

THESIS

APPLICATIONS OF INORGANIC NANOPARTICLES IN DIABETES

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ABSTRACT

APPLICATIONS OF INORGANIC NANOPARTICLES IN DIABETES

Diabetes Mellitus (DM) is an endocrine and metabolic disease that has become a global emergency because of the rapid rise in morbidity and mortality rates worldwide. Since the direct delivery of biomolecules, such as insulin, to treat DM is inefficient and subjected to enzymatic degradation, nanotechnology and nanomedicine research have been devoted to the development of more effective methods to treat DM. Nanoparticles (NP), organic, inorganic, or hybrid, have served as potential carrier for safe and efficient transport for insulin. Additionally, several NP have biological activities that help treat and/or prevent DM and diabetes complications, such as antioxidant, anti-apoptotic, or insulin-mimetic activities. Moreover, physicochemical properties of some NP allow them to be used in diagnostic tools for potential diagnosis or monitoring purposes. This work highlights the applications of inorganic NP such as, gold, selenium, silver, calcium phosphate, zinc oxide, cerium oxide, and iron oxide and in the treatment or diagnosis of DM.

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DEDICATION

I dedicate this work for my beloved family. My husband Haithem Eshami for his constant support and assistance and for my adorable son Taher my precious gift and blessing from God.

My parents, Atiya Elhabush and Fawziya Shagloof, it is never enough to say “thank you” because you have never stopped supporting me and believing that my dreams could be achieved and come true. My brothers and sisters, thank you for your endless encouragement and motivation. To my deceased mother in law, Rabiaa Elrifai, and father in law, Taher Eshami for your kindness and thoughtfulness towards me, I miss you. For all my relatives and friends who kept praying for me to reach to the place where I am right now, thank you. And a little word for my nephews and nieces: I love you all.

This isn't the end of the path that I have decided to take; this is just a step forward, toward the future that I have imagined.

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CHAPTER 1/ INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disease characterized by Hyperglycemia (elevated blood glucose levels) (Kharroubi and Darwish, 2015). There are three main types of DM. In type 1 diabetes mellitus (T1DM) the body cannot produce insulin (Sharma et al., 2015). In type 2 diabetes mellitus (T2DM) the body produces insulin but the cells are resistant to it (American Diabetes Association, 2014). Gestational diabetes mellitus (GDM) is diagnosed when hyperglycemia is first detected during pregnancy (Wu et al., 2016)

According to the International Diabetes Federation, 44.3 million people are living with diabetes in the U.S and 415 million people worldwide. This number is expected to rise by 2040 to reach 642 million (IDF Diabetes Atlas. 2015). This number is higher than the combined number of mortality caused by infectious diseases such as HIV/AIDs (1.5 million), tuberculosis (1.5 million), and malaria (0.6 million) in 2013 (WHO. 2013).

Long term complications that may associate with diabetes are caused by chronic hyperglycemia (elevated blood glucose level). These complications include retinopathy (damage to the retina of the eye) that can progress and cause blindness, nephropathy (damage to the kidneys) which can lead to renal failure and death, neuropathy (damage to the neurons), cardiovascular disease that represent the most common cause of death to diabetic patients (Nathan, 1993), foot ulcers, and amputations.

DM is diagnosed by the symptoms polyuria (excess, diluted urine) and polydipsia (excessive thirst) and by measuring plasma glucose concentration (American Diabetes

Association, 2014). Diabetic patients have Impaired Fasting Glucose (IFG) levels of ≥ 126 mg/dl (7.0 mmol/L) and impaired casual plasma glucose levels (measured any time during the day) of ≥ 200 mg/dl (11.1 mmol/L) (Seino et al., 2010). GDM is diagnosed by oral glucose tolerance test (OGTT) during 24 - 32 weeks of pregnancy. Impaired glucose tolerance after 2 hours of administering 75g of glucose solution is ≥ 140 mg/dl (7.8 mmol/L) (Meek et al., 2015). Some people have undiagnosed DM (Hoang et al. 2014). It is estimated that one in two persons has undiagnosed DM (IDF Diabetes Atlas. 2015).

Diabetes patients are currently treated with subcutaneous injections of insulin to reduce hyperglycemia. It is painful and uncomfortable to patients. Furthermore, it should be adjusted manually according to meal time. Incorrect dosage or time of this treatment may result to a dangerous hypoglycemia that result in coma and death if not treated promptly (Raj and Sharma, 2003). To avoid these complications, an alternative route to administer insulin to patients in a more convenient way is needed. Nanoparticles (NP) have been extensively studied as a potential treatment as a drug delivery system in different diseases (Faraji and Wipf, 2009) and as a tool for diagnosis (Alam et al., 2014). There are various organic NP that have been used to study their efficiency as carriers of insulin to reduce blood glucose levels (BGL) such as alginate, chitosan, dextran, hyaluronic acid, poly(lactic acid), and solid-lipid NP (Fonte et al., 2014). There are also inorganic NP that have been studied to treat and diagnose DM s. This review is a cultivation of several studies on inorganic NP, such as gold, selenium, iron oxide, calcium phosphate, cerium oxide, silver, and zinc that have been used in various experiments to investigate their efficiency in treatment, diagnosis, monitoring, or prevention of DM.

Inorganic Nanoparticles as Carriers for Insulin or Therapeutic Peptides 2.1

Gold Nanoparticles 2.1.1

Recently, gold nanoparticles (AuNP) have been extensively studied as a potential drug carrier for their inert nature (Hui Chen et al., 2013), optical features, ability to be tuned to different shapes and sizes, ease of surface modification, and bioconjugation ability to bind to different biomolecules such as proteins, amino acids (a.a) and DNA (Ghosh et al., 2008; Giljohann et al., 2010).

AuNP used in research for potential treatment of DM are in the form of insulin-AuNP composite, where insulin is adsorbed on the AuNP surface either directly or bound to another molecule that cap the AuNP nanosphere. They were mostly prepared by chemical reduction of tetrachloroauric acid (HAuCl_4) to obtain the metal nanosphere. To study their efficiency to treat diabetes, they formulated in different sizes and administered in to diabetic animal models through different routes in different concentrations and measured the maximum reduction in blood glucose levels (BGL) and the duration of effect until the BGL began to increase (Table-1). It has been found that when insulin is bound directly to bare AuNP, the release rate of insulin is slow when compared to other formulations when insulin is bound to amino acid-capped AuNP. Bare AuNP-INS composite were found to reduce diabetic rats BGL 19% and 31% of the baseline value of blood glucose when administered by oral and intranasal routes, respectively, in a dose of 50 IU/kg. On the other hand, INS-aspartate-capped AuNP composite reduced BGL to 50% and 55% when administered by oral and intranasal routes, respectively, and in lower doses of 20

IU/kg. The variation in effect of the two AuNP composites is attributed to the interaction between insulin and AuNP. In bare AuNP-INS composite, insulin is bound to AuNP through strong covalent bonds leading to a slow release of insulin when administered to diabetic rats, while insulin in aspartate-capped AuNP composite is bound to aspartic acid through hydrogen bonds. They are weaker than covalent bonds allowing insulin to be released more easily and quickly. Notably, intranasal route was observed to reduce BGL more efficiently than oral route (Joshi et al., 2006). The anatomical and physiological distinction (pH, enzymes, membrane barrier, thickness and surface area of the mucosa) between the oral and intranasal routes contributed to the variation in efficiency of AuNP in reducing BGL. It has been further studied by using chitosan to reduce H_{AuCl}₄, stabilize the nanocomposite, and enhance permeation/absorption to deliver insulin in animal model through oral and intranasal routes. BGL was significantly decreased to 31.41% and 34.12% when administered through oral (50 IU/kg) and intranasal (10 IU/kg) routes, respectively. Oral medicines are subjected to digestive enzymes that may degrade a considerable amount of the ingested dose. That explains how a larger dose of chitosan-reduced AuNP-INS composite contributed to less BGL reduction compared to a lower dose of intranasal route. Moreover, when this experiment was done *in vitro* using fresh goat nasal membrane, chitosan-reduced AuNP-INS showed 4-5 times greater permeation when compared to free insulin. Furthermore, the nanocomposite were proved to be stable for 6 months when visualized with transmission electron microscope (TEM), making a reliable carrier for insulin delivery (Bhumkar et al., 2007).

Chitosan have been used as natural polymers to coat different NP for their unique biodegradable, biocompatible, and mucoadhesive properties, as well as permeation enhancing

capability for the absorption of hydrophilic molecules such as insulin, allowing chitosan to transiently open tight junctions between the intestinal cells to pass through paracellular pathway of absorption when administered orally. Through intranasal route of administration, chitosan increase residence time of the used NP through its bioadhesive properties, allowing chitosan NP to adhere to the mucosa and increase drug bioavailability to enhance its absorption (Damgé et al., 2008; Sharma et al., 2015). There are other molecules that have been used in reduction of HAuCl_4 as well as coating and stabilizing AuNP, such as chondroitin sulfate (CS). A network of CS coats the AuNP and entraps insulin for stability. When administered orally to diabetic rats, BGL was significantly reduced up to 32.1% of baseline value in 60 min after application. Plasma insulin concentrations were 6.61 folds greater than free insulin-treated rats. The CS-reduced AuNP-INS composite proved to be stable for 7 weeks making it another reliable insulin carrier for glucose control therapy (Cho et al., 2014).

Efficiency of AuNP seems to depend on their shape, size, and surface charge. In the previously mentioned studies, the AuNP used were nanospheres. In another study, researchers used AuNP nanorods (AuNR) to deliver insulin through transdermal route *in vivo* and *in vitro* with or without the aid of near infrared (NIR) irradiation to explore how deep these AuNR can penetrate skin barrier. The *in vitro* experiment showed that AuNR-INS where able to penetrate to 500 um in skin depth. These results were verified with *in vivo* experiment that showed a significant reduction in BGL to 10% of baseline value, even though the maximum decrease was reached after 10h post application. NIR irradiation seemed to help AuNR to be administered through the skin when the absorbed light is converted to heat. This unique technique might be possible in delivering insulin through the skin to avoid digestive enzymes when insulin is taken

orally (Nose et al., 2012). Shilo et al. used different sizes and concentrations of AuNP to explore the possibility to design a personalized and adjustable treatment for hyperglycemia. It was found that variation in AuNP-INS sizes and concentrations showed different BGL reduction response in animal model that may work as a potential adjustable treatment to diabetes (Shilo et al., 2015).

Calcium Phosphate Nanoparticles 2.1.2

Although chitosan has desirable properties, as mentioned above, it precipitates in neutral to basic pH. To overcome this hurdle, calcium phosphate nanoparticles were uniquely formulated with chitosan and vitamin B12 coating to deliver insulin orally. Vitamin B12 conjugation with chitosan showed complete solubility in neutral and basic pH, making this nanoparticles formulation of chitosan-vitamin B12- calcium phosphate NP a potential carrier for therapeutic peptides such as insulin. This formulation showed a significant ability in intestinal uptake by paracellular pathway due to chitosan's permeation property, and through receptor-mediated endocytosis (A. Verma et al., 2016).

Iron Oxide Nanoparticles 2.1.3

A unique iron oxide nanocomposite was formulated to test their loading and release efficiency of biomolecules, specifically insulin. Graphene oxide nanosheets were previously studied for anticancer drug delivery. However, their loading and release rates were not efficient. Therefore, Turcheniuk and colleagues formulated graphene oxide nanosheets supported with modified iron oxide nanoparticles and loaded with insulin. This unique nanocomposite showed stability in acidic pH resembling gastric pH, and insulin was released

when the nanocomposites reach a more basic pH similar to intestinal pH. Insulin efficacy to lower hyperglycemia is yet to be studied; nonetheless, this new nanocomposite proves to be beneficial as potential carrier for biomolecules such as insulin to manage DM (Turcheniuk et al., 2014).

Selenium Nanoparticles 2.1.4

Selenium nanoparticles (SeNP) were used to add stability and increase the half-life of a T2DM therapeutic peptide, BAY 55-9837 (BAY). In pancreatic islets insulin-secreting beta-cells, VPAC2 receptors activate insulin secretion. BAY peptide is VPAC2 agonist that can stimulate insulin secretion in T2DM patients, making it a potential therapeutic peptide. Limitations of using BAY include their poor stability and short half-life due to their small size that undergo renal clearance, which in turn reduces their blood residence time. Chitosan-SeNP were used carriers for BAY to prolong their blood residence time and increase their bioavailability for a more sustained activity (Rao et al., 2014). Therefore, the use of SeNP to increase the size/molecular weight of therapeutic peptides could enhance their biological activity and stability in vivo, added to the beneficial biological function of selenium when conjugated with these peptides.

Antioxidant Inorganic Nanoparticles Reduce Diabetes-Induced Oxidative Stress 2.2

Selenium Nanoparticles 2.2.1

Selenium is a trace element that is known to have an insulin-like effect on cells, where it can translocate glucose transporters to cell membrane (Ezaki, 1990). When used as selenium nanoparticles (SeNP) to study their effect on streptozotocin-induced diabetic rats with or

without a combined insulin treatment, BGL was significantly reduced from 300 mg/dl to basal levels of 100 and 120 mg/dl when used as SeNP plus insulin treatment and as SeNP treatment alone, respectively. Moreover, the researchers studied the antidiabetic effect of these SeNP to revert the streptozotocin-induced damage pancreatic islets. It has been found that streptozotocin+SeNP-treated pancreatic islets were protected from degenerative damage compared to shrinkage of Langerhans islets in streptozotocin-treated rats. Furthermore, when the islets stained with insulin antibody for immunoassay, they revealed a considerable amount of insulin produced in the SeNP-protected islets compared to damaged islets (Al-Quraishy et al., 2015). Streptozotocin-induced diabetes is caused by reactive oxygen species (ROS) (Szkudelski, 2001). The generated ROS or free radicals damage cellular and organellar membranes, proteins and nucleic acids. In diabetes, chronic hyperglycemia is toxic to the body because of the auto-oxidation of glucose that produces hydrogen peroxide (H_2O_2), a potent ROS (Wolff and Dean, 1987). Normally, H_2O_2 is degraded by glutathione peroxidase (GPx_1) (Maritim et al., 2003). Glutathione peroxidase enzyme is one of the selenoenzymes that are regulated by selenium (Baker et al., 1993; Pepper et al., 2011). Therefore, selenium prevents the damage caused by glucose oxidation, ROS, and/or streptozotocin-induced damage in diabetes. The antioxidant and antiapoptotic activities of SeNP were further studied in diabetic nephropathy. It was reported in a study that 41% of Type 1 diabetes patients developed nephropathy at various ages, and only 10% of them survived (Andersen et al., 1983). Therefore, diabetic nephropathy was more extensively studied to stop the progression of renal damage. G.S. Kumar and colleagues reported that SeNP had a protective activity against kidney damage induced by STZ in rats by increasing the expression of antiapoptotic protein Bcl2 and decreasing expression of pro-

apoptotic protein Bax. Moreover, heat shock protein HSP70, known to prevent apoptosis, was overexpressed in SeNP-treated diabetic rats compared to diabetic control rats that showed significant reduction in HSP70 and Bcl2 and increased Bax parameters. Histopathological changes in renal tissues were assessed to further elucidate the protective activity of SeNP. Renal glomeruli suffered oxidative damage and cell degeneration due to apoptosis induced by increased oxidative stress, while SeNP-treated diabetic group maintained a relatively normal renal histological structure verifying that SeNP protected the diabetic kidneys from damage. Blood urea nitrogen and creatinine, kidney disease blood markers, were markedly reduced to normal levels with SeNP treatment compared to a significant elevation of those markers in diabetic control group (G.S. Kumar et al., 2014).

Although high concentrations of selenium was reported to generate ROS, this process was exploited in anticancer treatment (Stapleton et al., 1997).

Cerium Oxide Nanoparticles 2.2.2

Cerium oxide is another inorganic antioxidant that has the ability to scavenge ROS due to the large surface area to volume ratio that creates reactive sites to scavenge free radicals (Hirst et al., 2009). Cerium oxide NP and sodium selenite were used in diabetic rats to evaluate their ability to reduce oxidative stress caused by diabetes. Cerium oxide NP increased total antioxidant power in diabetic rats. Total antioxidant power measures the antioxidant ability to scavenge free radicals. Oxidative stress has decreased heart, kidneys and lungs in diabetic animals treated with cerium oxide nanoparticles. Moreover, cerium oxide NP decreased lipid peroxidation and ROS in the brain, kidney and heart. Sodium selenite was found to act

synergistically with cerium oxide NP by enhancing the antioxidant activity (Navaei-Nigjeh et al., 2012).

Oxidative stress is involved in the chronic diabetes complications including retinopathy, where ROS resulted from chronic hyperglycemia damage the microvasculature of the retina and subsequently damage the cells due to compromised perfusion (Madsen-Bouterse and Kowluru, 2008). ROS are also formed in photoreceptors in response to light damage. Photoreceptors consume a high amount of oxygen; therefore generate a high amount of free radicals that must be neutralized. Cerium oxide NP, were shot intravitreally to evaluate their efficiency in scavenging the free radicals in the eye and their protective activity from the degenerative damage induced by light. The results showed that photoreceptors were protected from degeneration, thus cerium oxide NP has the potential to prevent oxidative damage caused by chronic diabetes. Cerium oxide NP can transfer from +4 to +3 states and vice versa when scavenging free radicals. This regenerative ability reduces the need for repetitive doses for treatment (Chen et al., 2008).

Gold Nanoparticles 2.2.3

Although AuNP worked efficiently as insulin carrier, they also have been studied to treat diabetes for their antioxidant effect. Barathmanikanth and colleagues reported that administering AuNP to diabetic mice has significantly restores GSH, GPx and SOD parameters that were markedly reduced in diabetic control mice. Moreover, AuNP decreasead oxidative stress parameters (lipid peroxidation and ROS). Even though AuNP were tested for their cytotoxicity in this study(Barathmanikanth et al., 2010), it has been reported that they can

increase oxidative stress and cause cellular damage (Ferreira et al., 2015; Khan et al., 2012).

Therefore, more the toxicity and antioxidative effect of AuNP should be further studied.

Inorganic Nanoparticles with Insulin-mimetic Activity 2.3

Zinc Oxide Nanoparticles 2.3.1

Zinc also has an insulin-mimetic activity. It can enhance phosphorylation of serine/threonine protein kinase (Akt) and activate glucose transporter-4 (GLUT4) translocation from the intracellular vesicle to the cell membrane to increase glucose uptake. Additionally, zinc can stimulate the auto-phosphorylation of cytosolic subunit of insulin receptor (IR) increasing insulin sensitivity and initiating insulin signaling. Moreover, zinc inhibits protein tyrosine phosphatase 1B (PTP1B), an enzyme that terminates insulin signaling (Jansen et al., 2009). A study used zinc oxide nanoparticles (ZnONP) and silver nanoparticles (SNP) separately to evaluate their efficiency in reducing BGL in diabetic rats. An oral dose of 10mg/kg of each NP where administered daily. It has been found that ZnONP and SNP significantly reduced BGL by 75.8% and 68.2%, respectively. Moreover, ZnONP elevated serum insulin concentration by 79.4%. This determined that both ZnONP and SNP have antidiabetic activity even though ZnONP were more potent (Alkaladi et al., 2014). The antidiabetic effect of SNP isn't clearly understood, but the mechanism by which ZnONP reduce BGL is clear to some extent, as described above.

Other Nanoparticles 2.3.2

Selenium was found to activate translocation of GLUT4 to the cell membrane to increase glucose uptake by enhancing the activation of Akt, a mechanism that is activated by insulin

binding to IR, thus selenium stimulates cell sensitivity to insulin in a way similar to zinc activity (Al-Quraishy et al., 2015; Hatfield et al., 2011).

Detection of Type 1 Diabetes Autoantibodies 3.1

T1DM is an autoimmune disease where autoantibodies are directed against one or more pancreatic antigens (insulin, glutamic acid decarboxylase, and/or tyrosine phosphatase islet antigen 2). The chip is made of islands of nanostructured gold, to enhance the electrical response, and pancreatic islet antigens microarrays on a glass surface. A finger prick of 2 μ l of human whole blood or serum is sufficient to run the test. If the patient tested positive for T1DM autoantibodies, then these antibodies will bind to their respective islet antigens. Then the antibodies are detected using specific, fluorescently-labeled antihuman antibodies. The fluorescence intensity is then measured. This test is sensitive, inexpensive, and doesn't require an intravenous blood draw compared to the ordinary lab test that require a radiation immunoassay to detect the autoantibodies. The gold chip autoantibody test is also able to distinguish between T1DM and T2DM because the latter is positive to hyperglycemia but negative to autoantibodies. The test can also determine which pancreatic islet antigens the autoantibodies are directed to. Since not all cases of T1DM are early-onset, the gold chip can be a reliable, quick and easy diagnostic test for undiagnosed late-onset T1DM in adults. Furthermore it is a practical test to be used in screening populations for T1DM. (R.B. Kumar et al., 2015; Zhang et al., 2014).

Detection of Acetone in Diabetic Exhaled Breath 3.2

In T1DM, insulin concentration is very low in the blood or, in some instances, is absent. Because glucose can't be consumed for energy, the body attempts to produce energy through

lipolysis. During this process ketone bodies such as beta-hydroxybutyrate and acetoacetate are formed and converted to acetone. Due to the resulting metabolic acidosis, the body attempts to excrete acetone via respiration. Therefore, a higher concentrations of acetone is detected as a biomarker in diabetics' exhaled breath (Tassopoulos et al., 1969; Turner et al., 2009). Normal acetone concentrations in a healthy individual's breath is 0.3 – 0.9 ppm, while it exceeds 1.8 ppm in diabetic's breath. A new device made of AuNP and three-dimensional indium oxide (In_2O_3) inverse opals electrode can be used to detect organic volatile gases including acetone. AuNP are loaded on the In_2O_3 films to increase the electrical conductivity, thus enhancing the signal. The AuNP film is very sensitive to trace concentrations of acetone. It can detect concentrations as low as 20ppb rendering this new technique a reliable test to monitor and diagnose diabetes in a noninvasive way (Xing et al., 2015).

Acetone is generated in diabetic ketoacidosis as well as alcoholic ketoacidosis. Both are dangerous to the patient due to acidemia and hypokalemia that may cause death if untreated. AuNP/ In_2O_3 test cannot distinguish between diabetic and alcoholic ketoacidosis through detection of acetone. Thus further diagnostic measures to analyze hyperglycemia should be taken to exclude ketoacidosis due to alcoholism (Hockenfull et al., 2012).

Detection of Pancreatic Islets Cell Mass 3.3

It is known that T1DM patients suffer from immune-mediated beta cells destruction leading to no insulin production, while T2DM patients produce normal amount of insulin but their cells are resistant to it leading to hyperinsulinemia at the beginning and eventually insulin deficiency. It has been found that T1DM may still have some functioning beta cells. These

functioning cells have been studied as a target to stimulate their proliferation to produce more cells (Akirav et al., 2008). On the other hand, T2DM patients may suffer from reduction in beta cell mass. These findings argue the need for specific diagnostic imaging for pancreatic beta cells to determine the proper treatment according to the stage of the disease or according to beta cell mass, or as a monitoring imaging to maintain or adjust the treatment (Yagihashi, 2012). Glucagon-like peptide 1 receptor (GLP-1R) is highly and exclusively expressed in pancreatic beta cells of mice, rats and humans. It is not expressed in alpha or in delta cells. This makes GLP-1R a targeted biomarker for specific beta cell imaging. Exendin-4 is known to be GLP-1 analog that specifically binds to beta cell's GLP-1R. B. Zhang and colleagues used superparamagnetic iron oxide NP labeled with exendin-4 as a potential beta cell-specific MRI probe from diagnostic imaging of beta cell. Their results showed specific targeting of the labeled NP to islet beta cells and insulinoma cells *in vitro* that also express GLP-1R. These findings proved that this beta cell specific targeting mechanism could be beneficial for monitoring purposes of islet transplantation and/or diagnosis of the clinical stage of DM (B. Zhang et al., 2013).

Monitoring Diabetes-Associated White Blood Cell Count 3.4

It has been reported in a clinical study that chronic high white blood cells (WBC) count could be associated with the development and/or progression of Type 2 diabetes due to a relationship between high WBC count and insulin resistance (Vozarova et al., 2002). The high WBC count in T2DM could also lead to the development of cardiovascular disease. Therefore, the necessity to constantly monitor WBC count with a low cost, instrument-free, rapid and convenient way without the need of medical knowledge has inspired Zhang and colleagues to develop a portable, self-administering WBC counting device for patients. A very small amount

of blood (15 μ l) is sufficient to run the test. The blood sample is labeled with AuNP using biotin-labeled CD-45 antibodies. CD-45 is a common surface marker found on all WBCs. The blood sample flows vertically in a small orifice in the device. WBCs that are conjugated to AuNP would be trapped in the paper mesh positioned right under the orifice. The test spot would appear dark due to the intensity of the labeled AuNP trapped in the paper. If there were no cells, the test spot would appear white, because the free AuNP (40 nm) flowed through the paper. The test spot color intensity could reveal if the patient suffer from leukocytosis (high WBC count) or leukocytopenia (low WBC count) to seek medical assistance and prevent any complications. Because the test is based on using antibodies against CD-45 surface marker, it is designed to give a total WBC count but not a differential WBC count (Y. Zhang et al., 2015).

CHAPTER 4/ SUMMARY & CONCLUSION

Recently, inorganic nanoparticles have gained more interest for their effect in treating, preventing, or diagnosing diabetes because of their availability and significant biological and physical properties. Inorganic nanoparticles have significant biological and/physical properties that allow them to be a potential treatment for diabetes. They have been used to offer a safe and efficient transport to insulin to protect it from enzymatic degradation or to enhance its absorption. Gold nanoparticles are unique metal NP with desirable properties that have proved to be efficient and stable carriers of insulin to treat hyperglycemia. They can be synthesized in different formulations to enhance their absorption, stability, and bioavailability. When given through different routes, they were effectively absorbed by the cells and reduced blood glucose concentrations. Additionally, calcium phosphate and iron oxide NPs have also proved to be reliable and safe carriers for insulin. Other inorganic nanoparticles were found to have hypoglycemic effect when administered, such as selenium, silver, and zinc oxide NP that have efficiently reduced hyperglycemia. While the mechanism by which silver nanoparticles reduce blood glucose levels is still not clear, zinc oxide and selenium nanoparticles possess insulin-mimetic activity that allow them to translocate glucose transporter 4 to cell membrane to increase glucose uptake. Since oxidative stress increases with chronic diabetes leading to diabetic complications, antioxidant treatments in the form of nanoparticles have been used to reduce oxidative stress. Selenium and cerium oxide NPs have antioxidant activities and they have efficiently protected tissues from oxidative damage and prevented the progression of the disease. Moreover, selenium nanoparticles proved to have antiapoptotic activity by

upregulating antiapoptotic proteins expression and downregulating pro-apoptotic proteins to prevent cell death caused by diabetes-induced oxidative damage.

Various inorganic NP have been studied to be used in detection or monitoring DM for their physicochemical, electrical and optical properties. AuNP were used in a chip that can detect T1DM autoantibodies. A unique inexpensive and sensitive test that can be used for screening populations to detect early or late-onset T1DM, and it is capable of differentiating between type 2 and type 1 DM. Moreover, AuNP were used an organic volatile gas sensor to detect acetone in exhaled breath of diabetics. Since acetone is produced and excreted through respiration in diabetic ketoacidosis, a very dangerous complication of DM, the gas sensor is able to detect trace amounts of acetone, thus can diagnose DM and serve as a monitor to detect metabolic acidosis and prevent dangerous outcome. Furthermore, iron oxide NP proved to be efficient in targeting beta cells for specific islet beta cells MR imaging when labeled with exendin-4, a protein that has high affinity to specific beta cell receptor. It is used in diagnostic as well as monitoring purposes. Another monitoring device that utilizes AuNP is used to count WBC that has been found to be associated with diabetic complications, specifically heart disease.

Table 1 - Nanoparticles formulations and their pharmacological effect on blood glucose levels in in vivo or in vitro. BGL, blood glucose level; I/V, intravenous injection; NP, nanoparticles; PEG, polyethylene glycol; S/C, subcutaneous injection. (Continued)

Nanoparticle composition	Preparation Method/ Chemical Composition	Size	Study Model	Study Type	Route of administration	Dose/Conc	BGL reduction (of baseline value)	Max Decrease	Duration of BG reduction	Reference
Gold-insulin NP	HAuCl ₄ + NaBH ₄	/	Diabetic, Wistar rats	<i>in vivo</i>	oral	50 IU/kg	19%	After 3h	/	Joshi et al., 2006
Gold-insulin NP	HAuCl ₄ + NaBH ₅	/	Diabetic, Wistar rats	<i>in vivo</i>	Intranasal	50 IU/kg	31%	After 3h	/	Joshi et al., 2006
Gold-Aspartate-insulin NP	Gold NP (HAuCl ₄ + NaBH ₄) + Aspartic Acid	/	Diabetic, Wistar rats	<i>in vivo</i>	Oral	20 IU/kg	50%	After 3h	/	Joshi et al., 2006
Gold-Aspartate-insulin NP	Gold NP (HAuCl ₄ + NaBH ₄) + Aspartic Acid	/	Diabetic, Wistar rats	<i>in vivo</i>	Intranasal	20 IU/kg	55%	After 2	/	Joshi et al., 2006
Gold-insulin NP	HAuCl ₄ (citrate-reduced) + PEG	20 nm	Normal, Balb/c male mice	<i>In vivo</i>	I/V	30mg/ml =1.12 IU	20%	2h	BGL increased after 2h	Shilo et al., 2015
Gold-insulin NP	HAuCl ₄ (citrate-reduced) + PEG	50 nm	Normal, Balb/c male mice	<i>In vivo</i>	I/V	30mg/ml =1.12 IU	10% (90% reduction)	1-2h	6h	Shilo et al., 2015
Gold-insulin NP	HAuCl ₄ (citrate-reduced) + PEG	50 nm	Normal, Balb/c male mice	<i>In vivo</i>	I/V	7.5 mg/ml =0.28 IU	20%	1h	1h	Shilo et al., 2015
Gold-insulin NP	HAuCl ₄ (citrate-reduced) + PEG	50 nm	Normal, Balb/c male mice	<i>In vivo</i>	I/V	1.875 mg/ml =0.07 IU	57%	1h	1h	Shilo et al., 2015
Gold-insulin NP	HAuCl ₄ (citrate-reduced) + PEG	50 nm	Diabetic, Balb/c male mice	<i>In vivo</i>	I/V	30mg/ml =1.12 IU	20%	2h	6h	Shilo et al., 2015
Gold-insulin NP	HAuCl ₄ (citrate-reduced) + PEG	50 nm	Diabetic, Balb/c male mice	<i>In vivo</i>	S/C	30mg/ml =1.12 IU	15%	2h	6h	Shilo et al., 2015
Gold-Insulin NP	HAuCl ₄ (citrate-reduced) + PEG	70 nm	Normal, Balb/c male mice	<i>In vivo</i>	I/V	30mg/ml =1.12 IU	10%	1h	4h	Shilo et al., 2015

Nanoparticle composition	Preparation Method/ Chemical Composition	Size	Study Model	Study Type	Route of administration	Dose/Conc	BGL reduction (of baseline)	Max Decrease	Duration of BG reduction	Reference
Gold-insulin NP	AuNP + mPEG + sucrose esters of F.A	/	Diabetic, Sea:ddY, male mice	<i>in vivo</i>	Transdermal	3.7 mg/ml	10%	10h	10h	Nose et al., 2012
Gold-insulin NP	HAuCl ₄ + CH ₃ COOH + Chitosan	/	Diabetic, Wistar, male rats	<i>in vivo</i>	Oral	50 IU/kg	31.41%	2h	/	Bhumkar et al., 2007
Gold-insulin NP	HAuCl ₄ + CH ₃ COOH + Chitosan	/	Diabetic, Wistar, male rats	<i>in vivo</i>	Intranasal	10 IU/kg	34.12%	3h	/	Bhumkar et al., 2007
Gold-insulin NP	HAuCl ₄ + Chondroitin sulfate	123 nm	Diabetic, Sprague-Dawley males	<i>in vivo</i>	Oral	50 IU/kg	32.10%	1h	/	Cho et al., 2014
Gold NP (no insulin)	HAuCl ₄	50nm	Diabetic, male albino mice	<i>in vivo</i>	I/P	2.5 mg/kg	Reduced from 230 to 110	1.5 h	/	Barathmanikant h et al., 2010
Selenium NP (no insulin)	Reduction of sodium selenite by glutathione	10 - 80 nm	Diabetic, Wistar, albino, malr rats	<i>in vivo</i>	Oral	0.1 mg/kg	Reduced from 300 mg/dl to 120mg/dl	21d	28d	Al-Quraishy et al., 2015
Selenium NP + insulin	Reduction of sodium selenite by glutathione	11 - 80 nm	Diabetic, Wistar, albino, malr rats	<i>in vivo</i>	Oral SeNP S/C insulin	SeNP: 0.1 mg/kg Ins: 6U/kg	Reduced from 300 mg/dl to 100mg/dl	28d	28d	Al-Quraishy et al., 2015
Zinc Oxide NP	/	/	Diabetic, Sprague-Dawley males	<i>in vivo</i>	Oral	10 mg/kg daily	Reduced from 505 mg/dl to 122.2 mg/dl	/	/	Alkaladi et al., 2014
Silver NP	/	/	Diabetic, Sprague-Dawley males	<i>in vivo</i>	Oral	10 mg/kg daily	Reduced from 505 mg/dl to 160.6 mg/dl	/	/	Alkaladi et al., 2014
Calcium Phosphate-INS NP	CaPO ₄ NP (microemulsion method) + Chitosan + Vit. B12	<250nm	Diabetic, male Wistar rats	<i>in vivo</i>	Oral	/	Reduced from 350 to 120	6h	6h	A. Verma et al., 2015
Iron Oxide-INS NP	Laser ablation of ultrapure iron slug		Diabetic, female Wistar rats	<i>in vivo</i>	Oral	/	51%	/	/	Kebede et al., 2013

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