1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder affecting millions of people worldwide. According to the World Health Organization, the number of diabetic patients increased from 108 million in 1980 to 422 million in 2014 and is set to continue rising up to 693 million by 2045 [1]. As a result, global DM prevalence is estimated to reach 10.3% by 2045 [2].

DM is classified into two major types [3]: type 1 DM, which is a genetic condition characterized by the lack or reduction of insulin production by pancreatic β cells due to their destruction by the immune system, and type 2 DM, which is a lifestyle-related condition associated with age and caused by the development of resistance to the action of insulin [4]. Insulin is an anabolic polypeptide hormone secreted by β cells in the pancreatic Langerhans islets. It maintains physiological glycemia at around 100 mg/dL, so its absence or inability to function leads to hyperglycaemia. Insulin receptors are primarily located in skeletal muscle, liver and white adipocytes, where the binding of insulin promotes glycogen synthesis and lipogenesis [5]. Although insulin is continuously secreted at a basal level (i.e., 60 mg/dL), its secretion increases postprandially (up to 140 mg/dL) to restore glycemia levels [6].

Insulin replacement therapy is the first-line treatment for type 1 DM and is also used in advanced stages of type 2 DM when oral antidiabetic treatments fail [7]. With oral delivery severely hindered due to insulin degradation in the digestive tract, diabetic patients mostly self-inject insulin subcutaneously multiple times per day, depending on their self-monitoring of glucose levels through finger-prick tests [8,9]. However, the subcutaneous route is associated with disadvantages such as patient discomfort, needle phobia or even, in the case of chronic treatment, lipodystrophy [10–12].

To address these limitations, transdermal insulin delivery has...
become an interesting alternative approach, as it is more user-friendly in comparison with subcutaneous injections and enables treatment cessation upon detection of hypoglycaemia in case of insulin overdose [13–15]. For a drug to achieve efficient transdermal delivery, it must permeate through the skin barrier into the systemic circulation. With 2 m² area in a human adult, the skin is the largest organ in the body [16]. Its most external layer, the stratum corneum (SC), acts as an effective barrier to the entrance of xenobiotics [17]. Only a few drugs show the adequate physicochemical properties to diffuse passively through the SC. Given its high molecular weight, the low intrinsic permeability of insulin across the skin barrier has traditionally hindered its transdermal delivery [18]. Both chemical and physical approaches have been explored to overcome this barrier for transdermal insulin delivery [19]. Overall, the use of chemical enhancers shows limited permeability enhancement for high molecular weight molecules such as insulin [20]. In addition, chemical enhancers tend to diffuse out of the SC, reaching deeper tissues and often causing skin irritation and erythema [21]. As a result, physical methods are preferred for this purpose. Among them, microneedle (MN)-based systems are considered as a straightforward, consolidated (and very promising approach, due to their simplicity and exceptional results achieved in comparison with other physical strategies (i.e., iontophoresis, sonophoresis or electroporation)) [13,22,23].

Microneedle array patches are devices provided with micron-sized needle-like structures capable of physically piercing the SC to facilitate transdermal drug delivery [24]. Microneedle-assisted transdermal delivery is a minimally invasive and painless method of delivering drugs systemically, since MN length (in the range of 50 to 1000 μm) prevents them from reaching the nociceptive receptors present at the dermis layer [25]. Indeed, given their high translational potential, the World Economic Forum listed MNs within the ‘Top 10 Emerging Technologies of 2020’ [26]. Microneedles can be classified according to different criteria, but the most common is using their structure, which highly influences their performance [27]. In this regard, various microneedle types, including solid, hollow, coated, dissolving, and swelling microneedle types have been described, as reviewed in [22].

Although conventional MNs present many advantages for transdermal insulin delivery [28–31], they still cannot regulate the administration of this drug according to blood glucose levels. A delivery system capable of releasing insulin in response to these values would improve the quality of life of diabetic patients by not requiring frequent finger-prick tests and by providing better glycaemic control with lower risk of hypoglycaemia. Hence, glucose-responsive MN-based systems for transdermal insulin delivery are currently under development for the treatment of DM [32,33]. These act as an artificial pancreas releasing insulin only in response to hyperglycaemic states. So far, chemically and electrochemically controlled MN-based systems have been developed for this purpose. Chemically controlled MN-based systems are classified depending on the glucose-sensing elements that they incorporate, including glucose oxidase, phenylboronic acid, and glucose-binding transporters.

As the development of a self-regulated system for transdermal insulin delivery would represent an unprecedented breakthrough in the field of DM treatment, the latest advances in glucose-responsive MN-based systems for transdermal insulin delivery are here compiled with a thorough analysis of the delivery mechanisms and challenges lying ahead in their clinical translation. Among these challenges, special emphasis is placed on the insulin loading capacity of each system to achieve clinically relevant doses, their suitability to control both post-prandial blood glucose levels and basal glycaemia, the configurations that provide the most prolonged normoglycaemic times alongside a reduced risk of hypoglycaemia and the biocompatibility of both glucose-sensing elements and biomaterials used to fabricate MN systems. On this basis, this review includes all glucose-responsive MN-based systems as of October 2023 with published results on their antidiabetic effect tested in animal models of diabetes. Altogether, this analysis from both a mechanistic and translational perspective will provide rationale and guidance for future trends in the research hotspot of glucose-responsive MN-based insulin delivery systems.

2. Glucose oxidase-based MN systems

Glucose oxidase is one of the most widely used glucose-sensing materials in the formulation of smart MN-based transdermal insulin delivery systems. Glucose oxidase is an enzyme that catalyses the oxidation of glucose to gluconic acid and hydrogen peroxide (H₂O₂) (Eq. (1), Fig. 1) [34].

\[
\text{Glucose} + \text{O}_2 \xrightarrow{\text{Glucose oxidase}} \text{Gluconic Acid} + \text{H}_2\text{O}_2
\]  

(1)

Oxygen consumption during this process creates a localized hypoxic environment, which can be exploited to trigger insulin delivery in response to hyperglycaemia. The generation of hydrogen peroxide during glucose oxidation can also be exploited to trigger insulin release from MNs. Lastly, the formation of gluconic acid generates a local pH reduction, which can be alternatively exploited to induce insulin release by incorporating pH-sensitive materials in the transdermal delivery system. Thus, smart transdermal insulin delivery systems based on glucose oxidase need to incorporate stimuli-responsive materials (to hypoxia, H₂O₂ or pH) to attain glucose-responsive insulin release (Fig. 1) [35].

Hydrogen peroxide produced during enzymatic oxidation of glucose can both reduce the activity of glucose oxidase and induce free-radical oxidative stress to skin tissue. Therefore, H₂O₂-scavenging enzymes (i.e., catalase) are often included in the formulation of these systems to optimize insulin delivery. Table 1 summarizes all glucose oxidase-based MN systems incorporating stimuli-responsive materials for transdermal insulin delivery with published results on their antidiabetic effect tested in pharmacologically induced diabetic animal models.

2.1. Hypoxia-triggered glucose oxidase-based MN systems

The first glucose-responsive transdermal insulin delivery system ever described exploited the glucose oxidase-induced hypoxic microenvironment to trigger rapid insulin-release in response to hyperglycaemia. The few existing hypoxia-responsive materials normally contain 2-nitroimidazole groups [36]. These groups are reduced in a hypoxic environment, leading to changes in the aqueous solubility of the materials and ultimately triggering insulin release.

Yu et al. designed a hyaluronic acid-based MN system incorporating hypoxia-responsive nanovesicles made from hyaluronic acid conjugated with 2-nitroimidazole and loaded with insulin and glucose oxidase [37]. In a hypoxic environment, the hydrophobic 2-nitroimidazole group is reduced to a hydrophilic 2-aminoimidazole group, leading to the dissociation of the nanovesicles and subsequent insulin release (Fig. 2a). This hypoxia-triggered insulin release mechanism from the nanovesicle-loaded MNs was first demonstrated in vitro in dissolution media containing different glucose concentrations. Then, the antidiabetic effect of these MN systems was assessed in streptozotocin-induced type 1 diabetic mice. Animals received a 10 mg/kg insulin dose and were divided into five groups: i) empty MN arrays (control group), ii) MN arrays containing free insulin, iii) MN arrays containing hypoxic-responsive nanovesicles loaded with both insulin and glucose oxidase (1 mg/kg), iv) MN arrays containing hypoxic-responsive nanovesicles loaded with both insulin and half of the dose of glucose oxidase (i.e., 0.5 mg/kg), and v) MN arrays containing hypoxic-responsive nanovesicles loaded only with insulin. Results showed that only the MN arrays containing hypoxic-responsive nanovesicles loaded with both insulin and glucose oxidase at the full dose decreased blood glucose levels below 200 mg/dL within 30 min and maintained this normoglycaemic state over 3.5 h. This demonstrated that in the absence of glucose oxidase (group v), the hypoxic-responsive nanovesicles maintained their integrity, precluding insulin release and leading to a similar blood glucose profile to the one
observed for the control group. Similarly, when the glucose oxidase dose was halved (group iv), blood glucose levels below 200 mg/dL were not reached as the glucose oxidase dose was insufficient for the nanovesicles to be dissociated in response to hyperglycaemic values. Lastly, for MN arrays containing free insulin (group iii), although normoglycemic levels were rapidly achieved, hyperglycaemic levels were recovered much shortly thereafter (Fig. 2b). Altogether, this study showed the need for a minimum glucose oxidase dose to achieve efficient hypoxia-mediated insulin release from the described systems. An intraperitoneal glucose tolerance test conducted 1 h post-MN administration in diabetic mice further demonstrated the rapid responsiveness of the MNs containing hypoxic-responsive nanovesicles loaded with both insulin and glucose oxidase. Indeed, the diabetic mice treated with this system showed a similar behaviour to healthy mice in terms of blood glucose levels. Lastly, these glucose-responsive MNs significantly minimized the risk of hypoglycaemia in healthy mice in comparison with MNs containing only free insulin. These results evidenced the in vivo safety and efficacy of this glucose oxidase-based MN system for hypoxia-triggered smart transdermal insulin delivery in response to hyperglycaemic values.

The same researchers then utilized an analogous crosslinked hyaluronic acid-based MN patch to modulate insulin secretion in pancreatic β-cells in response to blood glucose levels [38]. The pancreatic β-cells were encapsulated in a biomimetic alginate microgel that was placed on the back of the MN patch. Conversely, the MN tips contained self-assembled nanovesicles such as the ones described previously, entrapping up to three enzymes (i.e., glucose oxidase, α-amylase and glucoamylase). Together, α-amylase and glucoamylase catalyse α-amylase hydrolysis to glucose. An α-amylase layer separated both parts of the MN patch. Release of the encapsulated enzymes was achieved through the hypoxia-triggered process described previously. The released enzymes subsequently hydrolyse the α-amylase embedded in the MN structure, generating a locally high glucose-concentrated site, which amplifies the initial hyperglycaemic signal. This hydrolysis also allows the diffusion of glucose to the externally located β-cells, ultimately triggering insulin secretion in response to hyperglycaemia. The antidiabetic effect of this MN patch was assessed in streptozotocin-induced type 1 diabetic mice, divided into six groups: i) empty MN patches (control group), ii) MN patches containing encapsulated β-cells but no enzymes, iii) MN patches containing the complete enzyme cocktail but no β-cells, iv) MN patches containing both β-cells and the complete enzyme cocktail, v) microneedle patches containing β-cells and an enzyme cocktail without glucose oxidase, and vi) microneedle patches containing β-cells and an enzyme cocktail without α-amylase. Results showed that only the approach in group iv significantly decreased blood glucose levels to nearly 200 mg/dL within 2 h and maintained these levels over 4 h. This demonstrated that in the absence of one or more of the enzymes (groups ii, v and vii), insulin secretion by β-cells remained at basal level, which could only account for a decrease in blood glucose levels within the first hour. Accordingly, in the absence of insulin-secreting β-cells (group iii and control group) no reduction of glycaemia could be observed. The MN patches containing β-cells and the complete enzyme cocktail also showed no risk of hypoglycaemia in healthy mice, achieving analogous blood glucose values to those obtained in the control group. Lastly, an intraperitoneal glucose tolerance test conducted 2 h post-MN administration in diabetic mice further demonstrated that treatment with MN patches containing β-cells and the complete enzyme cocktail led to similar blood glucose levels as those observed in healthy mice.

2.2. \( \text{H}_2\text{O}_2 \)-triggered glucose oxidase-based MN systems

Alternative transdermal smart insulin delivery systems responding to the glucose oxidase-induced \( \text{H}_2\text{O}_2 \) production in hyperglycaemic contexts were subsequently developed. Hydrogen peroxide-responsive materials that can be used for this purpose include functional groups like phenylboronic acid, thiol ethers and thioacetals [36,39]. Oxidation or hydrolysis of these groups in response to elevated \( \text{H}_2\text{O}_2 \) levels can lead to changes in the aqueous solubility of the materials, ultimately triggering insulin release.

The same authors who developed the previously described hypoxia-triggered transdermal insulin delivery system pioneered a \( \text{H}_2\text{O}_2 \)-triggered approach [40]. Hu et al. incorporated insulin and glucose oxidase-loaded polymersomes in the previously described hyaluronic acid-based MN patch. The polymersomes were composed of the amphiphilic block copolymer polyethylene glycol-polyserine. The pendant hydroxyl groups of the serine residues were conjugated via a carbonate linkage to the hydrophobic phenylboronic pinacol ester to achieve \( \text{H}_2\text{O}_2 \)-responsiveness. The carbonate linker is oxidized and hydrolysed by \( \text{H}_2\text{O}_2 \), ultimately rendering the copolymer water-soluble once it loses its phenylboronic pinacol ester side chains, leading to polymersome disassembly and subsequent insulin release. Similarly to what was described in the previous study, the antidiabetic effect of these MN arrays was assessed in streptozotocin-induced type 1 diabetic mice, receiving a single 10 mg/kg insulin dose and divided into four groups: i) empty MN arrays (control group), ii) MN arrays containing free insulin, iii) MN arrays containing \( \text{H}_2\text{O}_2 \)-responsive polymersomes loaded only with insulin, and iv) MN arrays containing \( \text{H}_2\text{O}_2 \)-responsive polymersomes loaded with both insulin and glucose oxidase. Only the MN arrays in group iv led to a decrease in blood glucose levels to 90 mg/dL within 0.5 h, with these levels being maintained below 200 mg/dL over 4.5 h. Microneedles containing free insulin (group ii) rapidly decreased blood glucose levels to 90 mg/dL, but failed to maintain them in the following hour. Analogously to the hypoxia-triggered study, this one demonstrated that in the absence of glucose oxidase (group iii), the \( \text{H}_2\text{O}_2 \)-responsive polymersomes maintained their integrity, precluding insulin release and leading to a similar blood glucose profile to the one observed in the control group. The intraperitoneal glucose tolerance test conducted 1 h post-MN administration in diabetic mice confirmed the resistance of animals treated with MNs containing \( \text{H}_2\text{O}_2 \)-responsive
Table 1
Compilation of the glucose-responsive microneedles based on glucose oxidase for smart transdermal insulin delivery. *Normoglycemic levels refer to blood glucose levels below or around 200 mg/dL for rodents in each case. PEG: polyethylene glycol; ip: intraperitoneal. N/A: not applicable.

<table>
<thead>
<tr>
<th>Trigger stimulus for smart insulin release</th>
<th>Microneedle material</th>
<th>Glucose-responsive element</th>
<th>Animal model</th>
<th>Insulin dose</th>
<th>Onset of action</th>
<th>Duration of normoglycemic levels*</th>
<th>Resistance to glucose challenge (administration route)</th>
<th>Risk of hypoglycaemia in healthy animals</th>
<th>Ref.</th>
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<tr>
<td>Hypoxia</td>
<td>Crosslinked methacrylated hyaluronic acid</td>
<td>Hypoxia-sensitive hyaluronic acid nanovesicles</td>
<td>Streptozotocin-induced diabetic male C57B6 mice</td>
<td>10 mg/kg</td>
<td>0.5 h</td>
<td>3.5 h</td>
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<td>No</td>
<td>[37]</td>
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<td>Hypoxia</td>
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<td>Hypoxia-sensitive hyaluronic acid nanovesicles loaded with enzymatic glucose-signal amplifiers</td>
<td>Streptozotocin-induced diabetic male C57B6 mice</td>
<td>N/A</td>
<td>2 h</td>
<td>4 h</td>
<td>Yes (ip)</td>
<td>No</td>
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</tr>
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<td>H₂O₂</td>
<td>Crosslinked methacrylated hyaluronic acid</td>
<td>H₂O₂-sensitive polyethylene glycol-polyserine polymersomes</td>
<td>Streptozotocin-induced diabetic male C57B6 mice</td>
<td>10 mg/kg</td>
<td>1 h</td>
<td>5 h</td>
<td>Yes (ip)</td>
<td>No</td>
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<td>H₂O₂-sensitive grafted mesoporous silica nanoparticles</td>
<td>Streptozotocin-induced diabetic male Sprague Dawley rats</td>
<td>40 IU/kg</td>
<td>2 h</td>
<td>3 h</td>
<td>Not evaluated</td>
<td>Not evaluated</td>
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<td>Mixture of crosslinked polyvinyl alcohol, methacrylated polyvinyl alcohol and hyaluronic acid</td>
<td></td>
<td>H₂O₂-sensitive anchorage of insulin to the gel matrix and H₂O₂-sensitive gel crosslinking</td>
<td>Streptozotocin-induced diabetic male C57B6 mice</td>
<td>50 mg/kg</td>
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<td>Streptozotocin-induced diabetic male Sprague Dawley rats</td>
<td>20 IU/kg</td>
<td>2 h</td>
<td>3 h</td>
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<td>[43]</td>
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<td>pH-sensitive grafted mesoporous bioactive glasses</td>
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<td>Stainless steel</td>
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<td>pH-sensitive grafted porous coating</td>
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<td>7 h</td>
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<td>Not evaluated</td>
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<td>Mixture of polyvinyl alcohol and polyvinylpyrrolidone</td>
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<td>pH-sensitive branched poly β-amino ester nanovesicles</td>
<td>Streptozotocin-induced diabetic male Sprague Dawley rats</td>
<td>3.48 IU/kg (as free insulin) + 2.88 IU/kg (loaded in the nanovesicles)</td>
<td>1 h</td>
<td>12.5 h</td>
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<td>Not evaluated</td>
<td>[46]</td>
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<td>Polycrylic acid</td>
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<td>pH-sensitive methacrylic acid-derived PEGylated nanoparticles</td>
<td>Streptozotocin-induced diabetic mice Sprague Dawley rats</td>
<td>10 mg/kg</td>
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<td>8 h</td>
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<td>pH-sensitive dynamically grafted mesopores</td>
<td>Alloxan-induced diabetic male NMRI mice</td>
<td>0.8 mg (+1.2 mg as reservoir)</td>
<td>1 h</td>
<td>5 h</td>
<td>Yes (ip)</td>
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<td>Silk fibroin</td>
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<td>pH-sensitive swelling hydrogel</td>
<td>Streptozotocin-induced diabetic mice Sprague Dawley rats</td>
<td>10 IU</td>
<td>6 h</td>
<td>Not evaluated</td>
<td>Not evaluated</td>
<td>Not evaluated</td>
<td>[49]</td>
</tr>
<tr>
<td>Dual (hypoxia and H₂O₂)</td>
<td>Crosslinked methacrylated hyaluronic acid</td>
<td>Hypoxia- and H₂O₂-sensitive polyethylene glycol-polyserine polymersomes</td>
<td>Streptozotocin-induced diabetic male C57B6 mice</td>
<td>10 mg/kg</td>
<td>1 h</td>
<td>5 h</td>
<td>Yes (ip)</td>
<td>No</td>
<td>[50]</td>
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<tr>
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<td>pH-sensitive micelles</td>
<td>Streptozotocin-induced diabetic male C57B6 mice</td>
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<td>3.5 h</td>
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</table>
polymersomes loaded with both insulin and glucose oxidase to the challenge. This resistance was analogous to that of healthy mice and superior to the one observed in the group treated with MNs containing free insulin. These $\text{H}_2\text{O}_2$-triggered glucose oxidase-based MNs also reduced the risk of hypoglycaemia in healthy mice in comparison with MNs containing free insulin.

Also using a $\text{H}_2\text{O}_2$-triggered insulin release mechanism, Xu et al. developed polyvinylpyrrolidone (PVP)-based dissolving MN patches containing $\text{H}_2\text{O}_2$-responsive mesoporous silica nanoparticles (MSNs) [41]. Insulin and glucose oxidase were encased into the nanopores of the particles, which were then capped via formation of an inclusion complex between $\alpha$-cyclodextrin and phenylboronic pinacol ester. This pendant phenylboronic pinacol ester group was conjugated to the surface of the MSNs via a carbamate linkage. Glucose oxidase-induced production of $\text{H}_2\text{O}_2$ under hyperglycaemic conditions leads to the degradation of the carbamate linkage, resulting in the destruction of the inclusion complex and subsequent insulin release through opening of the MSN pores. The antidiabetic effect of the $\text{H}_2\text{O}_2$-responsive MNs was evaluated in streptozotocin-induced diabetic Sprague Dawley rats, divided into four groups: i) empty MN arrays (control group), ii) subcutaneous insulin injection (24 IU/kg), iii) MN arrays containing $\text{H}_2\text{O}_2$-responsive MSNs loaded only with insulin (40 IU/kg), and iv) MN arrays containing $\text{H}_2\text{O}_2$-responsive MSNs loaded with both insulin (40 IU/kg) and glucose oxidase. The rationale behind the higher insulin dose loaded in the MNs in comparison with subcutaneous injection was to match the relative bioavailability of the drug, since MNs could not release their full insulin dose. Blood glucose levels decreased both in diabetic rats treated subcutaneously with insulin and in those treated with glucose-responsive MNs within 1 and 2 h, respectively, after a single administration. However, glucose-responsive MNs were able to maintain the blood glucose levels below 200 mg/dL over a slightly longer period (i.e., 3 h versus 2 h) in comparison with subcutaneous insulin. The lack of anti-hyperglycemic effect observed with the non-glucose responsive insulin-loaded MNs demonstrated that, in the absence of glucose oxidase, the inclusion complex cap maintained the nanopores closed, hence preventing insulin release and leading to a similar blood glucose profile as
the one observed for the control group.

Alternatively, Wang et al. created a core-shell MN patch that supplemented H$_2$O$_2$-triggered insulin release with H$_2$O$_2$-scavenging properties to mitigate tissue damage from H$_2$O$_2$-mediated oxidative stress [42]. The shell of the MNs consisted of a thin layer of crosslinked polyvinyl alcohol (PVA) embedded with acrylated nanogels containing catalase. The core of the MNs consisted of a PVA-based gel crosslinked by a H$_2$O$_2$-cleavable linker based on a phenylboronic ester. This core was loaded with insulin and glucose oxidase. Insulin was chemically linked to the PVA via a H$_2$O$_2$-labile carbamate linkage also based on a phenylboronic ester. Glucose oxidase was encapsulated into acrylated nanogels to restrict its leakage across the polymer matrix. Under hyperglycaemic conditions, the locally generated H$_2$O$_2$ hydrolyses both the PVA crosslinkers and the carbamate linker, triggering the rapid release of insulin (Fig. 2c). Using a streptozotocin-induced mouse model of type 1 diabetes, a single administration of these core-shell MN arrays at an insulin dose of 50 mg/kg was able to reduce blood glucose levels to normoglycaemic values within 30 min and maintain those levels below 200 mg/dL over 3.5 h. Notably, when catalase was included in the MN core, it did not affect the local concentration of H$_2$O$_2$ in the MN core, since analogous blood glucose profiles were observed for MN patches without the catalase shell. Nonetheless, when the catalase nanogels were included in the MN core, premature in situ exhaustion of H$_2$O$_2$ prevented insulin release and, consequently, no reduction in blood glucose levels was observed (Fig. 2d). Altogether, these results highlighted the need for separation of catalase in the shell to maintain a high local H$_2$O$_2$ level in the core of these insulin delivery systems. The catalase shell, however, overall improved the biocompatibility of the MN array by scavenging H$_2$O$_2$ tissue levels. Moreover, these H$_2$O$_2$-triggered core-shell MNs also confirmed their resistance to an in vitro hyperglycaemic glucose tolerance test conducted in diabetic mice 1 h post-MN administration. The results obtained were analogous to those achieved with healthy mice. These glucose-responsive MN arrays also showed a reduced risk of hyperglycaemia in healthy mice in comparison with subcutaneously administered insulin.

2.3. pH-triggered glucose oxidase-based MN systems

The glucose oxidase-induced acid microenvironment associated with high glucose concentrations can also be used to trigger insulin release. Materials that are responsive to changes in pH can be incorporated in delivery systems for this purpose, and this includes those with ionizable groups as well as acid-labile linkers [36]. Protonation or hydrolysis of these materials in an acid environment can lead to changes in charge properties and/or aqueous solubility that ultimately trigger insulin release.

Xu et al. developed PVP-based dissolving MNs containing pH-responsive mesoporous bioactive glass nanoparticles [43]. Insulin, glucose oxidase and catalase were encased into the mesopores of the bioactive glass material, which were then copped via electrostatic interactions with zinc oxide (ZnO) quantum dots. The glucose oxidase-induced acid microenvironment achieved under hyperglycaemic conditions resulted in the dissolution of the ZnO quantum dots, opening the pores and subsequently releasing insulin. The antidiabetic effect of the pH-responsive MN arrays was evaluated in streptozotocin-induced diabetic Sprague Dawley rats. Blood glucose levels decreased both in animals treated subcutaneously with insulin and transdermally treated with glucose-responsive MN arrays within 1 and 2 h, respectively, after single administration of the same insulin dose (20 IU/kg). This showed that the MNs were completely dissolved following penetration in the skin, with nearly complete relative bioavailability in comparison with subcutaneous insulin injection. The lack of antihyperglycaemic effect observed with the non-glucose responsive insulin-loaded MN arrays demonstrated that, in the absence of glucose oxidase, the ZnO quantum dot cap maintained the mesopores closed, hence preventing insulin release. This resulted in a similar blood glucose profile to the one observed in the group treated with drug-free MN arrays. Furthermore, the risk of hypoglycaemia after treatment with the pH-responsive MN arrays was evaluated in healthy Sprague Dawley rats. Blood glucose levels of healthy rats treated with the pH-responsive MN arrays were maintained around 130 mg/dL, while the glycaemia of healthy rats treated with MNs containing free insulin at the same dose (20 IU/kg) was decreased to 60 mg/dL, indicating a reduced risk of hypoglycaemia when using the glucose-responsive transdermal insulin delivery system.

Subsequently, these authors assessed an alternative approach of coating the insulin-loaded mesoporous bioactive glass nanoparticles with a multifunctional enzyme layer consisting of polyethyleneimine (PEI, ionizable group), glucose oxidase and catalase [44]. In this case, the glucose oxidase-induced acid microenvironment achieved upon glucose diffusion through the PVP-based MN arrays leads to PEI protonation, which ultimately causes the disruption of the multifunctional coating layer and subsequent insulin release. The antidiabetic effect of these pH-responsive MN arrays was also evaluated in streptozotocin-induced diabetic Sprague Dawley rats. Blood glucose levels decreased both in diabetic rats subcutaneously treated with insulin and transdermally treated with glucose-responsive MN patches within 1 and 2 h, respectively, after single administration at the same insulin dose (15 IU/kg). Notably, this efficacy study lacked a control group treated with non-glucose responsive insulin-loaded MN patches to gain insight into the role played by the glucose-responsive mechanism. Interestingly, the minimum blood glucose levels achieved with glucose-responsive MN patches were higher than those achieved with subcutaneous insulin injection, which could ultimately help prevent the occurrence of hyperglycaemia as a side effect. This trend was maintained in a multiple dose regimen over 24 h with the transdermal insulin delivery system. The reduced risk of hypoglycaemia after treatment with the pH-responsive MN patches was confirmed in healthy Sprague Dawley rats. In fact, whereas the blood glucose levels of healthy rats treated with non-glucose-responsive insulin-loaded MN patches dropped to 56 mg/dL, the use of the pH-responsive insulin MN patches was able to maintain glycaemia around 120 mg/dL at the same insulin dose (30 IU/kg).

Ullah et al. designed stainless-steel MN patches coated with a pH-responsive porous polyactic-co-glycolic acid (PLGA) layer [45]. Insulin, glucose oxidase and sodium bicarbonate were initially embedded in the porous polymer layer. Then, a thinner PLGA layer was applied so that, under hyperglycaemic conditions, glucose can diffuse through this outer layer and be converted to gluconic acid by glucose oxidase. This process decreases the local pH, which ultimately is set to trigger decomposition of sodium bicarbonate into CO$_2$. The generation of CO$_2$ increases the pressure of the system until bursting the external thin PLGA layer and consequently promptly releasing the encapsulated insulin. In vitro insulin release from MN arrays was performed at 37 °C in phosphate buffer saline (PBS) with four different glucose concentrations (0, 100, 200 and 400 mg/dL) over 12.5 h. Notably, insulin release was directly proportional to the glucose concentration in PBS: whereas insulin release was negligible in glucose-free PBS, it reached around 30 %, 70 % and 95 % at 100, 200, and 400 mg/dL, respectively. The antidiabetic effect of these MN patches was evaluated in streptozotocin-induced diabetic Sprague Dawley rats. Blood glucose levels decreased to normoglycaemia over at least 7 h upon transdermal administration of the glucose-responsive MN arrays at an insulin dose of 10 mg/kg. However, the control group was just left untreated, limiting straightforward observations about the efficiency of the glucose-responsive release mechanism.

The PVP/PVA dissolving MN patches developed by Hsu et al. were intended for a pulsatile two-stage transdermal insulin release [46]. On the one hand, free insulin was loaded into the MNs for immediate release to reduce blood glucose levels to normoglycemic levels. On the other hand, the MNs also contained pH-sensitive nanovesicles composed of branched self-assembled poly β-amino esters (ionizable groups) encapsulating both insulin and glucose oxidase for a delayed release in response to recovery of hyperglycaemic levels. With the enzymatically-
induced acidification produced under hyperglycaemic conditions, poly β-amino esters protonated and became more hydrophilic, triggering nanovesicle disassembly and insulin release (Fig. 2e). This pH-triggered release mechanism was firstly demonstrated in agarose gels containing different glucose concentrations, following insertion of MN arrays. Then, the antidiabetic effect of this approach was evaluated in streptozotocin-induced type 1 diabetic Sprague Dawley rats. Notably, rats receiving subcutaneous insulin had to be given 2.88 IU/kg every 6 h to attain normoglycemic levels, whereas the glucose-responsive MN patches maintained normoglycemic levels for up to nearly 13 h after a single dose administration (split into 3.48 IU/kg free insulin and 2.88 IU/kg as insulin-loaded glucose-responsive nanovesicles) (Fig. 2f). This transdermal system enabled a 2.2-fold higher insulin dose to be administered in comparison with the subcutaneous route, but with no apparent risk of hypoglycaemia. Importantly, this efficacy study included a group treated with non-glucose responsive insulin-loaded nanovesicles incorporated in the MN patches. In this group, blood glucose levels followed the same profile as the one observed following subcutaneous insulin injection, with a rapid drop to normoglycemia and a recovery of hyperglycaemia 6 h after treatment. This profile correlated with the release of non-encapsulated insulin, which serves as a loading dose, but not with the release of insulin from the nanovesicles, since poly β amino esters maintained their integrity in the absence of glucose oxidase. Altogether, this demonstrated the benefits of pH-responsive glucose oxidase-mediated transdermal insulin delivery. Furthermore, this study pioneered the incorporation of a second glucose oxidase-loaded MN patch with H2O2-sensitive properties to timely monitor blood glucose levels by a colour change in the presence of hyperglycaemia.

Luo et al. developed an alternative transdermal pH-sensitive MN patch [47]. Dissolving MNs were fabricated with PVP and simultaneously incorporated two types of nanoparticles. On the one hand, insulin was loaded into pH-sensitive nanoparticles made from a methacrylic acid-derived PEGylated polymer with pH-responsiveness (i.e., poly-(2-(hexamethylenimino) ethyl methacrylate)). The tertiary amines of this polymer protonate in the mild acidic environment induced by glucose oxidase under hyperglycaemic conditions and this hydrophobic to hydrophilic transition triggers insulin release through nanoparticle dissociation. On the other hand, glucose oxidase and catalase were loaded into pH-insensitive nanoparticles to minimize the leakage of both enzymes. These pH-insensitive nanoparticles were made from a methacrylic acid-derived PEGylated polymer devoid of pH-responsiveness as it lacked tertiary amino groups (i.e., poly-(2-cyclohexylethylmethacrylate)). The antidiabetic effect of these MN patches was evaluated in streptozotocin-induced type 1 diabetic mice. The mice were divided into five groups treated with: i) empty MN arrays (control group), ii) alumina MN arrays containing insulin, iii) uncapped core-shell MN arrays containing insulin, iv) capped core-shell MN arrays containing insulin with static caps (i.e., prepared with an enzyme-free chitosan hydrogel), and v) capped core-shell MN arrays containing insulin with dynamic caps (i.e., prepared with an enzyme-loaded chitosan hydrogel). The insulin dose was 2 mg in all groups. The efficacy study demonstrated that the treatment in group v was the only one able to regulate blood glucose levels within normoglycemic values (i.e., below 200 mg/dL) for 5 h, before gradually increasing back to hyperglycaemic levels. In the absence of glucose oxidase (group iv), the static cap maintained its “off” configuration, preventing insulin release, and leading to a similar blood glucose profile to the one observed in the control group. As expected, for both uncapped MN arrays (groups ii and iii), although normoglycemic levels were rapidly achieved, hyperglycaemic levels were recovered shortly after. To gain further insight into the glucose responsiveness of the insulin-loaded alumina MN arrays and dynamically-capped core-shell MN arrays, an intraperitoneal glucose tolerance test was conducted 1 h post-administration of the MNs. The insulin dose was 10 mg/kg in both groups for this test. Remarkably, diabetic mice treated with the glucose-responsive MN array behaved similarly to the control healthy mice in terms of blood glucose levels, whereas the mice treated with the uncapped MN array showed continuously rising values until reaching hyperglycaemic levels. Lastly, the risk to cause hypoglycaemia due to insulin release under normoglycemic conditions was tested in healthy NMRI mice. The dynamically-capped insulin-loaded core-shell MN arrays maintained normoglycemic levels over 2 h, whereas the uncapped insulin-loaded MN array caused severe hypoglycaemia in the animals, with blood glucose levels below 50 mg/dL. Altogether, the results from this study thoroughly evidenced both in vitro and in vivo the glucose-responsive gating effect of the enzyme-loaded chitosan hydrogel.

More recently, Tan et al. designed glucose-responsive swelling MNs made of a cationic polylysine-modified silk fibroin hydrogel, loaded with insulin and glucose oxidase [49]. Under hyperglycaemic
conditions, the glucose oxidase-induced acid environment weakens the electrostatic interaction between insulin and cationic silk fibroin due to the progressively increasing positive charge of the amino groups in lysine residues and decreasing negative charge of insulin (isoelectric point of 5.3), ultimately leading to insulin release. This pH-triggered mechanism of insulin release from the cationic silk fibroin MNs was first demonstrated in vitro in dissolution media containing different glucose concentrations. When subsequently assessed in vivo in a streptozotocin-induced rat model of type 1 diabetes, these MNs were more efficient to prevent the sudden increase in blood glucose levels after feeding than an insulin injection. Unfortunately, the study does not provide details on the route of administration for insulin injection, making it difficult to analyse the results in comparison with other studies.

2.4. Dually triggered glucose oxidase-based MN systems

To further enhance the glucose responsiveness of smart transdermal insulin delivery systems based on glucose oxidase, some MN patches have been designed to sense more than one stimulus (i.e., hypoxia, H$_2$O$_2$ and pH).

The first of these studies combined the hypoxia-responsive mechanism described for 2-nitroimidazole groups with the H$_2$O$_2$-driven translocation of thioether linkages to hydrophilic sulfones. In this case, Yu et al. developed a dual hypoxia- and H$_2$O$_2$-triggered hyaluronic acid-based MN patch [50]. For that purpose, MNs contained polymersomes made of a diblock polyethylene glycol (PEG)-polysilane copolymer and encapsulating both insulin and glucose oxidase. The polysilane backbone was modified with 2-nitroimidazole (for hypoxia responsiveness) via a thioether linker (for H$_2$O$_2$ responsiveness), so that under hypoglycaemic conditions the resulting sulfone groups would increase the water solubility of the copolymer and lead to polymersome dissociation and subsequent insulin release. In a streptozotocin-induced mouse model of type 1 diabetes, this dually-triggered mechanism was able to slightly prolong over 1–2 h the time that blood glucose levels remained below 200 mg/dl in comparison with their mono-triggered counterparts [37,40].

Subsequently, the same authors supplemented a H$_2$O$_2$-responsive mechanism with a pH-triggered insulin release system using core-shell MN patches [51]. The core of the MNs embedded nanosized complex micelles loaded separately with insulin and glucose oxidase, whereas the shell of the MNs embedded catalase-loaded nanogels. The insulin-loaded micelles were made of a cationic copolymer via a H$_2$O$_2$-labile phenylboronic ester linkage. Under hypoglycaemic conditions, this highly cationic polymer was hydrolysed by H$_2$O$_2$ and became weakly positively-charged. Together with the reduction of negative charge density on insulin in acidic environment, this weakens the electrostatic interaction between insulin and the copolymer, hence triggering its release. Remarkably, this dually-triggered mechanism was not able to prolong the time during which blood glucose levels remained below 200 mg/dl in a streptozotocin-induced mouse model of type 1 diabetes in comparison with their H$_2$O$_2$-only triggered counterpart [42].

3. Phenylboronic acid-based MN systems

More recently, glucose-responsive MN patches based on phenylboronic acid have been developed as an alternative to those based on glucose oxidase. Phenylboronic acid is a synthetic molecule that can act as a glucose sensor given its Lewis acid features, which enables it to reversibly form esters with cis-1,2 or cis-1,3 diols, such as glucose [52]. In aqueous media, phenylboronic acid exists in equilibrium in both an uncharged trigonal configuration and an anionic tetrahedral boronate structure. Despite both configurations being able to reversibly bind with glucose and form cyclic phenylborate complexes, the anionic boronate structure exhibits a much higher binding affinity. Given the high pKa of phenylboronic acid (8.6), at physiological pH (7.4) the equilibrium is mostly shifted towards the uncharged form, resulting in low glucose responsiveness. Hence, to boost glucose responsiveness at physiological pH, the phenylboronic acid included in glucose-responsive MNs is chemically modified to make it more acidic and eventually reduce its pKa. To this end, both electron-withdrawing moieties (i.e., halogens, nitro or carbonyl groups) and ortho-substituents able to form intramolecular coordination bonds (i.e., between B and N or B and O) have been introduced in the aromatic ring of the phenylboronic acid [53–55].

Until now, the smart insulin delivery mechanisms exploited with phenylboronic acid-based MNs include charge switch-triggered swelling, glucose competitive displacement and crosslinking dissociation (Fig. 3). Table 2 compiles all phenylboronic acid-based MN systems for transdermal insulin delivery with published results on their antidiabetic effect evaluated in pharmacologically induced diabetic animal models.

3.1. Charge switch-triggered phenylboronic acid-based MN systems

The first transdermal glucose-responsive insulin delivery systems based on phenylboronic acid exploited the equilibrium shift from the uncharged planar configuration to the anionic tetrahedral boronate structure upon glucose binding. Accordingly, these systems consist of polymers grafted to uncharged phenylboronic acid moieties that increase their negative charge density in the presence of glucose. This leads to polymer swelling due to electrostatic repulsion between polymer chains and a weakening of the binding strength with negatively charged insulin. As a result, this glucose-triggered swelling enables insulin release (Fig. 3a).

In fact, in a proof-of-concept study, Wang et al. developed a dissolving PVP MN patch containing a polymer-insulin complex based on this mechanism [56]. The polymer incorporated both amino groups, which are positively charged at physiological pH, and 3-fluorophenylboronic acid moieties, which are negatively charged only upon glucose binding in hyperglycaemic conditions. As a result, under hyperglycaemia, the decrease in the net positive charge of the polymer weakens its electrostatic attraction to insulin, facilitating insulin release. First, the glucose-responsive insulin release from these MN patches was demonstrated in vitro. Then, the antidiabetic effect of the MN patches was assessed in a streptozotocin-induced type 1 diabetes mouse model at a dose of 2 mg of insulin per patch. Notably, blood glucose levels of diabetic mice decreased to nearly 100 mg/dl and remained below 200 mg/dl for 5 h, which was longer than for mice treated with non-responsive insulin-loaded MN patches. Moreover, this smart transdermal insulin delivery system was able to respond to an intraperitoneal glucose tolerance test conducted 3 h post MN administration.

Subsequently, Yu et al. developed an alternative glucose-responsive swelling MN patch made of a charge-switchable crosslinked copolymerized matrix of 1-phenyl-2-pyrrolidinone, 2-(dimethyl-amino) ethyl acrylate and 3-(acyr-l-amido) phenylboronic acid [57]. In this case, the MNs were non-degradable and the pKa of the 3-(acyl-amido) phenylboronic acid moieties was reduced with the introduction of the Lewis base (dimethyl-amino) ethyl acrylate in the copolymer backbone, which stabilizes the boronate ester via electrostatic interactions with protonated dimethyl-amino groups. Notably, a single administration of the glucose-responsive MN patches maintained blood glucose levels below 200 mg/dl in diabetic mice for longer than 9 h, at an insulin dose of 0.5 mg. Moreover, this study pioneered the evaluation of the antidiabetic effect of this approach in a larger animal model of diabetes (i.e., Göttingen minipigs). In diabetic minipigs, MN patch administration regulated blood glucose levels over 20 h under normal feeding conditions, at an insulin dose of 7 mg. In both animal models, glucose tolerance tests performed 4 h after MN administration revealed that the glucose challenge triggered insulin release from glucose-responsive MN patches to recover normoglycemic values.

Based on an analogous design, the same authors developed a nondegradable swelling MN patch for dual glucose-responsive release of both insulin and glucagon. The aim of this approach is to function as a
synthetic artificial pancreas that can release both hormones upon hyperglycaemic and hypoglycaemic conditions, respectively [58]. This would eventually preclude the risk of acute hypoglycaemia. The hybrid patch comprises 25% of MNs loaded with glucagon and 75% of MNs loaded with insulin to account for the percentages of α- and β-cells in human pancreas islets, respectively. Both types of MNs are made of a copolymerized matrix of 3-(acryl-amido) phenylboronic acid, 2-aminoethyl methacrylate hydrochloride and 1-vinyl-2-pyrrolidinone but at a different monomer ratio to be selectively responsive to either hyper- or hypoglycaemia. Accordingly, the insulin-loaded MNs were prepared at a higher 2-aminoethyl methacrylate hydrochloride/3-(acryl-amido) phenylboronic acid ratio than the glucagon-loaded ones (2.6 vs 1.4). Positively charged 2-aminoethyl methacrylate hydrochloride interacts with negatively charged insulin whereas electrically neutral glucagon does not. Under hyperglycaemic conditions, the formation of negatively charged cyclic boronate esters with glucose decreases the net positive charge of the polymer network. In the insulin-loaded MNs, this triggers insulin release due to electrostatic displacement. However, in the glucagon loaded-ones, it prevents glucagon release due to matrix shrinkage by neutralization of the originally positively charged polymer network with the negatively charged boronate esters. Conversely, under hypoglycaemic conditions, the insulin-loaded MNs recover their initial net charge retaining the cargo, whereas the glucagon-loaded ones release their cargo due to matrix swelling by electrostatic repulsion between positively charged 2-aminoethyl methacrylate hydrochloride moieties, in the absence of negatively charged cyclic boronate esters with glucose. In a streptozotocin-induced diabetic mouse model, the integration of both types of MNs within a single patch reduced the risk of hypoglycaemia in comparison with the insulin-only counterpart. Moreover, this hybrid patch minimized the risk of hypoglycaemia in comparison with the insulin-only patch in the setting of a simulated delayed meal. Lastly, in the diabetic mouse model, the dual patch also minimized the risk of hypoglycaemia in comparison with the insulin-only patch in the setting of a simulated delayed meal.

The most recent charge switch-triggered glucose-responsive transdermal insulin delivery system based on phenylboronic acid used a dissolving MN patch made of a PVA and PVP hydrogel embedded with phenylboronic acid-modified chitosan particles containing insulin [60]. Under hyperglycaemic conditions, the ionization of the 4-carboxy-3-fluorophenyl boronic acid derivative leads to swelling of the chitosan particles and drives insulin release. To prolong the glucose-responsive insulin release process, the particles were embedded in the PVP/PVA hydrogel. This MN patch achieved blood glucose control within 4 h for up to 8 h in a streptozotocin-induced diabetic rat model at an insulin dose of 9.75 IU.

Fig. 3. Schematic illustration of the glucose-responsive insulin delivery mechanisms based on phenylboronic acid: a) charge switch-triggered swelling, b) competitive displacement and c) crosslinking dissociation. All images have been created with biorender.com.
Table 2
Compilation of the glucose-responsive microneedles based on phenylboronic acid for smart transdermal insulin delivery. * Normoglycemic levels refer to blood glucose levels below or around 200 mg/dL for rodents in each case. ip: intraperitoneal, po: peroral, iv: intravenous, PBA: phenylboronic acid.

<table>
<thead>
<tr>
<th>Trigger stimulus for smart insulin release</th>
<th>Microneedle material</th>
<th>Glucose-responsive element</th>
<th>Animal model</th>
<th>Insulin dose</th>
<th>Onset of action</th>
<th>Duration of normoglycemic levels*</th>
<th>Resistance to glucose challenge (administration route)</th>
<th>Risk of hypoglycaemia in healthy animals</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Charge switch</td>
<td>Polyvinylpyrrolidone</td>
<td>Charge-switchable polymer complex</td>
<td>Streptozotocin-induced diabetic male C57B6 mice</td>
<td>2 mg</td>
<td>≥1 h</td>
<td>≥5 h</td>
<td>Yes (ip)</td>
<td>Not evaluated</td>
<td>[56]</td>
</tr>
<tr>
<td>Crosslinked polyvinylpyrrolidone</td>
<td>Charge-switchable polymer matrix</td>
<td>Streptozotocin-induced diabetic male C57B6 mice and streptozotocin-induced diabetic male Gottingen minipigs</td>
<td>0.5 mg (mice) 7 mg (minipigs)</td>
<td>1 h (mice) 2 h (minipigs) 9 h (mice) 20 h (minipigs)</td>
<td>Yes (ip for mice; po and iv for minipigs)</td>
<td>Not evaluated</td>
<td>Not evaluated</td>
<td>[57]</td>
<td></td>
</tr>
<tr>
<td>Crosslinked polyvinylpyrrolidone</td>
<td>Two charge-switchable polymer matrixes</td>
<td>Streptozotocin-induced diabetic male C57B6 mice</td>
<td>0.729 mg (mice) 4.8 mg (minipigs)</td>
<td>2 h (mice) 2 h (minipigs) 8 h (mice) 22 h (minipigs)</td>
<td>Yes (ip for mice; po for minipigs)</td>
<td>Not evaluated</td>
<td>Not evaluated</td>
<td>[59]</td>
<td></td>
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<tr>
<td>Crosslinked polyvinylpyrrolidone</td>
<td>Charge-switchable chitosan nanoparticles</td>
<td>Streptozotocin-induced diabetic Sprague Dawley rats</td>
<td>9.75 IU</td>
<td>4 h</td>
<td>8 h</td>
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<td>Not evaluated</td>
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<td>Competitive displacement</td>
<td>Mixture of polyvinylpyrrolidone and polyvinyl alcohol</td>
<td>Streptozotocin-induced diabetic Sprague Dawley rats</td>
<td>17.5 nmol</td>
<td>1 h</td>
<td>≥24 h</td>
<td>Not evaluated</td>
<td>Not evaluated</td>
<td>Not evaluated</td>
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</tr>
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<td>Crosslinked alginate</td>
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<td>Streptozotocin-induced diabetic C57B6 mice</td>
<td>35 nmol</td>
<td>1 h</td>
<td>≥24 h</td>
<td>Not evaluated</td>
<td>Not evaluated</td>
<td>Not evaluated</td>
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</tr>
<tr>
<td>Crosslinked alginate</td>
<td>Glucose-responsive PBA-insulin diol complex</td>
<td>Streptozotocin-induced diabetic Sprague Dawley rats</td>
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<td>1 h</td>
<td>11 h</td>
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<td>Not evaluated</td>
<td>Not evaluated</td>
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<td>Crosslinking dissociation</td>
<td>Mixture of crosslinked methacrylated hyaluronic acid and 3-aminomethyl phenylboronic acid</td>
<td>Streptozotocin-induced diabetic Sprague Dawley rats</td>
<td>0.049 IU/g</td>
<td>2 h</td>
<td>6 h</td>
<td>Not evaluated</td>
<td>Not evaluated</td>
<td>Not evaluated</td>
<td>[65]</td>
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<td>Crosslinking dissociation</td>
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<td>0.042 IU/g</td>
<td>6 h</td>
<td>4 h</td>
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<td>Not evaluated</td>
<td>Not evaluated</td>
<td>[67]</td>
</tr>
<tr>
<td>Crosslinking dissociation</td>
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<td>12 IU/kg</td>
<td>3 h</td>
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<td>[68]</td>
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<td>Streptozotocin-induced diabetic Sprague Dawley rats</td>
<td>25 IU/kg</td>
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<td>Not evaluated</td>
<td>[69]</td>
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<td>Streptozotocin-induced diabetic Sprague Dawley rats</td>
<td>0.062 IU/g</td>
<td>4 h</td>
<td>5 h</td>
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<td>No</td>
<td>[70]</td>
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</table>
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3.2. Competitive displacement-triggered phenylboronic acid-based MN systems

Alternative transdermal glucose-responsive insulin delivery systems based on phenylboronic acid have subsequently been designed. In these systems, insulin analogues modified with diol-containing groups were conjugated to phenylboronic acid analogues so that the binding of glucose to phenylboronic acid competitively displaces insulin and ultimately leads to its release (Fig. 3b) [61].

The first system exploiting this mechanism was developed by Wu et al. [62]. To benefit from the ability of phenylboronic acid derivatives to reversibly bind to cis-diol moieties, the authors conjugated gluconic acid-modified insulin to two different phenylboronic acid derivatives (4-carboxyl-3-fluorophenylboronic acid and 3-aminophenylboronic acid) to develop two distinct MN patches. As phenylboronic acid forms more stable cyclic boronic esters with the cis-diols of glucose than with acyclic diols, under hyperglycaemic conditions phenylboronic acid derivatives preferably bind to glucose, competitively displacing and releasing the insulin analogue. Conversely, under hypoglycaemic levels, only a limited amount of glucose competes with the bound insulin and consequently insulin release is prevented. Both MN patches were made of an alginate matrix, with swelling properties that ultimately enabled glucose diffusion and insulin release. The performance of this release mechanism from both MN patches was first demonstrated in vitro, where insulin release showed a strong dependency on glucose concentration in the release medium. Then, the antidiabetic effect of the smart MN patches was assessed in a streptozotocin-induced type 1 diabetes mouse model at a dose of 17.5 nmol of insulin per patch. Notably, with a single administration, the MN patches maintained normoglycemic levels in diabetic mice after only 1 h for up to 17 h (in the case of 4-carboxyl-3-fluorophenylboronic acid-based patches) or 24 h (in the case of 3-aminophenylboronic acid-based patches) with no associated risk of hypoglycaemia. The authors reported slightly longer normoglycaemia times than those 17 and 24 h values, respectively, because they considered a higher upper limit for normoglycemic levels, fixed herein at 200 mg/dL for the sake of consistency among all studies. Moreover, this approach was able to alleviate some disease symptoms in the diabetic mice, namely thirst, polyuria, and body weight loss, together with reduced glycated albumin levels observed 4 days after administration.

In an analogous study, the same authors developed swelling alginate-based MN patches integrated with glucose-responsive gold nanoclusters to enhance the mechanical strength of the MNs while maintaining a glucose-responsive release mechanism [63]. For this purpose, gluconic acid-modified insulin was conjugated with up to three different phenylboronic acid derivatives (4-carboxyphosphorylboronic acid, 4-carboxyl-3-fluorophenylboronic acid or 4-aminophenylboronic acid) to develop three distinct MN patches (Fig. 4d). In all cases, in vitro insulin release from the MN patches highly correlated with glucose concentration in the release medium due to competitive displacement. A single administration of each of these MN patches was able to maintain normoglycemic levels in streptozotocin-induced diabetic mice for up to 24 h (Fig. 4e), while alleviating the disease symptoms observed in these mice. Based on the same premise, the authors also integrated the glucose-responsive gold nanoclusters into dissolving MNs made of a mixture of gelatin and starch [64]. In this case, a single administration of the dissolving MN patches was also able to maintain normoglycemia in type 1 diabetic mice for approximately 24 h regardless of the phenylboronic acid derivative used.

Following the same trend, Zong et al. prepared swelling MNs made of hyaluronic acid modified with methacrylic acid and 3-aminomethyl phenylboronic acid [65]. Glucic acid-modified insulin was conjugated to the phenylboronic acid derivative to exploit the competitive displacement glucose-responsive release mechanism. In vitro, these MN patches showed approximately a 2-fold increase in both the amount and rate of insulin release under simulated hyperglycaemic conditions in comparison with normoglycaemic conditions. The antidiabetic effect of these MN patches was evaluated in a streptozotocin-induced diabetic rat model. Results showed that, after a single administration, the glucose-responsive MN patches were the only treatment that maintained normoglycemic levels for up to 11 h under feeding conditions with no risk of hypoglycaemia, despite using an insulin dose 10 times higher than that of the subcutaneous insulin and non-glucose responsive MN groups (i.e., 4 U vs 0.4 U).

3.3. Crosslinking dissociation-triggered phenylboronic acid-based MN systems

A third type of transdermal glucose-responsive insulin delivery systems based on phenylboronic acid has been developed more recently (Fig. 3c). These systems rely on the competitive dissociation of reversible phenylboronate ester bonds formed between phenylboronic acid derivatives and diol moieties on the carrier material in the presence of high levels of glucose. This crosslinking dissociation ultimately leads to glucose-triggered insulin release [55].

The first systems exploiting this dissociation mechanism were developed by Chen et al. and did not show differential insulin release under different glucose concentrations in vitro. These authors developed, on the one hand, a MN patch composed of a hydrogel made of PVA and sodium hyaluronate grafted with 4-(2-aminoethyl)carbamoyl-3-fluorophenylboronic acid [66], and, on the other hand, a MN patch made of a hydrogel formed by PVA and polyallylamine grafted with 3-carboxy-4-fluorophenylboronic acid [67]. In both cases, the gel was crosslinked through boronate ester bonds between the phenylboronic acid derivative and the diol groups of the PVA. Theoretically, under hyperglycaemic conditions, glucose would competitively reduce this crosslinking density to enable insulin to be released in a concentration-dependent manner. In both cases, the in vivo antidiabetic study in type 1 diabetic rats lacked a non-responsive insulin-loaded MN group to evidence the efficiency of the glucose-responsive release mechanism. As a result, these studies only showed a prolonged release effect for these MNs...
patches.

Alternatively, Fu et al. used this competitive dissociation approach to dynamically drive the opening of an on/off gating system in MSNs. The authors developed a dissolving hyaluronic acid-based MN patch containing insulin-loaded MSNs modified with glucosamine and capped with zinc oxide nanoparticles [68]. The glucose-responsive on/off gating system was constructed by utilising dynamic ester bonds between diol groups of glucosamine and an arachnate-functional phenylboronic acid derivative grafted to the zinc oxide dots. Under normoglycemic conditions, the boronic ester bond is stable and the mesopores remain capped. Conversely, under hyperglycemic conditions the bond is competitively cleaved, leading to dissociation of zinc oxide dots, uncapping of the pores and insulin release. The antidiabetic effect of this MN patch was tested in streptozotocin-induced type 1 diabetic mice. Mice were divided into three groups treated with: i) empty MN patches, ii) subcutaneous insulin injection, and iii) MN patches with dynamically-capped insulin-loaded MSNs. The insulin dose was 12 IU/kg in all groups. Results showed that the group treated with the glucose-responsive MN patches was able to maintain normoglycemia within 3 h and for approximately 3 h longer than the group treated with subcutaneous insulin, before gradually recovering hyperglycemic levels. In addition, an intraperitoneal glucose tolerance test was conducted 3 h post-MN administration. Interestingly, diabetic mice treated with the glucose-responsive MN patch behaved similarly to control healthy mice in terms of blood glucose levels in response to the glucose challenge, whereas mice treated with subcutaneous insulin showed rising blood glucose levels until reaching hyperglycemia (> 400 mg/dL). The hypoglycemia risk due to insulin release under normoglycemic conditions was also tested in healthy mice. Glucose-responsive insulin-loaded MN patches maintained normoglycemic levels over 4 h, whereas subcutaneous insulin caused hypoglycemia (55 mg/dL) in 1.5 h. Lastly, this treatment was tested in a multiple dose regimen following daily administration over 4 weeks. Notably, mice treated with the glucose-responsive insulin-loaded MN patches exhibited similar glycosylated haemoglobin levels to healthy mice. However, all these in vivo studies also lacked a non-responsive insulin-loaded MN group to rule out the contribution of the administration route to the overall results and ultimately evaluate the efficiency of the glucose-responsive gating effect.

Subsequently, Ye et al. developed another transdermal glucose-responsive insulin delivery system exploiting the crosslinking dissociation mechanism [69]. The MN patch consisted of a hydrogel made of a synthetic polymer modified with pyridiniumboronic acid motifs, which were complexed with diol groups from a PEG macromer. The crosslinking was supposed to be dynamically reversible to provide a glucose-responsive trigger for insulin release. However, in vitro release studies were not conducted with the final MN patches to demonstrate it. As it occurred in the previous studies of this section, the in vivo antidiabetic study in type 1 diabetic rats lacked a non-responsive insulin-loaded MN group to evidence the efficiency of the glucose-responsive release mechanism in vivo, which only allowed a prolonged release effect over up to 8 h to be observed for these MN patches.

More recently, Chen et al. developed a core-shell MN patch based on this crosslinking dissociation mechanism that showed an insulin release profile responsive to different glucose levels in vitro [70]. The shell of these MNs was composed of PVA and ε-polylysine modified with 4-carboxy-3-fluorophenylboronic acid via reversible borate ester bonds, whereas the core was composed solely of insulin-loaded PVA. Under hyperglycemic conditions, the crosslinking density of the shell is reduced by competitive dissociation of the borate ester bonds in the presence of glucose. As a result, insulin in the core can diffuse through the shell (Fig. 4f). Notably, the authors demonstrated that by reducing shell thickness, the in vitro insulin release rate was increased as a function of glucose concentration, probably due to a decrease in the diffusion path. The antidiabetic effect of this MN patch was tested in streptozotocin-induced type 1 diabetic rats. Results showed that groups treated with glucose-responsive MN patches needed longer times to reduce blood glucose levels to normoglycemic values than the ones treated with subcutaneous insulin (Fig. 4g). This was ascribed to the onset time that MNs need upon insertion to achieve a sufficient cross-linking density reduction that enables insulin release. Indeed, this onset time was directly correlated with the thickness of the MN shell, being higher with the thickest shell. Moreover, MN shell thickness inversely correlated with the risk of hypoglycemia upon treatment with glucose-responsive MN patches, since the thinnest shell led to a massive insulin release, as observed in vitro. In addition, this treatment given as a single dose was tested in a daily blood glucose management setting with alternating fasted and fed periods. During the second fasted period following feeding, antihyperglycemic rate of animals treated with glucose-responsive MN patches outperformed not only that of those receiving subcutaneous insulin but also the one from the same group during the first fasted period. This indicated that insulin was no longer available for a second fasted period when given subcutaneously, due to potential peptide degradation. This also indicated that insulin release rate from glucose-responsive MN patches was limited by the onset time needed for crosslinking dissociation during the first fasted period and accelerated during the second fasted period thanks to partial previous crosslinking dissociation. Lastly, the risk to cause hypoglycaemia due to insulin release under normoglycemic conditions was also tested in healthy rats. Glucose-responsive insulin-loaded MN patches maintained normoglycemic levels over 8 h, whereas subcutaneous insulin caused hypoglycaemia (and even death) in 1 h. These results demonstrated that insulin release from the glucose-responsive MN patches was restrained by the MN shell under normoglycemic conditions.

4. MN systems based on the combination of glucose oxidase and phenylboronic acid

To attempt a synergistic effect between both mechanisms, Tong et al. developed a dialyze smart transferdernal insulin delivery system based on both glucose oxidase and phenylboronic acid [71]. For this purpose, polymersomes loaded with insulin and glucose oxidase were embedded into dissolving MN patches made of PVP and PVA. The polymersomes were self-assembled from an amphiphilic trilobekopolymer including PEG, poly (phenylboronic acid pinacol ester) and poly (3-acylamidophenylboronic acid). On the one hand, the phenylboronic pinacol esters endow the polymersomes with H2O2-responsiveness since ester bonds are hydrolysed by the H2O2 generated during the glucose oxidase-catalysed oxidation of glucose. Ultimately, this renders the copolymer water soluble once it loses its phenylboronic pinacol ester side chains, contributing to polymersome disassembly and insulin release. On the other hand, the 3-acylamidophenylboronic acid groups endow the polymersomes with charge switch-triggered swelling. Indeed, the initially uncharged phenylboronic acid moieties increase their negative charge density upon glucose binding under hyperglycemic conditions, leading to insulin release due to both polymer swelling and weakening of the binding strength between the polymer and negatively charged insulin.

The antidiabetic effect of these MN patches was assessed in streptozotocin-induced diabetic rats, divided into four groups: i) MN patches with empty polymersomes (control group), ii) subcutaneous insulin injection (20 IU/kg), iii) MN patches containing dual glucose-responsive polymersomes loaded only with insulin (40 IU/kg), and iv) MN patches containing dual glucose-responsive polymersomes loaded with both insulin (40 IU/kg) and glucose oxidase. Treatment with MN patches in this last group maintained glucose levels below 200 mg/dL for longer than their counterparts loaded only with insulin (i.e., 4 h versus 2 h), whereas subcutaneous insulin (group ii) rapidly decreased blood glucose levels to 80 mg/dL but failed to maintain them in the following hour. Altogether, these results proved that in the presence of glucose oxidase (group iv), the dual glucose-responsive polymersomes could accelerate insulin release thanks to their H2O2-responsiveness. This resulted in an improved blood glucose profile in comparison with
the one observed for the group with glucose-responsive polymersomes loaded only with insulin.

5. MN systems based on other mechanisms

Alternative glucose-responsive MN-based technologies have recently emerged and are in the pipeline (Table 3). Among these smart glucose-responsive strategies, electrochemical closed-loop systems integrate electrochemical glucose sensors and electronic devices enabling insulin delivery to ultimately serve as real-time personalized theranostic tools for diabetes management. Moreover, to overcome some of the concerns around phenylboronic acid-based systems, such as long-term toxicity, analogous competitive displacement-triggered delivery systems based on physiological glucose-binding molecules have been designed. Nevertheless, even though these novel methods show a new perspective on glucose-responsive strategies for transdermal insulin delivery, results are still far from the efficacy rates of the other strategies presented above. Further research should be conducted to improve these systems.

5.1. Electrochemical closed-loop MN systems

Li et al. synergistically coupled MN patches with i ontophoretic technologies to electrically enhance transdermal permeation of glucose and insulin [72]. Indeed, the authors developed the first MN-based transdermal closed-loop system for simultaneous in situ glucose monitoring and insulin delivery. Coupling with i ontophoretic technologies significantly enhanced both glucose extraction and insulin delivery. The electrochemically tunable closed-loop system consisted of three interconnected modules: i) a glucose monitoring sensor based on mesoporous MNs supplemented with reverse i ontophoretic extraction and electrochemical sensing based on the glucose oxidase-catalysed oxidation of glucose; ii) a flexible printed circuit board capable of electrically controlling the i ontophoretic release of insulin from the third module when hyperglycaemic state was detected, and iii) an insulin delivery component based on mesoporous MNs supplemented with i ontophoresis. Importantly, insulin could be freely loaded in the insulin delivery module without dose limitation. Insulin release from the therapeutic module was tested in vitro using a skin tissue surrogate built with water-impermeable paraffin (to mimic the SC) on top of an agar gel. Insulin release was measured in the presence and absence of an i ontophoretic current (i.e., 0 vs 0.5 mA, respectively). In the absence of i ontophoretic current, only 5.8 ± 0.15 % insulin was released after 3 h, due to continuous free diffusion of insulin through the mesoporous MNs and accounting for the basal delivery of insulin. Conversely, in the presence of an i ontophoretic current of 0.5 mA, insulin release was 2.3-fold higher, which represents an electrically-driven insulin bolus that would minimize postprandial fluctuations in blood glucose levels. Moreover, this closed-loop system demonstrated the ability to accurately track glycaemia fluctuations and responsive release insulin to regulate hyperglycaemia in a diabetic rat model. Overall, blood glucose levels of diabetic rats treated with the mesoporous MNs supplemented with i ontophoretic current maintained normoglycemic levels for longer (3.3 h) than rats treated with either subcutaneous insulin injection (1.7 h) or mesoporous MNs without i ontophoretic current (0.3 h).

More recently, Luo et al. developed an alternative electrochemically controlled transdermal closed-loop system for insulin delivery which, unlike the previous one, unprecedentedly integrated the glucose sensing and insulin delivery systems within a single MN patch [73]. The system consists of hollow MNs with two layers. The outer layer functions as a glucose electrochemical sensor based on the glucose oxidase-catalysed oxidation of glucose. In the inner part, a flexible electroosmotic pump based on a polycarbonate film with nanoprobes is integrated and connected to a drug reservoir for insulin delivery. A printed circuit board controls the operation of the glucose monitoring sensor and the pump so that insulin delivery rate through the inner channels of hollow MNs is controlled by interstitial glucose levels detected by the biosensor. This closed-loop system was evaluated in a diabetic rat model. In this model, the device efficiently maintained normoglycemic levels under both fasted conditions and following an intraperitoneal glucose challenge, as compared to a single insulin injection. Results showed that, in the absence of the electrochemically-driven closed-loop device, blood glucose levels rapidly increased to hyperglycaemic values, whereas the ones of rats treated with the closed-loop device restored normoglycaemic values after each small rise in blood glucose levels.

Altogether, these studies evidence the benefits of transdermal closed-loop systems to adjust the insulin delivery rate to real-time glucose monitoring by an electrochemical biosensor. However, these sophisticated systems have yet to show results as promising as those obtained with phenylboronic acid and glucose oxidase-based MN patches. Additionally, caveats such as a signal delay caused by limited glucose diffusion from the interstitial fluid to the sensor, inaccurate glucose measurements by the sensor potentially leading to an incorrect insulin levels.

Table 3

Compilation of the glucose-responsive microneedles based on other mechanisms for smart transdermal insulin delivery. * Normoglycemic levels refer to blood glucose levels below or around 200 mg/dL for rodents in each case. ip: intraperitoneal, GLUT: glucose transporter.

<table>
<thead>
<tr>
<th>Trigger stimulus for smart insulin release</th>
<th>Microneedle material</th>
<th>Glucose-responsive element</th>
<th>Animal model</th>
<th>Insulin dose</th>
<th>Onset of action</th>
<th>Duration of normoglycemic levels*</th>
<th>Resistance to glucose challenge (administration route)</th>
<th>Risk of hypoglycaemia in healthy animals</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2O2 (glucose-oxidase-mediated) and charge switch (PBA-mediated)</td>
<td>Mixture of polyvinylpyrrolidone and polyvinyl alcohol</td>
<td>H2O2 and glucose-sensitive triblock copolymersomes</td>
<td>Streptozotocin-induced diabetic Sprague Dawley rats</td>
<td>80 IU/kg</td>
<td>2 h</td>
<td>4 h</td>
<td>Not evaluated</td>
<td>Not evaluated</td>
<td>[71]</td>
</tr>
<tr>
<td>Electrochemical closed-loop (i ontophoresis-based)</td>
<td>Crosslinked polyyglycidyl methacrylate</td>
<td>Mesoporous microneedle-reverse i ontophoretic glucose sensor biosensor</td>
<td>Streptozotocin-induced diabetic male Sprague Dawley rats</td>
<td>5 IU</td>
<td>4 h</td>
<td>3.3 h</td>
<td>Not evaluated</td>
<td>Not evaluated</td>
<td>[72]</td>
</tr>
<tr>
<td>Electrochemical closed-loop (electroosmotic pump-based)</td>
<td>Chitosan</td>
<td></td>
<td>Streptozotocin-induced diabetic male Sprague Dawley rats</td>
<td>7.5 U</td>
<td>2.3 h</td>
<td>1 h</td>
<td>Yes (ip)</td>
<td>Not evaluated</td>
<td>[73]</td>
</tr>
<tr>
<td>Competitive displacement based on GLUT</td>
<td>Crosslinked methacrylated hyaluronic acid</td>
<td>Glucose-responsive GLUT-insulin complex</td>
<td>Streptozotocin-induced diabetic male C57BL/6 mice</td>
<td>10 mg/kg</td>
<td>1.5 h</td>
<td>4.5 h</td>
<td>Yes (ip)</td>
<td>No</td>
<td>[76]</td>
</tr>
</tbody>
</table>
dosing, or the existence of possible interferences must also be taken into account [74,75].

5.2. Competitive displacement-triggered MN systems based on glucose transporter GLUT

Although alternative glucose-binding molecules have been described to trigger glucose-responsive insulin release [36], the physiological glucose-binding transporter GLUT is currently the only molecule that has been used for formulation of glucose-responsive MN systems tested in vivo.

Chen et al. have developed the only transdermal glucose-responsive insulin delivery system based on glucose transporter GLUT described so far [76]. In this system, glucosamine-modified insulin is bound to the glucose transporter GLUT expressed on red blood cell vesicles, loaded into the tips of crosslinked methacrylated hyaluronic acid MNs. Binding of glucose to the transporter competitively displaces insulin and ultimately leads to glucose-triggered insulin release. To overcome the limited amount of glucosamine-modified insulin bound to red blood cell vesicles due to the one-to-one specific interaction between glucosamine and GLUT, additional glucosamin-modified insulin was added to the upper layer of the MN patch to serve as an insulin reservoir. This reservoir is expected to bind to GLUT once it is depleted of glucose (after recovery of normoglycemic levels) for subsequent glucose-responsive insulin release. Moreover, this GLUT-based glucose-responsive insulin release strategy has been extended to liposomes by anchoring recombinant GLUT receptors to their membrane. Interestingly, this formulation strategy exhibited a higher concentration of GLUT receptor in comparison with red blood cell vesicles. The antidiabetic effect of these MN patches was tested in streptozotocin-induced type 1 diabetic mice. GLUT-based MN patches were able to maintain normoglycemia within the first 1.5 h and for approximately 2.5 and 4.5 h in the case of liposomes and red blood cell vesicles, respectively, before gradually increasing back to hyperglycaemic levels. In both cases, normoglycemic levels were maintained for longer than in the group treated with subcutaneous insulin injection. In addition, an intraperitoneal glucose tolerance test was conducted 1 h post treatment. Interestingly, diabetic mice treated with either glucose-responsive MN patch achieved similar blood glucose levels to control healthy mice in response to the glucose challenge, whereas those of mice treated with subcutaneous insulin injection continued to rise until reaching a hyperglycaemic state. The risk of causing hypoglycaemia due to insulin release under normoglycemic conditions was also tested in healthy mice. Both glucose-responsive MN patches demonstrated a reduced risk of hypoglycaemia in comparison with subcutaneous insulin injection.

6. Outlook

Glucose-responsive diabetes treatment has traditionally been regarded as the holy grail of drug delivery. Current insulin replacement therapy requires lifelong subcutaneous injections multiple times per day, depending on glucose levels which are self-monitored through frequent finger-prick tests. This, together with the risk of acute hypoglycaemia derived from the narrow therapeutic index of insulin, often leads to poor glycaemic control and reduced quality of life.

Glucose-responsive insulin delivery systems that emulate β-cell function by releasing insulin in response to elevated glucose levels in a closed-loop manner have been developed to improve glycaemic control and reduce injection frequency [35]. In this regard, glucose-responsive MN array patches have become a research hotspot in this field, since transdermal administration is more user-friendly than subcutaneous injections and enables treatment cessation in case of hypoglycaemia [77]. These smart transdermal insulin delivery systems release insulin in response to interstitial glucose levels, which are well-matched with blood glucose levels [78,79]. As reviewed herein, both chemically- and electrochemically-controlled MN-based transdermal insulin delivery systems have been described (Fig. 5).

Chemically-controlled glucose-responsive systems regulate insulin release via structural transformations (i.e., swelling, disassembly, on/off gating or competitive displacement), which are triggered by elevated glucose levels. To this end, these systems incorporate a glucose-sensing element and a stimuli-responsive material. The most widely used glucose-sensing elements in MN-based systems are glucose oxidase and phenylboronic acid derivatives, despite more recent reports of glucose-binding receptors having also been introduced (Fig. 5a). Stimuli-responsive materials can initiate structural transformation in response to indirect signals correlated with elevated glucose levels, such as hypoxia, elevated H$_2$O$_2$ levels or local acidic pH (when using glucose oxidase as the sensing element) or directly in response to glucose levels (when phenylboronic acid derivatives are used for sensing). Materials can be either loaded in stimuli-responsive vesicles which are then embedded in unresponsive MNs or directly included in MNs that respond to the stimulus themselves (i.e., all-in-one approach). Compared with bulk all-in-one stimuli-responsive systems, vesicles generally exhibit a more pronounced stimulus responsiveness due to their smaller size and bigger specific surface area. However, should the stimuli-responsive vesicles have poor controlled release capacities, hypoglycaemia due to insulin burst release might occur.

The main advantage of using glucose oxidase as the sensing element

Fig. 5. Descriptive statistics of research articles on glucose-responsive micro-needle-based systems for transdermal insulin delivery with their antidiabetic effect tested in pharmacologically induced diabetic animal models. a) Percentage distribution of the various glucose-sensing elements used in glucose-responsive micronneedle patches; b) Percentage distribution of the trigger stimuli exploited for glucose oxidase-based transdermal insulin delivery; c) Percentage distribution of the trigger stimuli exploited for phenylboronic acid-based transdermal insulin delivery.
in glucose-responsive MN patches is its high catalytic selectivity for glucose, which greatly minimizes interferences by other saccharides or diol structures. Hypoxia- [37,38], $\text{H}_2\text{O}_2$- [40–42], pH- [43–49], or dual-responsive [50,51] materials have been included in glucose-responsive MN patches based on glucose oxidase to trigger glucose-responsive insulin release (Fig. 5b). Due to the strong buffering capacity of interstitial skin fluid, pH changes induced by glucose oxidase-catalysed glucose oxidation have traditionally been reported as subtle and slower than the generation of a hypoxic or $\text{H}_2\text{O}_2$-enriched microenvironment. However, a meta-analysis of reported onset times does not reveal significant differences among the various stimuli-responsive materials, even with the use of dual-responsive materials (Fig. 5b).

Notably, most glucose-responsive MN patches based on glucose oxidase use stimuli-responsive nanoparticles, such as polymeric vesicles [37,40,46,47,50,51] or MSNs [41,43,44] embedded in unresponsive MNs. The insulin release mechanism is triggered in most cases by nanoparticle disassembly or on/off gating. Most of these systems co-encapsulate glucose oxidase and insulin within the nanoparticles, leading to simultaneous release of both molecules. This has two associated risks: first, it can cause immunogenicity and, second, it can lead to the loss of glucose responsiveness in the system. Hence, to minimize both risks, some studies have encapsulated glucose oxidase into non-responsive nanostructures separately from insulin [42,47,48,51]. Alternatively, insulin release mechanisms of the scarce all-in-one MN patches based on glucose oxidase include glucose-triggered hydrogel crosslinking dissociation [42] or swelling/deswelling on/off gating of mesopores [48]. One of the biggest challenges of glucose-responsive MN systems based on glucose oxidase is its instability, as the enzyme degrades easily under certain pH or temperature conditions, hindering its activity and glucose-sensing ability over time [80]. This is an aspect that most authors have overlooked and that would be important to address when moving towards clinical translation of these systems.

Alternatively, phenylboronic acid is a small synthetic molecule capable of forming reversible boronate ester bonds with polyols. The main advantage of glucose-responsive MN patches based on this molecule as a glucose-sensing element is that it is less likely to trigger unwanted immune responses, given its non-biological nature, and it is more stable and suitable for long-term storage in comparison with glucose oxidase. However, the biggest challenges of these systems are associated with their limited glucose-sensing properties at physiological pH and low selectivity for glucose, making it susceptible to interferences with other saccharides or diol structures. The first challenge has been overcome by using phenylboronic acid derivatives with a lower pKa, whereas the second challenge remains. The trigger stimuli exploited for smart insulin release with MN patches based on phenylboronic acid derivatives include charge switch-mediated swelling [56–60], competitive displacement [62–65], and crosslinking dissociation (Fig. 5c) [66–70]. Although no overall differences in onset times can be observed for systems based on phenylboronic acid derivatives in comparison with those based on glucose oxidase (Fig. 6a), devices exploiting crosslinking dissociation mechanisms showed significantly higher onset times than those reported for the other trigger stimuli (Fig. 6c). Glucose-responsive MN patches based on phenylboronic acid derivatives can be divided into all-in-one swelling MN patches [57–59,62,65–67,69,70] and

Fig. 6. Outlook of glucose-responsive microneedle-based systems for smart transdermal insulin delivery: a) Onset time to normoglycaemia achieved by the various glucose-sensing elements utilized in glucose-responsive microneedle patches; b) Onset time to normoglycaemia achieved by the trigger stimuli exploited for glucose oxidase-based transdermal insulin delivery; c) Onset time to normoglycaemia achieved by the trigger stimuli exploited for phenylboronic acid-based transdermal insulin delivery; d) Normoglycaemia time achieved by the various glucose-sensing elements utilized in glucose-responsive microneedle patches; e) Normoglycaemia time achieved by the trigger stimuli exploited for glucose oxidase-based transdermal insulin delivery; f) Normoglycaemia time achieved by the trigger stimuli exploited for phenylboronic acid-based transdermal insulin delivery; g) Timeline of publications describing the various glucose-sensing elements used in glucose-responsive microneedle patches; h) Timeline of publications describing the trigger stimuli exploited for glucose oxidase-based transdermal insulin delivery; i) Timeline of publications describing the trigger stimuli exploited for phenylboronic acid-based transdermal insulin delivery. This figure is based on data from research articles reporting glucose-responsive microneedle-based systems for transdermal insulin delivery with their antidiabetic effect tested in pharmacologically induced diabetic animal models. Data were analysed using GraphPad Prism software (version 10.1.0). Statistical analysis was performed using one-way ANOVA followed by a post-hoc Tukey multiple comparison test. Statistical significance was fixed as *: $p < 0.05$, **: $p < 0.01$. 

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balance must be kept between dosing interval and insulin loading capacity, leakage and contamination during the refilling phase. Altogether, a responsive MN patches, as the glucose-responsive component consists of more appropriate, particularly when assisted with a backup insulin dose, to help control hyperglycaemia at the point of administration, due to burst insulin release. It is also important to prolong normoglycemic time by preventing insufficient insulin supply when the MN insulin content is nearly exhausted. On the one hand, to avoid hypoglycaemia, the glucose-responsive material should ideally restore its original configuration when normoglycemic levels are reached. In this regard, all-in-one MNs have an inherent advantage over MNs embedded with glucose-responsive nanovesicles, as these are difficult to reassemble after dissociation. Similarly, charge switch-mediated swelling/deswelling MNs are best suited to restore their initial configuration upon recovery of normoglycemia without crossing an insulin-based or competitive displacement-based systems. The risk of hypoglycaemia can also be minimized by developing a glucose-responsive MN patch for dynamic delivery of insulin and glucagon under hyperglycaemic and hypoglycaemic conditions, respectively [58,59]. This hybrid glucose-responsive MN-based system tunes the release of each hormone through the charge switch-mediated swelling/deswelling of the polymer matrix at distinct glucose concentrations, ultimately acting as a fully functional artificial pancreas. On the other hand, to avoid insufficient insulin supply, patches provided with an externally refillable insulin depot have been developed [48,72,73]. In all three cases, porous hollow MNs were used to take advantage of the void volume provided by the pores for efficient insulin loading. Moreover, these external reservoirs are deemed to have a higher translational potential for those MNs that respond themselves to elevated glucose levels, since commercially available liquid insulin formulations can be directly used to fill the reservoir. The possibility of extending the lifespan of each patch with these depots may also contribute to prolong normoglycemic times, thereby increasing dosing intervals. Notably, the time for maintaining normoglycemic levels needs to be prolonged from current reported values (Fig. 6d–f). Overall, phenylboronic acid was more efficient as a glucose-sensing element to maintain normoglycemia than glucose oxidase [61,84,85]. The longest normoglycemic time achieved with glucose-responsive MN patches based on glucose oxidase was 12 h (Fig. 6e). This system consisted of a dissolving MN patch loaded with both free insulin and insulin encapsulated into pH-responsive nanovesicles for a two-stage pulsatile insulin release [46]. Conversely, several glucose-responsive MN patches based on phenylboronic acid maintained normoglycemia for over nearly one day in distinct animal models (Fig. 6f). Among them, some exploited the charge switch-mediated swelling/deswelling effect of quinone methides are proteins and DNA which could result in toxic effects through their covalent modification. Specifically, it has been reported that the covalent modification of endogenous macromolecules via quinone methides can lead to hepatotoxicity, nephrotoxicity, or both bolus and basal insulin delivery, owing to the possibility to precisely control insulin flow rate through their inner channels.

Since the insulin release rate directly correlates with insulin concentration in the patch and with glucose levels, it is essential to avoid hypoglycaemia at the point of administration, due to burst insulin release. It is also important to prolong normoglycemic time by preventing insufficient insulin supply when the MN insulin content is nearly exhausted. On the one hand, to avoid hypoglycaemia, the glucose-responsive material should ideally restore its original configuration when normoglycemic levels are reached. In this regard, all-in-one MNs have an inherent advantage over MNs embedded with glucose-responsive nanovesicles, as these are difficult to reassemble after dissociation. Similarly, charge switch-mediated swelling/deswelling MNs are best suited to restore their initial configuration upon recovery of normoglycemia without crossing a dissolution-based or competitive displacement-based systems. The risk of hypoglycaemia can also be minimized by developing a glucose-responsive MN patch for dynamic delivery of insulin and glucagon under hyperglycaemic and hypoglycaemic conditions, respectively [58,59]. This hybrid glucose-responsive MN-based system tunes the release of each hormone through the charge switch-mediated swelling/deswelling of the polymer matrix at distinct glucose concentrations, ultimately acting as a fully functional artificial pancreas. On the other hand, to avoid insufficient insulin supply, patches provided with an externally refillable insulin depot have been developed [48,72,73]. In all three cases, porous hollow MNs were used to take advantage of the void volume provided by the pores for efficient insulin loading. Moreover, these external reservoirs are deemed to have a higher translational potential for those MNs that respond themselves to elevated glucose levels, since commercially available liquid insulin formulations can be directly used to fill the reservoir. The possibility of extending the lifespan of each patch with these depots may also contribute to prolong normoglycemic times, thereby increasing dosing intervals. Notably, the time for maintaining normoglycemic levels needs to be prolonged from current reported values (Fig. 6d–f). Overall, phenylboronic acid was more efficient as a glucose-sensing element to maintain normoglycemia than glucose oxidase [61,84,85]. The longest normoglycemic time achieved with glucose-responsive MN patches based on glucose oxidase was 12 h (Fig. 6e). This system consisted of a dissolving MN patch loaded with both free insulin and insulin encapsulated into pH-responsive nanovesicles for a two-stage pulsatile insulin release [46]. Conversely, several glucose-responsive MN patches based on phenylboronic acid maintained normoglycemia for over nearly one day in distinct animal models (Fig. 6f). Among them, some exploited the charge switch-mediated swelling/deswelling effect of quinone methides are proteins and DNA which could result in toxic effects through their covalent modification. Specifically, it has been reported that the covalent modification of endogenous macromolecules via quinone methides can lead to hepatotoxicity, nephrotoxicity, or
Swelling/Hydrogel-forming

- Higher insulin loading capacity
- Suitable for basal insulin delivery
- Lower risk of burst release (all-in-one responsive microneedles), hence lower risk of hypoglycaemia
- Longer normoglycaemia times
- Suitable for glucagon co-loading (hydrogel charge switch)
- Removable, hence reduced posttreatment safety issues
- Able to restore their initial configuration when normoglycaemic levels are restored
- Posttreatment safety issues associated with residues remaining under the skin after use
- Unable to restore their initial configuration when normoglycaemic levels are restored

Coating disassembly [45,46,47,50,51]

Hydrogel charge switch [49,57-59]

Competitive displacement [62,63,65,76]

Hydrogel crosslinking

dissociation [42,66,67,68,70]

Coated

- Suitable to control postprandial glucose levels
- Reduced sharpness of needle tips
- Limited insulin loading capacity
- Less suited for insulin refilling

Coating disassembly [45]
be an increasing trend towards the use of pH-responsive materials (Fig. 6h), there is no clear trend within phenylboronic acid-based systems in terms of stimuli-responsive materials (Fig. 6i).

In terms of MN type, most glucose-responsive MN-based systems designed for transdermal insulin delivery are composed of dissolving or swelling MNs, although hollow and coated MNs have also been described. A summary of the strengths and caveats of each MN type in the realm of glucose-responsive transdermal insulin delivery is shown in Table 4.

Finally, to fully exploit the translational potential of these MN-based systems for glucose-responsive transdermal insulin delivery, their regulatory status must be thoroughly analysed, in terms of their classification as medical devices or medicinal products, and of the quality standards that need to be met for commercialization [94,95]. Importantly, this analysis should be done on a case-by-case basis, since their classification may differ depending on the MN type.

Overall, glucose-responsive MN patches have high clinical translational potential for insulin replacement therapy. They have been successfully tested preclinically in rodent and swine animal models. Swine models have been utilized as a surrogate larger animal model because the structure, thickness, hair sparseness, collagen and lipid composition of porcine skin are highly analogous to those of human skin [96–99]. However, due to interspecies differences, glucose responsiveness of these systems still needs to be further investigated in humans. As a result of the substantial research efforts made in the field over the last years, a clinical trial (NCT05089942) [100] has recently been registered to evaluate the efficacy and safety of a recombinant human insulin MN patch (ZISRM2021). This patch consists of a nondegradable swelling glucose-responsive matrix that uses phenylboronic acid as the glucose-sensing element and charge switch-mediated swelling as trigger stimulus [57]. While the trial is not yet recruiting patients, the estimated enrolment is only 16 patients, which may preclude statistically relevant analysis to be conducted. Hence, further studies with larger sample sizes will be needed.

Glucose-responsive MNs for transdermal insulin delivery are continuously under development to increase glucose responsiveness and improve biocompatibility. As the development of a self-regulated transdermal system for insulin delivery will represent an unprecedented breakthrough in the field of insulin replacement therapy, the latest advances presented and analysed in this review provide the stepping stones to move the field forward and open exciting new avenues for on-demand glucose-regulated transdermal insulin delivery.

CRediT authorship contribution statement

Miquel Martínez-Navarrete: Writing – original draft. Alexandre Pérez-López: Writing – original draft. Antonio José Guillot: Writing – original draft. Ana Sara Cordeiro: Writing – review & editing. Ana Melero: Writing – review & editing. Juan Aparicio-Blanco: Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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