Read the following article: Yu et al. Microneedle-array patches loaded with hypoxia-sensitive vesicles provide fast glucose-responsive insulin delivery. *PNAS* (2015). For some questions, you may need to look into referenced papers or outside material for additional information. [http://www.pnas.org/content/112/27/8260.long](http://www.pnas.org/content/112/27/8260.long)

44 total points.

a) (2 points) The authors were aiming to develop a ‘closed-loop’ system for insulin delivery. What is meant by ‘closed-loop’ system, in regards to insulin delivery and diabetes treatment?

2 points

For insulin delivery, a ‘closed-loop’ system is a system that would sense the amount of glucose in the blood, and adjust the amount of insulin delivered based on the glucose concentration.

b) (5 points) The glucose responsive vesicles (GRVs) form through a self-assembly process that encapsulates the contents when the complete mixture is equilibrated in water. (i) What modification was made to hyaluronic acid that causes the formation of these vesicles? (ii) What chemical reaction was performed to make this modification? (iii) Why does this modification cause self-assembly of the vesicles?

5 total points

(i) (1 point) 2- nitroimidazole (NI) was conjugated to HA

(ii) (2 points) Amine-containing NI was reacted with HA using EDC and NHS

(iii) (2 points) NI is hydrophobic. The remainder of HA is more hydrophilic. In water, the modified proteins will assemble into vesicles with NI groups in the center and rest of HA protein presented to the outside, in order to minimize the interaction of NI groups and the water on the outside.
c) (2 points) The authors mention that previous glucose-responsive systems using glucose oxidase (GOx) utilized pH-sensitive materials. What product of the GOx-mediated reaction would a pH-sensitive material ‘sense’?
   2 points
   Gluconic acid

d) (2 points) What reason do the authors cite for pH-sensitive systems not being ideal?
   2 points
   Slow responsiveness in buffered physiologic environments

e) (2 points) What specific aspect of the GOx-mediated reaction do the authors develop their system to ‘sense’?
   2 points
   The depletion of O2. O2 is consumed in the reaction.

f) (6 points) Briefly explain how the GRVs, composed of the modified HA, act as sensor for glucose.
   6 total points
   (2 point) GOx converts glucose to gluconic acid, and depletes O2 in this process
   (2 points) In low oxygen (hypoxia), NI is converted from hydrophobic to hydrophilic.
   (2 points) Once NI is hydrophilic, the GRVs disassemble, which releases insulin.

g) (6 points) Briefly describe both the experimental assays and findings for the data shown in (i) Figure 2F and (ii) Figure 2G.
   4 total points
   (i) (3 points) Measurement of oxygen concentration within GRVs using an oxygen sensitive phosphorescent probe. Data show that oxygen concentration decreases (phosphorescence increases due to reduction in oxygen quenching) upon treatment with glucose. More rapid change and lower oxygen concentration for 400 mg/dl compared to 100 mg/dl.
(ii) (3 points) Measurement of NI groups within GRVs using UV absorbance (NI peak at A330)
Data show that glucose treatment reduces presence of NI groups. This reduction is most pronounced in 400 mg/dl condition compared to 100mg/dl condition.

h) (2 points) What does Figure 3 demonstrate regarding the effect of glucose concentration on the (i) release rate and (ii) total amount of insulin released.
2 total points

(1 point) Increased glucose concentration increased release rate.
(1 point) Increased glucose concentration increased total amount released.

i) (2 points) The authors further demonstrated a way in which they could tailor (specifically reduce) the amount of insulin released in response to a certain concentration of glucose. What was this alteration that would reduce insulin release?
2 total points
Reduce the amount of GOx in the GRVs (one-half the amount)

j) (2 points) What base hydrogel material was used to fabricate the array of microneedles, and how were the microneedle structures formed?
2 total points

(1 point) Hyaluronic acid (methacrylated)
(1 point) Molding using a silicone mold.

k) (3 points) UV crosslinking of the microneedle array was shown to enhance the mechanical properties. What were the 3 important chemical additions/modifications that were introduced during the fabrication process so that the UV crosslinking would work?
3 total points

(1 point) Methacrylate modification of HA
(1 point) Addition of cross-linker N,N’-methylenebisacrylamide
(1 point) Addition of photoinitiator
l) (2 points) Trypan blue staining of the mouse skin was used to illustrate the microneedle penetrations. What does this stain specifically demonstrate?

2 points

Trypan blue stains dead cells. There is local cell death in the tissue near the microneedle penetrations.

m) (5 points) For Figure 5B, (i) what are the following conditions: GRV (E+I), GRV (I), and Insulin. From the data in this figure, (ii) what is one of the main benefits of the complete glucose responsive vesicle system compared to microneedle-based delivery of insulin (without vesicles)

5 total points

(i) (3 points)
GRV (E+I) = microneedles loaded with GRVs containing both enzyme (GOx) and insulin
GRV (I) = microneedles loaded with GRVs containing insulin, but no enzyme
Insulin = microneedles loaded with recombinant insulin, but no GRVs

(ii) (2 points) The GRV system leads to a sustained (for longer time) reduction in blood glucose level compared to insulin without vesicles. The insulin only condition reduces blood glucose concentration temporarily, but then blood glucose concentration rapidly increases.

n) (3 points) What is the main motivation for performing the experiment illustrated in Figure 5H? What does this data show?

3 total points

(1 point) The motivation here was to test if the GRV system would affect blood glucose level in normal mice (non-diabetic mice).

(2 points) The GRV system does not affect blood glucose concentration in normal mice, however, insulin does decrease blood glucose (hypoglycemia).