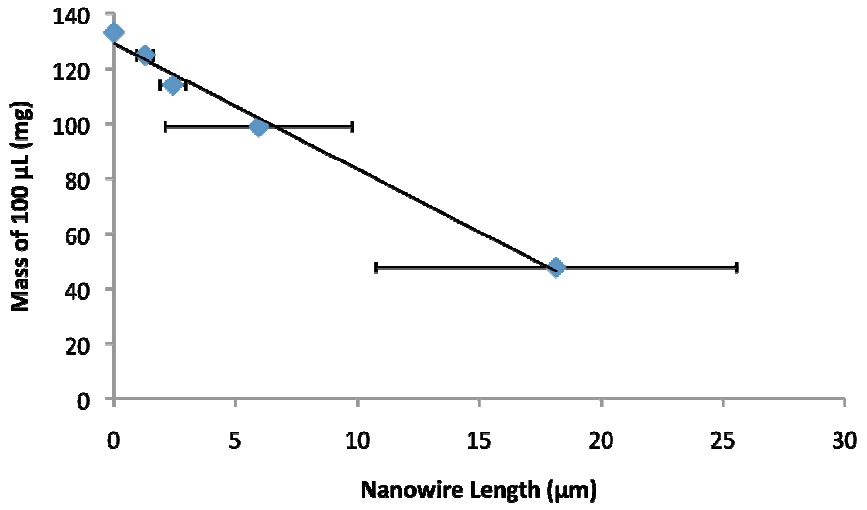
Supporting Figure 1. Surface area calculations and measurements. A) Calculations for various lengths of nanowires. B) Increase in surface area per device over un-coated devices based on nanowire length.

Surface Area and Packing Characterization:

To determine the increase in absorptive surface area from an uncoated control particle to a nanowire coated particle, the surface areas were measured using a multipoint surface area analysis using Krypton gas. Control beads with no nanowires had a measured surface area of 0.0654 m^2/g; long nanowires (approximately 12.1 µm length) had a measured surface area of 0.5919 m^2/g, whereas shorter nanowires (approximately 1.4 µm length) had a measured surface area of 0.0817 m^2/g. Assuming that nanowire lengths were constant, based on the

above model, nanowire coverage for the devices was about 0.6 % for the shorter nanowires and about 1.5 % for the longer nanowires (see Supporting Figure 1). Given 1% surface coverage, roughly 26,000 nanowires cover the surface of a device. For 5% and 10% coverage, the number of nanowires per device is 130,000 and 260,000. The assumption that nanowire lengths are constant is not necessarily accurate because measurements were taken from SEM images, which only show surface information. Thus, the path of longer nanowires may have been obscured and shorter nanowires may not have been visible at all. Nonetheless, the increase in surface area scales linearly with the length of the nanowires as predicted.

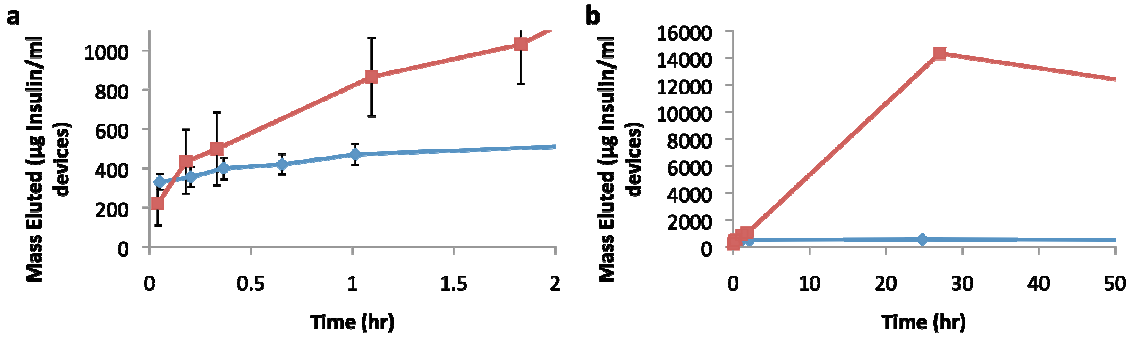
Additionally, because the nanowires change the packing characteristics of nanowire-coated beads as compared to uncoated beads, the mass per volume of devices (ie: the packing density of devices) was determined for several batches of different geometric proportions (Supporting Figure 2). Longer nanowires prevented the devices from packing as densely, thus reducing the mass of 100 µL of devices.



Nanowire Length	Mass of 100 µL of devices (g)
18.1 +/- 2.1 µm	47.7
5.9 +/- 1.2 µm	98.9
2.4 +/- 0.1 µm	114.0
1.3 +/- 0.1 µm	124.9
0 – no nanowires control	133.1

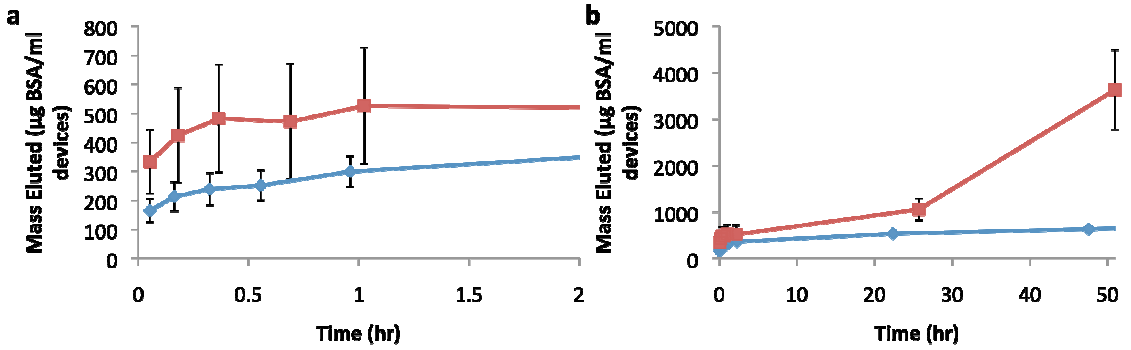
Supporting Figure 2. Variations in packing density by geometry.

Discussion of Elution Curves



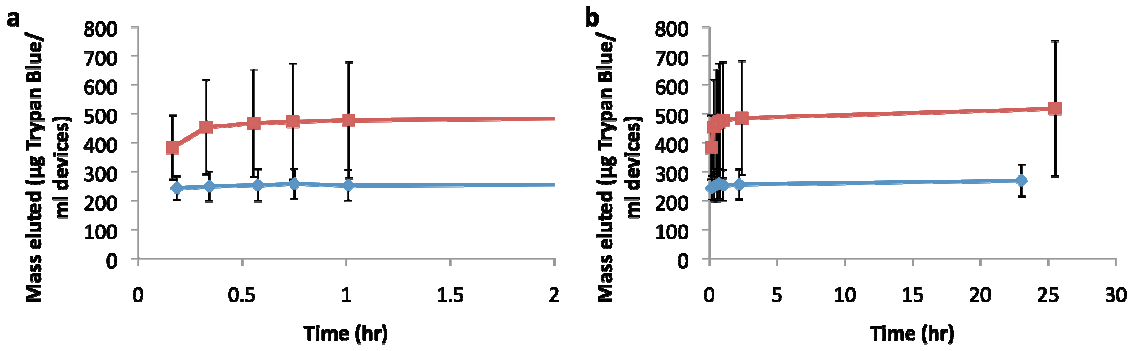
Supporting Figure 3. Elution of insulin from loaded particles over 2 hr (a) and 48 hr (b). Red square curve is Nanowire-coated CPG, blue diamond curve is uncoated CPG. Error bars are standard error of the mean.

As discussed in the body of this work, larger molecules, such as insulin, BSA, and IgG exhibit significantly increased loading and near-linear kinetics to 24 hours (Supporting Figures 3 and 4). This may be due to two separate reservoirs for drug loading: the nanowire base and the porous structure underneath. Overall, the nanowire coating increases the overall amount of drug delivered.



Supporting Figure 4. Elution of BSA from loaded particles over 2 hr (a) and 48 hr (b). Red square curve is Nanowire-coated CPG, blue diamond curve is uncoated CPG. Error bars are standard error of the mean.

In contrast, trypan blue exhibits a classic burst release (Supporting Figure 5), not particularly affected by nanowire coatings, although the amount of drug eluted is increased by having a nanowire reservoir.



Supporting Figure 5. Elution of Trypan Blue from loaded particles over 2 hr (a) and 24 hr (b). Red square curve is Nanowire-coated CPG, blue diamond curve is uncoated CPG. Error bars are standard error of the mean.

Stability of Loaded Therapeutics

This system is intended as a prototype, to demonstrate proof of the concept that macromolecular drugs can be loaded into the void between nanowires. A vacuum freeze dry/lyophilization system could be used to ensure the protein conformation stays stable[2, 3]. Furthermore, as with tablets and other powdered therapeutics, nearly any protein or macromolecule would be formulated with a stabilizing agent, which could be selected to keep the protein functional using this loading procedure. In this manuscript, we chose to focus on a proof of concept, device optimization, and the effects of loading on nanowire adhesion.

Comparison of Device Loading Capacity and Therapeutic Efficacy

The loading capacity of NEMPs is compared to an estimate of the necessary doses of insulin and antibodies (which are modeled by IgG).

For insulin, a mid-level diabetic would dose approximately 0.4 international units per kg of body weight per day (according to http://www.swmedicalcenter.com/documents/CME/GlycemicControl/Physician%20dosing%20guidelines.doc). Estimating 3.8e-5 grams per unit, and a 70 kg person, a typical dose of insulin is roughly 1 mg per day.

For the antibody drug etanercept (trade name is Enbrel, Amgen/Pfizer), the typical dose is 50 mg per week (according to Enbrel.com dosing instructions for Rheumatoid Arthritis), which constitutes 7 mg/day. Infliximab (trade name is Remicade, Centocor Ortho Biotech Inc.), a typical dose is 5 mg/kg every 2 weeks (according to rxlist.com/remicade-drug.htm, for Crohn's Disease). Assuming a 70 kg person, this constitutes a dose of 25 mg/day.

While these estimates are rough, considering that the pharmacokinetics and pharmacodynamics are likely to be different for each method of delivery, they give a starting approximation for comparison of loading capacities. In elution studies, 1

mL of devices (which would fit into a 00el size capsule from Capsugel), delivered 15.0 mg of IgG or 18.9 mg of insulin in a day (see Supporting Table 1). Assuming a 5 % oral bioavailability, which would theoretically be improved by the adhesion of the NEMPs to the gastrointestinal tract, 0.95 mg of insulin and 0.75 mg of IgG would be available in the bloodstream.

Supporting Table 1: Comparison of Drug Elution and Dosage Guidelines

Drug	Model Drug	Approx. Daily Dose	Mass of Drug Eluted by NEMPs in 24 hr	Mass of Drug Bioavailable at 5 % Oral bioavailability
Insulin	Insulin	1 mg	18.9 mg	0.95 mg
Etanercept	IgG	7 mg	15.0 mg	0.75 mg
Infliximab	IgG	25 mg	15.0 mg	0.75 mg

1. Fischer, K. E.; Aleman, B. J.; Tao, S. L.; Daniels, R. H.; Li, E. M.; Bunger, M. D.; Nagaraj, G.; Singh, P.; Zettl, A.; Desai, T. A., Biomimetic Nanowire Coatings for Next Generation Adhesive Drug Delivery Systems. *Nano Lett.* **2009,** *9* (2), 716-720.
2. Foraker, A. B.; Walczak, R. J.; Cohen, M. H.; Boiarski, T. A.; Grove, C. F.; Swaan, P. W., Microfabricated porous silicon particles enhance paracellular delivery of insulin across intestinal Caco-2 cell monolayers. *Pharm. Res.* **2003,** *20* (1), 110-116.
3. Freitas, S.; Merkle, H. P.; Gander, B., Microencapsulation by solvent extraction/evaporation: reviewing the state of the art of microsphere preparation process technology. *J. Controlled Release* **2005,** *102* (2), 313-332.