Alternative treatments for diabetes are currently being investigated to improve both patient comfort and avoid complications due to hyperglycaemia episodes. In the absence of a cure like pancreas or beta-islets transplants, the ideal method would be an artificial "closed-loop" system able to mimic pancreas activity. This would operate continuously and automatically, causing appropriate response to losses and gains in glucose levels. Chemically controlled closed-loop insulin delivery has been explored by two main strategies. The first one consists in delivering insulin with a glucose-responsive matrix. Polymeric hydrogels that swell or shrink according to the glucose concentration allow delivering insulin doses adapted to the glucose concentration. The second strategy consists in modifying insulin itself with glucose-sensitive functional groups that trigger its activity. Recent developments made in these areas represent significant progress in terms of biocompatibility, selectivity, pharmacokinetics, and easiness of administration, as required for in vivo applications. Although some issues still have to be overcome, this field of research is promising as a possible alternative to other approaches for diabetes treatment.

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Type 2 diabetes arises from disorders of both insulin resistance and secretion [2,3]. The treatment of type 2 diabetes concerns mainly oral antidiabetic drugs, but in some cases, insulino-therapy is needed when insulinopenia arises [4]. The usual treatment for type 1 diabetes is achieved by multiple subcutaneous insulin injections, administered daily. However, this method does not maintain normoglycaemia. In 1993, the results of a 10-year landmark study Diabetes Control and Complications Trial (DCCT) were published [5]. There were two important conclusions: first, that tight control of glucose closer to the normal level resulted in significant reductions in complications such as limb amputation, blindness, and kidney failure. Second, the tight control led necessarily to increased incidence of low blood sugar (severe hypoglycaemia). To avoid such abnormal episodes, curative therapy can be considered. It consists in the replacement of functional insulin-producing pancreatic beta-cells, with pancreas or islet-cell transplant. However, in addition to the difficulty to find adequate donor cells, pancreatic graft rejection has to be avoided. Therefore, transplantation has to be coupled to lifelong immunosuppression, which also presents severe side effects. This could be improved by encapsulating islets in a semi-permeable membrane, which could protect the grafts from the host immune systems [6]. A field of research has recently been developed in this area [7,8].

In the absence of a cure for diabetes, the next best alternative would be a “closed-loop” system, able to mimic the pancreatic activity of healthy people. An artificial glucose-responsive insulin delivery system that works by an automated feedback would therefore have obvious advantages and has been explored. In this concept, the insulin dosage and delivery would be governed by the blood glucose levels on an automatic and continuous basis. It is tempting to develop a closed-loop system based on the association of an insulin pump, capable of delivering insulin continuously, and a monitoring device, capable of sensing glucose continuously. However, another issue arises here, which is the delays in glucose sensing and insulin delivery. An insulin delivery algorithm is therefore required to link an existing glucose sensor and an external insulin pump. Recent technological progress in continuous glucose sensors [9] has led to prototypes of closed-loop systems based on the combination of a continuous glucose monitor, a control algorithm and an insulin pump [10,11]. However, the feasibility of this solution remains to be confirmed.

Among various strategies aimed at mimicking pancreas activity, a great deal of research was devoted to glucose-responsive hydrogels, which undergo a modification of their physical properties in response to a variation of glucose concentration. In particular, the variation of the mechanical and permeation properties, associated to a modification of their swelling state and/or viscosity, make them suitable for delivering amounts of insulin according to the glucose concentration. Therefore, the first part of this review article will provide an overview of current research in the field of glucose-responsive materials and their potential use as insulin self-delivering systems. In a second part, another strategy will be presented where insulin itself is modified and its availability depends on glucose concentration.

2. Glucose-responsive hydrogels

Hydrogels are cross-linked polymeric networks that absorb large amounts of water and swell. To maintain the three-dimensional structure, they can be either chemically or physically cross-linked. Some hydrogels belong to the class of the stimuli-responsive materials and present the unique property of undergoing abrupt volume changes from their collapsed to swollen states in response to environmental changes [12–15]. Sometimes called “intelligent” materials, stimuli-responsive materials display both sensor and effector functions. They can sense a stimulus as a signal and transduce it via the structural changes, but the structural change itself can play the role of effector. Various stimuli might be responsible for such modifications. The effect of stimuli such as pH, temperature, electric or magnetic field or light, have been studied both experimentally and theoretically. More recently, the concept of biomolecule-sensitive hydrogel [16] has been developed as biomaterials aiming at mimicking natural feedback systems. Just like natural ones, they can detect specific ions or biomolecules, which induce conformational changes and modify their biological functions. Hydrogels can sense a biomolecule (glucose) and respond to it by a release of the hormone (insulin).

Insulin release from hydrogels can occur mainly through two different pathways (Fig. 1). The hydrogel can be used as a membrane with a controlled permeability, which is triggered by glucose. The membrane separates a reservoir full of insulin from the outside [17–20]. It can have different geometries, for example planar in the case of membranes or spherical in the case of capsules [21]. The hydrogel can also be the reservoir itself thanks to its porous structure [22–24]. In both pathways, insulin is released when the hydrogel swells. More sophisticated release mechanisms can be imagined when the hydrogel is used in association with a device, for example when it is grafted in the pores of a membrane [25] or when it is inserted as a microvalve in a microfluidic delivery device [26].

The equilibrium volume of a neutral hydrogel is a balance between the osmotic pressure of the polymer network, which is governed by polymer–solvent interactions and the elasticity of the polymer network. If the polymer is soluble within the solvent, the corresponding hydrogel tends to swell in order to expand the contact between the polymer and the solvent. The elastic restoring force is controlled by the amount of cross-linkage: the higher the cross-linker density, the lower the swelling ratio is. When the hydrogels are constituted with charged polymers, they can present a very high swelling ratio. Their large swelling volume is mainly governed by a third contribution arising from the electrostatic repulsion between the charged monomer units and the osmotic pressure exerted by the mobile counter-ion concentration inside the gel [27,28]. The swelling ratio depends on the charge density of the gel but also on the ionic strength of the surrounding medium. Therefore, any factor affecting one of these contributions will induce a modification of the gel swelling volume.

At least, three issues have to be addressed by “intelligent” insulin delivery.

- Possession of glucose sensitivity, hydrogels have to contain a molecule that specifically interacts with glucose and senses its levels in the physiological range;

![Fig. 1. Illustration of the two possible ways for self-regulated insulin release from glucose-responsive hydrogels: (a) across a membrane; (b) the hydrogel plays the role of a reservoir. Insulin diffusion occurs when the hydrogel is swollen.](image-url)
- Modulation of insulin according to blood glucose levels;
- Biocompatibility since diabetes is a lifelong, chronic disease.

2.1. Glucose oxidase modified hydrogels

2.1.1. Insulin release by diffusion through a hydrogel membrane

Historically, the first glucose-responsive materials were made from combining glucose oxidase (GOD) as a sensing element with a pH-sensitive hydrogel. When GOD reacts with glucose and converts it into gluconic acid, the pH within the hydrogel is lowered. The gel responds to pH because its constituting element will capture protons and the charge density of the polymer is modified. This gives rise to the glucose-triggered response in terms of volume change.

Several groups have developed glucose-responsive hydrogels based on GOD entrapment or immobilization within the gel. In order to achieve insulin release, hydrogel swelling is required when glucose concentration increases, i.e. when the pH decreases due to the production of gluconic acid (Fig. 2). Ishihara et al. [17] were the first to report the regulation of insulin permeation across a glucose-sensitive membrane, which was itself made of two associated membranes: the pH-responsive one, a copolymer made of N,N-diethylaminoethyl methacrylate (DEAEM) and 2-hydroxypropyl methacrylate (HPMA), and a polyacrylamide one containing glucose oxidase. They further built up polymer capsules containing insulin using the interfacial precipitation method. The capsules, with a diameter of about 1.5 mm, were packed in a thermostated glass column studied. Insulin was shown to be released at a higher rate when glucose was present in the eluent compared to the buffer solution in the absence of glucose [21].

To achieve high insulin permeability, Albin et al. [18] designed a macroporous membrane made of a similar copolymer hydrogel entrapping glucose oxidase. The porosity of the gel was achieved through phase separation occurring during polymerization. However, the membrane did not show any experimental response for rises above normal blood glucose levels. A theoretical model taking into account the kinetics of glucose reaction was developed [29]. It described the steady state behavior of glucose-sensitive membrane. It was shown that the poor availability of the oxygen required for the enzymatic reaction was responsible for the limitation of the response to a particular range of glucose concentrations. In the case of thin membranes, the optimal range was found to be below the pathophysiological range. However, other device configurations were designed to create pathways for oxygen distribution inside the gel. Four configurations using silicone rubber in contact with the glucose-sensitive membrane were found to enlarge the range of glucose concentration sensitivity [30].

Oxygen depletion might also be overcome by the addition of catalase. Catalase reacts with hydrogen peroxide produced during the conversion of glucose into gluconic acid. This reaction liberates oxygen which can be used for glucose oxidation. The presence of catalase seemed to increase the swelling kinetic as observed by Traitel et al. with poly(HEMA-co-DMAEM) [31] and by Zhang et al. with p(NIPAM-co-MAA) nanoparticles dispersed within a hydrophobic polymer [32]. Another glucose-sensitive hydrogel, based on sulfonamide chemistry with covalently conjugated glucose oxidase and catalase showed a reversible glucose dependent swelling [33]. Traitel et al. also reported the first in vivo studies [31]. Matrices were implanted in the peritoneum of rats. No tissue encapsulation was observed and the released insulin seemed to keep at least part of its bioactivity. This result was confirmed very recently by Satish et al. [34].

2.1.2. Insulin release by other mechanisms

In the release mechanisms mentioned so far, insulin is released by diffusion through a hydrogel when the hydrogel expands. However, a more rapid response is expected from a squeezing effect. Therefore, pH-sensitive hydrogels with a shrinking behavior at low pH were combined with GOD. At low pH, poly(methacrylic acid-g-ethylene glycol), abbreviated as p(MAA-g-EG) exhibit intrapolymer complexation due to hydrogen bonds of the carboxylic acid groups and the ether group of the PEG chain [35,36]. Although oscillatory pH-swelling studies showed a promising rapid collapse supposed to squeeze insulin out of the gel, insulin release was studied via a model but not experimentally [37].

Instead of a hydrogel, a gating membrane can be used to deliver insulin in a self-regulated fashion. Here, the polymer expansion is used to close the pores of a membrane whereas the polymer collapse allows insulin diffusion through the pores of the membrane. In this case, polymers with carboxylic functions were used because they collapse when pH decreases, i.e. when gluconic acid is produced. This was achieved by Ito et al. using cellulose membranes grafted with poly(acrylic acid) (PAA) and immobilized GOD [25]. A poly(vinylidene) membrane was also modified by an acrylic acid copolymer derivative with covalently bound GOD and associated to a pressurized chamber [38]. Unfortunately, it did not satisfy the medical requirements due to a too high basal flow rate. The importance of the grafting yield was reported by Chu et al. [39]. It appeared necessary to adjust the grafting yield to design the ideal gating response.

All the examples listed above use the gel permeability variation to modulate insulin release. Another method consists in degrading the gel to release the peptide. Uchiyama et al. [40] synthesized a hydrogel which was degraded by hydroxyl and/or hydroperoxy radicals. Thus, the liberation of H₂O₂ produced by the enzymatic reaction between GOD and glucose, induces a degradation which is synchronized with glucose concentration.

2.1.3. Towards biocompatible hydrogels

Efforts were made to improve the formulation of glucose-swelling hydrogels in order to get more biocompatible materials. Incorporation of poly(ethylene glycol) grafts on the main chains of glucose-responsive

![Fig. 2. Schematic representation of pH-responsive hydrogel with entrapped GOD. After glucose diffusion inside the gel, glucose is enzymatically converted into gluconic acid. The pH decreases, and the polymer becomes charged due to the protonation of pendant amino groups. The hydrogel swells, facilitating the release of insulin by the diffusion mediated process.](image-url)
hydrogels was developed by Podual et al. [41–44]. These grafts were expected to retard the degradation of enzymes and proteins within the gel and to minimize the immunoreaction and subsequent rejection by the body [45]. The size of these hydrogels was scaled down to microparticles having a diameter between 30 μm and 300 μm [44]. They showed rapid swelling/deswelling dynamics in response to changes in pH, with a faster response for the smallest particles. A reversible swelling was achieved in less than 5 min in response to pulsatile variations of glucose [44].

Very recently, Kashyap et al. [22] reported glucose-responsive hydrogels made of a nontoxic, nonimmunogenic, biocompatible and biodegradable polymer. Chitosan, a structural amino polysaccharide found in invertebrate animals, can form an in situ gelling system when combined to a polyol counter-ionic dibase salt such as β-glycerophosphate disodium. A sol–gel transition occurs when the solution is heated to body temperature. A gel containing GOD and peroxidase – which converts H2O2 into oxygen and increases GOD activity – can be obtained. This gel is glucose-responsive and swells to a maximum when glucose concentration is around 3 mg/mL. The swelling response is not linear with the glucose concentration. However, these authors showed that loaded insulin was released from the gels in a pulsatile manner over 2-hour periods. The formulation was evaluated in vivo in diabetic rats. A dose of 3 IU/kg insulin was administered subcutaneously to the rats, as a loaded gel form to a group of animals and as plain insulin to a control group. A third group of animals was treated with a blank gel. Plasma insulin levels and glucose insulin levels were measured. The insulin-gel-treated group displayed a sustained and better hypoglycemic activity which illustrated the pharmacodynamic effect of the formulation. The pharmacokinetic profile was also better, since the residence time of insulin was longer for the insulin-gel-treated group (up to 16 h). A second oral glucose challenge was given after 24 h. Although the plasma glucose level of the insulin-gel-treated group was lower than the blanks, insulin was released over 24 h. As discussed above, glucose-responsive materials can be developed as drug delivery systems for glucose-responsive hydrogels for use in vivo. 

2.2. Lectin-modified hydrogels

Lectins are a family of carbohydrate binders, which can be used as natural receptors in the development of glucose-responsive hydrogels. These proteins are known to interact with glycoproteins and glycolipids on the cell surface, where they play a role in cellular adhesion or hormone regulation. The most studied of these lectins is concanavalin A (Con A), which presents four binding sites [46].

Glucose-regulated insulin release was first achieved using a glycosylated insulin derivative (and still biologically active) complexed with Con A. Insulin release occurred in the presence of glucose thanks to the binding competition between glucose and glycosylated insulin with Con A which led to the breakdown of the complexes [47–49]. This system was further enclosed within a polymer microcapsule [50]. Succinyl-amidophenyl-glucopyranoside insulin (SAPG-insulin) was shown to be released according to glucose variations. Both membranes and microcapsules were optimized to operate in vivo studies.

Different polymers bearing saccharide residues were complexed with Con A to form a gel and encapsulate insulin. Natural saccharide polymers [51] as well as novel polymers containing saccharide residues were synthesized [52] and their complexation with Con A was studied. For example, the polymerization of poly(2-glucosyl- yethyl methacrylate) (pGEMA) with the lectin receptors was shown to be broken down by the addition of monosaccharides [52]. pGEMA was further cross-linked in order to entrap Con A within a hydrogel [53]. Their swelling ratio increased when free glucose concentration increased, because Con A played the role of an additional cross-linker. As it was progressively dissociated from the gel upon adding glucose, the cross-linking density decreased and the gel swelled (Fig. 3).

Based on the concept of sol–gel transition, another type of polymer with glucose moieties, vinylpyrrolidinone-aldehydeglucose (VP/Ag) copolymer was synthesized. The complex with Con A formed a hydrogel, but it became a sol upon glucose addition. This sol–gel transition was shown to be reversible upon glucose removal [54,55]. This hydrogel was then sandwiched between two porous membranes and used as a gate between two diffusion chamber cells [19]. The release of model proteins (insulin and lysozyme) through the hydrogel membrane was studied as a function of the free glucose concentration in the environment. The release rate of model proteins through the glucose-sensitive hydrogel membrane was dependent on the concentration of free glucose. This study demonstrated that the glucose-sensitive phase-reversible hydrogels can be used to regulate the insulin release according to the free glucose concentration in the environment.

A severe limitation to these systems was the progressive loss of activity due to Con A leaking through the mesh, which also caused a problem of toxicity. To circumvent this problem and obtain a reversible glucose-responsive hydrogel, the lectin was covalently bound. Several strategies were developed. Miyata et al. obtained a covalent link with Con A by copolymerizing GEMA with Con A having vinyl groups [56]. Concanavalin A was covalently coupled to glycosgen using derivatives of Schiff’s bases [57,58] or using carbodiimide chemistry with carboxylic acid on Carbopol [59,60] or carboxylic acid modified dextran [20,61,62]. The carbodiimide coupling chemistry has the advantage that it introduces no potentially cytotoxic groups into the gels formed, rendering them more suitable for potential in vivo applications. Such materials showed a differential delivery of insulin in response to glucose with in vitro diffusion experiments [20,61].

2.3. Hydrogels and microgels modified with a phenylboronic acid moiety

As discussed above, glucose-responsive materials can be developed from the combination of a hydrogel matrix and a natural recognition element of glucose such as lectin or glucose oxidase. A very different method was opened by the pioneering work of Kataoka et al. [23,63], who first used phenylboronic acid (PBA) as a non natural receptor of glucose. PBA and its derivatives are known for their ability to form complexes with polyol molecules such as glucose in aqueous solution [64,65].

2.3.1. Glucose-swelling hydrogels

The pioneering studies reported a glucose-responsive system based on the binding competition between glucose and another polyol [63,66–68]. They synthesized copolymers bearing PBA moieties,
(poly(N-vinyl-pyrrolidone-co-PBA), and formed a complex with a long polypoly(vinyl alcohol) (PVA) [66]. The complex between the PBA entity and the polypoly(vinyl alcohol) was dissociated in the presence of glucose, which acted as a competing polypoly(vinyl alcohol) was linked to several sites and played the role of cross-linker. The gel swelled and/or eroded, and became a sol at high glucose concentrations. The concept was applied to the design of a glucose-sensitive electrode [69]. The electrode was coated with the poly(NVP-co-PBA)/PVA complex. The complex dissociation upon glucose addition induced current changes proportional to glucose concentration. However, this complex was not stable at physiological pH. The same team further showed that it could be stabilized by the presence of amino groups in the vicinity of the PBA moiety [63]. Therefore, a hydrogel with both functions was prepared and formed a complex with gluconated insulin (insulin chemically modified with gluconic acid) which dissociated upon glucose complexation [68]. Insulin release in response to the glucose concentration was achieved at physiological pH.

As well as the binding competition, PBA-derived hydrogels might swell upon glucose addition following another mechanism, which relies on an increase of their charge density upon glucose complexation. It is well-known that charged hydrogels exhibit higher swelling ratios than neutral ones, when the swelling medium has a low ionic strength. This is due to the presence of mobile counterions within the gel which exert an osmotic pressure [27]. Therefore, the volume of the gel increases when the charged density of a gel increases. This concept was applied to PBA-derived hydrogels to give a glucose-responsive material. Indeed, both the uncharged and the charged form of PBA exist in equilibrium (Fig. 4). Upon complexation, this equilibrium is shifted towards the charged form because the complex in the charged form is more stable than that in the neutral form, which is highly susceptible to hydrolysis [70]. Therefore, polymers bearing PBA moieties undergo an increase of their charge density when the pH is close to their pKa, and PBA-modified hydrogels can swell upon glucose complexation.

Although this electrostatic mechanism should be limited to media with low salt concentrations, the PBA-derivatives could keep their glucose-responsive properties at physiological salinity, when combined with thermoresponsive polymers. Thermoresponsive polymers, such as the family of poly(alkylacrylamides), are soluble at low temperature in water and precipitate when heated above a critical temperature, called critical solution temperature (LCST). When cross-linked, they are swollen at low temperature and shrink upon heating. The transition occurs at the volume phase transition temperature (VPTT). Kataoka et al. synthesized copolymers with N,N-dimethylacrylamide and PBA [63]. They showed that the presence of the PBA moiety decreased the LCST of the polymer, but the LCST increased upon glucose addition. This indicates clearly that the PBA derivative plays the role of a hydrophobic monomer but complexation with glucose imposes hydrophilicity. In the case of a cross-linked hydrogel, it tends to shrink without glucose but swells in the presence of glucose to expand the contact with water. Likewise, hydrogels obtained from N-isopropylacrylamide (NIPAM) and PBA swelled and shrank according to the glucose concentration [23]. This system was used to demonstrate that insulin could be released repeatedly with an on–off regulation in response to stepwise changes in the glucose concentration (Fig. 5).

This important proof of principle was demonstrated at pH 9. The system was more recently improved to operate at physiological pH conditions by modifying the chemical structure of the receptor with an electron-drawing group in the phenyl ring [71,72]. Beads of this hydrogel could be obtained using an inverse emulsion as a template for polymerization. Their diameter was about hundreds of micrometers [93]. In an attempt to design a glucose-responsive chemical-valve, cylindrical gels were also prepared and shown to effectively control the flow rate of the applied solution in response to a change in the glucose concentration [73].

The pioneering work of Kataoka et al. was followed by many developments in the area of PBA-derived hydrogels, in particular, autonomous colorimetric sensors that could be engineered from such hydrogels. Several research teams developed the concept of colorimetric sensors using a periodic array within the gel. This could be obtained via three strategies: the inclusion of a colloidal crystal within the gel [74,75], or a periodic array of voids within the gel [76–78], or the gel itself forms an array in the case of a hologram [79]. The array diffracts light according to Bragg’s law. When the distance between particles has the same order than the wavelength of light, one can observe a colored material. The color depends on the interparticle distance and therefore on the gel swelling. Any factor affecting gel swelling thus modifies the crystal color. In fact, colorimetric sensors can be obtained when combining the existence of a periodic array

![Fig. 4. Representation of the complexation between the (alkylamido)phenylboronic acid and glucose in aqueous solution.](image)

![Fig. 5. Repeated on–off release of fluorescently-labelled insulin from the gNIPAM-co-AAPBA gel at 28 °C, pH 9.0, in response to external glucose concentration (from ref [23] Fig. 3).](image)
within a molecule-sensitive hydrogel. This was achieved for glucose-sensing materials using the inclusion of latex particles [80,81], using an inverse opal [82,83] and making a holographic grating with the hydrogel [84–86]. In all cases, the glucose-sensitive hydrogel constituted the matrix. The use of an inverse opal was chosen to accelerate the response time resulting from the quicker diffusion through large voids. Holographic sensors are attractive for their mass fabrication. An optical sensor could also be obtained using an inverse opal without glucose sensitivity, which was filled with glucose-responsive nanoparticles [87].

2.3.2. Glucose-shrinking hydrogels

The totally synthetic gels discussed above, swell according to the target concentration and also according to the complexation constant with the PBA group. Competition might arise between different saccharides. For example, fructose has a stronger affinity than glucose with the PBA group. Phenylboronates are usually not selective and bind all molecules with a cis-diol group as mono-bidentate (1:1) complexes. However, bis-bidentate (2:1) complexes can form involving one sugar with two cis-diols and two boronates. Among the relevant physiological saccharides, glucose is the only molecule that presents two diols which can be involved in a bis-bidentate complex relevant physiological saccharides, glucose is the only molecule that presents two diols which can be involved in a bis-bidentate complex (Fig. 6). Therefore, glucose can be recognized selectively. The bis-bidentate complex forms additional cross-links within a hydrogel. Glucose-responsive hydrogel can thus shrink upon glucose addition.

Asher et al. were the first authors who reported a selective glucose-responsive hydrogel which shrinks when the glucose concentration increases [80,88]. The complex was stabilized by the presence of sodium ions in the vicinity of the boronate group. Using the concept of colorimetric sensors with a colloidal crystal inclusion, these authors developed a colorimetric sensor which blue shifted at glucose concentrations in the patho-physiological range, at physiological pH and salinity. Glucose determination could even be performed in tear fluid, which made it suitable for the development of an integrated colorimetric sensor in soft contact lenses [89].

Samoei et al. synthesized another hydrogel sensor which shrunk selectively when glucose concentration increased and operated in human blood plasma [90]. Their synthesis consisted in the chemical modification of a commercial polymer, PMMA, via a very simple and modular batch procedure. Hydrogel shrinkage occurred upon the formation of bis-bidentate complexes between the PBA receptors and glucose. This complex was thought to be favored by the presence of emerging cationic charges from water and glucose bound to boron, which may reduce the electrostatic repulsion and solvation by water that would lead to swelling. They would therefore stabilize the cross-linking through bis-bidentate binding to boron. The sensitivity and response time could be easily optimized to selectively sense glucose within the range of 0 to 10 mM, within 20 minutes.

The two previous examples were devoted to sensor applications. Glucose-shrinking hydrogels have been also investigated for their ability to deliver insulin. Siegel et al. have also shown that acrylamide hydrogels derived with PBA were able to either shrink or swell when the glucose concentration increased, depending on the pH conditions [26]. The hydrogels swelled upon glucose addition at pH 7.4 and shrank at pH 9.0 where they recognized glucose selectively compared to fructose. With this hydrogel, these authors designed a simple microvalve prototype inserted into a microfluidic device [91]. Insulin was delivered with an on–off regulation according to the glucose concentration. However, insulin flow was stopped when the gel was swollen, i.e. when glucose concentration increased at physiological pH and the response time was too long (20 min) (Fig. 7). They further improved the system and designed another microvalve, the basic structure of which is a silicon membrane having an array of orifices in which a hydrogel is anchored [92]. The hydrogel allows gating the flow across the membrane. In the swollen state, the hydrogel completely occupies the void space of the orifice, blocking pressure-driven fluid flow. In the shrunk state, the hydrogel collapses and leaves a void, allowing fluid to flow through an opened annular gap. The response to glucose variations occurs within 10 min.

2.3.3. Glucose-responsive microgels

Most of the hydrogels cited above present the defect of exhibiting very long response times. Even beads (with a diameter of several hundred of micrometers) displayed response times of several hours [93]. As mentioned previously, the kinetics could be improved to 20 min by incorporating voids or macropores within the gel [83]. The same order of magnitude was obtained by Samoei et al. [90]. It is well-known that the shrinking rate of the gel is inversely proportional to the square of the smallest dimension of the gel [27,28]. Therefore, the kinetics properties of macroscopic hydrogels do not seem suitable for fast insulin controlled release but these systems can be scaled down to colloidal stable particles made from hydrogels, which are referred to as microgels or nanogels. Their size is generally comprised between a few tens of nanometers to a few micrometers.

The first report concerning PBA-modified microgels was published by Hazot et al. [94]. They structure achieved the copolymerization of a PBA monomer with a thermoresponsive monomer. They proved the presence of the phenylboronate group at the microgel surface but did not show any glucose-triggered swelling response. Later on, several groups reported the synthesis of glucose- and thermoresponsive microgels bearing PBA functions [95–97]. Two synthesis methods are available: the PBA receptor can be grafted to a thermoresponsive microgel bearing carboxylic groups [95,97] via a carbodiimide coupling, or a PBA monomer can be synthesized and copolymerized with other monomers to be statistically incorporated within the microgel [96]. The post-modification method has the drawback that not all the
carboxylic groups are substituted because the PBA cannot diffuse deep inside the microgel and the chemical modification is effective mainly at the surface. However, the particles obtained by the two methods had a similar size, in the range of 200–400 nm. They swelled upon glucose addition at pH above 8.5. In addition, our group showed that they were still responsive at physiological salinity [96]. The synthesis of the microgels has been extended to hollow nanocapsules [98]. These objects were obtained by synthesizing core-shell microgels with a degradable core and a glucose-responsive shell. The core was made of cross-linked hydrogel with a degradable cross-linker. The debris of the degraded polymer was released through the shell, leaving a hollow capsule which could be further filled with insulin. Recently, Hoare and Pelton [24] produced amphoteric thermo-responsive microgels with PBA functional groups that were glucose-responsive at physiological temperature, salinity and pH. The composition of the microgels could be adapted to produce microgels either swelling or shrinking in response to glucose. These amphoteric microgels also showed interesting properties concerning insulin incorporation based on electrostatic interactions. Insulin uptake was high when the net charge of the microgels and insulin was opposite. A larger amount of insulin was released when the glucose concentration was raised to 2 g/L compared to no glucose.

3. Modified insulin

Self-regulated insulin delivery was addressed by another approach developed by Hoeg-Jensen et al. [99,100]. These authors used the glucose sensor as part of the insulin molecule. Insulin was modified with a phenyl boronate group. Their idea was to modify the diffusion rate of insulin according to the glucose concentration. It is known that the diffusion rate of insulin is strongly dependent on the form of the injected depot, because it is proportional to its half-time. For example, fast acting insulin is monomeric (t½–2–4 h), whereas long acting insulin is obtained from binding to high molecular weight proteins [102] or from soluble high molecular complexes (t½>10 h) [103]. By formulating insulin as a traditional Zn(II) hexamer, they demonstrated (Fig. 9) [99]. By formulating insulin as a traditional Zn(II) hexamer, a steeper glucose sensitivity and release profile was gained compared to monomeric insulin formulation.

4. Conclusion and perspectives

4.1. Treating diabetes with self-delivery materials

The goal of type 1 diabetes treatment is to achieve tight glucose control, to avoid chronic complications, while limiting the frequency of hypoglycemic episodes in day-to-day life. Although considerable efforts have been made to improve pharmacokinetikins of insulin and to develop user-friendly monitors, this goal remains difficult to achieve and an automated artificial pancreas is still required. Considerable work remains to be done to develop algorithms for the automated regulation of glucose by insulin. Furthermore, even if insulin pumps and glucose sensors have been miniaturized, such devices remain cumbersome for the patient.

Other strategies for the cure of diabetes are also on their way. The transplantation of islets of Langerhans was made possible by developing methods for the isolation of islets. Nevertheless, their rate of success is still low and the number of donors (two to three pancreases per patient) remains insufficient. With the progress of molecular biology, insulin secreting cells can be created but these cells are still far from having the fine-tuning of the genuine β-cell in response to glucose. The use of human embryonic stem cells still represents a scientific challenge and an ethical issue.

Alternative strategies have therefore to be considered. Progress in the field of biomaterials, drug delivery and insulin provide tools for constructing an “intelligent” insulin vector. The ideal vector would enable insulin delivery in a self-regulated and long acting but modulated manner. Self-regulated since in such a system the necessary amount of insulin could be administered in response to the blood glucose concentration and therefore replace the need of blood glucose monitoring for the patient. Long acting and modulated, to avoid the need of multiple day insulin injections. In other words, such bio-systems aim at mimicking natural feedback by combining sensor function (to perceive glucose modification) and effector function (insulin delivery) not to cure the patient, but at least to deliver him from the heavy burden of the disease in terms of day-to-day treatment but also of the possibility of chronic complication.

However, these glucose-responsive materials still require further research before any clinical application could be envisaged.

Fig. 8. Illustration of insulin self-assembly and disassembly under carbohydrate control (from ref [100]).

Fig. 9. Insulin release upon batch-wise washings with buffer or buffered D-glucose solutions (5, 25, 50 mM) at pH 7.4, from monomer (squares) and Zn(II) insulin hexamer (triangles) formulation immobilized on D-glucamine polymer, (from [99], Fig. 3).
4.2. Challenges for the future

The challenges remain to find a device which would combine:
- sensor specificity, which means that the sensor has to respond only to glucose variations;
- pharmacokinetics similar to normal pancreatic activity;
- easiness of administration, possibly by simple long lasting injections (ideally a weekly or monthly subcutaneous injection);
- biocompatibility without any in vivo toxicity, and possibly no long term side effect.

A classification of the existing methods is proposed in Table 1. It relates their advantages and disadvantages with regard to the above criteria. Comments about the future are given below.

It has been shown that three types of receptors could be used. It is generally admitted that natural receptors like proteins recognize specifically target molecules, whereas artificial receptors are non specific. However, this statement has to be refined in the area of glucose sensing. Among natural glucose receptors, only glucose oxidase specifically recognizes α-glucose, whereas lectins are non specific receptors. Artificial receptors are represented by the family of phenylboronate derivatives, able to form mono-bidentate (1:1) complexes as well as bis-bidentate (1:2) complexes with 1,2-diols such as carbohydrates. The 1:2 complexation promotes glucose selectivity over other common blood sugars such as fructose and galactose. In the case of 1:1 complexes, fructose is known to bind to boronate with a higher affinity constant than glucose. However, since the physiological concentration of glucose is approximately 100 times higher than the fructose one [104], this competition is likely not to be a major issue.

Several physical factors are known to affect the insulin release kinetics through hydrogels. Some of them have already been tested: it is expected that this will become more active within the next few years.

Insulin self-regulated delivery systems can also present several forms which will have to be related to the route of administration. A cross-linked gel is a highly elastic material, which cannot be administered via a simple injection but probably as an implant. The easiest route of administration is certainly the subcutaneous injection of a liquid, such as a suspension of microgels, a pre-gel solution or a glucose-responsive insulin depot. Ideally, this injection should enable the insulin vehicle to reach blood circulation, which would prevent from any time lag between glycemia variations and insulin release. Microgels are the best candidates to administer. Their size below 0.5 µm makes them suitable to go through blood capillaries without any obstruction. In return, microgels should have a prolonged circulation time to deliver insulin during several hyperglycemia episodes. A fast clearance from blood circulation has to be avoided and a major issue will be to render them stealthy. Another interesting form of insulin delivery is in situ gelling systems, which can be injected in the liquid state and form a gel at body temperature. This method has recently shown promising results. Nevertheless, if administered subcutaneously, the issue of time lag remains unsolved. Insulins with built-in boronated glucose sensors might also be injected subcutaneously. They are designed to be administered similarly to long acting commercially available insulin depots, except that their action time should depend on the glycemia. This field of research is

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Summary and classification of the different methods for chemically closed-loop insulin release</th>
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<tbody>
<tr>
<td>Sensor</td>
<td>Mechanism for self-regulated insulin delivery</td>
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<tr>
<td>GOD</td>
<td>Swelling of a pH-sensitive hydrogel bearing amino groups (with or without catalase [31–34])</td>
</tr>
<tr>
<td></td>
<td>With PEG grafts [41–44]</td>
</tr>
<tr>
<td>Gating membrane : cellulose [25] or poly(vinylidene) [38,39] membrane having pores grafted with PAA</td>
<td>Mechanical resistance</td>
</tr>
<tr>
<td>Degrading matrix : cross-linked poly(methacryloyloxyethyl phosphorylcholine) [40]</td>
<td>Blood compatibility and biocompatibility</td>
</tr>
<tr>
<td>In situ gelling system: chitosan+β-GPS [22]</td>
<td>Biocompatible Suitable for subcutaneous injection</td>
</tr>
<tr>
<td>Con A</td>
<td>Disruption of the complex with SAGP-insulin [47–50]</td>
</tr>
<tr>
<td></td>
<td>Dissolution of the complex with polysaccharides or polymers with saccharides residues such as p(GEMA) [51] or p(VP-co-AG) [54,55]</td>
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<tr>
<td></td>
<td>Swelling of a hydrogel by removal of Con A (cross-linker) [53]</td>
</tr>
<tr>
<td>PBA</td>
<td>Binding competition with PVA complexes with p(NVP-co-PBA) [66]</td>
</tr>
<tr>
<td></td>
<td>Binding competition with gluconated insulin complexed with amine containing PBA gel [68]</td>
</tr>
<tr>
<td></td>
<td>Change in the LCST of thermoresponsive polymers [23,63,71,72]</td>
</tr>
<tr>
<td></td>
<td>Exist as bulk hydrogels, beads [93] microgels [24,94–97] or nanocapsules [98] with possible faster response time and fluid preparation suitable for injection</td>
</tr>
<tr>
<td>Hydrogel shrinkage by bis-bidentate formation [24,26,80,88,90]</td>
<td>Selectivity compared to other physiological sugars</td>
</tr>
<tr>
<td></td>
<td>Dissociation of the hexamer–hexamer self-assembly of insulin modified with a PBA-carbohydrate pair [100]</td>
</tr>
</tbody>
</table>
still in its infancy and will require significant further work to become applicable. Biocompatibility and non-toxicity are finally the last but not the least requirements. Diabetes treatment is a long term treatment, which does not tolerate any possible side effects. Many hydrogels are known to be highly biocompatible. This can be explained by their high water content and their similarity of structure with the extracellular matrix, in particular for polysaccharide hydrogels. More generally, all natural biopolymers possessing a high degree of functional groups can be cross-linked to obtain three-dimensional structures, and further scaled down to the design of microgels. This is the case of chitosan-, hyaluronan-, or polyaminoacid-based hydrogels [105]. Successful in vivo applications have already been obtained with hydrogels [106]. Biodegradability is also desirable, at a larger time scale than that used for insulin delivery. Biodegradation of hydrogels can be achieved by several mechanisms including enzymatic, hydrolytic or environmental (like pH, temperature, etc). A number of degradable cross-linkers have already been reported, such as acetal, which can be degraded under acidic environment (pH < 6.5) [107], or disulfides, which can be degraded in the presence of a reducing agent including a tripeptide glutathione [108,109]. They might be incorporated into glucose-responsive hydrogels to build up the ideal insulin delivery system. Therefore, many tools are now available to design a chemically controlled closed-loop device for in vivo use. Although some issues still have to be overcome, this field of research is promising as a possible alternative to other approaches for diabetes treatment.

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