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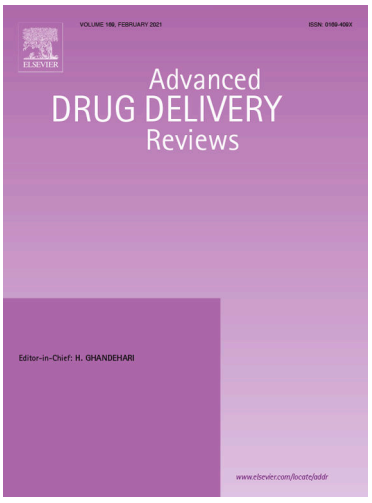

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Highlights

- Uncontrolled diabetes causes fibrosis which can lead to end-organ complications
- The delivery of antidiabetic medication using microneedle arrays is demonstrated
- Novel microneedle-mediated glucose-responsive insulin systems are described
- The use of microneedle arrays for glucose monitoring is highlighted
- Ongoing challenges in microneedle-mediated delivery of insulin are addressed

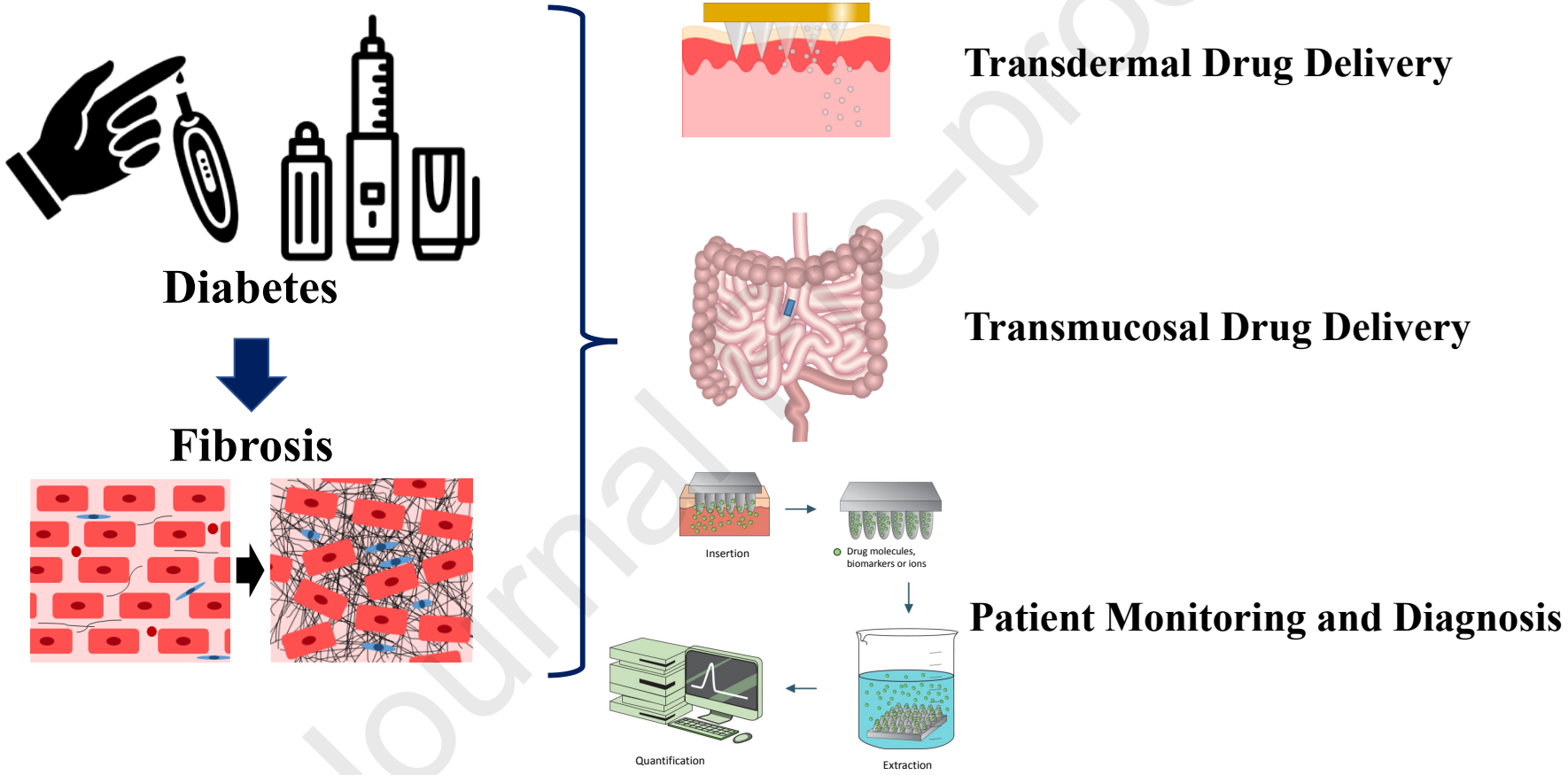
Abstract

Diabetes affects approximately 450 million adults globally. If not effectively managed, chronic hyperglycaemia causes tissue damage that can develop into fibrosis. Fibrosis leads to end-organ complications, failure of organ systems occurs, which can ultimately cause death. One strategy to tackle end-organ complications is to maintain normoglycaemia. Conventionally, insulin is administered subcutaneously. Whilst effective, this delivery route shows several limitations, including pain. The transdermal route is a favourable alternative. Microneedle (MN) arrays are minimally invasive and painless devices that can enhance transdermal drug delivery. Convincing evidence is provided on MN-mediated insulin delivery. MN arrays can also be used as a diagnostic tool and monitor glucose levels. Furthermore, sophisticated MN array-based systems that integrate glucose monitoring and drug delivery into a single device have been designed. Therefore, MN technology has potential to revolutionise diabetes management. This review describes the current applications of MN technology for diabetes management and how these could prevent diabetes induced fibrosis.

Keywords:

- Diabetes
- End-organ complications
- Microneedle-mediated transdermal delivery
- Minimally invasive devices
- Insulin delivery systems
- Glucose monitoring
- Interstitial fluid

Graphical Abstract



Contents

1. Introduction

1.1 Diabetes

Diabetes, or diabetes mellitus, is a chronic metabolic disorder. Diabetes occurs when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces [1,2]. This leads to hyperglycaemia, with high glucose levels ultimately causing irreversible damage and organ failure. As stated by the International Diabetes Federation, 4.2 million deaths worldwide were attributed to diabetes in 2019 [3]. Globally, approximately 463 million adults are living with diabetes, however, it is estimated that this figure will rise to 700 million by 2040 [3]. Thus, diabetes is considered one of the fastest growing health challenges. There are three main types of diabetes – type 1 diabetes, type 2 diabetes and gestational diabetes. This review will focus on type 1 and type 2 diabetes.

Formerly known as insulin-dependent, juvenile or childhood-onset diabetes, type 1 diabetes often begins in childhood [1]. This represents approximately 10% of diabetes cases and is a result of the autoimmune destruction of pancreatic β-cells, leading to absolute insulin deficiency. That said, 10% of type 1 diabetic patients display no evidence of autoimmunity, meaning the pathogenesis in these cases are considered idiopathic [4]. Nevertheless, a number of contributing factors in type 1 diabetes pathogenesis have indeed been determined. For instance, genome-wide association studies have shown that the main genes linked to type 1 diabetes are located on chromosome 6, specifically within the major histocompatibility (MHC) region, also known as the human leucocyte antigen (HLA) [5]. In particular, Class II HLA molecules, responsible for the recognition of antigens by CD4+ T helper lymphocytes, are linked with type 1 diabetes [6]. Substitutions at critical sites within the amino acid chains of Class II HLA molecules can increase the binding capacity towards pancreatic β-cells. This is seen with two particular HLA alleles, HLA-DQB1*0201 and HLA-DQB1*0302, which are present in more than 90% of type 1 diabetics and in only 40% of healthy individuals [7]. In these specific patients, β-cell autoantigens released during pancreatic cell turnover are presented to CD4+ T helper cells. This autoimmune response results in the activation of macrophages, CD8+ cytotoxic T cells and autoantibody producing B-cells, all of which act synergistically to destroy insulin-producing pancreatic

β-cells [8]. In contrast, HLA-DQB1*0602 is known to provide protection against type 1 diabetes and is only present in 1% of type 1 diabetic patients [9,10].

Other genetic associations with type 1 diabetes includes missense mutations in the protein tyrosine phosphatase non-receptor type 22 gene (PTPN22) [11], knockdown of the Huntingtin-interacting protein 14 gene (HIP14) [12] and single nuclear polymorphisms in the Erb-B2 receptor tyrosine kinase 3 (ERBB3) gene [5]. This results in uncontrolled T-cell activation, increased nuclear-factor kappa B (NFκB) activity and a heightened cytokine response respectively, all of which induce apoptosis of β-cells.

Type 2 diabetes, the most common type of diabetes, occurs when the body develops resistance to the effects of insulin, or the pancreatic β-cells are unable to produce sufficient insulin to maintain glucose within the normoglycemic range (4-7 mmol/L). Previously called non-insulin dependent or adult-onset diabetes, type 2 diabetes is most commonly diagnosed in older adults [1]. The incidence of type 2 diabetes is augmented due to rising levels of poor diet, physical inactivity and obesity. The main signs and symptoms of diabetes are polyuria, polydipsia, extreme fatigue, unexplained weight loss, general itching (or regular episodes of thrush), slow healing of cuts/wounds and blurred vision [13,14]. The signs and symptoms in type 1 diabetes are obvious and develop very quickly, normally over a few weeks, whereas in type 2 diabetes are often less obvious. As a result, several years after onset may pass before the disease is diagnosed. Together with the signs and symptoms, blood glucose tests are the gold standard for the diagnosis of diabetes [13,15]. In both type 1 and type 2 diabetes, blood glucose tests include random plasma glucose, fasting plasma glucose, haemoglobin A1c and oral glucose tolerance test [13,15]. Risk factors for developing type 1 diabetes are unclear whereas the risk factors for type 2 diabetes include obesity, age (45 years or older), family history of diabetes, history of gestational diabetes, an inactive lifestyle and ethnicity [3,15,16]. Attaining normal levels of blood glucose remains the main foundation in managing diabetes. Such approaches, including prompt initiation of diabetic treatment, play a fundamental role in delaying or preventing the onset and progression of potentially irreversible diabetic complications such as diabetic retinopathy and glomerular damage [17]. For the

treatment of type 1 and type 2 diabetes, guidelines produced by the National Institute for Health and Care Excellence and the British National Formulary in the UK are adopted [18–20]. Insulin, a polypeptide hormone, administered conventionally by subcutaneous (SC) injection, either *via* an insulin pump or pen, remains the first-line therapy for the primary management and treatment of type 1 diabetes. With exogeneous insulin therapy, the goal is to mimic the endogenous release of insulin [18]. At present, several insulin formulations are available on the market. To achieve different durations of efficacy, insulin products are classified according to their onset, peak and duration (**Table 1**). Rapid- or short-acting insulin are quickly absorbed, with a fast onset and high peak [19,21,22]. They are administered before and after meals to account for the high influx of glucose, to mimic endogenous insulin. Intermediate and long-acting insulins, are absorbed slower, with a prolonged onset and lower peak [19,21,22]. They maintain a constant background level of insulin throughout the day or night, and in between meals. Mixtures of insulin preparations may be required, and appropriate combinations are determined on a case-by-case basis. Commonly, treatment is started with a rapid- or short-acting insulin (given before meals) with intermediate- or long-acting insulin administered once or twice daily. Alternatively, pre-mixed rapid- or short-acting insulin analogue with an intermediate- or long-acting insulin are available, which can be administered once or twice daily. These are available for those who would rather not use, or find multiple injection regimens, challenging [22]. The dose of insulin is highly variable to individual requirements and needs to be adjusted as necessary according to the results of regular monitoring of blood-glucose concentrations. Factors affecting insulin requirements include taken or planned exercise, intended food intake, infection, stress, trauma or coeliac disease [18,19]. This self-management of insulin therapy therefore requires the patient to have the skills and confidence to manage the regimen.

Table 1 Types of insulin products approved by the FDA.

Insulin Preparation	Brand Name	Device	Taken	Onset of action (min)	Peak action (h)	Duration of action (h)
Rapid-acting						
Insulin aspart	• Fiasp®	Vial, FlexTouch pen, cartridge	Just before, with or just after food	10-20	1-3	2-5
	• NovoRapid®	Vial, FlexPen, FlexTouch, cartridge				
Insulin gulisine	• Apidra®	Vial, Solostar pen, cartridge				
Insulin lispro	• Humalog®	Vial, KwikPen, cartridge				
Short-acting						
Human regular insulin	• Actrapid®	Vial	15-30 min before food	30-60	1-5	5-9
	• Humulin® R	Vial, KwikPen				
	• Humulin® S	Vial, cartridge				
	• Hypurin® Porcine Neutral					
	• Insuman® Infusat	Cartridge				
	• Insuman® Rapid					
Intermediate-acting						
Isophane insulin	• Humulin® I	Vial, KwikPen, cartridge	About 30 min before food or bedtime	60-90	2-12	12-24
	• Hypurin® Porcine Isophane	Vial, cartridge				
	• Insulatard®	Vial, Innolet pen, cartridge				
	• Insuman® Basal	Vial, SoloStar pen, cartridge				
Long-acting						
Insulin degludec	• Tresiba®	FlexTouch pen, cartridge	Once daily	30-90	No peak	42
Insulin detemir	• Levemir®	FlexPen, cartridge	Once or twice daily	120-240	6-14	16-20
Insulin glargine	• Abasaglar®	KwikPen, cartridge			No peak	20-24
	• Lantus®	Vial, Solostar pen, cartridge				
	• Toujeo®	SoloStar pen, DoubleStar pen				
Intermediate-acting and rapid-acting						
Biphasic insulin aspart: 70% insulin aspart protamine, 30% insulin aspart	• NovoMix® 30	FlexPen, cartridge	Just before, with or just after food	10-20	1-4	Up to 24
Biphasic insulin lispro: Insulin lispro protamine, insulin lispro	• Humalog® Mix 25 • Humalog® Mix 50	Vial, KwikPen, cartridge				
Intermediate-acting and short-acting						
Biphasic isophane insulin: Insulin isophane human, insulin soluble human	• Humulin® M3	Vial, KwikPen, cartridge	15-30 min before food	30	1-4	12-24
	• Hypurin® Porcine 30/70 Mix	Vial, cartridge				
	• Insuman® Comb 15	Cartridge				
	• Insuman® Comb 25	Vial, SoloStar pen, cartridge				
	• Insuman® Comb 50	Cartridge				

A healthy lifestyle is the cornerstone of managing type 2 diabetes. A healthy lifestyle includes a healthy diet, regular physical activity, maintaining a healthy body weight and not smoking [23]. Due to the progressive nature of type 2 diabetes and the fact that maintaining a long term healthy lifestyle is difficult, patients normally require oral antidiabetic drugs [19,23]. Metformin, a biguanide, is the first-line therapy of choice for patients with type 2 diabetes, unless contraindicated [19,20]. If glycaemic control is not maintained on one oral antidiabetic drug, dual or triple therapy can be considered. When glycaemic control is inadequate with metformin and two other oral antidiabetic drugs, the addition of glucagon-like peptide-1 (GLP-1) analogues (incretin mimetics), which are administered subcutaneously, are considered. Treatment with GLP-1 analogues are advantageous for overweight patients because they are associated with the possible promotion of weight loss and prevention of weight gain [19]. For patients not adequately controlled by diet, oral hypoglycaemic drugs or subcutaneously administered GLP-1 analogues, insulin may be added to the treatment regimen or substituted for oral therapy [19]. For more than four decades, there were only two classes of oral antidiabetic drugs available, namely, biguanides and sulfonylureas, but in the last 20 years, several other glucose-lowering medications for the treatment of type 2 diabetes have been introduced [23]. **Figure 1** summarises the major classes of oral antidiabetic drugs and the subcutaneously administered GLP-1 analogue class. **Figure 2** shows the chemical structures of the FDA approved oral antidiabetic drugs.

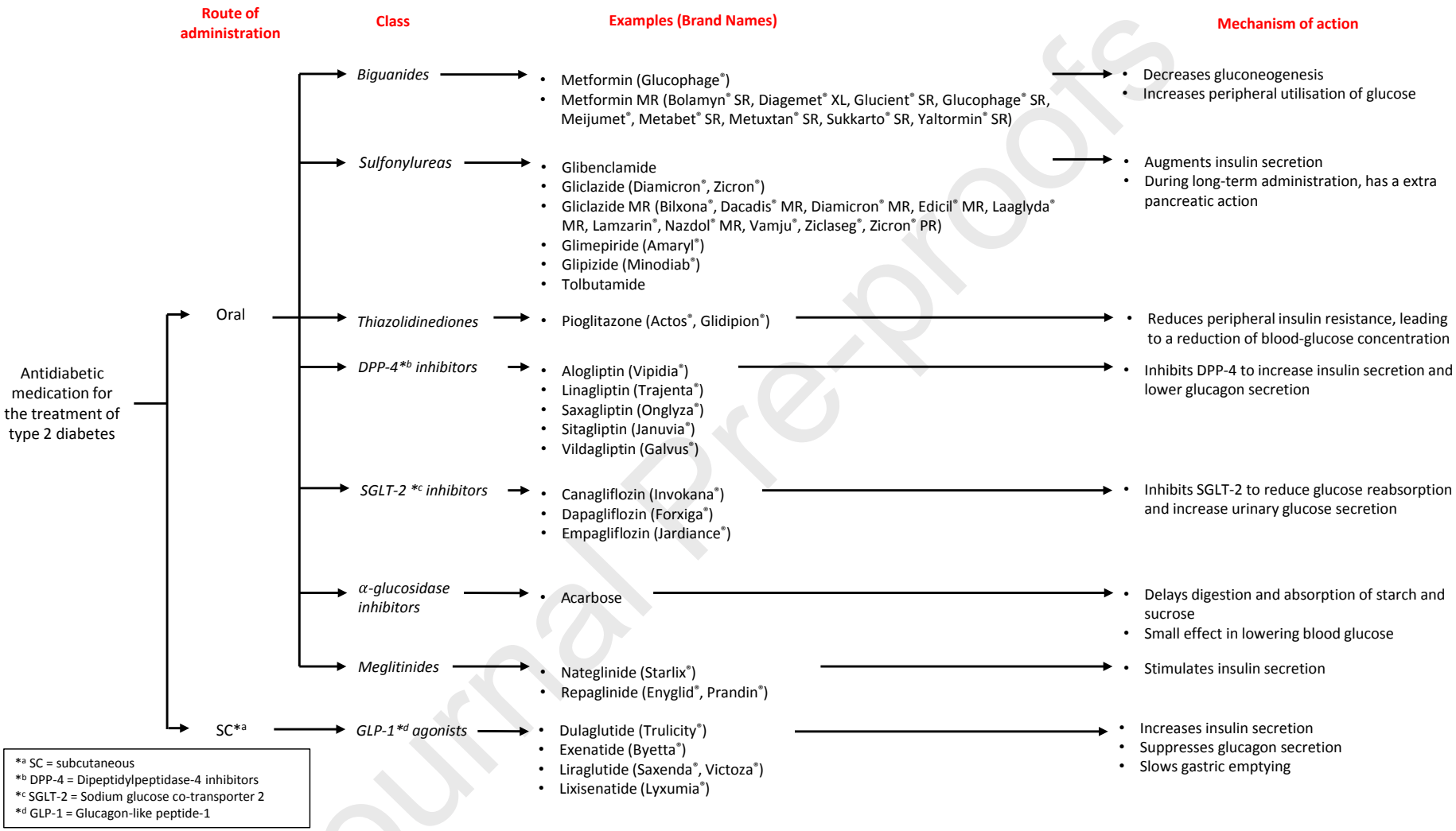


Figure 1 FDA approved antidiabetic medication for the treatment of type 2 diabetes.

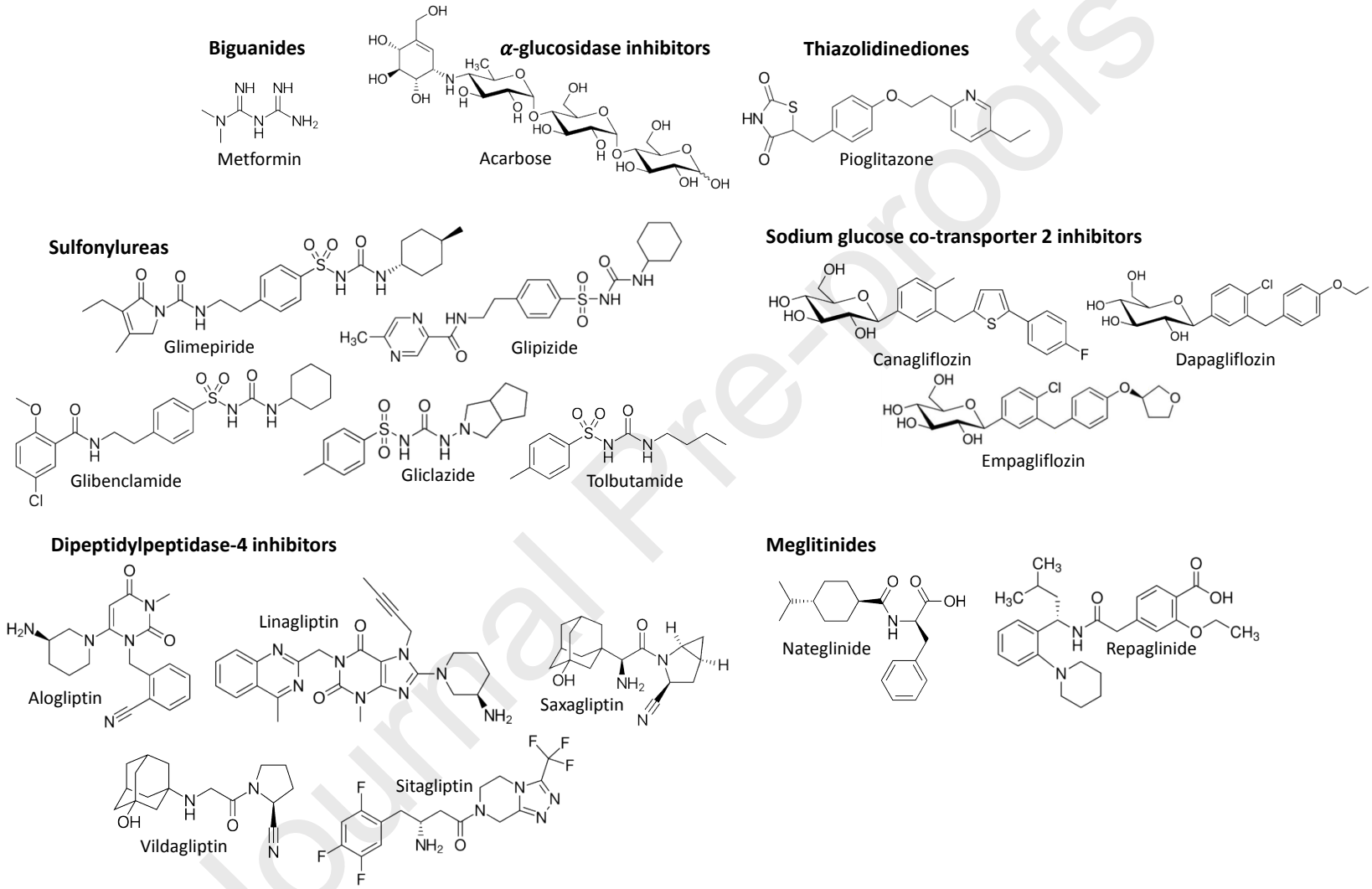


Figure 2 Chemical structures of FDA approved oral antidiabetic medication for the treatment of type 2 diabetes.

Blood glucose monitoring plays a pivotal role in the management of type 1 diabetes and where necessary, type 2 diabetes. To measure the concentration of glucose in the blood, blood glucose monitoring is used. Blood glucose monitoring can also detect hypoglycaemia and hyperglycaemia [19]. To date, self-monitoring of blood glucose by finger pricking remains the gold standard for point-of-care glucose measurement [24]. Most published studies in this review compare blood glucose levels to normoglycaemic state (<200 mg/dl, <11 mmol/l). However, specifically, current national guidelines recommend adults with type 1 diabetes to aim for a fasting plasma glucose level of 5-7 mmol/l on waking, a plasma glucose level of 4-7 mmol/l before meals, a plasma glucose level of 5-7 mmol/l after meals and a plasma glucose level of 4-7 mmol/l at other times of the day however these values vary accordingly to the patient [18]. These national guidelines also advise routine self-monitoring of blood glucose levels and, depending on the patient's lifestyle, recommend testing four to ten times per day [18]. As a potential replacement for self-monitoring of blood glucose, in 2015, the first continuous glucose monitoring device, namely the FreeStyle® Libre (Abbott Diabetes Care, Witney, UK) was introduced.

1.2 Diabetes induced fibrosis

Poor glycaemic control can consequently result in chronic hyperglycaemia and tissue injury. Fibrosis, a pathological response to tissue injury, subsequently leads to end-organ microvascular and macrovascular complications. The pathogenesis pathway resulting in end-organ complications is associated with a number of biochemical pathways and factors [25,26]. Key contributors include the renin-angiotensin-aldosterone system, the matrix metalloproteinase system, the plasminogen activator/plasmin system, influx of advanced glycation end-products (AGEs) and increased concentrations of growth factors [25,26]. Growth factors, namely transforming growth factor-β1 (TGF-β), play a central role in end-organ diabetic complications. However, it is important to note that it is not only over expression of growth factors which leads to fibrosis. In the liver, downregulation of

adipokines, namely adiponectin, has been shown to increase the risk of diabetic fibrosis and its associated complications [27]

Fibrosis, by definition is an excess or an accumulation in extracellular matrix (ECM) components, such as collagen [25]. The ECM is a three-dimensional, non-cellular structure present in all tissues and is essential for life. The main structural protein in the ECM is collagen [27]. Regarding the function of the ECM, not only does it provide physical support for tissue integrity and elasticity: it is a highly dynamic structure that is remodelled constantly to maintain tissue homeostasis [27]. Alterations in the ECM, which occur during diabetes induced fibrosis, are well-documented in the literature [25,28]. As previously mentioned, diabetes induced fibrosis affects many organs and ultimately leads to irreversible complications. There are two types of diabetes complications - microvascular (damage to small blood vessels) and macrovascular (damage to larger blood vessels). Microvascular end-organ complications include those that affect the eye (diabetic retinopathy), the kidneys (diabetic nephropathy) and the skin (and nerves). Macrovascular end-organ complications include those that affect the heart (diabetic cardiomyopathy), the liver (non-alcoholic fatty liver disease) and the lungs (diabetic pulmonary fibrosis). The most common organs affected by diabetes induced fibrosis are schematically represented in **Figure 3**. Each diabetic end-organ complication will now be discussed in turn.

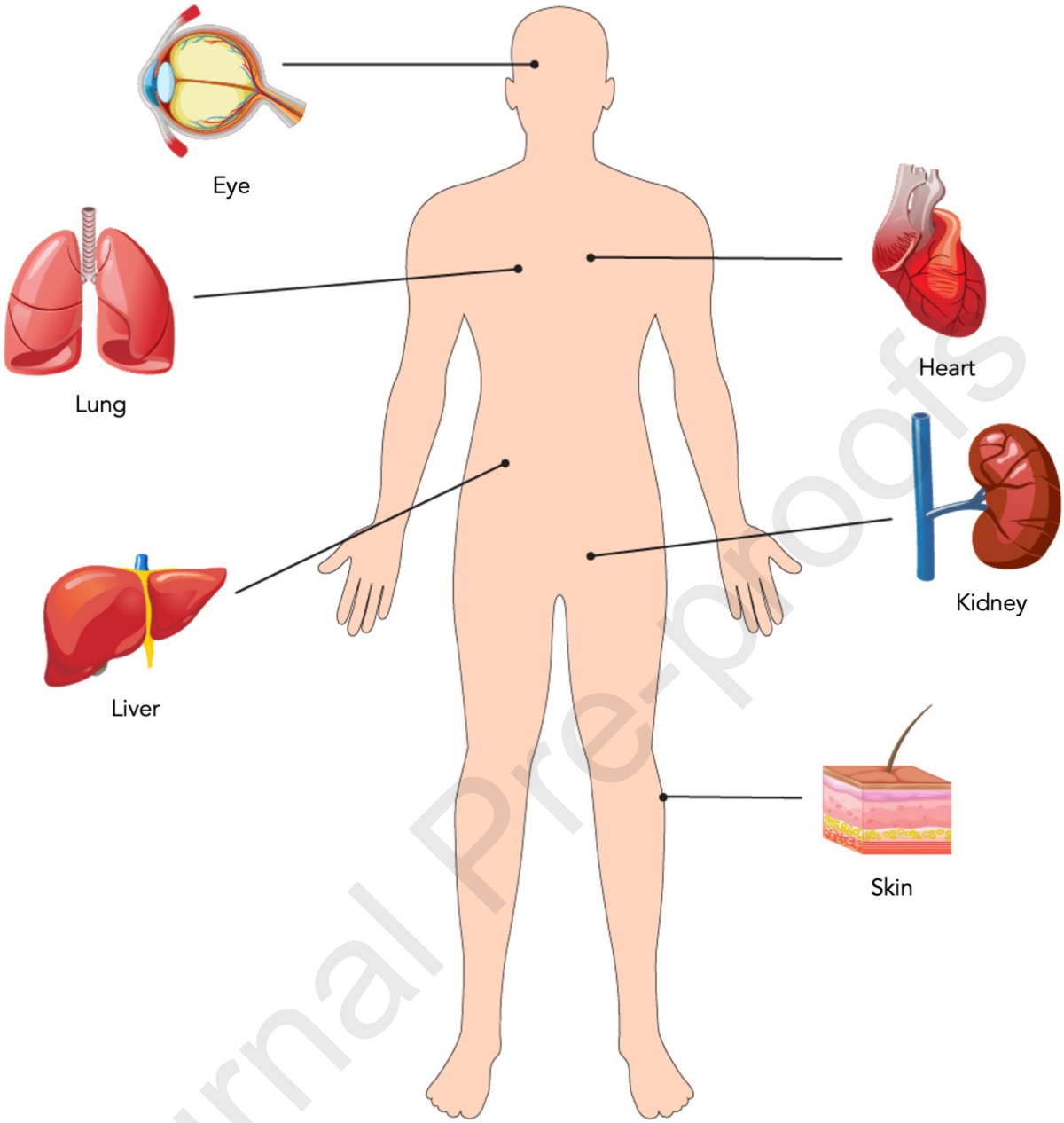


Figure 3 Common organs affected by diabetes induced fibrosis.

Diabetic retinopathy causes visual impairment and blindness, with one-third of diabetics (type 1 and type 2 diabetes) diagnosed with the condition [29]. Clinically, there are two stages of diabetic retinopathy - non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR). NPDR represents the early stage of diabetic retinopathy. At this stage, high glucose levels cause apoptosis of pericytes, the vascular smooth muscle cells responsible for providing structural support within capillaries. Leukostasis further contributes to endothelium damage through the apoptotic Fas-

ligand pathway [30]. This leads to capillary occlusion and ischemia, with hypoxia upregulating vascular endothelial growth factor (VEGF) through activation of hypoxia-inducible factor 1 (HIF-1) [31]. This particular growth factor is a key contributor to increased vascular permeability by inducing phosphorylation of occludin and other such tight junction proteins [32]. Angiopoietins also promote vascular leakage during this stage by interacting with endothelial receptor tyrosine kinase Tie2 [33]. Retinal neurodegeneration has also been observed in the early stages of diabetic retinopathy. Here, high glucose levels instigate mitochondrial fragmentation through the upregulation of cleaved caspase-3, Bax and Fas within retinal neurons [34,35]. In PDR, the more advanced stage of diabetic retinopathy, neovascularisation occurs, and the patient becomes symptomatic. Due to retinal detachment and vitreous haemorrhages (abnormal micro-vessels bleeding into the vitreous), patients experience severe vision impairment [25,36]. In this stage, types VI, VIII, XII, and XIV collagen as well as laminin, perlecan and fibronectin are over expressed [26]. When the blood-retinal barrier breaks down, thickening or swelling of the macula due to sub- and intra-retinal accumulation of fluid occurs, leading to diabetic macular oedema. The diagnosis of diabetic retinopathy is made by clinical manifestations of vascular abnormalities in the retina [36]. Monitoring and controlling both blood glucose levels and blood pressure have been shown to help delay the onset and progression of diabetic retinopathy [29]. In terms of treatment strategies for diabetic retinopathy, intravitreal administration of anti-VEGF agents is currently the first line therapy for both early and advanced stages of diabetic retinopathy [36]. Non-specific anti-angiogenic and intravitreal steroids are also used [37–39].

Diabetic nephropathy, also known as diabetic kidney disease, affects the kidney structure and renal function. Several metabolic pathways, namely polyol, hexosamine, AGEs and protein kinase C (PKC) are involved in diabetic nephropathy as a result of hyperglycaemia and increased glycolysis [40]. Fructose, the end product of the polyol pathway, is considered a potential nephrotoxin. Its presence reduces glomerular filtration rate (GFR), increases proteinuria and increases glomerular injury [40]. The hexosamine pathway leads to the production of TGF-β1 causing renal cell hypertrophy and increased mesangial matrix components [41,42]. AGEs, produced as a result of irreversible glycation of proteins, modify type IV collagen and laminin causing increased glomerular basement membrane

permeability. Such products also bind to proinflammatory receptors which activate downstream cytokines such as IL-1, IL-6 and TNF-α [43,44]. PKC activation increases prostaglandin E_2 and nitric oxide levels leading to vasodilation of the afferent arteriole in addition to increasing TGF-β1 levels, fibronectin and type IV collagen [45–47]. The end result is mesangial expansion, thickening of the basement membrane and nodular glomerulosclerosis (Kimmelstiel-Wilson nodules) [48,49]. In advanced stages of diabetic nephropathy, types 1 and III collagen are expressed and are also associated with the formation of the Kimmelstiel-Wilson nodules [25,26]. **Figure 4** pictorially shows masson's trichrome-stained sections of the kidney. **Figure 4A and Figure 4B** represent the control and a stained section from a diabetic rat showing increased amount of deposited collagen respectively. It is widely accepted that diabetic nephropathy can be divided into five stages, with patients only becoming symptomatic in stage four. Symptoms of diabetic nephropathy that occur during stage four include swelling of ankles, legs and hands due to water retention, blood in urine, fatigue and nausea [48]. If left untreated, diabetic nephropathy can lead to kidney failure, which can only be treated by dialysis or a kidney transplant. Diabetic nephropathy can be diagnosed by measuring the amount of albumin in the urine and testing the function of the kidneys. A holistic approach is taken to manage diabetic nephropathy. After maintaining blood glucose levels, the focus for the treatment of diabetic nephropathy constitutes mostly of an antihypertensive means [25,48]. Regular screening of the kidney function also remains imperative.

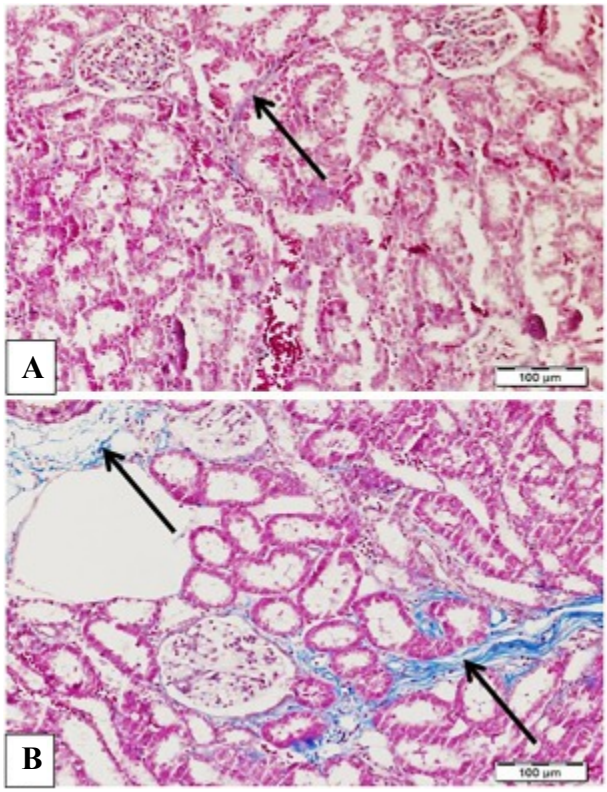


Figure 4 Masson's trichrome-stained sections in the kidney of **(A)** control and **(B)** chemically-induced diabetic rats using a single streptozotocin intraperitoneal injection (55 mg/kg) showing increased amount of deposited collagen (arrow) (Produced with permission from [50]).

Fibrosis contributes to the development of skin disorders or failure of wound healing. Dermatologic manifestations of diabetes varies, with health implications ranging from those that are aesthetically concerning to those that can be life-threatening. Diabetes damages skin function in a complex fashion and several skin disorders can occur [51]. To name a few, the formation of AGEs, provoking oxidative stress, endothelial dysfunction and inflammation, in turn accelerates the development of diabetic dermopathy (pigmented pretibial patches) and scleredema diabeticorum (thickening of skin on back and neck) [52]. Imbalance of insulin-like growth factor (IGF), epidermal growth factor receptor (EGFR), fibroblast growth factor receptor (FGFR), cytokines and α-melanocyte stimulating hormone (α-MSH), resulting from increased proliferation of melanogenesis, leads to the development of acanthosis nigricans (dark discoloration in body folds), acrochordons (growths on skin surface), acne and inflammatory dermatitis [52]. Combining neuropathy and vasculopathy, one common skin complication is diabetic foot syndrome. More prevalent in type 1 diabetics, diabetic foot syndrome

initially presents with dry skin and calluses. In later stages, ulcers develop, predominantly at pressure areas. If left untreated and along with secondary infections, this can result in gangrenous necrosis, osteomyelitis or require lower extremity amputation [53]. Treatment of diabetic foot syndrome involves an interdisciplinary team-based approach.

Diabetic cardiomyopathy is defined as the presence of abnormal cardiac structure and performance, encompassing metabolic changes, in the absence of other risk factors such as coronary artery disease and hypertension [54,55]. Diabetic cardiomyopathy subsequently leads to heart failure. Heart failure, a form of cardiovascular disease is the inability of the heart to pump blood around the body properly. The pathophysiology of diabetic cardiomyopathy is a growing interest, with its evolvement considered by many to be multifactorial. In brief, diabetic cardiomyopathy is characterised by left ventricle diastolic dysfunction (impaired filling), reduced left ventricular ejection fraction (inadequate blood volume ejection) and cardiac hypertrophy (enlargement of the left ventricle with interstitial fibrosis) [25]. The mechanisms and markers implicated in the pathogenesis of diabetic cardiomyopathy include a change in the metabolic status, impaired calcium homeostasis and energy production, increased inflammation and oxidative stress, as well as an accumulation of AGEs and type III collagen [26,54]. Patients are usually asymptomatic in the early stages of diabetic cardiomyopathy [56]. As the disease progresses, signs and symptoms include fatigue, shortness of breath, swelling in ankles/feet, dizziness, light-headedness, leg pain, confusion and paralysis. Treatment includes lifestyle modifications (diet and exercise) in addition to maintaining good glycaemic control, lipid-lowering drugs, and heart failure medication, as required.

The most common chronic liver disease associated with diabetes induced fibrosis is non-alcohol fatty liver disease (NAFLD). NAFLD describes the build-up of hepatic fat within the liver in the absence of alcohol. This is an umbrella term that represents a spectrum of conditions, with fibrosis and inflammation linked to the activation of NF-κB and the c-Jun N-terminal kinase (JNK) pathway [57]. Activation of NF-κB due to hyperglycaemia results in pro-apoptotic gene activation and elevation of pro-inflammatory molecules, such as IL-1β, IL-6, TNF-α and C-reactive protein [58,59]. NF-κB can further increase liver tissue damage by promoting the production of reactive oxygen species (ROS) and

AGEs [60]. The JNK pathway, which consists of stress activated mitogen-activated protein kinases (MAPKs), is triggered by pro-inflammatory cytokines. Stimulation can result in hepatocyte apoptosis, necrosis, proliferation and inflammation [61]. Therefore, NF-κB and JNK both present promising therapeutic targets for the treatment of NAFLD, if treated early. This can be difficult to achieve as patients are commonly asymptomatic during the early stages of NAFLD. Without the appropriate intervention, this can progress to non-alcoholic steatohepatitis which is associated with an increased risk of morbidity, end-stage liver disease, hepatocellular carcinoma and death [62]. Symptoms during these stages include right upper quadrant abdominal discomfort, fatigue, malaise, unexplained weight loss and weakness. Elevated markers due to NAFLD include liver enzymes, alanine aminotransferase and aspartate aminotransferase [63]. The gold standard for the diagnosis of NAFLD is a liver biopsy. Weight loss and dietary modifications are currently the first recommendation for NAFLD patients. In terms of pharmaceutical management, metformin, the first oral hypoglycaemic agent used to treat type 2 diabetes, is used, as it has been found to reduce the progression of liver disease [25].

The eye, kidney, skin, heart and liver have all been extensively studied as diabetes targeted organs affected by fibrosis. Recently, it has been suggested that the lungs, which consist of an abundant capillary network may be a further target by diabetic micro-vascular damage [64]. Emerging evidence confirms that diabetes increases the risk of pulmonary dysfunction, characterised by pulmonary fibrosis [65]. In type 1 and type 2 diabetes, pulmonary dysfunction has been identified through reduced lung volumes (forced expiratory in 1 sec and forced vital capacity). However, it is recognised that more attention and studies should now be given to diabetic pulmonary fibrosis as another end-organ diabetic complication.

1.3 Challenges of current treatment regimens for diabetes

One strategy to tackle diabetes induced fibrosis and end-organ complication is to maintain good glycaemic control. Conventionally, insulin is administered subcutaneously. However, the frequent injection of this molecule for the treatment of type 1 diabetes has several drawbacks. A painful

application and the risk of skin infection at the injection site are the main challenges associated with insulin given *via* the SC route [2,66]. Insulin-induced hypoglycaemia from insulin administered subcutaneously has also been observed [67]. Refrigeration is required for all insulin pens which is inconvenient. The insulin administration procedure for insulin presents some additional limitations [2,68]. This is because some older adults with cognitive decline may have difficulty operating insulin pens, especially changing cartridges. Due to insulin pens not having a memory for given doses, missed or incorrect doses are also problematic. The numbers on the insulin pen are sometimes hard to see for visually impaired patients. Adherence to medication for the pharmaceutical management of type 2 diabetes maintains glycaemic control, reduces the risk of serious complications and subsequent economic costs associated with this chronic disease [69]. However, retrospective studies in patients with type 2 diabetes reported adherence rates of 36-93% for oral antidiabetic drugs and 62-64% for insulin [70]. Medication adherence factors include social influences and psychological influences such as depression [71–73]. Thus, an alternative route of administration must be sought to ensure adherence of medication for the treatment of both type 1 and type 2 diabetes to prevent the risk, onset and progression of end-organ diabetic complications.

1.4 Alternative routes of administration

There is a growing interest in non-invasive routes of insulin administration. Delivery of insulin *via* the oral route is currently being investigated by both research institutions and pharmaceutical companies, however, poor bioavailability and enzymatic degradation in the gastrointestinal tract (GIT) and liver present significant challenges [74,75]. Inhaled drug delivery avoids the first pass effect and has a large absorption surface. The first FDA approved pulmonary delivered version of insulin was Exubera® (Pfizer/Nektar) in 2006. However, it was later discontinued in 2007 due to low sales, high costs, device design and concerns relating to decline in pulmonary function [66,76]. Afrezza®, a rapid-acting inhalable insulin by Sanofi and MannKind, was FDA approved in 2013. However, the safety profile of Afrezza® was concerning in that, similarly to Exubera®, long-term exposure of insulin in the lungs can

reduce pulmonary function [77]. With this, it was noted that Afrezza® and other inhaled insulins are not considered safe in patients with pulmonary disease, such as asthma or COPD. As a result, Sanofi has now withdrawn from Afrezza's® marketing agreement with MannKind.

For the delivery of insulin and other antidiabetic medications, intradermal and transdermal drug delivery present attractive alternatives. The primary consideration is that hepatic first pass metabolism is avoided. By circumventing the GIT, gastrointestinal degradation is also prevented. The large surface area makes it a convenient and patient-friendly target for drug delivery. Currently, diabetics can avail of intradermal insulin delivery through marketed insulin pump products such as the Medtronic MiniMed™ 670G and the Insulet Omnipod DASH®. These small electronic devices are worn by the user, usually on the upper arm, upper leg, or lower abdomen, and provide regular glucose level monitoring *via* the implanted cannula. If an adequate insulin supply is loaded by the user, these devices then deliver the required dose of insulin based on this feedback. Intradermal insulin delivery in this manner is highly invasive when compared to a similar system based on transdermal insulin delivery. Furthermore, cannula application can be difficult and painful in some cases, with users requiring training regarding correct cannula placement and application. The transdermal route of administration is limited currently to a relatively small number of drug molecules, due to the formidable barrier posed by the *stratum corneum,* the outermost layer of the skin. Several active technologies have been developed to modify the barrier properties of the *stratum corneum,* for systemic delivery of a wider range of drug molecules [78–80]. Microneedle (MN) arrays are one such active technology, which continues to garner attention.

1.5 Microneedle arrays

MN arrays are minimally invasive devices that can be used to bypass the *stratum corneum* and thus enhance transdermal drug delivery [81–84]. MN arrays consist of multiple micro-projections assembled on one side of a supporting base, ranging in height from 25-900 μm. MN arrays combine the potential delivery capabilities of a hypodermic needle with the patient friendly benefits of a transdermal patch. The unique feature of MN arrays is that they penetrate the skin barrier sufficiently to enable access to the skin's rich microcirculation, yet are short and narrow enough to avoid stimulation of nerve fibres or puncture of blood vessels that primarily reside in the dermal layer [85]. Pain and bleeding after MN array insertion are thus avoided [86–89]. Needle phobia from insulin pens is removed which is experienced by many people which can ultimately reduce medication adherence. The stability of drug molecules, delivered using MN arrays is enhanced, with the "cold chain," which is used for insulin pens, no longer a necessity [90,91]. MN arrays can be categorised into 5 main drug delivery strategies, namely solid (**Figure 5A**), coated (**Figure 5B**), hollow (**Figure 5C**), dissolving (**Figure 5D**) and hydrogel-forming (**Figure 5E**). MN arrays have been shown to facilitate successful transdermal delivery of a wide range of drug compounds [92–94], including low molecular weight drug molecules [91,95–102] and macromolecules [96,103–105]. Among many other macromolecules, insulin has been successfully delivered using MN technology [103,106–109]. We discuss, in this review, the MN-mediated transdermal delivery of insulin and non-insulin-based antidiabetic medication for the potential treatment of type 1 and type 2 diabetes. Studies surrounding the transmucosal delivery of insulin using MN arrays are described and current developments in the field of MN array-based minimally invasive glucose monitoring and diagnosis of diabetes are reviewed. Finally, ongoing challenges in the MN-mediated delivery of insulin are highlighted.

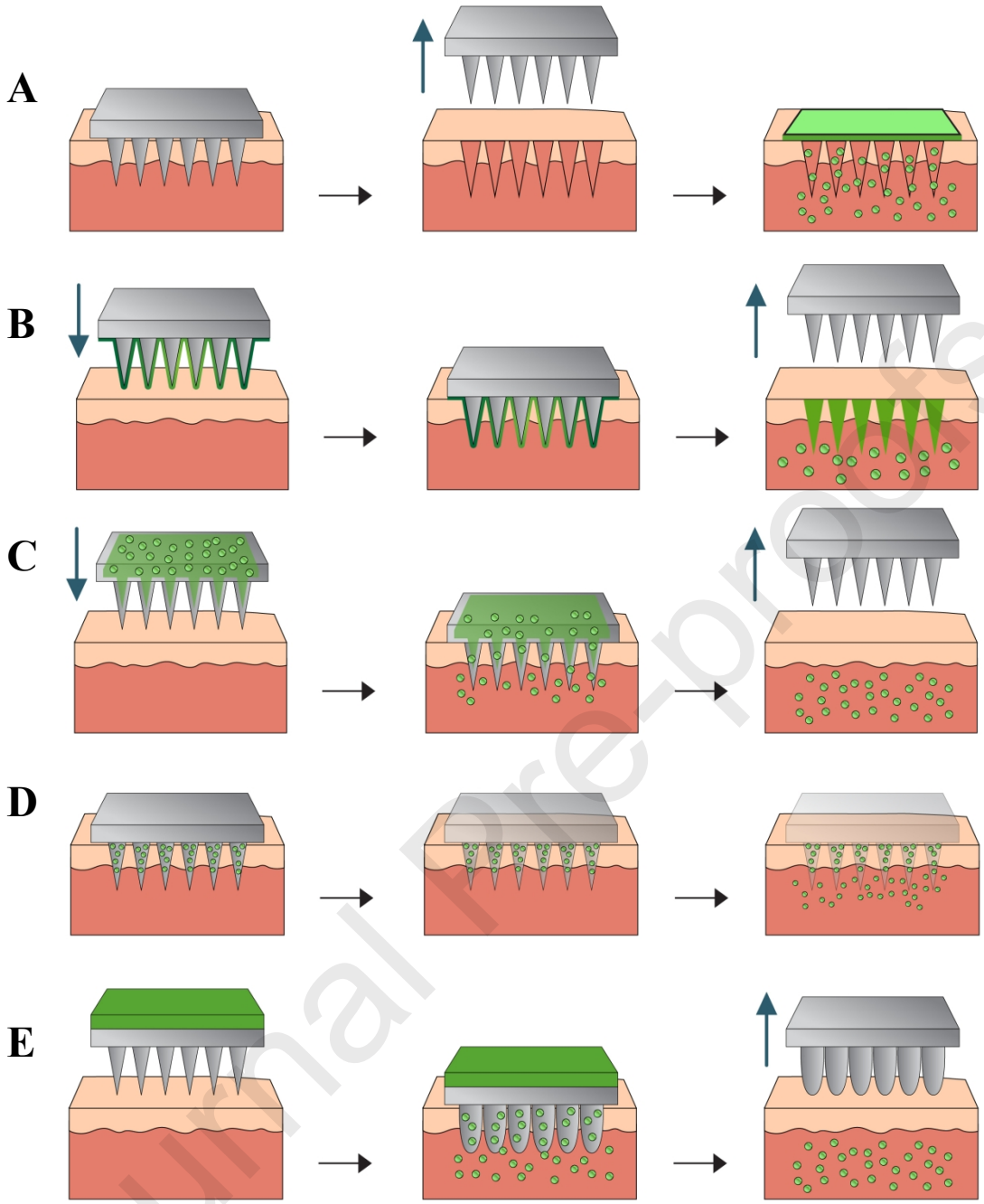


Figure 5 Schematic representation of five different MN array application strategies to facilitate transdermal drug delivery. **(A)** Solid MN arrays for increasing the permeability of a drug formulation by producing microholes across the skin pre-treatment followed by the application of a conventional drug formulation; **(B)** Coated MN arrays for deposition and dissolution of a drug-containing layer into the skin; **(C)** Hollow MN arrays for insertion into the skin and infusion of drug molecules *via* the central lumen of the MN array; **(D)** Dissolving MN arrays for delivery of incorporated drug into the skin and **(E)** Hydrogel-forming MN arrays that take up skin ISF, inducing diffusion of the drug from a drug-containing reservoir through swollen micro-projections.

2. Transdermal insulin delivery using MN arrays

2.1 Solid MN arrays

As the first MN array design to be conceptualised in 1998, solid MN arrays utilise a 'poke and patch' technique. This two-step application process has been employed extensively for the transdermal delivery of many therapeutics, including peptides such as insulin. Generally regarded as the pioneers in this area, McAllister *et al*. developed solid silicon MN arrays to aid the delivery of insulin across human skin *in vitro* [110]. This study established, for the first time, that solid MN arrays could deliver therapeutically relevant doses of insulin, capable of meeting the basal demands of many diabetics. Martanto *et al*. observed a similar effect in diabetic rats using solid metal MN arrays. In this instance, blood glucose levels were reduced by as much as 80% [109]. Owing to their painless administration, these preliminary studies proved that MN arrays had real potential in improving diabetes management.

Developing upon the aforementioned studies, and in an attempt to improve the commercial viability of a solid MN array device for the delivery of insulin, Li *et al*. created a biodegradable solid MN array composed of poly(lactic acid) [111]. Using diabetic mice, blood glucose levels were lowered to 29% of the initial level at 5 h, while for the SC injection, blood glucose decreased rapidly to 19% after 1.5 h, before gradually increasing to 100% after a further 1.5 h. The authors concluded that solid MN arrays can provide a means of painlessly delivering an effective level of insulin across the skin for a prolonged duration compared to the conventional parenteral route.

Despite the significant advances offered by solid MN-mediated delivery, a number of key challenges still need to be addressed. This includes the inconvenience of a two-step application process, storage of the insulin containing formulation to prevent microbial growth, the requirement for a higher drug loading to provide the same hypoglycaemic effect as a SC injection and difficulty in controlling the dose of insulin delivered [109,112,113]. With this, metal and silicon are not necessarily the most suitable material choices. There are concerns that such non-biodegradable materials, when inserted into the skin, may cause safety issues. Example of safety issues include silicone-related granulomas and reports of solid MN array tips remining in the skin after removal of solid MN arrays [114,115].

Nevertheless, these initial studies using solid MN arrays created a basis for the development of more complex MN systems as discussed below.

2.2 Coated MN arrays

Coated MN arrays are typically limited to the delivery of highly potent, low dose macromolecules such as vaccines [116–120]. This is primarily due to the limited loading capacity of this design [121,122]. For this reason, the number of studies involving insulin coated MN arrays are minimal and to the best of our knowledge, only one *in vivo* study has been performed using a diabetic mice model [123].

Several techniques are typically employed during the manufacture of coated MN arrays. This includes dipping, spraying, gas jet-drying and ink jet printing. Using the ink jet printing process, Ross *et al.* used a range of polymers, including gelatin, polyvinyl caprolactame-polyvinyl acetate-polyethylene glycol (SOL), poly(2-ethyl-2-oxazoline) (POX) and trehalose to coat insulin onto metal MN arrays [124]. Although this resulted in homogenous insulin-polymer layers, gelatin and POX had an adverse effect on insulin stability, with the authors observing possible conformation changes in the peptides secondary structure *via* circular dichroism analysis. Insulin release *in vitro* using porcine skin showed that SOL-insulin was the best performer, releasing its entire payload after 40 min.

In a similar study, Pere *et al.* employed a 3D printing stereolithographic technique to create MN arrays with various designs. Ink jet printing was then used to deposit insulin formulations with mannitol, trehalose or xylitol on the MN array surface [125]. Interestingly, cone and pyramid shaped 3D printed MN arrays had the same *in vitro* release profiles for all the coated formulations used in this study, with approximately 90-95% insulin release after 30 min. The same group recently examined the effects of this rapid release profile *in vivo* using diabetic mice [123]. The positive control (SC injection) in this study caused a fast increase in blood insulin concentration as expected, decreasing plasma glucose to approximately 30.1% from its initial value after 1 h. Interestingly, the 3D printed MN array group lowered blood glucose at the same rate as the SC injection, with comparable glucose levels after 1 h. In

addition, this MN array device provided a superior steady state hypoglycaemic effect compared to SC injection over the 4 h study period. This demonstrated that 3D printing can be used as a viable tool for the rapid delivery of insulin.

This coated MN strategy offers a patient-friendly, one step application process for transdermal insulin delivery than that described for solid MN arrays where skin pre-treatment is necessary. However, a technical challenge of this delivery strategy is the amount of drug/insulin formulation that can be coated (and indeed, uniformly dispersed) over the surface of the MN array itself. Owing to the small size of the needles, the loading capacity of such an array is small. Typically, the dosing is limited to microgram (μm) quantities. With this, as mentioned previously, a balance must be struck between drug quantity and needle strength. Increasing the amount of coated insulin might affect he mechanical strength of MN arrays [126]. Perhaps, with a more concentrated form of insulin, therapeutic doses using a relatively small, coated MN array device could be achieved.

2.3 Hollow MN arrays

In contrast to solid and coated MN arrays, hollow MN arrays have the ability to deliver large quantities of pharmaceuticals either by passive diffusion, pressure or electrical driven flow [127]. Using a 900 μm borosilicate glass hollow MN array, bevelled at a 30-degree angle with an oval-shaped opening, rapid insulin absorption and decline in glucose levels in human subjects (n=5) was obtained by Gupta *et al*. [128]. Using this single hollow MN array, which was connected to a 3 ml syringe and syringe pump, peak insulin concentrations were reached in approximately half the time. This led to a greater reduction in blood glucose levels compared to a 9 mm long SC catheter. The authors established that rapid pharmacokinetics and the minimally invasive infusion of intradermal insulin using MN arrays has the potential to improve patient compliance. Acknowledging the small population size of this study, Norman *et al.* conducted the same study with 16 participants, aged between 10-18 years old [129]. Using an identical MN array design, the results were consistent with the previous study in adults, showing that insulin administered with MN arrays provided greater control over blood glucose levels

compared to the SC catheter. The authors suggested that the accelerated pharmacokinetics was a result of the MN arrays targeting the rich capillary bed found in the superficial dermis. In addition to the reduction in pain upon application, this MN array device has the possibility to improve compliance in children and adolescents with type 1 diabetes. Furthermore, the fast onset and offset times of insulin action has enabled closed-loop insulin delivery systems to be exploited as discussed further in this review.

Hollow MN provides great control over the amount and timing of insulin delivery. However, there are a number of technical challenges associated with this drug delivery strategy. The design of hollow MN arrays is complex, with an inherently more challenging manufacturing process, in comparison to solid or coated MN arrays. In comparison to solid MN arrays, hollow MN arrays are inherently weaker in terms of mechanical strength and are therefore at a greater risk of breakage. Furthermore, insertion of hollow MN arrays into the skin could be more difficult because the bore opening for drug infusion reduces the overall sharpness of the needle.

2.4 Dissolving MN arrays

Dissolving MN arrays encapsulate their payload in a soluble matrix, typically a biodegradable polymer or carbohydrate. Upon application to the skin, interstitial fluid (ISF) causes the MN array to dissolve, releasing its contents into the rich microvasculature system housed in the superficial dermis [92]. As this creates a self-disabling device, there is no risk of needle re-insertion and no requirement for sharps disposal, an issue associated with solid, coated and hollow MN arrays. Dissolving MN arrays are typically fabricated from inexpensive polymeric materials that can be manipulated at ambient temperatures [84]. For example, chondroitin sulfate [130], hyaluronic acid (HA) [131] and poly-gamma-glutamic acid [130] have been used to manufacture insulin loaded dissolving MN arrays at room temperature using micromolding casting techniques. For instance, Liu *et al*. developed a HA-based dissolving MN array containing 0.13 IU and 0.44 IU of insulin which resulted in a 43% and 88% decrease in blood glucose levels respectively [131]. In a similar study, Migalska *et al*. prepared MN

arrays composed of poly (methylvinylether maleic anhydride) mixed with insulin [106]. This enhanced insulin transport and provided a dose-dependent hypoglycaemic effect in diabetic rats. However, the cumulative amount of insulin delivered across the skin accounted for approximately 40-55% of the original drug loading. As a result, the MN arrays in this study produced a hypoglycaemic effect which was 40 times lower than the SC injection. This highlighted the difficulty in delivering larger biomolecules, with delivery often limited to the drug loaded in the needles and producing undesirable drug wastage [106,132]. This has subsequently led to the development of a two-step manufacturing process in which the biomolecule is only housed within the needles themselves. Ling *et al*. developed a dissolving bilayer MN array composed of starch and gelatin, which contained insulin loaded needles and a drug free baseplate [133]. Importantly, the pharmacological activity of insulin from the MN arrays was maintained after encapsulation and release from the MN arrays. Pharmacokinetic and pharmacodynamic results showed a similar hypoglycaemic effect in rats receiving insulin loaded MN arrays and a SC injection. Ito *et al*. also developed a two-layered dissolving MN array, composed of chondroitin sulfate needles containing the intermediate-acting insulin, protamine sulfate insulin (PSI), and a drug-free chondroitin sulfate supporting baseplate [134]. Upon skin insertion, PSI was released from the MN array within 5 min. The relative pharmacologic availability of PSI from the MN arrays was 100.2 ± 9.8%, with no significant differences observed in the hypoglycaemic effects when compared to a SC injection *in vivo*, using rats.

Designing a rapidly dissolving MN system in which insulin is only loaded into the needle tips offers great potential for bolus or mealtime insulin administration. If sustained insulin delivery is required, biodegradable materials such as amylopectin, chitosan and carboxymethlycellulose can be used [135,136]. However, previous studies have shown that these materials require high processing temperatures thus affecting insulin stability [137–139]. One possible way to overcome this issue is to encapsulate the insulin into microparticles as detailed by Liu *et al* [140]. Here, a two-layered dissolving MN array was fabricated from insulin loaded $CaCO_3$ microparticles and a poly(vinyl pyrrolidone) (PVP) matrix. Using diabetic rats, the maximum decrease in blood glucose occurred after 2 h *via* SC injection (5 IU/kg). In contrast, a comparable reduction in glucose level was determined after 4-5 h following

insertion of the dissolving MN array (20 IU/kg). This slow decrease in glucose level was attributed to the slow diffusion rate of insulin from the MN array. The authors also showed that this MN array device-maintained blood glucose levels under the normoglycaemic state twice as long as the SC control.

Multiple daily insulin injections have been a common regime worldwide for the management of diabetes. In this context, single integrated MN array was developed by Chen *et al* with multiple release kinetics for mimicking the daily bolus release of insulin. [141] Chen *et al.* fabricated the MN from gelatin, cross-linked gelatin, and a mixture of cross-linked gelatin and hyaluronic acid in a single platform to offer three different insulin release profiles that could deliver the insulin on post-breakfast lunch and dinner trend. The *in vivo* results of this study using streptozotocin (STZ)-induced diabetic mice highlighted that the produced device was not only able to rapidly respond to elevated glucose levels but also provide the ability for rapid transdermal delivery of controlled release depots for basal-bolus combinatorial release of insulin. This further highlighted the versatility of the dissolving MN array system in delivering insulin for diabetic therapy.

2.5 Hydrogel-forming MN arrays

To overcome the challenges of drug-loading in other MN array types, self-disabling hydrogel-forming MN arrays have been developed. First described by Donnelly *et al.*, hydrogel-forming MN arrays are composed of cross-linked polymers which imbibe the ISF and swell, creating hydrogel micro-conduits within the skin layers. This subsequently permits drug delivery into the dermal micro-circulation [103]. In principle, this MN array type consists of drug-free micron-scale needles situated in perpendicular orientation on a base plate to which a separate drug containing reservoir is placed on top. As a result, drug loading is no longer restricted, a considerable drawback with dissolving and coated MN arrays. For this reason, hydrogel-forming MN arrays have successfully delivered high doses of peptide and protein-based molecules [103]. In particular, delivery of insulin from a 600 μm height, 19 x 19 poly(methylvinylether/maleic anhydride) crosslinked with poly(ethyleneglycol) (PEG) MN array resulted in a 52.5% increase in skin permeation *in vitro* relative to a standard insulin loaded adhesive

patch. This was further enhanced when the MN array was coupled with iontophoresis which as discussed previously, using electronic systems to enhance drug delivery has true potential provided they are of suitable size to enable incorporation into a MN array device. Application of this MN array *in vivo* resulted in a controlled reduction in blood glucose levels to 90% of its original value in diabetic rats after 2 h [103]. Blood glucose levels decreased further to 37% by the end of the experimental period (12 h). This demonstrated the ability of this MN array design to maintain sustained doses of insulin for improved glycaemic control.

An alternative hydrogel-forming MN array design was proposed by Yang *et al.* in which a poly(vinyl alcohol) (PVA) hydrogel system was created by forming microcrystalline domains as the crosslinking junctions [142]. This was achieved through a freeze-thaw method which enabled the insulin to be encapsulated within the needle tips without denaturing [142]. In this study, the relative availability of insulin delivered *via* the MN array to diabetic pigs was approximately 20% of the total insulin loading. Therefore, to determine the relative bioavailability *in vivo*, two groups of diabetic pigs received insulin through SC injection (0.4 IU/kg) and two MN arrays (2 IU/kg) respectively. Blood insulin curves for the MN arrays and SC injection were comparable to each other, signifying that this MN array design can deliver predictable doses of insulin. Further analysis showed that the onset peak of the MN array was approximately 20 min slower than the SC injection, however this was followed by a sustained release of insulin over a subsequent 3 h period. This was not observed with the SC injection, which resulted in a rebound in blood glucose concentration 2 h after administration. The authors suggested that this sustained effect has the potential to control basal blood glucose levels thus reducing the number of insulin doses required each day. It is evident that this MN array, in addition to the MN arrays described previously by Donnelly *et al*., require a greater insulin loading to achieve the same therapeutic effect as the SC injection. This is perhaps due, in part, to the relative infancy of this MN array type. Further development may indeed yield a MN array that is more comparable to the proven traditional SC injection.

Although these conventional drug delivery strategies using MN arrays are painless, one limitation is that they lack a sensing ability of blood glucose concentrations and therefore require frequent

monitoring of blood glucose levels and timely application of insulin-containing MN arrays to maintain normoglycaemia. Therefore, a system that can mimic the physiological feedback provided by the pancreatic *β*-cell normally, is critical for achieving successful outcomes in the management of diabetes is desirable [67,143]. One such system to fulfil these requirements are glucose-responsive closed-loop MN arrays.

2.6 Glucose-responsive MN arrays

Extensive efforts have focused on glucose-responsive closed-loop insulin delivery systems. Closed-loop drug delivery systems are considered a healthcare innovation because they are able to provide medical intervention using the complex network of feedback loops on which human physiology depends [143,144]. These systems combine three fundamental components: a continuous analyte or signal sensor, a control algorithm that receives the feedback from the sensor and calculates the needed drug dose for the patient, and a drug delivery mechanism that administers such drug [144,145]. These systems are commonly used for the anaesthesia management and for the continuous infusion of insulin required for diabetes treatment [144].

Closed-loop insulin delivery systems are medical devices consisting of a continuous glucose monitor sensor, a control device and an external insulin infusion pump that delivers insulin continuously/automatically as a direct response to glucose levels (**Figure 6**) [143,145,146]. As well as containing glucose-responsive components, this platform includes polymeric MN arrays [147]. The aim of this system is reduce the development of insulin-induced hypoglycaemia while obtaining tight control of blood glucose levels [143,145,146]. Apart from the integration of polymeric MN arrays, the novelty of this system resides in the real time feedback between blood glucose levels and the insulin release, similar to that provided by the pancreatic β-cell [143], which increases the efficacy of the treatment while reducing adverse side effects [148–150]. Accordingly, closed-loop insulin delivery systems represents a promising alternative to the conventional injections and insulin pump treatment [146], improving quality of life and health in diabetic patients.

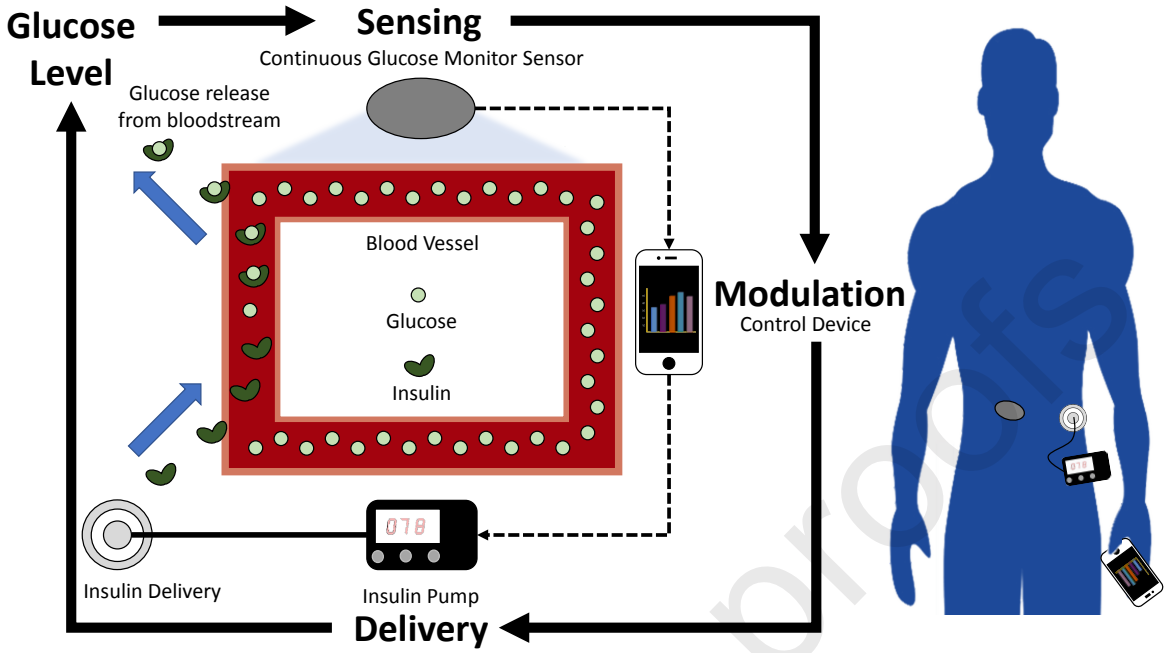


Figure 6 The functioning scheme for a closed-loop insulin delivery system.

Glucose-responsive MN arrays are able to monitor the blood glucose levels and release the required amount of insulin or any other antidiabetic therapeutics to closely control blood glucose levels, thereby maintaining normoglycaemia, achieving a smart closed-loop system for insulin delivery. For instance, Yu *et al.* developed a HA-based MN array containing nanoscale glucose-responsive vesicles (GRVs) loaded with insulin and the enzyme glucose oxidase (GOx) (**Figure 7A**) [151]. Cross-linked HA was used as the polymeric MN array matrix backbone to ensure that strong needles were formed. The GRVs were prepared by the self-assembly of HA modified with hydrophobic 2-nitroimidazole groups, which can be reduced to hydrophilic 2-aminoimidazole groups under hypoxic conditions [151]. GOx catalyses the oxidation of glucose to gluconic acid and hydrogen peroxide (H_2O_2) in the presence of oxygen (**Equation 1**). Therefore, in the presence of high blood glucose levels, oxygen was consumed due to the previous enzymatic conversion, causing a local hypoxic, acidic and H_2O_2 rich environment, and reducing hydrophobic 2-nitroimidazole groups. This resulted in the disassembly of GVRs and release of the encapsulated insulin (the therapeutic payload) [151]. This glucose-responsive device showed

promising *in vivo* results, maintaining a normoglycaemic state in mice for up to 4 h, while reducing the risk of hypoglycaemia. Based on the previous approach, Ye *et al.* reported the testing of a HA-based MN array containing pancreatic *β*-cells and synthetic glucose-signal amplifiers (GSAs) and loaded with α-amylose [152]. These GSAs were prepared similarly to the previous described hypoxia-responsive vesicles and encapsulated three enzymes: GOx, α-amylase, and glucoamylase. Therefore, when the glucose level increased, a hypoxic local environment was generated, leading to the disassociation of the vesicles, and thus releasing the three encapsulated enzymes. Then, α-amylose was hydrolysed into disaccharides and trisaccharides by α-amylase, which was then converted to glucose by glucoamylase. This amplified glucose signal reached the *β*-cells capsules (that were loaded on the back of the needles), stimulating insulin secretion. The *in vivo* results of this study using STZ-induced diabetic mice highlighted that the produced devices were able to closely control the glucose levels for a extend period of time (10 h) [152].

$$Glucose + O_2 + H_2O \xrightarrow{GOx} Gluconic\ acid + H_2O_2 \qquad \text{(Equation 1)}$$

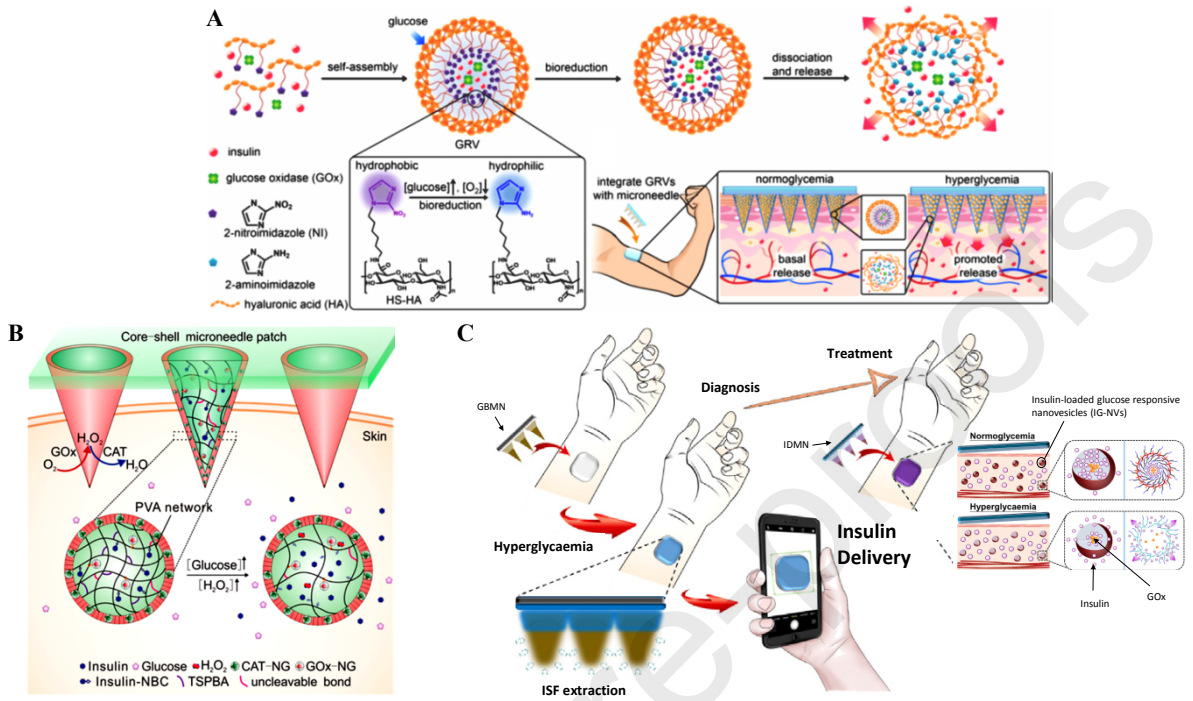


Figure 7 Schematic representations of **(A)** Hyaluronic acid (HA)-based MN arrays containing glucose-responsive vesicles (GRVs) loaded with insulin and the enzyme glucose oxidase (GOx); formation and dissociation of such GRVs; **(B)** Cross-linked poly(vinyl alcohol) (PVA)-based core-shell MN array for insulin delivery. Insulin was released from the core part of MN array when hyperglycaemia conditions occur and excess H_2O_2 was consumed in the shell part by enzyme catalase (CAT), and **(C)** A dual platform strategy comprised of a glucose-biosensing MN array (GBMN) and an insulin-delivery MN array (IDMN) for diagnosis and treatment, respectively. Reproduced and adapted with permission from [151,153,154]**.**

To improve the sensitivity of glucose-responsive devices, Yu *et al.* reported a HA-based MN array integrating both H_2O_2 and hypoxia responsiveness [155]. In this work, the authors developed a polymersome, comprising of PEG and polyserine modified with 2-nitroimidazole *via* a thioether moiety, loaded with insulin and GOx [155]. As reported in the previous approaches, the hypoxic and H_2O_2-rich local environments induced by the GOx-mediated glucose oxidation finally promoted the disassembly of the polymersomes and release of the encapsulated insulin [155]. Furthermore, the excess of H_2O_2 removal can support the activity of GOx, while mitigating the skin inflammation caused by this compound. This study showed favourable *in vivo* results regulating blood glucose levels for 6 h [155], while reducing the risk of hypoglycaemia using a diabetic mice model. Despite reducing the risk of

insulin-induced hypoglycaemic, one limitation from the outcome of this study [156], was the concerns over long-term biocompatibility [153]. Therefore, at this stage, it was suggested that the next generation glucose-responsive closed-loop delivery systems would prioritise biocompatibility and safety in their development.

Prioritising ease of preparation, administration and rapid responsiveness, biocompatibility and safety, Wang *et al.* reported a cross-linked biodegradable PVA-based core-shell MN array to regulate blood glucose levels (**Figure 7B**) [153]. The core-shell MN array is a unique innovation. The core of this glucose-responsive device contains insulin and GOx, while the shell component incorporates the enzyme catalase (CAT), which catalyses the breakdown of H_2O_2 to water and oxygen, thereby reducing the risk of inflammation produced by H_2O_2 and shielding the cells from oxidative damage [157,158]. Insulin was modified with 4-nitrophenyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) benzyl carbonate (insulin-NBC) to obtain H_2O_2-responsive insulin release. Moreover, PVA was also crosslinked by an H_2O_2-labile linker, N^1-(4-boronobenzyl)-N^3-(4-boronophenyl)-N^1,N^1,N^3,N^3-tetramethylpropane-1,3-diaminium to further facilitate transport of insulin and increase the responsiveness of the system [153]. In this report, the *in vivo* performance of the core-shell MN array was assessed in a type 1 diabetic mouse model. A rapid decrease of blood glucose levels in mice was observed 30 min post MN array application. Blood glucose levels were also effectively regulated for almost 6 h, which was longer than the subcutaneously administered insulin (control). Following a similar approach, Zhang *et al.* developed another PVA-based core-shell MN array incorporating both H_2O_2 and pH responsiveness [159]. The core component of this device was loaded with GOx-encapsulated nondegradable micelles and insulin-encapsulated degradable micelles (Ins-NCs), while the shell part incorporated CAT, as previously reported by Wang et al. [153]. Ins-NCs were prepared by the self-assembly of H_2O_2-labile and positively charged amphiphilic block copolymers, thus responding to the acidic and H_2O_2-rich local environments created by the GOx-mediated glucose oxidation. This led to the disruption of the Ins-NCs, promoting the insulin release. Using a type 1 diabetic mouse model, the authors highlighted that this MN array could release insulin rapidly and safely under hyperglycaemic conditions owning to both, an oxidative and acidic environment.

Promising results were shown whilst using this MN array in a mouse model of type 1 diabetes, regulating blood glucose levels for up to 4 h, while minimising the risk of hypoglycaemia [159]. Moreover, the use of CAT significantly alleviated the skin inflammation produced by the generated H_2O_2, thus improving the safety and biocompatibility of these previously discussed MN arrays [153,159].

Developed to treat both type 1 and type 2 diabetes, a glucose responsive insulin containing MN array integrating pH-responsive insulin-loaded nanoparticles (NPs) was manufactured [160]. The authors developed insulin loaded NPs loaded along with GOx catalase (CAT)-loaded pH-insensitive NPs. The *in vivo* study on STZ-induced diabetic mice demonstrated that the platform could effectively regulate blood glucose levels within normal ranges for a prolonged period (10 h). Manufactured specifically for the treatment of type 2 diabetes, a glucose-responsive alginate-based MN array was developed [161]. This device incorporated a dual mineralised particle-based system separately containing exenatide (exendin-4) and GOx. This dual system enabled the rapid release of exendin-4 as a result of the acidic conditions induced by the GOx-mediated glucose oxidation, while GOx remained immobilised, thus regulating blood glucose levels for a prolonged time period. *In vivo* results also showed a relatively mild inflammation reaction, thus confirming the high biocompatibility of this system in diabetic mice [161]. This study will be further discussed in section 3 of this review.

A dual platform strategy comprised of a glucose-biosensing MN (GBMN) array and an insulin-delivery MN (IDMN) array was recently developed by Hsu *et al.* for realising on-demand insulin in response to changes of blood glucose levels (**Figure 7C**) [154]. The GBMN array consisted of GOx-conjugated manganese oxide/graphene oxide nanozymes (GOx-MnO2@GO) and swelling methacrylated gelatin (MeGel), which was then sprayed with a 3,3′,5,5′-tetramethylbenzidine (TMB) solution on the semi-dry MN array. When the glucose level increased, gluconic acid and H_2O_2 were generated by reacting the glucose with GOx of GOx-MnO2@GO. H_2O_2-rich local environment then induced the oxidation of TMB in combination with MnO_2, causing a colour change that can be distinguished by the naked eye or using a smartphone. Subsequently, once a hyperglycaemic state was detected, the IDMN array was applied onto the skin surface. The PVA/PVP MN array was loaded with free insulin and insulin-loaded

glucose-responsive nanovesicles (IG-NVs). These NVs were prepared by the self-assembly of poly(*β*-amino esters), encapsulating both GOx and insulin. Thus, when the MN array was inserted, the free insulin and the IG-NVs were released with the subsequent disassembly of the latter, responding to the acidic conditions created by the GOx-mediated glucose oxidation. The IDMN array showed favourable *in vivo* results regulating blood glucose levels and a maintaining normoglycaemic state for up to 13 h, while reducing the risk of hypoglycaemia using STZ-induced diabetic rats [154].

As previously discussed, the closed-loop insulin therapy system generally consists of a glucose sensing element, a control system that adjusts insulin delivery according to blood glucose concentrations determined by the glucose sensor and an insulin pump. Liu *et al.* designed a piezoelectric (PZT) micropump, made from biocompatible materials, for the precision delivery of insulin [162]. This novel closed-loop insulin delivery system consisted of a silicon MN array which was used as the injection component and a non-invasive glucose monitor with wireless communication. The PZT micropump was compact, lightweight, disposable, biocompatible and had high resolution of flow rate [162]. Despite high backpressures obtained, *in vivo* experiments using diabetic rats verified the capability of this system to effectively regulate blood glucose levels. In addition, a touch-actuated MN (TAMN) array was manufactured for transdermal delivery of insulin [163]. This TAMN array consists of a solid MN array, medical tape, an anti-seepage gasket and a drug-containing medical sponge. Interestingly, the drug is in liquid form in the medical sponge. For transdermal delivery of insulin, the solid MN array is used to puncture the *stratum corneum.* Insulin subsequently passively diffuses into the skin and then into systemic circulation using the temporary microchannels produced by the solid MN array [163]. *In vivo* data showed that this system was able to maintain a normoglycaemic state for a prolonged period (11.63 h), using diabetic rats [163]. In this work, authors claimed that the system in this study could be potentially converted into a glucose-responsive MN array by changing the microchannels to self-close and re-open, based on the feedback of blood glucose levels, or integrating a MN based biosensor for glucose measurements.

Despite the benefits of the aforementioned approaches (glucose-responsive closed-loop insulin delivery systems), there are still some challenges to be considered. The glucose-responsive closed-loop delivery

systems mainly rely on GOx for glucose sensing. However, in the literature, there are concerns that GOx is associated with immunotoxicity and insulin denaturation, along with possible inflammation caused the toxic by-product H_2O_2 [164]. Glucose-responsive closed-loop insulin delivery systems which utilises the synthetic molecule, phenylboronic acid (PBA) is now being widely exploited [165].

2.7 Glucose-responsive MN arrays: Phenylboronic acid

PBA is a synthetic molecule that can interact with diols to form a 5-or 6-membered ring cyclic boronate ester. In order to increase the insulin-loading capacity in closed-loop delivery systems, a non-degradable polymeric matrix was used for the development of a facile glucose-responsive MN array (**Figure 8A**) [166]. Accordingly, this matrix was manufactured from an insulin-preloaded monomer mixture of *N*-vinylpyrrolidone, 2-(dimethylamino)ethyl acrylate, 3-(acrylamido)phenylboronic acid and ethylene glycol dimethacrylate by *in situ* photopolymerization. In this work, PBA was employed as the glucose-sensor component. In hyperglycaemic conditions, reversible boronate esters were formed, and due to their increased negative charge density, induced altered interactions in the polymeric matrix, this triggered the rapid release of the pre-encapsulated insulin into the skin. The increased charge differences further weaken the electrostatic interaction between the negatively charged insulin molecules and the polymeric units, further stimulating the insulin release. *In vivo* studies using minipigs confirmed the ability of these MN arrays to regulate blood glucose levels for a prolonged period of time (20 h) as well as the capability of preserving the insulin bioactivity at room temperature for over 8 weeks. Moreover, the lack of GOx as the glucose sensor component in this system avoided the toxic generation of H_2O_2 during the GOx-mediated glucose oxidation [166].

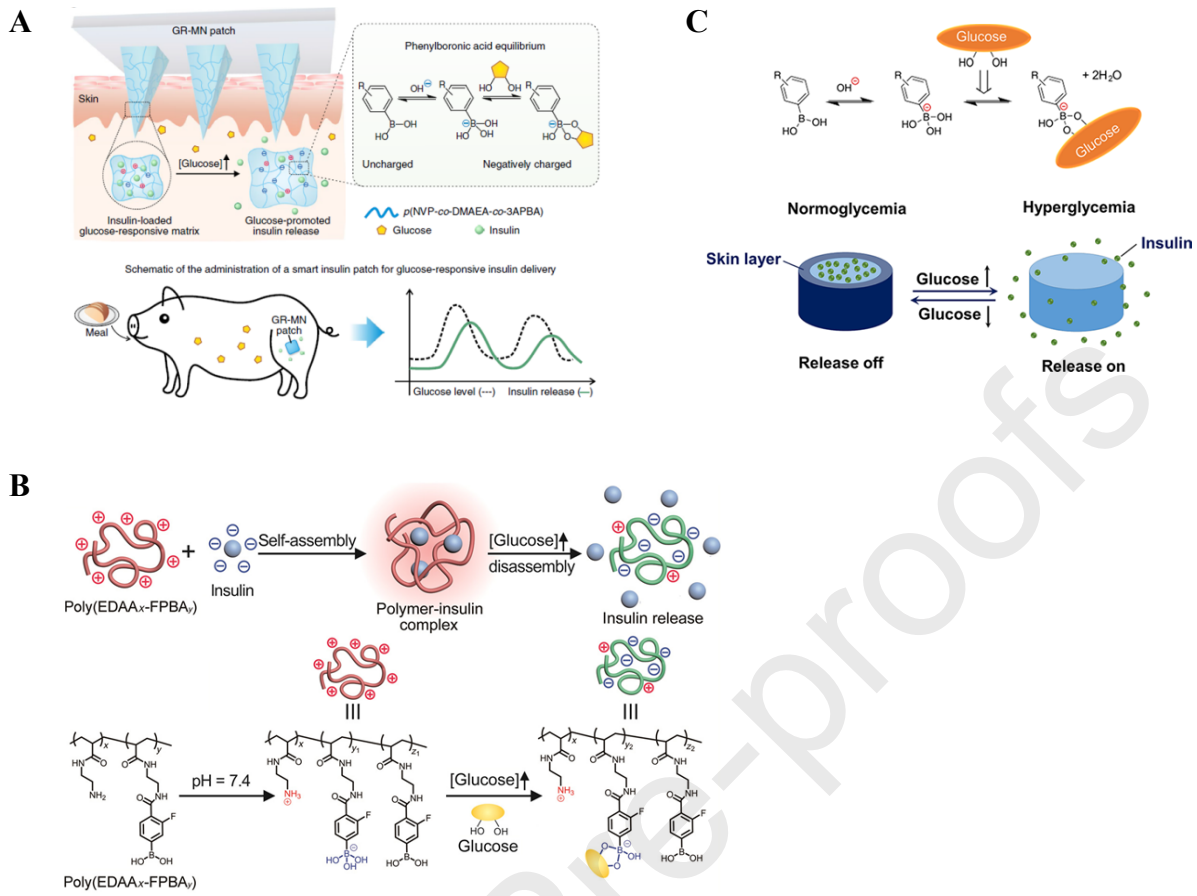


Figure 8 (A) Glucose-triggered insulin release from GR-MNs mechanism; **(B)** Schematic of glucose-responsive charge-reversal polymers for insulin delivery and **(C)** Schematic representation of (top): glucose-dependent equilibria of PBA derivatives and (bottom): skin-layer-regulated glucose-responsive insulin release from the hydrogel. Reproduced with permission from [164,166,167].

In order to increase the release rate of insulin from glucose-responsive insulin delivery systems, a novel charge switchable polymeric complex was prepared by Wang *et al.* [167]. This charge-switchable insulin-containing polymeric complex was fabricated from a positively charged polymer and negatively charged insulin *via* electrostatic interactions (**Figure 8B**). Incorporating amino and PBA groups, the positively charged polymer formed a stable micro-sized suspension with a high insulin loading capacity (49%) and a high loading efficiency (95%). Under hyperglycaemic conditions, glucose binds to the glucose-responsive PBA which is incorporated into the MN array. This converts the charge of the polymeric moiety from positive to negative, thereby facilitating insulin release from the complex. In a type 1 diabetic mouse model, *in vivo* hyperglycaemia-triggered insulin release with a rapid response is established (peak plasma insulin level was observed at 30 min) after the complex is administered by

either a SC injection or a MN array. *In vivo* studies also confirmed the ability of these novel MN arrays to maintain normoglycaemia for up to 6 h using mice [167].

Zhang *et al*. developed gelatin–starch mixture-based MN arrays embedded with insulin-loaded gold nanocluster (AuNC) nanocarrier to achieve minimally invasive and glucose responsive insulin release *in vivo*. In this study, two types of high drug-loading AuNCs for glucose-responsive insulin release were prepared. PBA groups were incorporated into both AuNCs. By encapsulation of these AuNC nanocarriers into MN arrays enabled a glucose-responsive insulin-release manner. With a single transdermal application on the dorsal skin of mice, the MN arrays efficiently controlled the blood glucose levels of STZ induced diabetic mice in normoglycemic ranges for 1 to 2 days [168].

Hu *et al.* developed H_2O_2-responsive polymeric vesicles loaded with insulin and GOx, based on HA MN arrays [156]. These vesicles were prepared by the self-assembly of amphiphilic block copolymers, PEG and phenylboronic ester (PBE)-conjugated polyserine, leading to the formation of a hollow spherical structure (also known as polymersomes). Due to the oxidation of glucose to gluconic acid, H_2O_2 was produced, oxidising and hydrolysing the grafted PBEs of the amphiphilic copolymer. This led in the dissociation of the polymeric vesicles and release of the encapsulated insulin [156]. To improve the biocompatibility, Tong *et al.* designed a PVP/PVA-based MN array integrating glucose- and H_2O_2-responsive polymeric vesicles loaded with insulin and GOx [169]. These vesicles were self-assembled from an amphiphilic triblock copolymer involving with PEG, poly(phenylboronic acid), which is the glucose-responsive block, and poly(phenylboronic acid pinacol ester) (PPBEM), which is the H_2O_2-responsive block. Therefore, the increase in the glucose levels led to the disassembly of the vesicles, releasing the loaded insulin in these hollow spherical structures, and can be further promoted due to the H_2O_2 generated during the GOx-mediated glucose oxidation, which hydrolyses the ester linkages of PPBEM. Likewise in the previous studies, this system also showed positive results in regulating blood glucose levels [169].

Produced in response to the challenges of the previously described conventional drug delivery strategies, hydrogel-forming MN arrays are removed from the skin completely intact, leaving no measurable

polymer residue [103]. Prepared from a hydrogel matrix, Chen *et al.* developed a glucose-responsive MN array composed of a semi-interpenetrating network hydrogel prepared by biocompatible silk fibroin and PBA/acrylamide for insulin delivery [164]. Therefore, when the glucose level increased, boronate-glucose complexes were formed, and due to their negative charge, electrostatic repulsion between them and the loaded insulin occurred, promoting the release of insulin (**Figure 8C**). Moreover, this release was regulated by a dehydrated layer ("skin layer") induced on the surface of the MN arrays by abrupt and quick change of hydration state, as previously reported in their previous works [170–174]. Thus, the performed system provided a sustained and glucose-responsive insulin release in safe way [164].

Glucose-responsive insulin closed-loop delivery systems hold remarkable promise for diabetes treatment with regards to maintaining normoglycaemia [14]. Despite achievements thus far regarding the glucose-responsive MN arrays, they are still at the preclinical in vivo studies involving small rodents. To facilitate the translation of these promising MN arrays to clinical use, detailed investigation of these systems on large animal models and humans is critically required.

3. Transmucosal insulin delivery using MN arrays

The oral route remains the preferred drug administration route by both prescribers and patients [175]. However, the delivery of biological macromolecules like insulin in the GIT is particularly challenging due to the high variation in pH and the presence of proteases, endonucleases, and bacteria, which lead to drug degradation [176]. For almost one century, researchers have been attempting to deliver insulin orally. In 1923, only two years after the discovery of insulin, G.A. Harrison documented the oral administration of high concentrations of insulin using an alcoholic solution, which was effective in one out of four patients [177]. Since then, a plethora of published articles described the use of an array of strategies to increase the oral bioavailability of insulin, including chemical modifications and increasing tissue permeation by incorporation of the drug into micro and nanoparticles, achieving only limited

success [178–185]. Despite this knowledge, researchers have explored the use of MN arrays for insulin delivery directly in the GIT mucosa.

Traverso *et al.* demonstrated for the first time the potential of MN arrays to increase the bioavailability of insulin in an animal model of Yorkshire pigs [186]. In this proof-of-concept work, the authors made endoscopy-assisted injections of insulin in three different regions of the GIT, the stomach, duodenum, and colon, using SC injections as a comparator. The blood glucose levels were monitored every 2 min after SC injection, and a considerable reduction in the onset time was observed for the gastric and duodenal treatments. In parallel, a prototype consisting of a cylindrical MN array pill (1 cm in diameter, 2 cm in length) was deployed into the stomach and its safe passage through the GIT of pigs was confirmed by radiography and post-mortem macroscopic evaluation of the GIT mucosa. This preliminary study demonstrated the feasibility and safety of the gastrointestinal delivery of insulin using MN arrays.

Aiming to deliver biological drugs directly into the gastric mucosa, Abramson *et al.* designed a self-orienting millimeter-scale applicator (SOMA) [187]. This work was motivated by the ability of a leopard tortoise to reorient itself passively. The SOMA was able to autonomously position itself in order to engage with the stomach's mucosa and deploy milliposts loaded with insulin, safely penetrating the mucosa without perforating the stomach wall. When assayed *in vivo* in fasted swine, the novel SOMA showed a similar pharmacokinetic behaviour than that observed in the control cohort treated with SC injections. In another work from the same group, a luminal unfolding MN array injector (LUMI) for oral administration of insulin as a model macromolecule drug was described [188]. The novel device depicted in **Figure 9A-E**, consisted of an ingestible capsule containing insulin-loaded dissolving MN arrays, which were unfolded their arms with a view to pierce intestinal tissue and deliver the drug loads. *Ex vivo* human and *in vivo* swine studies demonstrated the ability of the MN array device to efficiently deliver insulin without causing full-thickness perforations of the duodenal wall. Pharmacokinetic studies revealed that the experimental LUMI resulted in a profile which demonstrated a more rapid pharmacokinetic uptake. Furthermore, the systemic uptake profile was found to be >10% of that of a SC injection across 4 h.

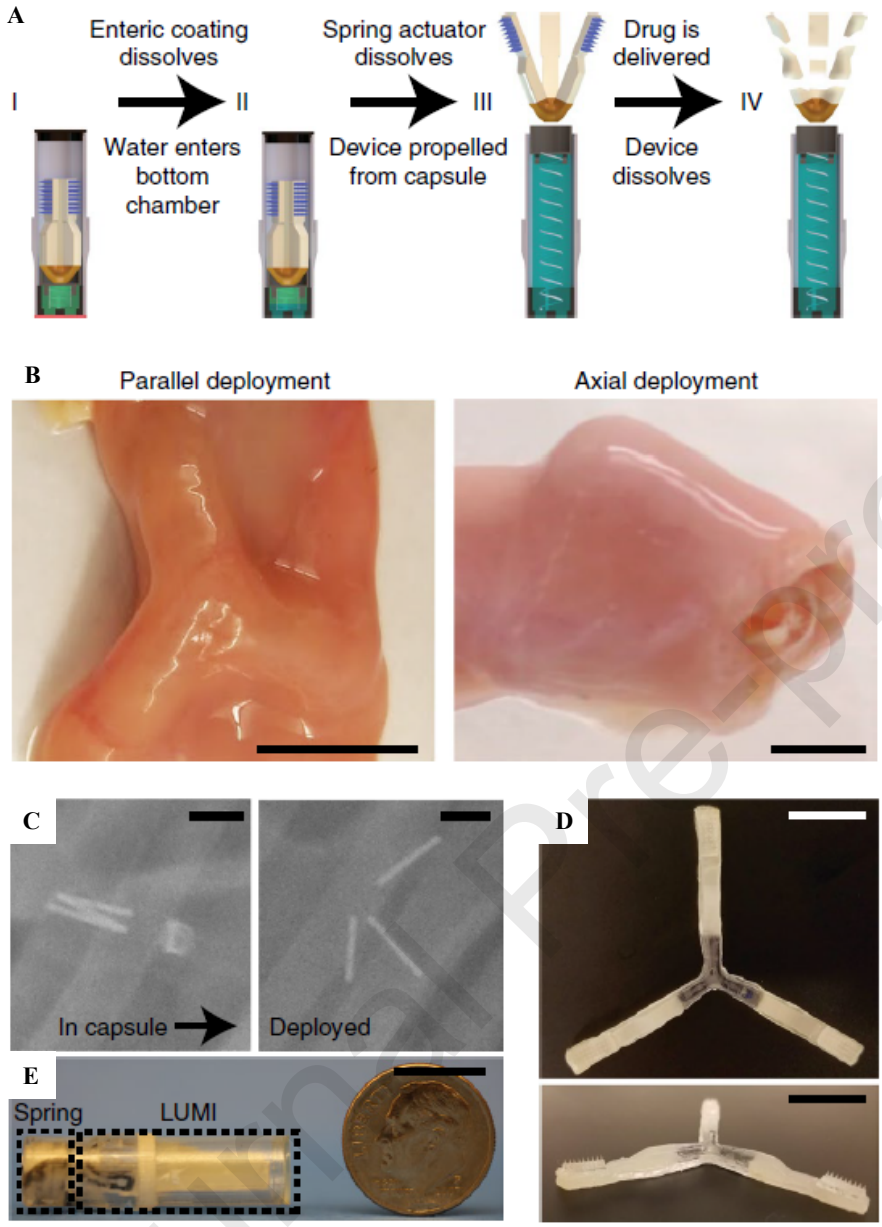


Figure 9 Design and specification of the luminal unfolding MN array injector. **(A)** Actuation scheme of the device; **(B)** Multiple orientations of the dispositive in the duodenum, (left) parallel and (right) axial; **(C)** Radiographic images revealing the actuation of the capsule in vivo within two hours; **(D)** Images of the unfolded device observed from the top and side and **(E)** Unfolding dispositive inside the capsule. (Reproduced with permission from [188]).

It is important to highlight that all *in vivo* studies previously discussed in this section were performed on Yorkshire pigs that were fasted overnight prior to the beginning of the study. In Abramson *et al.,* the effect of food and liquid in the animals' stomach was investigated [187]. In conclusion, the SOMA was not able to operate effectively when food and liquid was present in the stomach. Nevertheless, these

studies together demonstrate that these robotic milli and MN array devices are an attractive platform to deliver insulin orally, for the treatment of insulin-dependent diabetes. By using these platforms, patients' compliance toward medication and quality of life may be increased. Despite the undoubted positive impact that such smart drug delivery systems could make on the treatment of diabetes, their potential production at the industrial scale may be challenging and lead to high treatment costs.

4. Transdermal non-insulin delivery using MN arrays

Although considerable research has been undertaken for MN mediated transdermal delivery of insulin, this novel minimally invasive delivery platform has not been widely investigated for non-insulin antihyperglycaemic agents. Of the few other pharmacological applications explored with MN arrays, metformin and GLP-1 analogues have received the most attention. Metformin is the first line therapy for treatment and management of type 2 diabetes, following patient education and dietary advice [20], and therefore its delivery using MN arrays has been widely investigated. Delivery of metformin using MN arrays ranges from needles made from simple polymer blends [189] to needles that trigger the release of loaded drug only when exposed to certain conditions, such as infrared light [190,191] or heat [192,193] using photo/thermal conversion agents.

The ability of hydrogel-forming MN arrays to deliver metformin transdermally was first explored by Migdadi *et al*. [95]. MN arrays were made from an aqueous blend of 20% w/w poly (methylvinylether-*co*-maleic acid) cross-linked with 7.5% w/w PEG 10,000 Da by an esterification reaction, with a lyophilised wafer situated on top of the array. *In vitro* permeation studies using dermatomed neonatal porcine skin indicated that metformin permeation from the hydrogel-forming MN arrays was significantly greater than when delivered from the lyophilised wafer alone (p<0.05). Furthermore, *in vivo* studies performed in rats investigated the ability of hydrogel-forming MN arrays (each lyophilised wafer containing 50 mg) to deliver metformin into the systemic circulation compared to oral drug delivery (100 mg/kg). Transdermal bioavailability was 30%, with a maximum achieved concentration of 3.77 ± 2.09 μg/ml at 24 h, compared to 6.25 ± 2.94 μg/ml from the oral dose. Plasma concentrations

of metformin were sustained over 24 h from the hydrogel-forming MN array. The authors cautiously extrapolated *in vivo* results to determine whether a reasonable patch size could be feasibly created for delivery of metformin in a human and found that a metformin dose of 500 mg in a human subject would be achievable with a patch size of approximately 8 cm^2, which would contain 833 mg metformin.

In a different approach, Yu *et al.* fabricated dissolving MN arrays which containing metformin and a photothermal conversation agent (Prussian blue nanoparticles) for on-demand, controlled delivery of the drug [190]. It was demonstrated that by exposing the MN array to near-infrared light irradiation (NIR), a rapid thermal ablation reaction occurred, transitioning the nanoparticles from a solid into a liquid state. This transition caused the encapsulated metformin to be released. It was further found, that increasing the duration of irradiation, considerably increased release of metformin with negligible thermal damage, as it was demonstrated that 21.1-24.1% of metformin loaded into the nanoparticles was released, compared with 9.9-11.8% for 2 min and 1 min exposure, respectively. The hypoglycaemic effect of the MN arrays was further assessed *in vivo*. Mice treated with MN arrays loaded with metformin displayed a trend of slow reduction in blood glucose levels, which was maintained for a longer period of time compared to the SC injection group. For example, the concentration of blood glucose in the MN array group (0.6 mg) decreased to <150 mg/dl within 3.5 h, and was maintained at a low level thereafter. The ability of the NIR to control the drop in blood glucose was attributed to the needle tips, which melted and released metformin following the irradiation step. Following removal of the NIR light, the needle steadily cured, thus delaying further drug release.

The ability of NIR for metformin delivery *via* MN arrays was also investigated by Zhang *et al.* [191]. In this instance, photothermal conversion factor (Cu_7S_4 nanoparticles) and metformin were encapsulated into lauric acid and polycaprolactone arrowheads. A fast yet consistent release of metformin was observed (14.6% and ~30.5% of metformin was released after each irradiation period of 2 and 4 min respectively). *In vivo*, a 1 mg SC injection of metformin was found to cause a rapid reduction in blood glucose levels (82.3 ± 6.5 mg/dl) at 2 h, which quickly returned to the original high blood glucose level. MN arrays loaded with 0.5 mg and 1 mg of metformin resulted in blood glucose

levels of 180.7 ± 8.2 mg/dl and 125.2 ± 4.7 mg/dl respectively at 6 h, levels which were maintained for a greater duration compared to the SC injection group.

Apart from MN arrays that respond to infrared radiation to deliver their drug cargo, thermoresponsive MN arrays have also been fabricated in an attempt to control drug release. For example, Lee *et al*. created a wearable, graphene-based electrochemical device with integrated MN arrays which respond to thermal stimuli for the monitoring of diabetes [194]. The wearable device consisted of sweat-control (a waterproof film combined with a layer to absorb sweat), sensing (pH, humidity, tremor and glucose sensors) and therapeutic (MN arrays, heater, temperature sensor) components. The device was designed so that polymer-based MN arrays coated with the phase-change material released the drug into the bloodstream when the programmed threshold temperature was exceeded. Therapeutic *in vivo* efficacy studies indicated that the MN array treatment group suppressed blood glucose levels compared to the controls thatwere considered statistically significant ($p< 0.05$). However, the use of diabetic rats meant that the device could not be "worn" as intended for use in humans.

Dissolving MN arrays with a thermoresponsive or thermal-responsive (lauric acid) coating for transdermal delivery of metformin were produced by Liu *et al*. [192]. When NIR was applied to the dissolving MN arrays, bismuth nanodots induces a light-to-heat transduction reaction. This reaction resulted in the melting of lauric acid, dissolving the polymer matrix was dissolved following ISF absorption, enabling the release of metformin from the MN arrays. Compared to a SC metformin injection (1 mg, which caused a maximal 74% blood glucose level reduction at 2 h), application of the dissolving MN array (1 mg metformin) resulted in a maximal 69.4% reduction in blood glucose levels at 6 h. This is compared to a 9.4 % drop in blood glucose levels when the MN arrays were applied without the application of NIR to induce the light-to-heat transduction.

Zhang *et al*. published two studies investigating the ability of metformin to be delivered into the systemic circulation using MN arrays [189,193]. In the first study [189], polydopamine was used as a photothermal conversion agent, lauric acid was used as a phase change component, which was coated onto hollow mesoporous SiO_2 nanoparticles to form NIR responsive nanocarriers. Compared to a SC

injection of metformin (1 mg, which resulted in a 81.8 ± 4.5 mg/dl minimum blood glucose level at 2 h), application of the dissolving MN array (1 mg metformin) resulted in a 167.7 ± 5.2 mg/dl minimum blood glucose level at 8 h using diabetic rats. These blood glucose levels were maintained below normoglycaemic state (<200 mg/dl) for approximately 4.0 h in the MN array group, compared with 2.5 h for the SC group. The second study [193] used polycaprolactone/PVA MN arrays with metformin-loaded polycaprolactone arrowheads. When thermal stimuli was applied, the metformin-loaded MN arrowheads would separate from the array and release metformin into the skin. Metformin-loaded MN arrays (8 and 16 mg) were applied to rats and compared against a SC injection of 8 mg metformin. Similarly to the previous study, the SC injection group resulted in a rapid decline in blood glucose levels, reaching their minimum level at 2 h. MN arrays loaded with metformin demonstrated a trend of slow reduction in blood glucose levels in rats, reaching their minimum level at 6 h, and maintained a significantly (p<0.05) lower level for an extended duration (~3.5 h) when compared to the SC group (~1.5 h). Both studies appear to show the same trend, in that application of a MN array *in vivo*, results in a significant (p<0.05) reduction in blood glucose levels, though this does not occur as rapidly as a SC injection. However, the MN arrays appear to have the advantage of prolonged hypoglycaemic effects. . Therefore, one may argue that MN-mediated delivery of metformin may be ideal for patients who traditionally use a once daily or twice daily insulin regimen due to the prolonged hypoglycaemic effects attributed to MN-mediated delivery of metformin. This is in contrast to bolus injections of insulin, which are typically administered before meals when a large intake of glucose is anticipated. Therefore, there may be a place in diabetes therapy in the future for metformin MN arrays for a generalised reduction in blood glucose, combined with insulin administration before mealtimes.

The investigation of MN arrays for the potential treatment of diabetes has not solely been limited to the delivery of insulin and metformin. The systemic delivery of GLP-1 analogues using MN arrays has recently been investigated [161,195–197]. One GLP-1 analogue is exendin-4, a therapeutic peptide, approved by the FDA, used for the treatment of type 2 diabetes. Zhu *et al*. explored MN mediated delivery of exendin-4 transdermally using sodium hyaluronate MN arrays [195]. The C_{max} (22.18 ± 4.33 ng/ml) and relative bioavailability (97.06%) of the exendin-4 MN arrays was found to be comparable

to the SC injection (C_{max} 24.20 ± 4.79 ng/ml, relative bioavailability 100%). Furthermore, observed blood glucose levels demonstrated that both the SC and MN route of exendin-4 administration rapidly decreased blood glucose levels, resulting in minimum plasma glucose levels of 42.32 ± 7.90% (SC) and 43.89 ± 9.81% of their initial concentration (MN). Following the observed reduction in blood glucose levels, a return to pre-test levels were observed at 6 h, and typical hyperglycaemia was observed 12 h post exendin-4 administration.

Lahiji *et al*. [197] optimised the physical, chemical, and thermal requirements for the manufacture of exendin-4-containing dissolving MN arrays. Enhancing the dissolving MN array manufacture parameters was found to maintain exendin-4 activity in the dissolving MN arrays by up to 98.3 ± 1.5%. Exendin-4 (10 μg) was delivered to diabetic rats either by the optimised dissolving MN system or *via* a traditional SC injection. Blood-glucose levels were reduced to 39.68 ± 8.05% and 26.26 ± 3.23%, for the SC injection and exendin-4 dissolving MN array group, respectively, after 3 h. C_{max} was achieved at 1 h in the SC group and 2 h in the exendin-4 dissolving MN group. This was thought to be caused by slower diffusion of exendin-4 from dissolving MN arrays compared to the SC injection. A similar study that focused on the use of dissolving MN arrays formulated from HA for the delivery of exendin-4 was reported by Liu *et al.* [196], this time, with exendin-4 loaded in the MN array tips. The secretion of insulin was noticeably increased by a SC injection of 10 μg/kg and 50 μg/kg exendin-4, and was found to be similar in the exendin-4 tip-loaded MN array groups (C_{max} values of 3.10 ± 0.56 ng/mL and 3.13 ± 0.24 ng/mL for the SC and MN groups respectively from 10 μg/kg exendin-4 administration).

A novel, dual mineralised particle MN array system was designed to simultaneously deliver exendin-4 with GOx as a glucose responsive catalysing enzyme to degrade the MN polymeric tip matrix while insertion [161].. They evaluated the *in vivo* antidiabetic effect of MN arrays for type 2 diabetic in mice. MN arrays containing both mineralised exendin-4 and mineralised GOx showed promising blood glucose level control. Even after 72 h treatment, blood glucose levels in treated mice were still lower than the original level. This was attributed to the responsiveness of mineralised GOx and fast dissociation of mineralised exendin-4. These MN arrays resulted in 5 days of normoglycaemia

maintenance, and levels slowly returned to their original stage after 9 days, which is considered somewhat more effective than long-acting formulations currently available [198,199].

The various studies presented here show promise for the possible use of MN arrays in the future for the needle-free delivery of antihyperglycaemic agents, thus, potentially eradicating the necessity for adherence to daily oral medication regimens. However, to realise the full potential of this novel delivery platform, more comprehensive preclinical and clinical pharmacokinetic studies and, the investigation into patient usability and acceptability are now required to drive the technology towards successful commercialisation.

5. Patient monitoring using MN arrays

5.1 Patient Monitoring: What is it, and why is it important?

Therapeutic monitoring in patients is the analysis of biological matrices, typically plasma or serum, to detect and quantify drugs and/or endogenous markers. In clinical settings, most commonly hospitals and GP surgeries and community pharmacies, and therapeutic monitoring is used to assist health care professionals in providing care from diagnosis to outcome [200]. In the community pharmacy, therapeutic monitoring is used for the purpose of monitoring a patient's carbon monoxide levels in the nationwide smoking cessation scheme. In the GP setting, a wider range of conditions can be diagnosed and monitored through the analysis of blood, urine, stool and sputum samples. In the primary care setting, therapeutic monitoring can be used as a diagnosis tool for both acute and chronic conditions. For example, pathophysiological events such as acute myocardial infarction can be diagnosed and monitored in real time through effective use of therapeutic monitoring of endogenous marker levels i.e. troponin. Oftentimes the outcome of an emergency case, such as this, is vastly improved due to the rapid detection and treatment facilitated by therapeutic monitoring. Whilst disease diagnosis is an important aspect of patient monitoring, the ability to detect and quantify drug levels in a patient after administration provides vital information regarding disease treatment. This information allows health

care professionals to optimise treatment based on clinical evidence and ensures that instances of sub-optimal and toxic dosing are avoided. Therapeutic monitoring of drug levels is all the more important when a patient is being treated with a drug that has a narrow therapeutic index such as lithium, gentamicin or warfarin. Patient monitoring can also underline pharmacokinetic and pharmacodynamic differences within a given population. Variation between patients in terms of drug absorption, distribution, metabolism and elimination (ADME) is common. For providence of optimum treatment, care will often require personalisation. Other uses of therapeutic monitoring include evaluation of patient adherence to treatment regimens as well as abstinence from alcohol and illicit drugs. As it is a requirement for the diagnosis and successful treatment of many conditions, the value of therapeutic monitoring in a clinical healthcare setting cannot be understated.

5.2 Conventional sampling techniques for glucose monitoring

Various sample types exist for therapeutic monitoring, such as breath, stool, sputum and urine samples. However, the vast majority of therapeutic tracking is carried out using whole blood samples, extracted traditionally using a hypodermic needle and syringe. The analysis of whole blood can provide precise and accurate information about drug and endogenous marker levels before, during and after treatment. Regular and accurate quantification of glucose levels in diabetic patients is essential for the appropriate management of this condition [201]. As previously mentioned, the universal method for glucose monitoring in people with diabetes is achieved by pricking his/her finger with a lancet before the resultant blood is added to a glucose test strip *via* capillary action and then analysed with the corresponding hand-held device. In 2005, a cross-national Diabetes, Attitudes, Wishes and Needs (DAWN) study was performed to establish adherence and identify barriers in self-monitoring of blood glucose [202]. It is important to note that although completed in 2005, this is the latest national adherence study surrounding diabetes management. From this study, it is estimated that only 44% of type 1 diabetes patients and 24% of type 2 diabetes patients routinely test as per guidelines [202]. Multiple barriers have been identified surrounding effective self-monitoring of blood glucose. These

include pain, inconvenience, fear of blood or needles, stigma associated with monitoring and diabetes, social pressure and psychological issues e.g. depression and anxiety [203–205]. Indeed, previous research has identified that 10% of the population are thought to suffer from needle phobia [206]. As stated previously, elderly and neonatal patients often require increased levels of therapeutic monitoring.

5.3 Alternative sampling techniques for glucose monitoring

To overcome the barriers associated with the conventional sampling technique for glucose monitoring, there is a need for the advancement of minimally invasive, or ideally, non-invasive techniques that permits both fast and repeatable monitoring opportunities for patients. Accordingly, a paradigm shift away from whole blood sampling has re-focused substantial research efforts on the extraction of ISF for therapeutic monitoring. ISF can be extracted from the outer layers of the skin in a minimally invasive or non-invasive manner, without the need for a hypodermic needle and syringe. As a result, a number of new and innovative sampling techniques have been developed in recent years, which are based on the principle of extracting ISF from the skin by overcoming the barrier properties that the skin possesses. Moreover, glucose concentrations in ISF have been shown to be accurately reflective of those in whole blood samples [207,208]. Two skin ISF extraction methods have been detailed in the literature. The first method is reverse iontophoresis which is considered non-invasive. This method involves the application of a small electric current to the skin using two probes. Extraction of both neutral polar molecules and cations from skin ISF can be obtained by this method, which is then available for analysis [208]. Unfortunately, there is a need for highly trained personnel for reverse iontophoresis for its operation, and equipment used for iontophoresis is often costly. The second method is known as clinical microdialysis, which may be considered minimally invasive. This method involves the insertion of a microcatheter probe into the skin whereby the principles of conventional chemical dialysis results in the extraction of analytes. This main challenge associated with this method of analyte extraction is the positioning of the microcatheter, which can easily be incorrectly inserted, even by a healthcare professional who has undergone appropriate training [207].

Alternative to traditional finger prick testing, continuous glucose monitoring (CGM) is the uninterrupted measurement of an individual's glucose levels throughout the course of a day. CGM provides a more complete picture of the fluctuation in a patient's glucose levels and therefore their diabetic control [209]. Rather than rely on intermittent blood sampling, CGM is based on constant chemical microdialysis of analytes in the ISF. An example of a CGM device is the FreeStyle® Libre glucose monitoring system, mentioned previously and shown in **Figure 10.** In the form of white round disc that can worn for up to 14 days on the arm, the FreeStyle® Libre senses interstitial glucose by using a wired GOx enzyme co-immobilised on an electrochemical sensor [210,211]. In this instance, users can obtain instant feedback on glucose levels in the ISF by scanning the system with a handheld reader or their mobile phone. While this system can sometimes present difficulties, i.e. pain and inaccurate readings, it serves to remedy many of the drawbacks associated with traditional blood glucose testing due to microcatheter positioning. Therefore, through its ease of use, this device represents a leap forward in the domain of minimally invasive glucose monitoring and has led to heightened demand for this novel technology in the UK. CGM has the capability to drastically improve the level of care that a diabetic patient receives and as a result, those working in the MN array field have recognised the novelty of this technology.

Figure 10 The FreeStyle® Libre glucose monitoring system composed of a wearable sensor and a handheld reader which can send real-time data to devices such as mobile phones (Reproduced with permission from [212]).

5.4 MN array innovations for glucose monitoring

Since their first conceptualisation in the 1970s, the majority of research into MN arrays has focused on the delivery of drugs and vaccines into and across the skin. This innovation has also led to the realisation of a number of alternative applications for MN arrays that diverge from their traditional use in drug delivery. One such application is the use of these minimally invasive and pain-free devices for therapeutic monitoring [213–216]. Upon application to the skin, MN arrays have the ability to obtain ISF, from which relevant biological information can be obtained [207,208]. It has been shown that MN arrays can be used to obtain blood samples through modification of MN array geometry i.e. longer needle length, however, the likelihood of pain receptor stimulation is increased compared to ISF sampling [213].

Considering the mechanisms involved in MN array-based therapeutic monitoring, there are two parameters that highly influence effective ISF extraction, namely mechanical and fluidic considerations. Firstly, MN arrays must be able to penetrate the *stratum corneum* upon application. Hence, during the MN array fabrication, it is important to ensure that MN array tips are suitably strong and sharp enough so that the resistive properties of the skin are overcome during insertion [217]. Secondly, it is essential that sufficient fluid flow through the MN array tips is maintained.

Numerous publications exist in the literature related to MN-mediated monitoring of drug, metabolite and biomarker levels through analysis of ISF For instance, MN arrays have been developed for the continuous monitoring of levodopa [218], phenoxymethylpenicillin [219], β-lactam antibiotics [220] and opioid agent [221] concentrations post-administration. Interestingly, MN arrays have been used for the monitoring of electrocardiography signals [222], electroencephalography signals [223] and lymphatic drainage [224], in efforts to detect instances of abnormal function. The proceeding section will focus on the use of MN technology for glucose monitoring, either through MN arrays as sensing probes or MN arrays for ISF collection.

5.4.1 MN arrays as sensing probes

One of the first devices patented for ISF glucose monitoring using MN arrays involves the use of an iontophoretic device. Yuzhakov *et al.* patented a hollow MN-reverse iontophoresis device that included a reservoir, anodal and cathodal electrodes, an electrical power source, and a control system, which combined a visual indicator with a central processing system [225]. A third electrode was also included and acted as a bio-electrochemical sensor. From this, a MN array for ISF collection is most commonly coupled with a sensor, such as amperometric, potentiometric, electronic or electrochemical sensors, to detect and analyse glucose [209]. Specifically in amperometric sensors, the needles are designed into three electrode types; a working, reference and counter electrode [226]. The basic strategy of a glucose sensor with associated MN array is to use the amperometry measurement with the immobilised enzyme, GOx for the detection of H_2O_2 generated by the reaction previously described in **Equation 1** and shown

in **Figure 11A**. The working electrode then detects H_2O_2 (and subsequently glucose levels) based on the number of electrons produced, following the reaction provided in **Equation 2**. To explain this further, the electrons generate electrical currents that can be detected by the working electrode. As a result, the change of current corresponds to the change of glucose concentration in the ISF.

$$H_2O_2 \xrightarrow{+700\ mV} O_2 + 2H^+ + 2e^- \qquad \text{Equation 2}$$

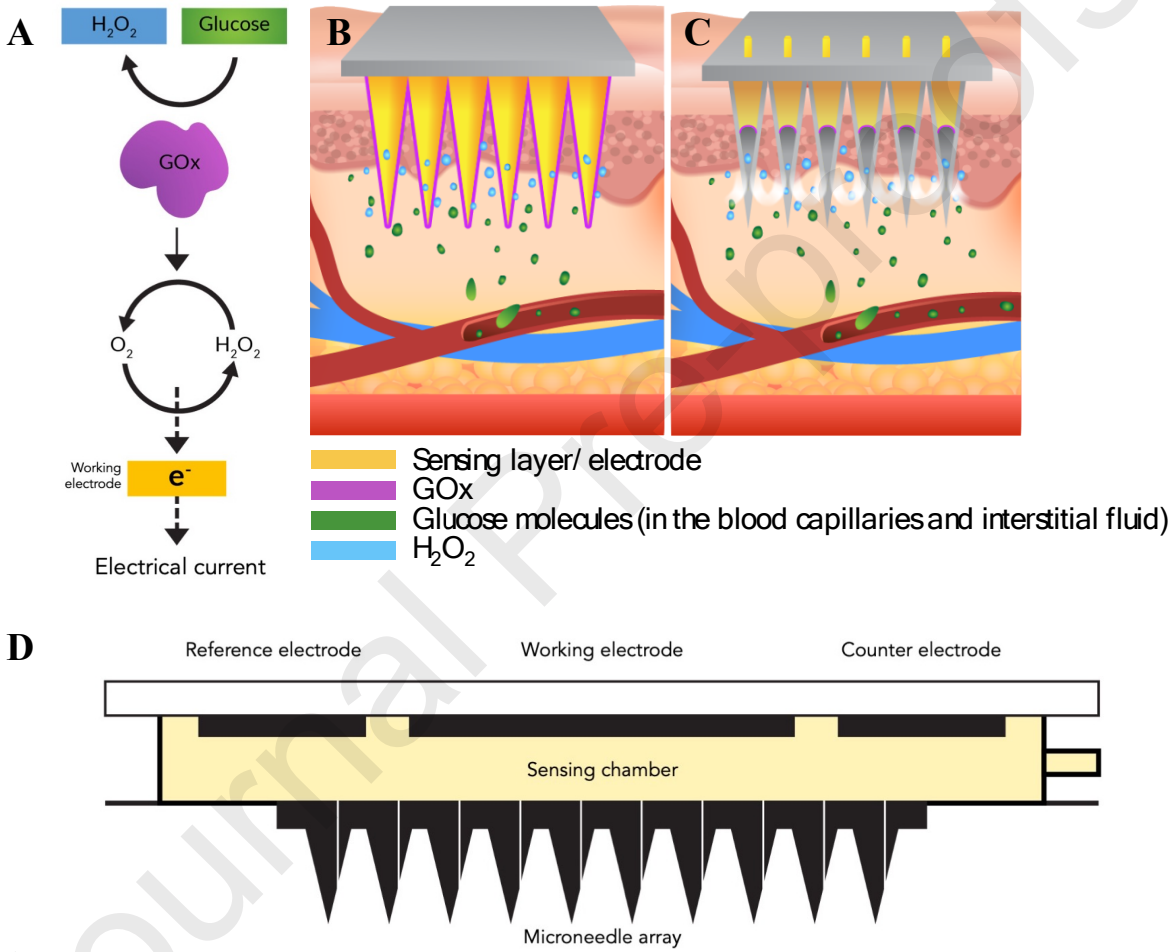


Figure 11 (A) A working mechanism of a glucose monitoring sensor; **(B-C)** Two typical designs of MN array devices used for CGM **(B)** MN arrays coated with conductive materials and **(C)** Hollow MN arrays combined with electrochemical electrode and **(D)** Schematic representation of a prototype MN array device for CGM.

For continuous glucose detection, MN arrays are either modified into or combined with electrochemical electrodes which consist of, in the majority, conductive electrodes and sensing materials [209,227]. Coating the MN arrays with electrochemical components is one strategy used to provide the MN arrays

with an electric function. In such systems, MN arrays are usually coated with a conductive material, such as silver or platinum which indirectly measures glucose levels by detecting H_2O_2 (**Figure 11B**) [209]. Polycarbonate-based MN arrays coated with platinum (as the working electrode) was developed by Sharma *et al*. [228]. The polycarbonate-based MN arrays were evaluated for their ability to measure blood glucose concentrations in type 1 diabetics for 24 h. The current output of these specific MN arrays correlated well with venous blood glucose concentrations, in addition to being well tolerated by the user. Another approach to utilise MN arrays as a sensing probe is to integrate non-MN array shaped electrodes into hollow MN arrays (**Figure 11C**). In this method, the hollow MN arrays protects the electrode and immobilised enzyme from damage upon skin insertion. Ribet *et al.* demonstrated that silicon-based hollow MN arrays could be fabricated with side openings that enclosed the platinum working electrode [229]. In this approach, the side openings allow ISF permeation, which is measured by the working electrode. In this work, this novel MN array device successfully detected glucose according to the given glucose concentration.

Using other approaches, Jina *et al.* have recently demonstrated the potential of a prototype device consisting of an array of hollow MN arrays attached to a sensor pod and an external amperometric sensor for glucose monitoring (**Figure 11D**). This MN array device has reported clinically accurate glucose levels (tested against blood glucose levels) for over 72 h [230]. Another approach used by Ribet *et al.* utilised a silicon-based hollow MN array integrated with an ultra-miniaturised electrochemical sensing probe to measure dermal glucose. It was documented from *in vivo* tests in humans, that the device was able to accurately track glucose levels, although there was a 10 min delay [231]. Work completed by the Shim group has focused on the use of a silver-based MN arrays coupled with a wireless signal transmitter. This work achieved a promising result, demonstrating enhanced repeatability and stability of glucose monitoring, whilst using Bluetooth to send the measured values to the patient's mobile phone [232].

5.4.2 MN arrays for ISF collection

ISF extraction through the micro channels produced by MN arrays is an alternative way of collecting and accessing biological information. Hollow MN arrays were first investigated to collect ISF [209]. This was most likely due to their ability to function in a similarly to a conventional hypodermic needle and syringe. To date, a number of hollow MN array systems have been proposed as potential devices for the ISF extraction from the body [233–236] Hollow MN arrays extract ISF by external negative pressure or *via* capillary action [209]. In terms of polymeric MN arrays, Ito and colleagues prepared dissolving MN arrays from sodium chondroitin sulphate to form channels in the skin upon application. Upon MN array dissolution, a wet unwoven cloth was applied to the treated area. The results of this study illustrated that the content of water in the cloth was linearly correlated with the amount of glucose extracted from the rat, which was approximately 3.25 μg after 1 min of cloth application [237]. Another report from Caffarel-Salvador *et al.* first reported successful ISF extraction from human volunteers using hydrogel-forming MN arrays in order to assess glucose levels following oral dosing of glucose powder (75 g) [216]. The MN arrays were composed of poly(methyl vinyl ether–co–maleic acid) and PEG, which were then cross-linked using heat. The MN arrays come into contact with ISF following skin insertion, which allows ISF diffusion to occur into the MN array. The result of this process is a swollen hydrogel. In the work, caffeine and glucose was detected *in vitro* and *in vivo via* uptake into the MN arrays. After the MN arrays had been inserted into for 1 h, the MN arrays were removed and immediately submerged in a buffer solution, after which the analyte of interest was analysed using a validated HPLC method (**Figure 12**). In human volunteers, glucose levels were monitored using MN-mediated ISF analysis over 0–1 h,1–2 h, 2–3 h and 0–3 h intervals following glucose administration. The blood glucose levels were compared against whole blood glucose measurements taken using the traditional combination of the finger prick lancet and glucometer. Through its convenient wear and ease-of-use, this study provides preliminary evidence and a high degree of promise that hydrogel-forming MN arrays have a viable application in glucose monitoring for diabetic patients.

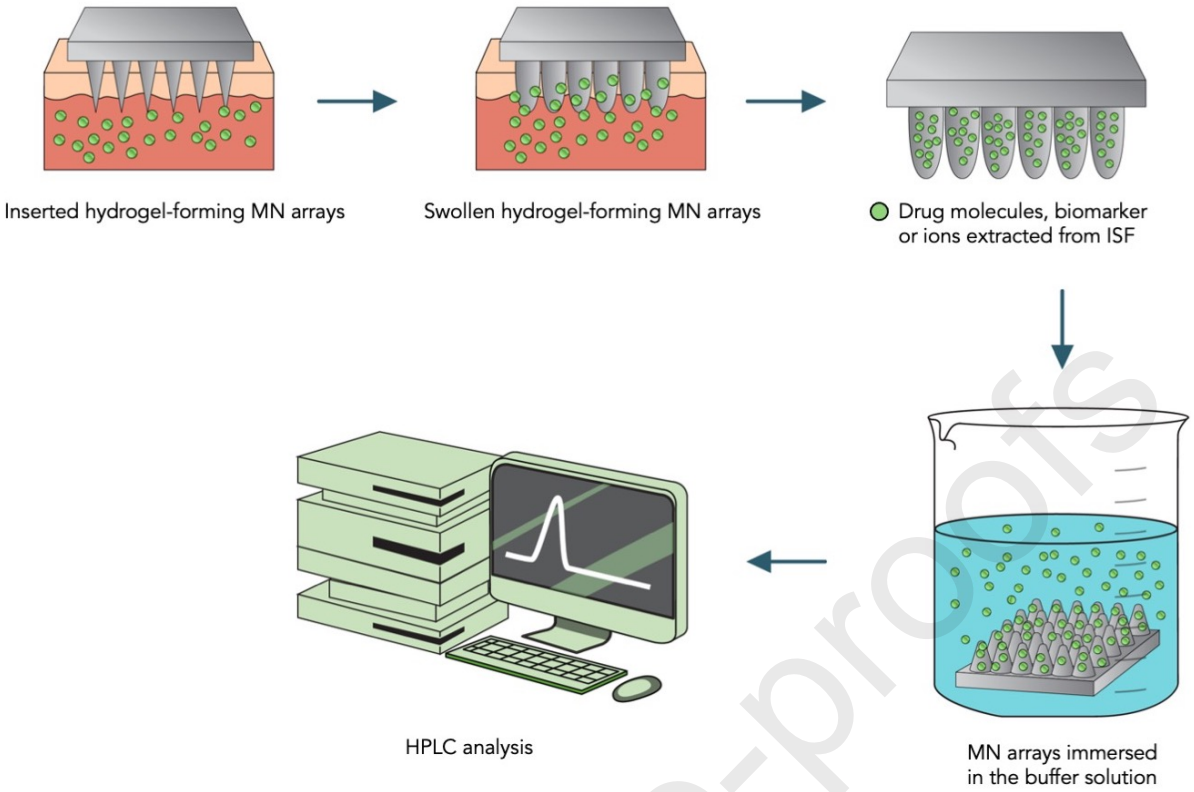


Figure 12 Schematic illustration of hydrogel-forming MN arrays used for ISF glucose monitoring.

Despite a large volume of research in this field, there is currently only one marketed MN array product (known to the authors) that extracts ISF. The Renephra device penetrates skin sites using MN arrays where oedema is present, such as the ankles. A negative pressure is applied in the form of a vacuum, and excess ISF is removed. This company focuses on removing excess ISF from the body caused by various diseases, such as peripheral oedema and heart failure [238]. In 2014, Seventh Sense Biosystems launched a product known as the TAP™ (Touch Activated Phlebotomy) blood collection device [239]. In the same year, similar blood collection devices were released by Tasso Inc. namely the Tasso-M20 and the Tasso-SST [240]. These devices utilise the concept of microfluidics to draw blood into a collection tube under negative pressure. However, these devices extract whole blood samples rather than ISF. Nevertheless, these marketed devices outlined above possess considerable promise in terms of patient-friendly collection of bodily fluids for therapeutic monitoring. Great encouragement can be drawn by those working towards a MN array-based device for therapeutic monitoring from the fact that

some devices have already reached the market. Continued work regarding MN array design, will serve to increase confidence in such a product and, most importantly, increase the likelihood of its success.

6. Conclusion

SC injection of insulin and oral antihyperglycaemic agents remain first line for the primary management and treatment of diabetes. However, many patients experience treatment fatigue associated with long-term chronic therapy, consequently affecting regime adherence. One complication of reduced treatment adherence is chronic hyperglycaemia, which if left untreated, can result in the development of diabetes induced fibrosis and eventual end-organ microvascular and macrovascular complications. In an attempt to improve treatment adherence, alternative routes of administration have been sought, such as nasal and pulmonary delivery. However, these alternative routes of drug administration are each associated with their own unique complications which has thus far resulted in discontinuation of previous FDA approved devices [66,76,77].

Over the last decade, transdermal drug delivery systems, specifically, MN arrays, have gained increasing attention and have become known as a potential alternative for the treatment of diabetes owing to the benefits that they offer compared to invasive injection and daily oral dosage forms. This review has demonstrated that MN arrays have successfully delivered a variety of drugs used to treat diabetes at doses that are considered therapeutic. Furthermore, since their conception [241], MN array designs have progressed significantly to include sophisticated technologies such as glucose-responsive closed-loop delivery systems [146,151,152], reverse iontophoretic devices [208,209], and coatings which release drug only when triggered by light or heat [190,191,194]. This continual and rapid development from the initial simplistic MN array design has served to increase the capabilities of the technology and resolve many of the issues associated with their initial use.

Despite the rapidly growing and expanding use of MN arrays for drug delivery purposes, hurdles remain which must be overcome before a MN array-based device will be accepted onto the market. A common

theme throughout this review is the successful delivery of antidiabetic drugs using MN arrays *in vivo*, however there is a distinct lack of long-term studies investigating repeated MN array polymer deposition into the skin, something which would be commonplace for diabetic patients requiring daily insulin administration and frequent glucose monitoring. Recent studies have addressed this gap with promising results [242,243], but for MN arrays to reach the market and benefit patients, biocompatibility and safety must be at the forefront of development considerations, in addition to patient usability and acceptance studies.

The potential for MN array technologies for treatment and monitoring of diabetes is apparent by the increasing number of studies investigating their use, their increasing presence in clinical trials and the increasing regulatory attention [244]. With continued and focused collaboration between regulatory bodies, industry, academia and the end user, it is possible that the benefits of MN arrays may soon be fully realised for investors, healthcare professionals, and most importantly, the patient.

7. Future Perspectives

Since their conception in 1971 [241], the field of MN arrays has grown considerably. MN arrays have also proven their utility in multiple disorders [245]. One of the key fields in which MN arrays are quickly progressing is the delivery of anti-HIV drugs and contraceptives as recently reviewed by Paredes *et al* [246]. This could significantly improve women's sexual and reproductive health, especially in sub-Saharan countries, where women are disproportionally bearing the burden of the current HIV pandemic. The delivery of vaccines, including coronavirus vaccines has also been intensively explored and it is another area where MN arrays can have a significant impact by facilitating immunization in a minimally invasive manner [247,248]. The fact that MN arrays are able to deliver drugs with long-acting profiles, makes this platform an attractive alternative for the treatment of chronic diseases, and great research effort is being devoted by scientists working in the field [249].

The treatment of diabetes and its associated end-organ complications caused by fibrosis remains one of the largest fields targeted by this drug delivery device. Yet, there remains several hurdles that must be overcome before MN arrays can make the transition from benchtop to bedside. The value of the worldwide transdermal market is increasing, market growth between 2019 and 2023 is predicted to reach $1.79 billion [250]. Furthermore, the World Economic Forum has included MN array patches as number one on the top 10 most relevant emerging technologies of 2020 [251]. This urgent need to utilise transdermal delivery systems into upcoming treatments may be, in part, attributed to the increased prevalence of chronic diseases, such as diabetes, which require long term treatment. Furthermore, over half of the current top 20 blockbuster drugs are biopharmaceuticals, creating a large market share which is predicted to grow to be worth $388 billion by 2024 [252]. With the huge growth potential in both the biopharmaceutical and transdermal markets, it is apparent that the treatment of diabetes using transdermal delivery devices has the potential to be extremely profitable in addition to having a positive impact on patients' lives. This drive to deliver drugs for diabetes treatment using MN arrays is perhaps best demonstrated by a ClinicalTrial.gov search, which produced 110 results for the search term "microneedle" (June 2020), 25% of which involved treatment for diabetes and diabetic complications (such as diabetic retinopathy and retinal degeneration). Furthermore, NanoPass Technologies have already developed a hollow MN array, MicronJet™, for insulin delivery in type 2 diabetes [253]. The company is also conducting a Phase I clinical trial for delivery of a "proinsulin" derived peptide for the treatment of type 1 diabetes [254]. Interest in MN arrays for the treatment of diabetes and its associated complications, therefore, cannot be denied. However, there are currently no MN array drug delivery devices on the market, thus the hurdles currently preventing their clinical use must be considered if MN-mediated delivery of antidiabetic drugs is to be a real possibility.

The hurdles which must be overcome for MN array adoption can be divided into three categories, namely, safety, acceptability, and regulatory, all of which has been covered elsewhere [255,256], though without focus on MN-mediated treatment of diabetes specifically.

The safety of MN arrays is paramount to their acceptance by both prescribers and patients. Studies appear to demonstrate that the risk of a skin infection caused by the use of MN arrays is minimal;

significantly lower microbial penetration was observed when skin was pierced using MN arrays, when compared to a traditional hypodermic needle [257,258] and hydrogel-forming MN arrays have found to exhibit antimicrobial properties [259], in addition to showing no evidence of skin inflammation [242]. Additionally, the small size of MN arrays mean that it is highly unlikely that the same exact points on the skin surface will ever be pierced. This could be further assured through regular rotation of the application site. However, for chronic conditions such as diabetes, it should be considered that if a successful MN array-based device containing an antidiabetic drug is created, it is likely to be used for long periods, and potentially multiple times a day. For dissolving MN arrays, which have already been studied for insulin delivery [133,134,260], polymer is deposited into the viable skin layers. As previously described, approximately 50-100 mg of polymer from a dissolving MN array may be deposited within the skin following application of a 10 cm^2 patch [255]. The implications of long term, repeated polymer deposition in the skin has not been fully investigated. It will be advisable that biodegradable or low molecular weight polymer be employed for dissolving MN arrays that will be applied repeatedly. Hydrogel-forming may be also be useful here, as they do not deposit polymer within the skin and are removed intact. *In vivo* studies revealed that repeat application of both dissolving (once daily for 5 weeks) and hydrogel-forming (twice daily for 3 weeks) MN arrays did not alter skin appearance or barrier function and caused no measurable disturbance of serum biomarkers of infection, inflammation or immunity [243]. More recently, Al-Kasasbeh *et al.* (2020) investigated the clinical impact of repeated application of hydrogel-forming MN arrays [242]. The results demonstrated that repeat hydrogel-forming MN application does not lead to prolonged skin reactions, prolonged disruption of skin barrier function and concentrations of systemic inflammation biomarkers were all found to be within the normal range. Therefore, the impact of repeatedly puncturing the skin using MN must be considered, as the delivery of insulin using conventional hypodermic needles has been associated with lipohypertrophy [261]. It is unlikely that the delivery of insulin using MN arrays would carry this risk, as MN arrays do not penetrate past the dermis. Additionally, rotation of the injection site may minimise the risk of lipohypertrophy in patients using either hypodermic needles or MN arrays for the delivery of insulin.

In addition to those concerns discussed above, there is evidence of an immune response being triggered following protein and peptide infusions [262,263], which theoretically may extend to MN-mediated delivery of insulin for diabetes treatment, particularly when considering such drugs will be delivered into the dermis, where a wealth of immune cells reside [264]. Immune cells within the dermis are of a much greater concentration than in SC tissue, where insulin is typically delivered. Ultimately, long term studies will be required to determine entirely the likelihood of and severity of an immune response from repeat MN array application, particularly when the MN arrays contain biological drugs such as insulin.

Many of the studies referenced within this review demonstrate the ability of novel polymers or polymer-drug conjugates for the successful delivery of anti-diabetic drugs. Whilst these results are positive in regard to the ability of novel formulations to successfully deliver anti-diabetic drugs, the translation of such novel formulations from a regulatory standpoint will likely be expensive, and these associated costs may therefore reduce the likelihood of commercialisation. From this perspective, MN arrays which are fabricated from biocompatible polymers [93] may be the most likely to succeed and pave the way for more complex, novel systems in the future.

Patients must want to use MN arrays as an alternative to their current drug delivery method. For a condition such as diabetes, a painless method of delivering insulin is an obvious benefit that traditional SC injections cannot offer. Furthermore, delivery of drugs such as GLP-1 analogues for the treatment of type 2 diabetes circumvents first pass metabolism, lowering the risk of potential side effects, potentially improving patient compliance. It is likely then that patients would be willing to use a MN array if one became available on the market [86]. For patient acceptance, MN array must be as convenient, if not more so, than a patient's current treatment regimen, and efforts have been made to ensure patients can self-apply MN arrays [89], particularly when using a "pressure-indicating sensor film" [265].

Units of insulin required for effective diabetes control may differ inter-day and intra-day, depending on the patient's blood glucose levels. The required dosage of insulin can be easily controlled using an insulin syringe. Numerous factors affect the absorption of insulin administered *via* the SC route, related

to the specific preparation of insulin, the injection technique, and the patient receiving the insulin [266]. These factors remain the case for insulin delivered by MN arrays. Hydrogel-forming MN arrays connected to an iontophoretic system or hollow MN arrays could deliver a tailored dosing. As previously discussed in the review, glucose-responsive MN arrays allow for tailored dosing. Some studies have developed MN arrays which demonstrate a relative bioavailability which is considered comparable to a SC injection of insulin [267,268], thus there is promise for the future.

Despite the huge growth in the biopharmaceuticals market, driven by potential for biopharmaceutics to treat diseases which were previously thought impossible, challenges remain. These challenges must be overcome for biopharmaceuticals to reach their full market potential [256]. For example, the majority of biopharmaceuticals currently must be stored and transported under the "cold chain", which is expensive to maintain, and this limits the potential of biopharmaceutics in developing countries, where such conditions are not commonplace. Formulating drugs within MN arrays in the dry state [269] not only improves their chemical and physical stability, but removes the need for the cold chain. For MN arrays to reach their full potential and gain regulatory approval, there may be a number of requirements, which have been summarised elsewhere [90,256,270,271], though are not unique to the formulation of drugs for the treatment of diabetes. Such considerations include, but are not limited to, upscaling and mass production of MN arrays, the potential need for sterility, MN array packaging and disposal, and long-term stability of drugs, particularly biopharmaceutics, within MN arrays. It should be noted that several companies are now able to manufacture MN arrays under aseptic conditions, with scalable methods, according to GMP standards if this is deemed necessary by regulatory authorities. Three examples of such companies are LTS Lohmann, Kindeva and FujiFilm.

It is clear that MN arrays hold the potential to transform how we deliver drugs transdermally. However, their full potential in terms of market value and value to the patient cannot be realised without commercialisation. Focused collaboration between industry, academia, patients and regulators will be required to ensure that the considerable benefits of MN arrays are able to outweigh development costs. This collaboration has already begun and is ongoing through PATH's "Centre of Excellence For Microarray Patches" [244].

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Graphical abstract