Optimization of a Glucose-Responsive Implant Microdevice for ‘Smart’ Insulin Therapy

Michael K.L. Chu, Claudia R. Gordijo, Azhar Z. Abbasi, Jason C. Li and Xiao Yu Wu

Leslie Dan Faculty of Pharmacy, University of Toronto, Canada, M5S 3M2
michaelk.chu@utoronto.ca

ABSTRACT SUMMARY
This work was aimed to optimize the design of a bioinorganic material-based and nanotechnology-enabled glucose-responsive insulin microdevice developed by our group to minimize immune response of host and to prolong the application time. The modified microdevice showed minimal acute and chronic immune response in host rat model and sustained in vivo efficacy in glycaemia control over 18 days when implanted subcutaneously in diabetic rats.

INTRODUCTION
Diabetes affects 366 million people worldwide in 2011 and projected to 552 million in 2030.1 All Type 1 diabetes patients require insulin, while ~20% of Type 2 patients manage their disease by insulin therapy. The goal of insulin therapy is to provide exogenous insulin at defined time points to achieve euglycemia and minimize periods of hyper- and hypoglycemia. Typically, periodic insulin injections are performed by patients to provide compensatory insulin for glucose spikes from meals. However, this can be inconvenient due to inconsistent meal and delivery schedules. An ideal method would involve a delivery system that provides basal insulin at low levels, along with responsive, pulsatile release during periods of high glucose.

Continuous delivery systems are receiving much more praise recently due to constant basal delivery of insulin and rapid bolus delivery during mealtimes. On-demand insulin pumps and IV catheters are common forms of these systems, which provide direct exogenous insulin to the bloodstream2. However, they are linked with potential biocompatibility issues from catheter infection, mechanical blockage or failure and difficult to use with an active lifestyle. Other systems, such as osmotic pumps, patches and implant pumps have been tested, and while showing promising patient outcomes, still have similar mechanical issues and do not provide insulin in response to real-time physiological glucose levels3.

Our lab has developed an implantable, glucose-responsive insulin microdevice system that provides ‘smart’ insulin delivery according to glucose levels. Insulin is delivered via reservoir through porous hydrophilic membrane that modulates permeability4. Previously, we have achieved good glucose-responsiveness, with in vivo efficacy in a streptozotocin-induced diabetic rat model over 5 days when implanted intraperitoneally (IP). This IP implant was still susceptible to fibrous capsule formation, even with a 2K PEG brush layer. As well, degradation of bioinorganic membrane occurred after two weeks, due to cellular adhesion and immune response.

In our present work, we have optimized the glucose-responsive microdevice implant system to reduce immune response from the host, which is a common issue with implant systems. Moreover, we have improved the system to provide glucose-responsive efficacy when subcutaneously implanted, rather than IP. Subcutaneous implantation is a much less invasive surgical procedure and lends itself to fewer complications for long-term in vivo study. Long-chain 20K PEG was tested to determine improvement in biocompatibility and microdevice integrity. This long-chain PEG brush layer provides a barrier against cell adhesion and, in turn, immune response. Histology and SEM analysis of the implanted microdevices ex vivo confirm this hypothesis over a 15 day period, while still retaining efficacy for blood glucose maintenance.

EXPERIMENTAL METHODS
Bioinorganic membrane microdevices and activation of PEG were prepared as previously described4. PEGylation of microdevices was performed by incubating in PBS pH 7.4 with 0.2M activated PEG solution for 24 h, and washed multiple times with DDI water to remove excess byproduct from PEG surface conjugation. From this method, PEG is covalently bound to the microdevice surface, creating a hydrophilic, inert brush layer.

For insulin release testing, human recombinant insulin solution dissolved in pH 7.8 HEPPS buffer was injected into microdevices prior to experiments. In vitro glucose-responsiveness testing was performed by incubating microdevices in 2 ml of PBS pH 7.4 buffer in glass vials at 100 mg/dL glucose for 0-2 h and increased to 400 mg/dL for 2-4 h, measuring insulin released into buffer with UV spec at 276 nm. In vivo release study was performed by subcutaneously implanting insulin-filled microdevices into streptozotocin-induced diabetic Sprague Dawley rats. Blood was taken for both glucose and insulin levels during fed state and compared with control sham device animals.
Devices retrieved after in vivo implantation were washed lightly in buffer and fixed with universal fix and formaldehyde for histology and SEM analysis, respectively. PEGylated and control devices were implanted for 15 and 30 days and analyzed ex vivo for histological cross-section and SEM analysis.

RESULTS AND DISCUSSION

In vitro insulin release rate studies showed an increase of approximately two-fold from glucose level of 100 mg/dL (normal) to 300 mg/dL (hyperglycemia) as shown with previous devices. Glucose-responsiveness was shown to be similar between all three groups (no PEG, 2K PEG and 20K PEG) and efficacy was not compromised by the addition of the long-chain PEG brush layer.

After long-term 15 and 30 day in vivo biocompatibility studies, with retrieved microdevices in three groups: no PEG, 2K PEG and 20K PEG, there was a clear distinction in between the amount of cell adhesion and inflammatory capsule formation around the subcutaneous implant. Heavy degradation of the bioinorganic membrane was seen with non-PEGylated devices, while 2K and 20K PEGylated devices showed maintenance of the porous hydrophilic membrane structure. From histology cross-sections, non-PEGylated devices had heavy cell adhesion and immune cell recruitment, such as eosinophils neutrophil clusters and plasma cells. As well, capsule formation was quite thick, comprised of a heavy fibrous layer surrounding the implant. Both 2K and 20K PEG had thinner capsule formation (moreso 20K) and overall reduced presence of immune cells, specifically eosinophils, both acute and chronic (Fig. 1).

Long-term in vivo efficacy studies of 20K PEGylated microdevices showed maintenance of glucose in a normal range, over a 18 day period. Compared to control rats, which were hyperglycemic as expected, the implanted rats maintained euglycemia until device removal, at which point the rats returned to hyperglycemia (Fig. 2). Insulin levels dropped as well, confirming glycemic control from exogenous insulin provided by the subcutaneous microdevice implant.

CONCLUSION

We have optimized a glucose-responsive microdevice implant system with a highly biocompatible 20K PEGylated brush surface. This PEGylated implant system is capable of providing on-demand insulin subcutaneously without external manipulation and shows minimal immune response or inflammatory reaction over an 18-day period.

Fig. 1. Comparison of histology cross sections of no PEG (top left), 2K PEG (top right), and 20K PEG (bottom) fibrous capsule formation at Day 30. Black line indicates thickness measurement of capsule. Image viewed at 10x magnification.

Fig. 2. Long-term blood glucose levels of diabetic rats with insulin microdevice-implant (black square) or sham device control (red circle). Double-sided arrows represent implant (day 0) and retrieval (day 21) time points. Shaded area indicates normal glucose range. Error bars represent standard deviation (n=4).

REFERENCES