Dual self-regulated delivery of insulin and glucagon by a hybrid patch

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Reduced β-cell function and insulin deficiency are hallmarks of diabetes mellitus, which is often accompanied by the malfunction of glucagon-secreting α-cells. While insulin therapy has been developed to treat insulin deficiency, the on-demand supplementation of glucagon for acute hypoglycemia treatment remains inadequate. Here, we describe a transdermal patch that mimics the inherent counterregulatory effects of β-cells and α-cells for blood glucose management by dynamically releasing insulin or glucagon. The two modules share a copolymerized matrix but comprise different ratios of the key monomers to be “dually responsive” to both hyper- and hypoglycemic conditions. In a type 1 diabetic mouse model, the hybrid patch effectively controls hyperglycemia while minimizing the occurrence of hypoglycemia in the setting of insulin therapy with simulated delayed meal or insulin overdose.

Pancreatic islets play a critical role in blood glucose homeostasis through the reciprocal regulation of insulin produced from β-cells and glucagon secreted from α-cells (1). Type 1 diabetes (T1D) is an autoimmune disease in which the pancreatic β-cells are destroyed and there is a deficiency in insulin secretion (2). Type 2 diabetes (T2D) is a metabolic disorder resulting from insulin resistance and β-cell dysfunction with impaired insulin secretion (3). Current treatment methods for both T1D and advanced T2D address insulin deficiency and include modalities such as subcutaneous (s.c.) insulin injection or infusion, endogenous insulin stimulation, and novel glucose-responsive insulin administrations (4–7). Although these treatments can be effective in treating hyperglycemia, they carry the risk of hypoglycemia and therefore require patients to monitor and rapidly respond to episodes of low blood sugar to prevent progression to seizure, coma, or death.

It has recently become recognized that the destruction of β-cells may disrupt other islet cell types and lead to the hyper- or hyposecretion of glucagon from the α-cells (8, 9). α-Cell dysfunction can further exacerbate hyperglycemia among individuals with diabetes and importantly, may increase the risk for severe hypoglycemia due to an abnormal counterregulatory response during insulin treatment. Therefore, researchers have focused on exploring approaches to reprogram and modulate α-cell function (10, 11). Unfortunately, compared with the advances of glucose-responsive insulin delivery systems to address insulin deficiency due to β-cell destruction or dysfunction (12–14), the development of therapeutic systems to treat α-cell dysfunction and mitigate the associated risk for acute hypoglycemia remains challenging.

Here, we report a hybrid microneedle patch that can deliver insulin and glucagon in a glucose-dependent manner. This patch is constructed of dual modules that contain either insulin or glucagon, therefore mimicking the functionality of pancreatic islet cells for the comprehensive regulation of blood glucose levels. The modules are copolymerized from the same monomers of 3-(acrylamido)phenylboronic acid (APBA), 2-aminoethyl methacrylate hydrochloride (AMH), and 1-vinyl-2-pyrrolidinone (VP) but contain different ratios of each monomer (Fig. 1A). A mask-mediated photopolymerization preparation strategy facilitates the integration of the two modules, loaded with insulin and glucagon, respectively, into a microneedle-array patch which mimics islet cell secretion of insulin or glucagon in response to the plasma glucose levels (PGls). The composition ratio of the two modules can be easily adjusted by arranging the loading pattern of the microneedles. The human islet comprises 50–60% of β-cells and 30–45% of α-cells (15–17). Similarly, one-quarter of the microneedles in this hybrid patch is loaded with glucagon, while the remaining microneedles are loaded with insulin.

Results

The needles of the hybrid patch comprise insulin- and glucagon-loaded polymeric matrix. To distribute the glucagon formulation into one-quarter of the needles, a polyvinylpyrrolidone microneedle “mask” was used to prevent liquid infiltration into the insulin modules of the microneedle mold. A glucagon-preloaded (7 wt %) monomer mixture of VP, AMH, APBA, photoinitiator, and polyethylene glycol, was used to prevent liquid infiltration into the microneedle mold and enhance microneedle retention.

Significance

The efficacy of current diabetes treatments designed to reverse insulin deficiency is often limited by the frequent occurrence of acute hypoglycemia. To this end, a hybrid glucose-responsive delivery patch is engineered to function as a synthetic artificial pancreas by delivering insulin and glucagon upon hyperglycemic and hypoglycemic conditions, respectively. A mask-mediated polymerization method is developed to accommodate the insulin and glucagon modules that share a copolymerized matrix but comprise different ratios of the key monomers. The synergistic counterregulation of the two modules is found to enhance the capability of the hybrid patch in maintaining normoglycemia during plasma glucose fluctuations. This platform strategy could facilitate the development of customized therapeutic patches for closed-loop delivery in a precise manner.
and cross-linker was then added to the mold followed by vacuum and photopolymerization on ice. After solidification of the glucagon-loaded needles, the mask was peeled off and the insulin-preloaded (7 wt %) mixture, containing the same monomer components but in altered ratios, was added to the mold and underwent the same vacuum and photopolymerization process. The pyramid-shaped microneedles with a width of 300 μm and a height of 700 μm were arranged in a 30 × 30 array. To give the patch a transparent flexible base, we used Norland Optical Adhesive 86, a commercial ultraviolet-curable material, on top of the needles for demolding. The fluorescence image of the hybrid patch revealed that fluorescein isothiocyanate (FITC)-labeled glucagon needles and Cy5-labeled insulin needles were separated into the predesigned pattern (Fig. 1B). The magnified photo and scanning electron microscopy (SEM) image (Fig. 1C) further confirmed the successful integration of the two modules with a negligible difference in surface morphology. Additionally, the fracture force of the glucagon and insulin microneedles was measured and found to be comparable at 0.26 and 0.28 N per needle, respectively (Fig. 1D), both sufficient for skin penetration (18) (SI Appendix, Fig. S1).

The glucose-responsive mechanism of insulin and glucagon delivery can be attributed to the synergistic net charge shift of the AMH/APBA polymeric network at various glucose concentrations, the difference in isoelectric points (pIs) of insulin and glucagon at physiological pH, and the consequent shrinkage or swelling of the surrounding polymeric gel matrix (Fig. 2A). Insulin has a pI of ~5.4 (19, 20) and therefore exhibits a negative charge and high solubility at physiological pH. By contrast, glucagon has low solubility in the pH range of 6–8 (21, 22) due to its pI of ~7.1 (23) (SI Appendix, Fig. S2). The APBA monomer can reversibly bind with glucose to generate negatively charged cyclic boronate esters (24). Meanwhile, the positively charged AMH monomer is used to adjust the charge of the polymeric matrix (25, 26); this monomer outperformed nine other positively charged monomers partnered with APBA at the ratios of 1.1 and 1.4 (SI Appendix, Figs. S3 and S4). The glucose-responsive release of insulin or glucagon is determined by manipulating the ratios of the major monomer components (SI Appendix, Figs. S5 and S6), with the AMH to APBA ratio of 1.4 for the glucagon needles and 2.6 for the insulin needles. When conditions are changed from normoglycemia to hyperglycemia, a more negatively charged glucose–boronate complex is formed (27). The decrease in net positive charge weakens the electrostatic attraction between insulin and the positively charged matrix, promoting the release of the insulin molecules from the insulin modules. For the glucagon matrix, increased negative charge neutralizes the initially positively charged matrix, which contracts the polymeric network and inhibits the diffusion of the glucagon molecules from the glucagon modules (28). Together, promoted...
insulin release and limited glucagon release were observed at a high glucose level of 400 mg/dL (Fig. 2 B and C). When surrounding conditions are associated with hypoglycemic level, the insulin matrix restores the initial net charge, slowing down the glucagon diffusion. (A, i) The repulsion force between the positively charged polymeric matrix is neutralized, inducing the polymer to shrink and slowing down the glucagon diffusion. (A, ii) The electrostatic attraction between negatively charged insulin and positively charged polymeric matrix is weakened to promote the release of insulin from the microneedles. (B and C) In vitro accumulated insulin release (B) and glucagon release (C) from the glucose-responsive polymeric matrix in varying glucose concentrations at 37 °C, pH 7.4. Data points are means ± SD (n = 3). (D and E) Pulsatile release of insulin (D) and glucagon (E) by alternating the glucose concentrations between 50 and 400 mg/dL by alternating the glucose concentrations between 50 and 400 mg/dL. The incubation time for glucagon and insulin in each solution is 15 and 30 min, respectively. Data points are means ± SD (n = 3). (F and G) Glucose-responsive release of insulin (F) and glucagon (G) from the hybrid patch in the indicated glucose concentrations. The accumulated release is quantified with ELISA. Data points are means ± SD (n = 3). Statistical significance was determined by two-tailed Student’s t test. **P < 0.01, ***P < 0.001, and ****P < 0.0001.

Fig. 2. Mechanism and in vitro evaluation of the glucose-regulated insulin/glucagon release. (A) In hyperglycemic conditions, the formation of the glucose–boronate complexes increases the surrounding negative charges. (A, i) The repulsion force between the positively charged polymeric matrix is neutralized, inducing the polymer to shrink and slowing down the glucagon diffusion. (A, ii) The electrostatic attraction between negatively charged insulin and positively charged polymeric matrix is weakened to promote the release of insulin from the microneedles. (B and C) In vitro accumulated insulin release (B) and glucagon release (C) from the glucose-responsive polymeric matrix in varying glucose concentrations at 37 °C, pH 7.4. Data points are means ± SD (n = 3). (D and E) Pulsatile release of insulin (D) and glucagon (E) by alternating the glucose concentrations between 50 and 400 mg/dL. The incubation time for glucagon and insulin in each solution is 15 and 30 min, respectively. Data points are means ± SD (n = 3). (F and G) Glucose-responsive release of insulin (F) and glucagon (G) from the hybrid patch in the indicated glucose concentrations. The accumulated release is quantified with ELISA. Data points are means ± SD (n = 3). Statistical significance was determined by two-tailed Student’s t test. **P < 0.01, ***P < 0.001, and ****P < 0.0001.

APBA resulted in an opposite pattern of glucose-responsive behavior (SI Appendix, Fig. S5).

We characterized the pulsatile release profiles for the insulin and glucagon formulations, respectively, and demonstrated several cycles of glucose-responsive hormone release by alternating incubation of the polymeric matrix in low glucose level (50 mg/dL) and high glucose level solutions (400 mg/dL) (Fig. 2 D and E). The release of insulin and glucagon started to decrease after three cycles of consumption. To assess the glucose-responsive behavior of the integrated insulin and glucagon formulations, hormone
levels in varying concentrations of glucose were quantified by the enzyme-linked immunosorbent assay (ELISA). As shown in Fig. 2 F and G, the glucose-dependent release performance was similar to the individual release profile. The release rate of insulin or glucagon from their corresponding module was regulated in a glucose-dependent but counterregulatory manner. Additionally, matrix-assisted laser desorption/ionization–time-of-flight mass spectrum analysis of the native insulin/glucagon and the insulin/glucagon released from the microneedles confirmed that the protein molecules stayed intact throughout the polymerization process (SI Appendix, Fig. S7).

Next, in vivo glycemic regulation abilities of the respective insulin-only and glucagon-only patches were assessed in a streptozotocin-induced insulin-deficient diabetic mouse model. PGLs in mice treated with the insulin patch (dose: 50 mg/kg) approached a normoglycemia level (<200 mg/dL) within 1 h and stayed in this range for up to 6 h. The plasma insulin level reached a peak at 1 h and was stabilized after 3 h (Fig. 3A). In vivo glucose responsiveness was assessed with an intraperitoneal (i.p.) glucose tolerance test (IPGTT) performed 4-h posttreatment at a dosage of 3.0 g/kg. A spike in PGLs was observed, followed by a decreasing trend in PGLs over the following period of observation (Fig. 3B). In response to the initial increase in PGLs, a significant spike of plasma insulin (Fig. 3B) was recorded within 1 h of the PGL spike.

We further characterized the release profile of the glucagon-only patch on two groups of diabetic mice: one with hyperglycemic PGLs (untreated) and one with hypoglycemic PGLs (treated with overnight fasting and an s.c. injection of 70 μg/kg insulin). Simultaneous study of the PGLs and plasma glucagon level showed a notably higher plasma glucagon level in the group with hypoglycemia 3-h postadministration. A subsequent increase of PGLs to the normal range was also achieved (Fig. 3 C and D). An intraperitoneal insulin tolerance test (IPITT) was performed by administering an i.p. injection of 2.5 mg/kg insulin 2 h post–glucagon-only patch administration, after which PGLs of the insulin-challenged group decreased and reached hypoglycemia in 2 h (Fig. 3E). A gradual increase in the plasma glucagon level was observed in response to the drop in PGLs. By contrast, the control diabetic mouse group without the insulin injection showed no fluctuation in the release rate of glucagon and therefore, did not cause a further rise in PGLs (Fig. 3F and SI Appendix, Fig. S8A). Taken together, both the insulin-only patch and
glucagon-only patch displayed glucose-regulatory release to maintain normoglycemia.

To substantiate the capability of the glucagon module to mitigate hypoglycemia, the diabetic mice in hypoglycemic conditions (induced by overnight fasting with an s.c. injection of 70 μg/kg insulin) were treated with the glucagon-only patch and kept under fasting conditions during the treatment period. The patch-treated mice restored normoglycemic conditions after 2 h, while the non–patch-treated group remained in hypoglycemic ranges (SI Appendix, Fig. S8B). To further characterize the safeguard capability of the glucagon patch, the diabetic mice were deprived of food throughout the experiment and subjected to an insulin injection (2.5 mg/kg). The glucagon-only patch slowed down the decrease in PGLs after the insulin injection and ultimately prevented PGLs from dropping below 50 mg/dL (SI Appendix, Fig. S8C). In contrast, the PGLs of the control group rapidly declined to hypoglycemic ranges where they remained for 2 h. When the diabetic mice under normal food intake conditions were challenged with an insulin injection (2.5 mg/kg), the patch-treated group still experienced a faster PGL recovering rate compared with the control group (SI Appendix, Fig. S8D).

The integration effect on the in vivo glucose responsiveness of the insulin module was validated in the hybrid patch with IPGTT. A spike in plasma insulin occurred 2 h post-glucose ingestion, similar to the insulin-only case, with a maximum insulin level at around 470 μU/mL. The effect of the increased plasma insulin levels was reflected in a rapid reduction of PGLs during the following observation period (SI Appendix, Fig. S9). Thus, the glucose-responsive insulin release behavior was retained in the hybrid patch. We then administered the hybrid patch and compared the glucose-responsive treatment performance with the performance of the separate insulin-only patch. As expected, the integration of the glucagon modules with the insulin modules delayed the decrease of the PGLs, and a fluctuating pattern in PGLs around the normoglycemic range was observed in the hybrid patch-treated group. Compared to the flat curve of the insulin-only patch (Fig. 4A), this result validates the alternating regulatory effect between the insulin and glucagon modules. Additionally, the hybrid patch showed a remarkably reduced hypoglycemic index (defined by the difference of the initial and nadir PGL readings divided by the time spent to reach nadir) average of 0.99, compared with 1.42 of the insulin-loaded

**Fig. 4.** In vivo evaluation of the hybrid patch safeguard effect. (A and B) PGLs (A) and hypoglycemia index (B) in diabetic mice (n = 5) after treatment with insulin-only patch (insulin dose: 50 mg/kg) or the hybrid patch (insulin dose: 50 mg/kg, glucagon dose: 17 mg/kg). (C and D) PGLs (C) and hypoglycemia index (D) in diabetic mice (n = 5) fasted for 6 h after treatment with insulin-only patch (insulin dose: 50 mg/kg) or the hybrid patch (insulin dose: 50 mg/kg, glucagon dose: 17 mg/kg). (E and F) PGLs (E) and hypoglycemia index (F) in diabetic mice (n = 5) s.c. injected with 70 μg/kg insulin 2-h (indicated with an arrow) post–insulin-only patch (insulin dose: 50 mg/kg) or the hybrid patch (insulin dose: 50 mg/kg, glucagon dose: 17 mg/kg) administration. Statistical significance was determined by two-tailed Student’s t test, *P < 0.05, **P < 0.01. Hypoglycemia index is defined by the difference of the initial and nadir PGL readings divided by the time spent to reach nadir PGL. Severe hypoglycemia region for mice (< 50 mg/dL) is shown in the shaded area. Dotted black line indicates the average normoglycemia level of mice (150 mg/dL).
We characterized the effect of a simulated delayed meal intake on the performance of the insulin-only and hybrid patch by subjecting the diabetic mice to 6 h of fasting. The hybrid patch regulated the PGLs within a normoglycemic range, while the insulin-only patch led to hypoglycemia (Fig. 4C). This notable safeguard effect for meal delay was also reflected in the maintained hypoglycemia index average of 0.91 for the hybrid patch and increased hypoglycemia index average of 2.25 for the insulin-only patch (Fig. 4D). We have further compared the safeguard performance of the insulin-only patch and hybrid patch on diabetic mice by challenging both devices with a simulated overdose of insulin (350 μg/kg s.c. injection) upon reaching normoglycemia at 2-h timepoint. A sudden drop of PGLs was observed in both groups, but only the insulin-only patch group alone approached hypoglycemia at the 3-h timepoint (Fig. 4E). Despite the increase of the hypoglycemia index in both groups, the insulin-only patch group exhibited a higher hypoglycemia index value of 1.79 than the average of 1.47 for the hybrid patch (Fig. 4F).

Regarding biocompatibility, a matrix–cross-linked and removable patch may eliminate posttreatment safety issues associated with dissolvable matrices or implantable devices. Hematoxylin and eosin (H&E) staining of the mice skin treated with each patch for 10 or 24 h was evaluated, respectively. There was no significant difference in histological appearance between the 10- and 24-h administration groups, or between the insulin-only patch and glucagon-only patch. Briefly, epidermal and dermal atrophy, as well as suppurrative inflammation, were present on day 1 after administration, followed by a normal to near-normal epidermal and dermal thickness with sparse mixed inflammation on day 3. Inflammation at the administration sites was mostly healed 1 wk after administration (SI Appendix, Fig. S10 and Table S1). For safe usage, a 1-wk recovery period may be recommended before reapplying the hybrid patch to the same skin site.

Discussion

In summary, we developed a dual glucose-responsive insulin and glucagon delivery device to function as an external “pancreatic islet” across a spectrum of glucose ranges. Treatment with this hybrid formulation may be particularly beneficial for individuals with diabetes in the setting of lifestyle changes, irregular schedules and meals, or inaccurate insulin dosing. Individual differences in insulin and glucagon requirements to maintain euglycemia can, in turn, determine the lifespan of the hybrid patch with regards to treatment (i.e., insulin) and safeguard (i.e., glucagon) capacities. There was some delay in the response rate of each module observed, which resulted in oscillation rather than entirely steady-state blood glucose levels. Therefore, opportunities remain to enhance release kinetics of the hybrid system through optimization of formulation and microneedles (MNs) design. To target euglycemic blood glucose levels among humans and facilitate further translation of the hybrid patch, the ratio of the insulin and glucagon modules can be altered during the fabrication procedure; this ratio can also be customized to fulfill the diverse needs of a wide range of users. Moreover, three-dimensional printing technologies could automate the design procedure and equip the patch with a stinglike applicator to standardize the skin penetration process. Finally, the masking and sequential photopolymerization approach employed in the present study may be expanded to other drug delivery applications for co-delivering multiple therapeutics with enhanced efficacy and safety.

Materials and Methods

Materials used in the paper, detailed preparation and characterization procedures for hybrid MN patch, in vitro release studies of insulin and glucagon, in vivo animal experiments, H&E staining, and statistical analysis are provided in SI Appendix. The animal study protocol was approved by the Institutional Animal Care and Use Committee at the University of California, Los Angeles.

Data and Materials Availability. All data needed to evaluate the conclusions in the paper are present in the paper and/or the SI Appendix.

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