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Inference of Nanotechnology in modern Medicine - The Example of Diabetes Therapy

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RESUMEN

Nanotecnología ha encontrado numerosas aplicaciones, incluso en el área de la medicina moderna. El concepto novedoso aquí presentado acerca de mediciones de monitoreo básico de glucosa permite una descripción integrada de glicemia en pacientes con diabetes dentro de un intervalo de tiempo dado, y adicionalmente incluye factores independientes para juzgar el control metabólico.

SUMMARY

Nanotechnology has already found entry in a variety of applications, including in modern medicine. The novel concept presented here on the basis of glucose monitoring measurements provides for an integrated description of glycemia in patients with diabetes over a given time interval, while it also includes independent factors for assessing metabolic control.

Nanotechnology in medicine

Nanotechnology is a very complex field of research. It is the technology which creates structures or objects in dimensions below 100 nanometres. This technology has already found entry in a variety of applications, including in modern medicine. Here nanosensors are used primarily in medical diagnosis and therapy. Because such sensors are very small in size they can be implanted directly into the organism for measurement purpose or as a therapeutical tool [1]. As examples we cite

- nanosensors with functional nanoparticles as detector reagents in bio analytical tests (biochips), for example nanoarrays for chemical, pharmaceutical, biochemical and genetic tests
- into the organism implanted nanosensors in support of physiological reactions and / or for therapy
    - sensors for continuous monitoring: such as measurement of glucose, lactate, urea, fatty acids, brain activity and autonomous functions,
    - sensors to control physiological processes in the organism, e.g. glucose sensors to control insulin pumps for type 1 diabetes patients,
    - nanosensors in cancer diagnostics which can identify specific proteins associated with a specific type of cancer.

An example of challenge in modern medicine: The Diabetes epidemiology

The number of people with diabetes worldwide is set to double in the next 20 years, as a result of increasing obesity and longevity [2]. While some of this increase will be observed in Europe and North America, it is clear that the bulk of the epidemic will be observed in non-European origin populations, in countries undergoing rapid westernization. If anything, the European origin populations are the anomaly, being substantially protected from type 2 diabetes compared to other world populations. This is reflected in our current understanding of the epidemiology of diabetes, derived mainly from the study of non-European populations, such as the Pima Indians and Naruans. But it is clear that diabetes risks, manifestations, natural history and even the criteria for the definition of diabetes itself, may vary considerably by population.

Not only the incidence of type 2 diabetes grows, also the incidence of type 1 diabetes has been steadily increasing worldwide since the middle of the 20th century [2]. The increase has so far been linear and predictable. A changing environment, infant and maternal diets in particular, would seem to be the most likely explanation for this alarming acceleration. It is on hand that the disease diabetes mellitus is the focus of interest of the health authorities in different countries. Following the disease is in the focus of interest of medical research for the development of new therapeutical methods and technological aids (e.g. insulin delivery devices, devices for blood sugar measurements).

Diagnostic in the diabetes therapy under daily life conditions

Self-monitoring blood glucose and measuring HbA_{1c} are the established methods of assessing glycemic control of insulin treated patients with diabetes. For these patients the primary reason for self-monitoring blood glucose levels typically 4 to 6 times a day is adaptation of their insulin dose to their food intake and levels of physical activity, and to correct for non-physiological glycemic excursions. However, the spot blood glucose measurements show only a small part of glucose history (Fig. 1).

Diabetologists are particularly interested in the HbA_{1c} value, which helps them to assess the average metabolic quality over several weeks. Glycosylated hemoglobin (HbA_{1c}, or A1C) is a form of hemoglobin used primarily to identify the average plasma glucose concentration over prolonged periods. It is formed in a non-enzymatic pathway by hemoglobin's normal exposure to high plasma levels of glucose. Glycosylation of hemoglobin has been implicated in nephropathy and retinopathy in diabetes mellitus. Monitoring the Hb_{A1c} in patients with diabetes may improve treatment [3]. In the normal 120-day life span of the red blood cell, glucose molecules react with hemoglobin, forming glycated hemoglobin. In individuals with poorly controlled diabetes, the quantities of these glycated hemoglobins are much higher than in healthy people. Once a hemoglobin molecule is glycated, it remains that way. A buildup of glycated hemoglobin within the red cell therefore reflects the average level of glucose to which the cell has been exposed during its life cycle. Measuring glycated hemoglobin assesses the effectiveness of therapy by monitoring long-term glucose regulation.

The HbA_{1c} value has an established position because of the documented correlation between the degree of protein glycosylation and the risk of developing diabetes related complications; a relationship that has been demonstrated in both groups of patients with diabetes in large clinical studies such as DCCT / EDIC and UKPDS [4-6]. This correlation is the main reason why the HbA_{1c} measurement has gained widespread acceptance as the main target parameter in practically all national and international diabetes treatment guidelines to judge the quality of metabolic control. However, it does not describe acute fluctuations in blood and tissue glucose levels (i.e., glycemic variability), due to the fact that glycosylated hemoglobin is present only in its labile aldimine state for the first 6 hours after formation. The stable ketoamine form only arises afterwards.

Diagnostic by using continuous glucose monitoring

In contrast to the self-monitoring blood glucose is the continuous measurement of glucose still not established. Continuous glucose monitoring provides information about the direction, magnitude, duration, frequency, and causes of fluctuations in blood glucose levels. Continuous monitoring provides much greater insight into glucose levels throughout the day. Continuous glucose readings that supply trend information can help identify and prevent unwanted periods of hypo- and hyperglycemia.

Several approaches to the continuous glucose monitoring are possible:

- non-invasive methods,.
- implanted sensors in the vascular system or subcutaneous tissue
- minimally invasive sensors (self insertion by patients in the subcutaneous tissue,
- delivery of interstitial liquid from the tissue to the glucose sensor by using of the micro-dialysis technique.

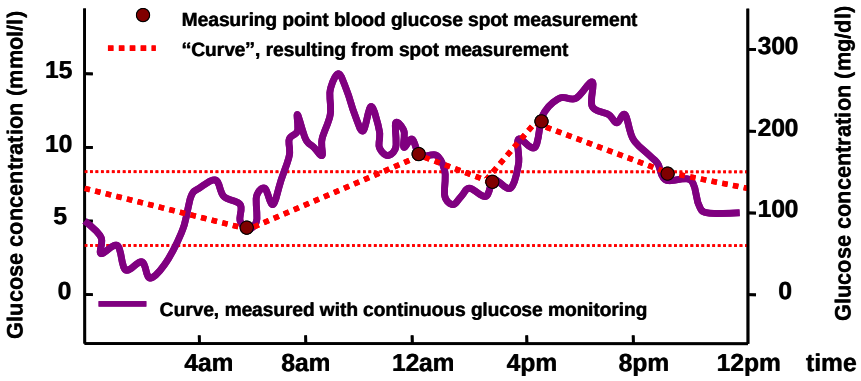

Fig 1: Comparison between conventional blood sugar spot measurements (intimated dotted line) and continuous glucose measurement (solid line). The discrepancy between both methods of glucose measurement is considerable.

Non-invasive methods for glucose monitoring require the nano technology. For example is the glucose detection possible by using nanotube-based optical sensors (7). Whose optical properties of commonly used organic and nanoparticle fluorescent probes are depending of quantum yield, human tissue penetration, and photobleaching stability. Single walled carbon nanotubes are cylindrical molecules based on graphene where the nanometer scale radius serves to quantum confine electrons, imparting the material with new and unique properties. A select number of carbon nanotubes fluoresce in the near infrared where human tissue penetration is maximum and biological autofluorescence is minimal. They are also infinitely photo-stable and are therefore one of very few fluorphores that are viable as long term optical biosensors.

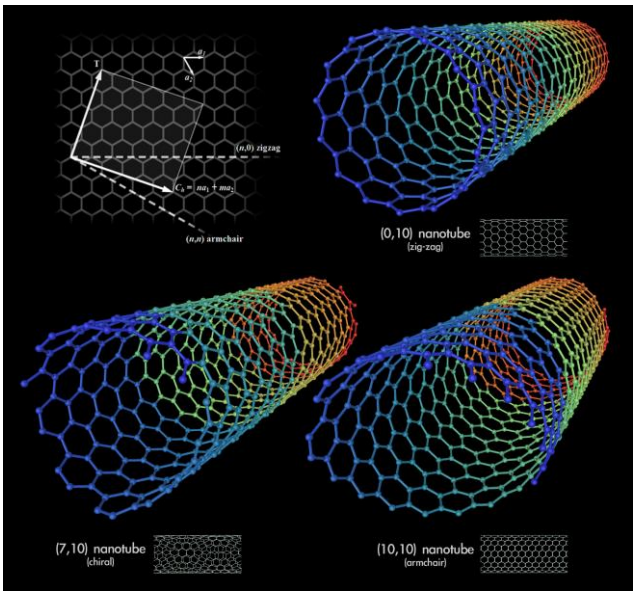

Fig. 2: Optical sensors for measurement of the glucose level in patients with diabetes have a nano-tube structure like that shown in the image. This technology is in development [7]

The currently available CGM sensors measure the glucose level minimal invasiveness through continuous measurement of interstitial fluid. The handling for bringing a sensor into contact with ISF include inserting an indwelling sensor subcutaneously (into the abdominal wall or arm) to measure ISF in situ or harvesting this fluid by various mechanisms that compromise the skin barrier and delivering the fluid to an external sensor [8]. After a warm-up period of up to 2 h and a device-specific calibration process, each device's sensor will provide a glucose reading every 5 min for up to 72 h. The data`s are transferred to a small monitor.

Fig. 3: Guardian®REAL-Time (Medtronic) for continuous Glucose monitoring. The small sensor measures the glucose in the interstitial tissue (enzymatic reaction by using glucose oxidase). The data's transferred with a transmitter to the monitor (radio transmission).

Impact of continuous glucose monitoring of diabetes diagnostic and therapy

HbA_{1c} values as "gold standard" for glycemic control does not describe acute fluctuations in glucose levels, but the glycemic variability is an important factor for diabetes prognoses. The clinical significance of such glycemic variations has more become of concern when it becomes clear that there is a correlation between swings in postprandial hyperglycemia and cardiovascular disorders, which was demonstrated as early as the 1990s in several studies [9-11]. The Kumamoto Study which was also performed during this time period, shows that increased postprandial glucose excursions are associated with microvascular complications in patients with type 2 diabetes [12,23]. *In-vitro* studies performed on cells in which fluctuating glucose levels produce the greatest degree of oxidative stress, along with the highest rate of apoptosis, underscore the significance of glycemic variability [14,15]. There are also indications that non-physiologically high postprandial excursions in patients with type 2 diabetes are at the center of a cascade of diabetogenic and atherogenic events such as increased insulin resistance, postprandial dyslipidemia, increased oxidative stress, a deranged coagulation equilibrium, endothelial dysfunction, etc. [16-18. This problem is also relevant to patients with type 1 diabetes [19-21.

Only continuous glucose monitoring (CGM) allows the characterization of the glycemic profile of patients with diabetes in detail over the course of at least a few days. The software integrated in the CGM systems calculates a variety of different summary measures from the recorded glucose profiles in order to characterize these profiles. CGM can determine all important parameters describing the glycemic variability. This is not possible by measurement of the HbA_{1c} value and the spot blood sugar measurement alone. In result of this, it change the perspective on the glycemic regulation and hence on diabetes therapy.
This includes the creation of a new model about glucose control. One possibility is the "Glucose pentagon" [22].

The model includes values of acute and long-term glycemia. To this end, the following parameters are calculated from glucose profiles. Each parameter forms a single axis of a five-sided figure, i.e., the "glucose pentagon" (Fig. 4):

- The mean glucose concentration
- The standard deviation of the mean glucose concentration
- The amount of time per day in which hyperglycemic values (>160 mg/dL = 8.9 mmol/L) were recorded.
- The area below the curve of hyperglycemic values (>160 mg/dL = 8.9 mmol/L)
- The HbA_{1c} value.

Taken together, the selected parameters provide an integrated description of glycemia over the period of time under observation.

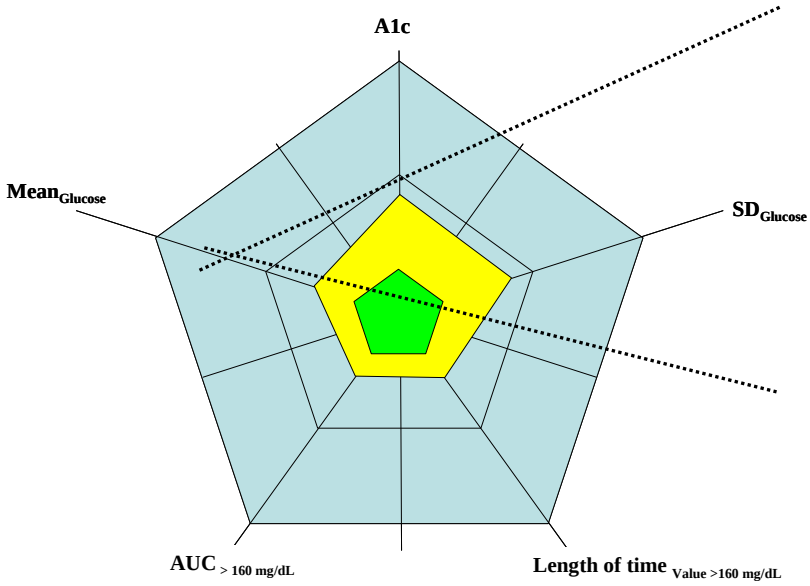

Fig. 4: The "glucose pentagon" illustrates five glycemic parameters on five axes. Connecting these points yields an enclosed area (left). For a patient with diabetes, these parameters result in a pentagon (yellow) that describes glycemia during one day. The green area (left and right) shows the same parameters for a person with healthy metabolism.

The values taken into consideration here cover a certain surface area that is easy to calculate and that can be viewed as an independent, integrated parameter for describing glycaemia in a given patient. A meaningful way of obtaining a dimensionless value is to normalize this area using the following values calculated from CGM profiles of healthy subjects (the resulting area is shown in green color in figure 4 [23,24]):

- Mean glucose concentration: 90 mg/dL (5 mmol/L)
- Standard deviation of the mean glucose concentration: ±10 mg/dL (0.55 mmol/L)
- Time per day >160 mg/dL (8.9 mmol/L): 0 min
- AUC >160 mg/dL (8.9 mmol/L): 0 mg/dL x day

and

- HbA_{1c} value: 5.5%.

The area calculated for the glucose pentagon of a patient with diabetes, divided by the standard area of healthy subjects, should provide a more meaningful overall description of metabolic control and also an assessment of a patient's risk of developing diabetic complications than it is possible with the HbA_{1c} value. The reason for this is that the pentagon also incorporates parameters providing information on glycemic variability. This is not the case with HbA_{1c} alone.
Taking the area within the glucose pentagon of a given patient with diabetes and normalizing it to the standard area of healthy subject's yields a non-dimensional characteristic value defined as the glycemic risk parameter (GRP):

$$GRP = \frac{\text{area within the glucose pentagon of a patient with diabetes}}{\text{area within the glucose pentagon for healthy subjects}}$$

This parameter allows a rapid assessment of a patient's metabolic control while taking significantly more factors into consideration than by looking solely at the HbA_{1c}.

The following examples use data from patients with type 1 diabetes and are intended to illustrate how the glucose pentagon can be used in practice. An HbA_{1c} of 7.5% had recently been measured for a 49-year-old female patient who suffers from diabetes since 40 years and is treated with insulin pump therapy combined with a rapid-acting analog insulin. Blood pressure and lipid parameters were well regulated with a beta-blocker, an ACE inhibitor, and a statin. Known diabetes related complications include retinopathy, nephropathy, peripheral neuropathy and peripheral arterial occlusive disease in stage 2. Fig. 5 shows the CGM profile over the course of 3 days and the corresponding glucose pentagon.
The data used for calculating the GRPs for this patient:

	Day 1	Day 2	Day 3
HbA_{1c} (%)	7.5	7.5	7.5
$MEAN_{glucose}$ (mg/dL)	191	188	278
$SD_{glucose}$ (mg/dL)	44	34	57
$AUC_{>160\ mg/dL}$ (mg/dL x day)	39	32	118
$Time/day_{>160\ mg/dL}$ (min)	1155	1230	1325
GRP	3.30	2.87	7.38

The average GRP of these three days is 4.52, which (according to the assumptions outlined above) indicates an increased risk to develop of diabetes related complications. One suspects that these values are typical for the patient, as indicated by the existing diabetes related complications. The pattern in the glucose pentagons from these three days indicate high glucose variability on all days (= high standard deviation of the mean glucose concentration). Another characteristic is that the mean glucose concentration does not fit to the most recently measured HbA_{1c}. If this holds true over a longer period of time, one might anticipate that the subsequent HbA_{1c} numbers will increase.

Discussion

The novel concept presented here on basic of glucose monitoring measurements provides for an integrated description of glycemia in patients with diabetes over a given time interval, while it also includes independent factors for assessing metabolic control. The time interval can be even just a single day, which is also a useful feature.

Determination of the GRP for each individual day of a given patient would allow the patient (and his treating physician) to assess his success to optimize metabolic control on a day-to-day basis. Subsequently, a mean GRP value can be calculated over longer periods of time. One advantage of the concept presented here is that it takes both long-term and acute metabolic control into account, i.e. it combines HbA_{1c} and glycemic fluctuations into a single model. Because it yields a characteristic numerical value, the GRP serves as a good starting point for assessing the risk of developing

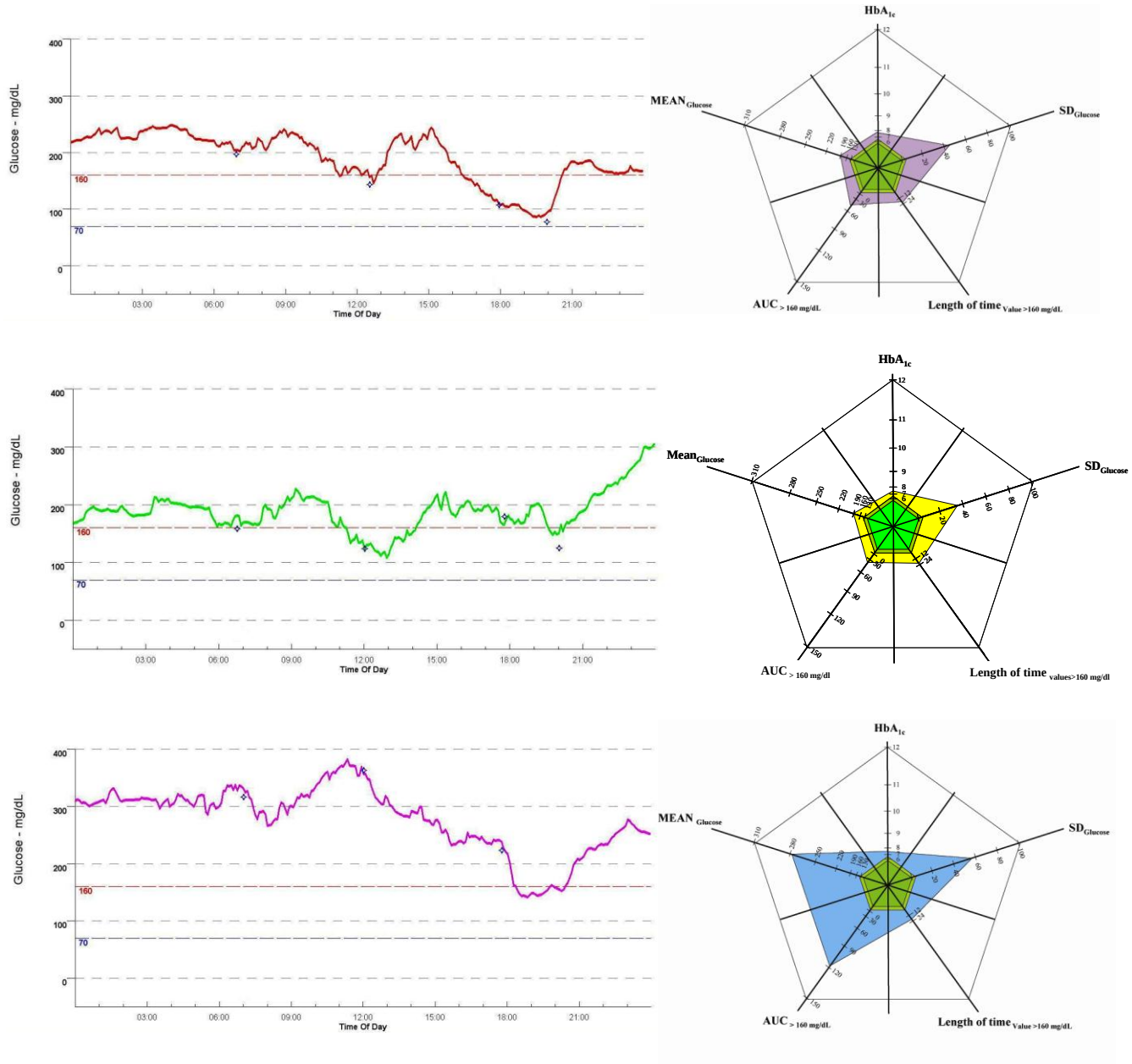

Fig. 5: Examples for CGM glucose profiles over three days of a given patient and the corresponding glucose pentagons.

diabetes related complications and presumably provides more prognostic value/information than the HbA_{1c} on its own.

The deploying of sensors for the continuous glucose monitoring would be impossible without nanotechnology. The currently available systems and devices are at the beginning of development. In the future more miniaturized glucose sensors will appear on the basis of nano- technology. In this way, new knowledge can be achieved for diabetes therapy.

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