REVIEW ARTICLE

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Recent Developments in Self-Regulated Insulin Delivery

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In the last two decades enormous research has been carried out to develop self-regulated insulin delivery. Self-regulated delivery system is designed to release the insulin in response to changes in glucose concentration. Various sensing mechanisms like pH dependent polymer erosion, substrate enzyme reaction, competitive binding, drug solubility and various types of devices like hydrogel membranes, matrix system and grafted porous membranes are used to develop self-regulated insulin delivery. These self-regulated insulin delivery systems have the potential to improve the quality of the patient by avoiding repetitive administration of insulin.

Diabetes mellitus is caused by the inability of the pancreas to control the blood glucose concentration. The acute hyperglycemia causes life-threatening ketoacidosis and also chronic hyperglycemia leads to conditions like retinopathy, nephropathy and cardiovascular symptoms. According to American Diabetes Association, Diabetes resulting from a deficiency of insulin secretion is called type 1, which occurs in childhood, and requires exogenous insulin for survival. Diabetes resulting from resistance to insulin is called type 2, which may or may not require exogenous insulin and occurs later in life[1,2]. During the treatment of diabetes, a necessary amount of insulin, a hormone secreted from the islets of pancreas that controls glucose metabolism must be administered while constantly monitoring the blood glucose concentration. However this approach is a poor approximation of normal physiological insulin secretion. As a result it is impossible to maintain consistent blood glucose levels within normal range. Therefore there is a need for the self-regulated delivery system having the capability of adopting the rate of insulin release in response to changes in glucose concentration in order to keep the blood glucose levels within the normal range[3,4].

Glucose-sensitive hydrogel:

There has been much interest in the development of self-regulated delivery systems that releases insulin in response to elevated glucose levels. Self-regulated delivery system is designed to release the insulin in response to changes in glucose concentration[5-10]. Various sensing mechanisms that include pH-dependent polymer erosion[11], substrate enzyme reaction[12-19], drug solubility[25], competitive binding[29-41] and various types of devices such as hydrogel membranes[11], matrix system[21] and grafted porous membranes[13] are used to develop self-regulated insulin delivery. For the purpose of this review, a self-regulated drug delivery system is defined as one capable of receiving feedback information and adjusting the drug output in response to that information.

Hydrogels loaded with glucose oxidase:

This type of system utilizes glucose oxidase (GOD) as the glucose sensor and pH sensitive hydrogel as the insulin release controller. In such system, glucose is oxidized to gluconic acid, catalyzed by GOD[21], as shown here. $Glucose+O_2+H_2O \rightarrow$ gluconic acid$+H_2O_2$. Because of the above reaction, pH inside the microenvironment decreases with the increase in the glucose concentration. This causes increase in the volume of the pH sensitive hydrogel, which results in the release of entrapped insulin. Since the above reaction consumes oxygen the pH decrease in the device is limited by the presence of oxygen, which is low compared to glucose concentration. Also the formation of hydrogen

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peroxide deactivates GOD, therefore catalase is added which converts hydrogen peroxide to oxygen. $H_2O_2 \rightarrow 1/2\ O_2 + H_2O$, Glucose + 1/2 $O_2 \rightarrow$ gluconic acid, in presence of GOD and catalase.

Horbett and coworkers[11,12,19] were the first to investigate systems containing immobilized GOD in a pH responsive polymeric hydrogel enclosing a saturated solution. The hydrogel is made of 2-hydroxyethyl methacrylate (HEMA), N,N-dimethylaminoethyl methacrylate (DMAEMA) and tetraethylene glycol dimethacrylate (TEGDMA) as cross-linking agent. The authors investigated several parameters that affect the swelling and permeability of the membrane; such as concentration of amine groups (DMAEMA), cross-linking agent (TEGDMA) and glucose oxidase. The sensitivity range expanded from 0-10 mg/dl to 0-100 mg/dl as the amine concentration was increased and also swelling increased with increase in amine concentration and decrease in cross-linking agent concentration. Also it was observed that the loading levels of glucose oxidase did not influence the swelling behavior of membranes at different glucose concentrations.

Goldraich and Kost[17] have evaluated a matrix system in which the drug and enzymes were uniformly distributed throughout the hydrogel poly(HEMA-co-DMAEMA). The results showed that the hydrogels with high amine content of DMAEMA (18.5 vol%) and low cross-linking agent concentration of TEGDMA (0.3 vol%) are the most sensitive to pH. This system overcomes the problem of possible leakage of all the insulin that can happen in reservoir type.

Traitel and coworkers[21] studied the same hydrogel in simulated *in vivo* conditions. Polymer morphology was modified by changing the cross-linking agent (TEGDMA) concentration (0-0.95%). The swelling rates and swelling extent at steady state increased with decreasing cross-linking agent concentration. The matrices without cross-linking agent were stable in water and did not dissolve in water for an extended period of few months. Also it was observed that physically cross-linked hydrogel showed faster insulin release rates than chemically cross-linked hydrogel. The *in vivo* experiment with physically cross-linked hydrogel showed significant reduction in blood glucose levels from 400 mg/dl to 100-200 mg/dl after 2.5 h and to 50-120 mg/dl after 6.5 h. Also no fibrotic encapsulation was observed for 2-3 weeks indicating biocompatibility of the hydrogel.

Podual *et al.*[22] studied the pH sensitivity of poly(diethylaminoethyl methacrylate-g-ethylene glycol) (P(DEAEM-g-EG)) hydrogel microparticles and observed a distinct transition behavior, characterized by steep change in swollen volume at pH 7.0. Below this pH, the microparticles exhibited high swelling ratios. The ionization of the tertiary amine side groups resulted in the swelling of the polymer due to (i) increase in hydrophilicity of the polymer and (ii) electrostatic repulsion between the positively charged groups. Microparticles of 30, 160 and 370 μm were studied for swelling time constant, rate of swelling and release rate. The swelling time constant varied from 800 s in the 370 μm particles to 30 s in 30 μm particles. It was also observed that the rate of swelling for 30 μm was 1 s^{-1} and for 160 and 370 μm particles it was 0.15 s^{-1} and 0.07 s^{-1} respectively. The rate of release was found to be dependent on rate of swelling. It was proposed that the incorporation of polyethylene grafts on the main chain of the hydrogel provided 'stealth' effect, which helps to reduce immunoreactions and subsequent rejection, by the body, which eventually maximizes the lifetime of the hydrogel in the body.

Further Podual *et al.*[23] studied the glucose sensitivity of P(DEAEM-g-EG) hydrogel microparticles. It was observed that the enzymes in the hydrogel were active even after polymerization and were able to reduce the pH inside the hydrogel to 3.5. Also increasing the crosslinking density from 0.02 to 0.03 and 0.02 to 0.04 decreased the average mesh size of the hydrogel by 29 and 51%, respectively. The swelling ratio was also found to decrease correspondingly.

Zhang and Wu[24] have prepared stimuli sensitive membrane system by a physical method (solution casting method). In this system nanoparticles of poly(N-isopropylacrylamide-co-methacrylic acid) (poly(NIPAm/MAA)) were dispersed in a hydrophobic polymer. Both GOD and catalase enzymes were incorporated in the membrane. The permeability of drugs across the membrane increases with decreasing pH or increasing temperature. The pH or temperature sensitivity is controlled by the swelling /shrinking of the nanoparticles, and the degree of sensitivity depends on the properties of solute and nanoparticles. This system is more sensitive to pH and has good mechanical strength. Since physical method is used in the preparation of the membrane, inactivation of the incorporated proteins and enzymes is greatly reduced.

Brown *et al.*[25] have characterized a glucose sensitive insulin delivery system in which solid particulate insulin was incorporated into an ethylene-vinyl acetate copolymer (EVAc) matrix. Feed back control was mediated by the

glucose oxidase enzyme, which was immobilized on sepharose beads, which were incorporated along with insulin into EVAc matrix. A decrease of 0.71 pH units was measured within 4 min after exposure to glucose at 10 mg/ml. The decrease in pH inside the matrix resulted in increase in solubility of the insulin and consequently a rise in insulin release rates from the matrix. The system response to repeated glucose challenges was consistent and at least 60 min was required between two glucose stimuli to observe an optimal response as the repeated glucose stimuli induced a degree of refractory behaviour.

Concanavalin immobilized systems:

Concanavalin A (Con A), a lectin is a glucose binding protein obtained from the jack bean plant, *Canavalia ensiformis*. The formulation of glucose responsive insulin delivery employing the plant lectin, Con A and specific polysaccharide has been reported widely[27-36]. A highly specific interaction between glucose and Con A was used to form physical cross-link between glucose containing polymer chains and Con A. Since Con A exists as tetramer at physiological pH and each subunit has a glucose binding site, Con A can function as a cross linking agent for glucose containing polymer chains. Because of the non-covalent interaction between glucose and Con A, the formed cross-links are reversible. Individual free glucose molecules can compete with the polymer-attached glucose molecules. Thus, the maintenance of the cross-link depends on the relative concentration of the free glucose in the environment. As the concentration of the free glucose increases in the environment, the gel becomes sol and insulin is released and when the environment glucose concentration decreases, gel is formed again.

Brownlee and Cerami[37] and Seminoff *et al.*[38] were the first to develop glucose sensitive insulin release system using Con A. In these systems biologically active glycosylated insulin derivatives able to form a complex with Con A was synthesized and in presence of free glucose, due to competitive binding, glycosylated insulin is released. Kim *et al.*[39] prepared self-regulated insulin delivery system based on the concept of competitive binding between synthetic glycosylated insulin (G-insulin) and glucose to Con A. G-insulin prepared were succinyl amidophenyl glucopyranoside insulin (SAPG-insulin) and succinyl amidophenyl mannopyranoside insulin (SAPM-insulin). The system was based on SAPG- or SAPM-insulin with water soluble Con A enclosed in pouches of porous poly(HEMA). This system was implanted into the peritoneal cavity of the pancreatectomized dogs. The fasting glucose level, which was 300 mg/dl at the time of implantation, returned to normal values within 10 h. The disadvantages of the system were the leakage of immunogenic Con A, and inadequate permeability of glucose and G-insulin. To overcome the problem of leakage of Con A, Con A was immobilized in sepharose beads. *In vitro* studies showed that a glucose concentration of 100 mg/dl was necessary to displace G-insulin. This type of system was heavy and suffered from lag time for the onset of displacement of Con A. An alternative system based on nylon microcapsules of size 30-250 μm and pore size of 1 μm were prepared which had rapid onset of action due to large surface area.

Lee and Park[40] studied a new type of hydrogel capable of sol-gel phase reversible transitions based upon changes in the glucose concentration. In this system vinylpyrrolidone-allylglucose copolymer was complexed with Con A, which led to formation of hydrogel. Addition of free glucose to this led to a phase transition from gel to sol phase.

Kim and Park[34] studied hydrogels prepared from PEGylated concanavalin A and glucose containing polymer. Con A grafted with five PEG molecules were used to enhance the stability of Con A. Glucose containing polymers were prepared by free radical copolymerization of allyl glucose with comonomers such as 3-sulfopropylacrylate-potassium salt, N-vinyl pyrrolidone, and acrylamide. Three different types of insulin delivery systems were examined namely diffusion-controlled reservoir, diffusion controlled matrix, and erosion controlled matrix systems. Only diffusion controlled reservoir and diffusion controlled matrix systems showed modulated insulin release in response to changing glucose concentration. The insulin diffusion experiments through glucose-sensitive hydrogel membranes using Franz diffusion cell showed that as the glucose concentration of receptor compartment was increased from 1 to 4 mg/ml, the insulin release rate increased four fold from 0.1 to 0.4 μg/cm^2/h. When the glucose concentration was lowered to 1 mg/ml the release rate decreased sharply to 0.16 μg/cm^2/h within 30 min.

Tanna *et al.*[41] prepared a glucose-responsive gel between Con A and polysaccharide, which was stabilized via coupling to a carbomer resin. This was done to prevent toxicity and to preserve the working mechanism of the formulation. Increased gel stability was achieved by covalently bonding amine groups present on the lysine residues of Con A to carboxylic moieties on carbopol 974NF using carbodiimide chemistry. Addition of dextran then

transformed the glucose responsive formulation from gel to sol in the presence of free glucose. *In vitro* experiments demonstrated that the system showed increasing and graded response to glucose triggering concentration of 0.2-1% w/v, which represent mildly to a fairly severe diabetic state. In these systems leaching of Con A from covalently coupled gels was reduced when compared to a non-coupled gel.

Hydrogels with phenylboronic acid moieties:

A polymer having phenylboronic acid group as a side chain can form a complex gel with polyol polymer such as poly(vinyl alcohol) through the covalent complex formation between the pendent phenylborate group and hydroxyl groups. When glucose is added to this complex gel, the gel swells due to decrease in the cross linking density caused by the substitution reaction of glucose with the pendent hydroxyl groups of the polymer towards borate groups. This results in the release of the loaded insulin and the swelling is reversible so that when glucose concentration decreases the gel shrinks due to the formation of borate-polyol complex[5].

Kitano *et al.*[42] have prepared a polymer containing phenylboronic acid by co-polymerizing N-vinyl-2-pyrrolidone (NVP) and 3-(acrylamido) phenylboronic acid (PBA). A gel was obtained by reversible complex formed between phenylboronic acid of poly(NVP-co-PBA) and poly(vinyl alcohol) (PVA). Competitive binding between phenylboronic acid and glucose was used to develop glucose sensitive system. The formation of complex between PBA groups and PVA, its dissociation and formation of PBA-glucose complex could be observed by measuring the changes in viscosity.

Kataoka *et al.*[43] have synthesized terpolymers of m-acrylamidophenylboronic acid, N,N-dimethylamino-propylacrylamide (DMAPAA) N,N-dimethylacrylamide. DMAPAA was introduced to increase the stability of PBA-polyol complex at the physiological pH. The effect of amino group on complex stabilization was estimated from viscosity as well as UV difference spectrum measurement. The effect of pH as well as amino group content in the copolymer on the formation of polymer complex was studied in detail to determine the optimum composition in order to achieve prompt release toward glucose. Also Shiino *et al.*[44] observed that amine containing phenylboronic acid gel exhibited higher stability at physiological pH.

CONCLUSIONS

A lot of research has been carried out to develop self-regulated insulin delivery, and for them to become clinically useful, some improvements need to be made. Though the hydrogels exhibit swelling in response to glucose it is not fast enough to meet the ever-changing glucose concentration in clinical situations. Also the hydrogels do not return to original state fast enough once the glucose concentration has come down. Con A immobilized systems suffer from the problem of leaching of mitogenic Con A. The polymers used in the development of self-regulated insulin delivery system should be biocompatible and the system should overcome the problem of accidental release. If the achievements of the past can be extrapolated to future, it is definitely possible that self-regulated insulin system can be developed.

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