White Paper

Continuous Glucose Measurement by Means of SENCELL Osmotic Pressure Sensor

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The purpose of this document is to provide an explanation about the mode of operation of the **SENCELL** Continuous Glucose Measurement Sensor, which is targeting a broader public audience.

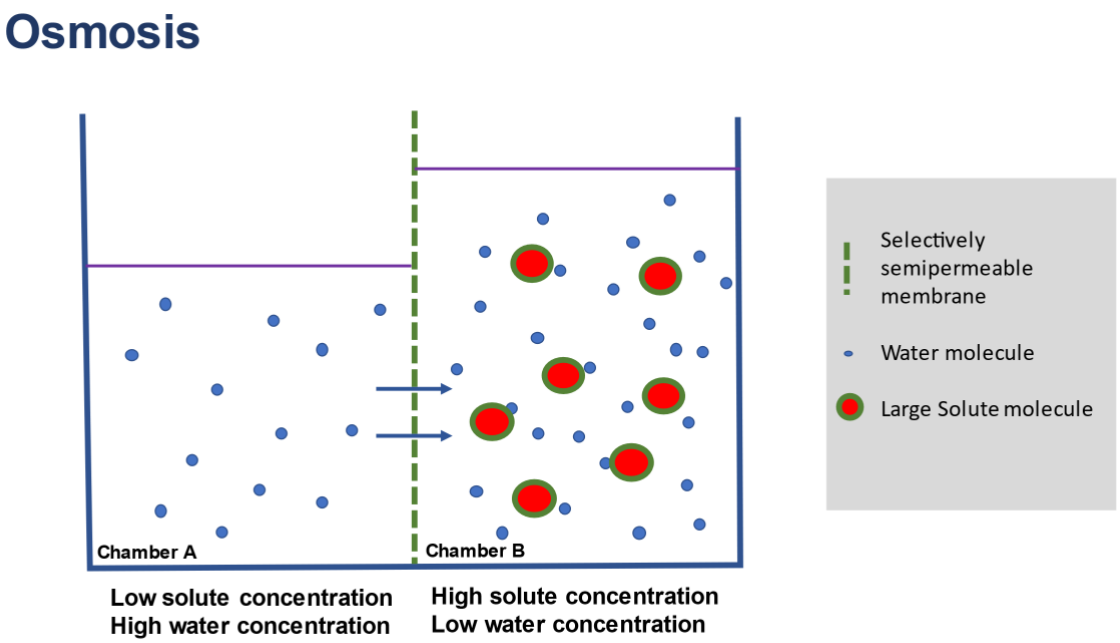
The text in the „Science Boxes“ in this document is intended for readers seeking a more advanced technical and scientific understanding.

Introduction – What is osmotic pressure?

Osmosis is defined as the movement of water/solvent from an area of low concentration of particles in solution (solute) to an area of higher concentration of particles, e.g., through a semipermeable membrane until an equilibrium is established. If some of the particles on one side cannot cross the semipermeable membrane, this means that the volume of the solution in the area of the previously higher concentration will become larger until the overall particle concentrations are equal on both sides.

Osmotic pressure is the pressure created by water moving across a membrane due to osmosis. The more water moving across the membrane, the higher the osmotic pressure. This situation is shown for an idealized situation in Figure 1.

Figure 1. Osmosis-induced movement of water through a semi-permeable membrane



In the human body, osmotic pressure is the pressure caused by water at different concentrations due to the dilution of water by dissolved molecules (solute), notably salts and nutrients. Biological cells use osmosis as a means to keep their intracellular volume constant and ensure proper functionality of the encapsulating outer cell membrane. Osmotic pressure is hence particularly important for the fluid transport and fluid balance of animal and plant cells.

The osmotic concentration (Cosm) indicates the mass concentration of the osmotically active particles of a solution. Size or type of particles do not play a role for the osmotic pressure, since it is not a chemical but a physical phenomenon. Only the number of particles (dissolved atoms and ions, but also other molecules, such as sugar, proteins, or ethanol) is decisive, Osmolarity indicates the number of osmotically active particles per liter of solution or test material. In medical analysis, osm/L or osmol/L is used as the unit of osmolarity, for lower concentrations; the notation osmol/l or mOsmol/l is also common. Each molecule is osmotically active, but if two osmotically active molecules are combined to one molecule, their osmotic values changes from 2 to 1. For example, dextrane is a molecule consisting of several glucose molecule bound to each other. One dextrane molecule causes just as much osmotic pressure as one isolated glucose molecule. It does not matter how many glucose monomers the dextrane consists of.

Science Box 1.: Osmotic pressure is independent from volume!

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Osmotic pressure can be determined using the following formula:

$$\pi = i \cdot M \cdot R \cdot T$$

Where:
π = osmotic pressure
i = van't Hoff's factor
M = molar concentration of the particles in solution
R = ideal gas constant
T = Temperature in Kelvin (K)

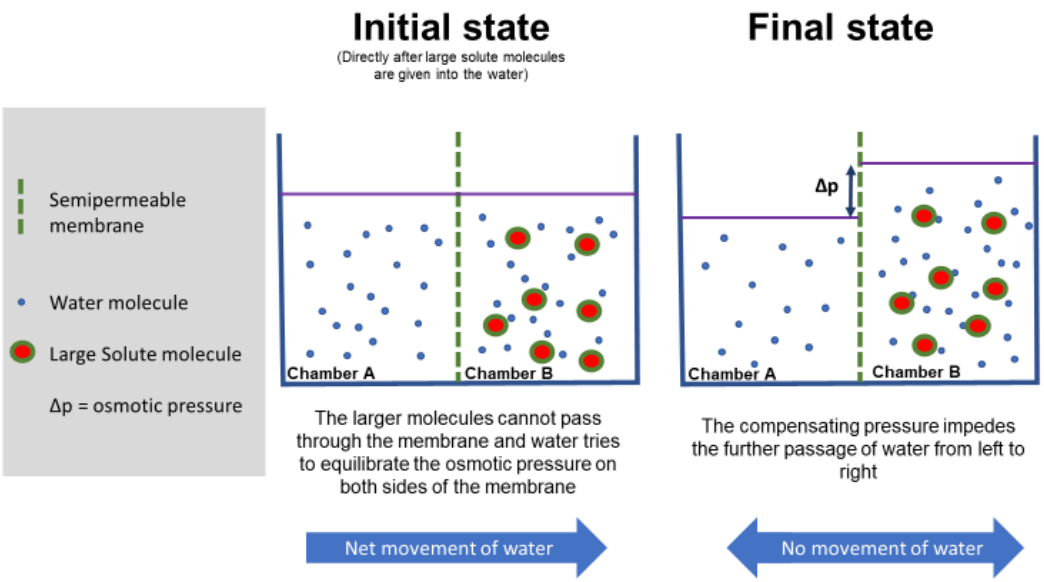
i/van't Hoff's factor: a factor describing the amount of particles occurring when one molecule of the solid substance is dissolved in water, e.g. i = 1 for glucose or i = 2 for NaCl (as it dissociates into Na+ and Cl- in water)

R/ideal gas constant = 0.08206 L x atm /mol x K

T = temperature in °C + 273.15 (30 °C = 303.15 K)

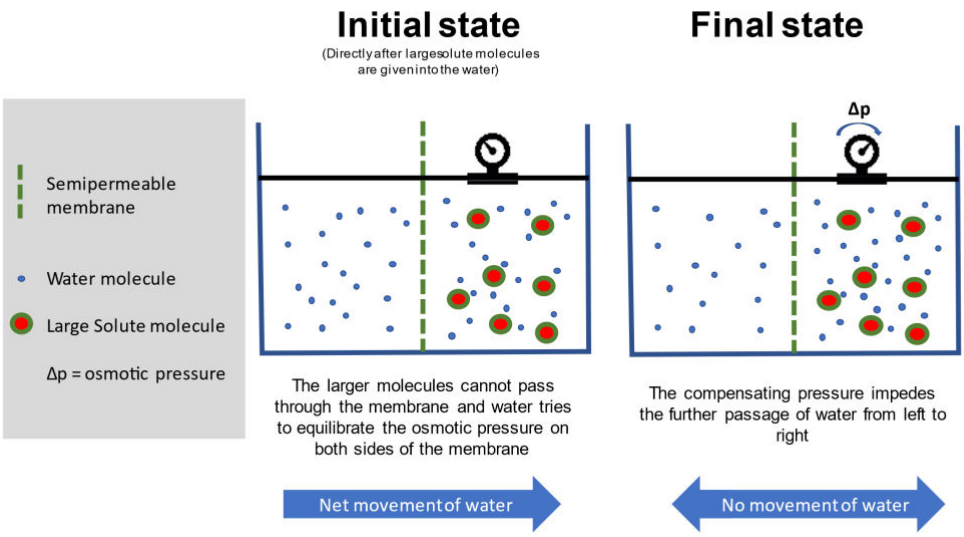
Osmolarity can be determined via the pressure difference between two chambers separated by a semipermeable membrane. One chamber is filled with a defined reference solution, the other with the solution to be investigated. Since the particles cannot penetrate the membrane, the solvent must diffuse into the chamber of higher concentration until the resulting hydrostatic pressure equalizes the osmotic pressure. The increased liquid level can be easily measured as shown in Figure 2.

Figure 2.: Experimental visualization of osmotic pressure in a standard experiment



If the system used for investigating osmotic pressure is not an open system as shown in Figure 2, but a completely closed and locked in system, then a change in osmotic pressure can only be visualized by employing a pressure sensor, because the level of the volume of the liquid in chamber B cannot expand anymore. This situation is shown in Figure 3.

Figure 3.: Osmotic pressure can be visualized with a pressure sensor in a closed chamber system.

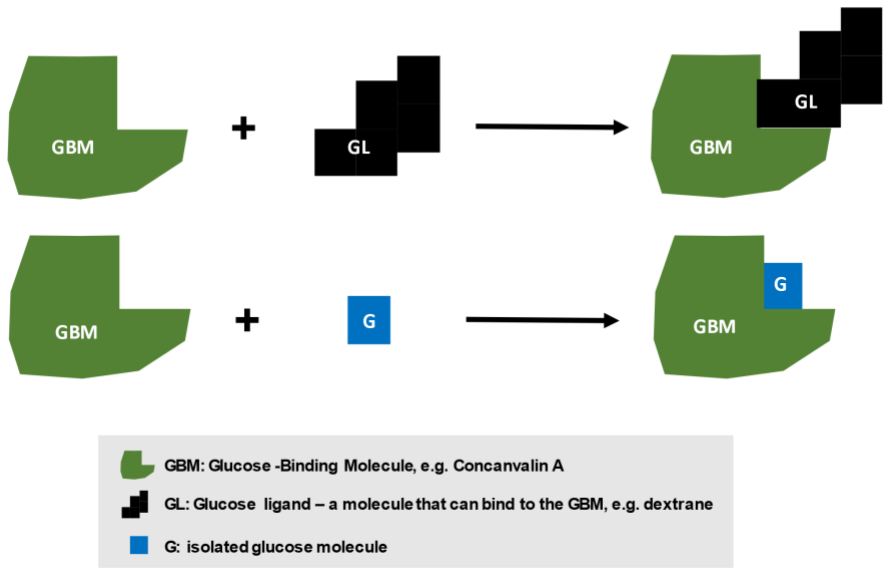


Any reaction that changes the amount of osmotically active larger particles within such a closed chamber system will hence result in changes in the osmotic pressure. This concept is used to measure glucose in an osmotic pressure chamber.

Use of SENCELL Osmotic Pressure Cell for Glucose Measurement

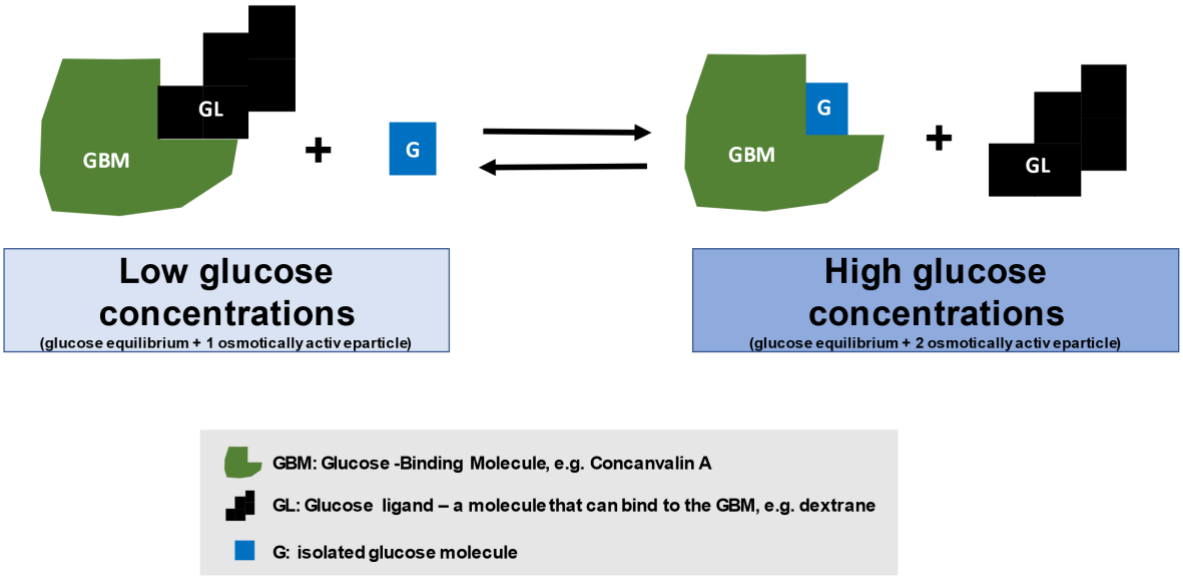
The glucose concentration of an analyte medium, e.g. in the inerstitial fluid, can be determined by measuring the osmotic pressure of a sensitive liquid, which is „captured“ by a transpermeable membrane within an otherwise closed chamber. „Sensitive liquid“ means that the diffusion of glucose into the chamber through the semipermeable membrane leads to a change in the number of osmotically active particles. The sensitive liquid of the SENCELL device consists of two major components, which are both dissolved in an aqueous solution with physiological ph inside of the sensor chamber: Concavalin A – a plant lectin with glucose binding sites (also referred to as GBM, glucose binding molecule) and dextrane, a macromolecule consisting of several glucose molecules (also referred as GL, glucose ligand). In a glucose-free solution, the GBM and the GL form a complex because of electrostatic binding of the GL to the GBM at a glucose-specific binding site. If glucose is present in the solution, it may also bind to the GBM. These two possible affinity binding reactions are shown in Figure 4.

Figure 4.: Possible affinity binding reactions in the sensitive liquid of Sencell



Based on the physico-chemical properties of the electrostatic binding conditions, the tendency of the GBM to bind glucose is slightly higher than the tendency to bind the GL. The differences in the binding forces are so subtle that the amount of GBM-glucose complexes is dependent on the glucose concentration in the liquid. The higher the glucose concentration, the more GBM-GL complexes will be separated and will be replaced by GBM-Glucose complexes. In essence, there is a competition between glucose and the GL for the GBM binding site, which is mainly dependent on the glucose concentration in the liquid as shown in Figure 5.

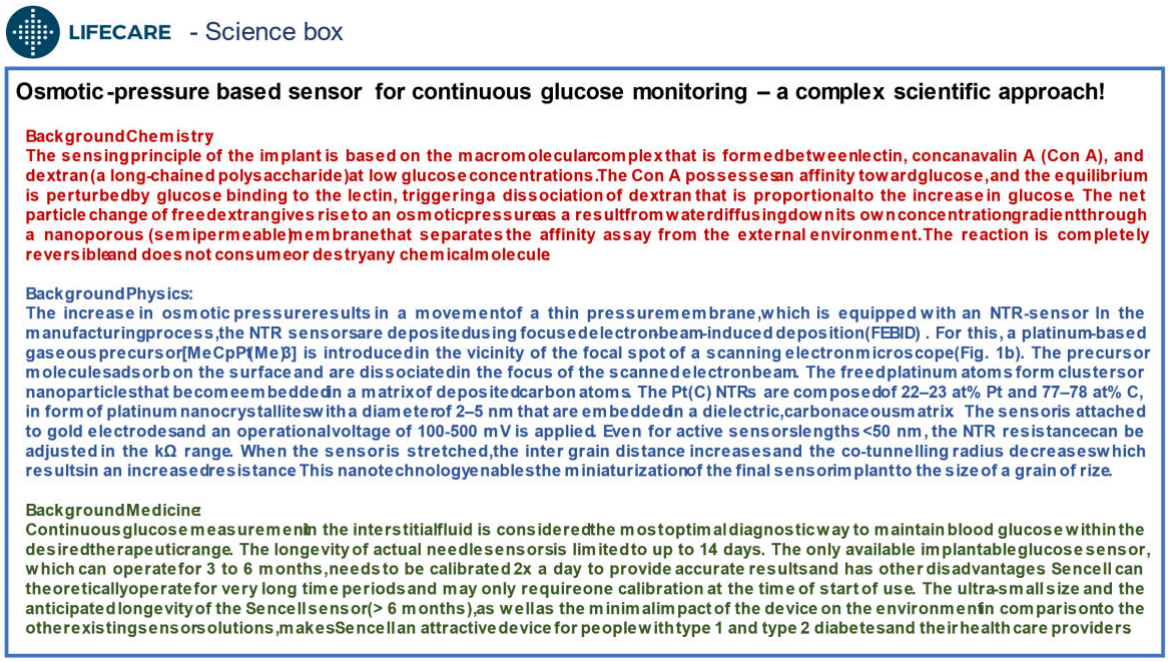
Figure 5.: Glucose concentration-dependent complex formation in the sensitive liquid inside the Sencell osmotic-pressure chamber.



As indicated in the graph, each GBM-glucose complex formation leads to an increase in the amount of osmotically active particles within the sensor chamber. When placed into the subcutaneous tissue this represents an entirely closed chamber system with the surrounding tissue liquid representing chamber A and the sensor chamber representing chamber B in the idealized Figure 3. In consequence, glucose-concentration dependent formation of GBM-glucose complexes leads to an increase in the osmotic pressure inside the sensor chamber. If the osmotic pressure is

measured with an appropriate pressure transducer, which translates the pressure into an electronic signal, an increase in the glucose concentration in the interstitial fluid will result in an appropriate change of the electronic sensor signal. It is important to note that the affinity binding reactions are both completely reversible, and that none of the participating chemical molecules is altered or destroyed during these reactions. This forms the scientific basis for a possible ultra-long and extensive usage time of a sensor employing this technology.

Science Box 2.: The SENCELL development is a complex interdiciplinary undertaken



LIFECARE - Science box

Osmotic-pressure based sensor for continuous glucose monitoring – a complex scientific approach!

Background Chemistry
The sensing principle of the implant is based on the macromolecular complex that is formed between lectin, concanavalin A (Con A), and dextran (a long-chained polysaccharide) at low glucose concentrations. The Con A possesses an affinity toward glucose, and the equilibrium is perturbed by glucose binding to the lectin, triggering a dissociation of dextran that is proportional to the increase in glucose. The net particle change of free dextran gives rise to an osmotic pressure as a result from water diffusing down its own concentration gradient through a nanoporous (semipermeable) membrane that separates the affinity assay from the external environment. The reaction is completely reversible and does not consume or destroy any chemical molecule

Background Physics:
The increase in osmotic pressure results in a movement of a thin pressure membrane, which is equipped with an NTR-sensor In the manufacturing process, the NTR sensors are deposited using focused electron beam-induced deposition (FEBID) . For this, a platinum-based gaseous precursor [MeCpPt(Me)3] is introduced in the vicinity of the focal spot of a scanning electron microscope (Fig. 1b). The precursor molecules adsorb on the surface and are dissociated in the focus of the scanned electron beam. The freed platinum atoms form clusters or nanoparticles that become embedded in a matrix of deposited carbon atoms. The Pt(C) NTRs are composed of 22–23 at% Pt and 77–78 at% C, in form of platinum nanocrystallites with a diameter of 2–5 nm that are embedded in a dielectric, carbonaceous matrix. The sensor is attached to gold electrodes and an operational voltage of 100-500 mV is applied. Even for active sensor slengths <50 nm, the NTR resistance can be adjusted in the kΩ range. When the sensor is stretched, the inter grain distance increases and the co-tunnelling radius decreases which results in an increased resistance This nanotechnology enables the miniaturization of the final sensor implant to the size of a grain of rize.

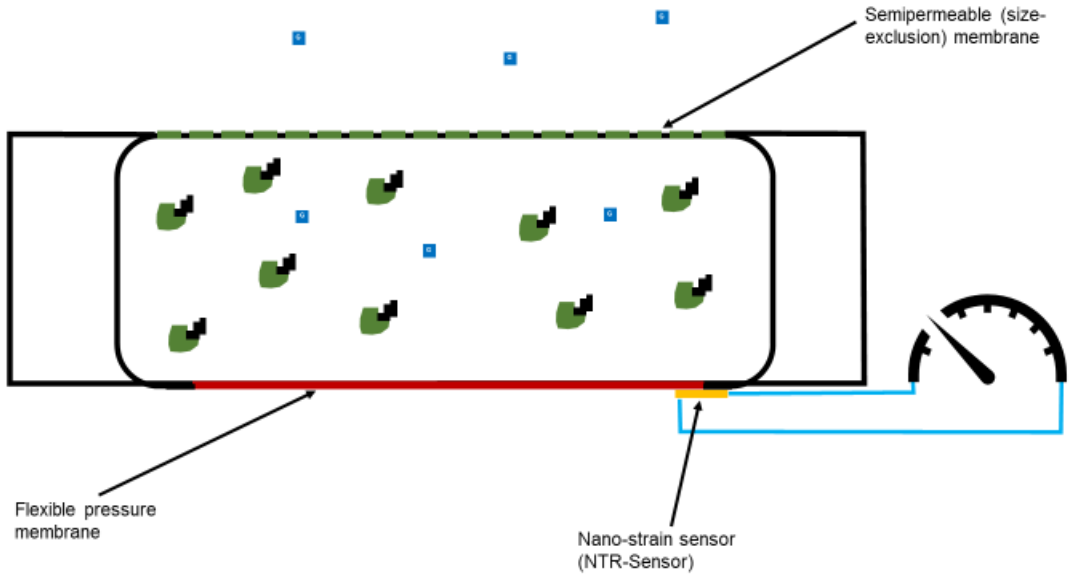
Background Medicine:
Continuous glucose measurement in the interstitial fluid is considered the most optimal diagnostic way to maintain blood glucose within the desired therapeutic range. The longevity of actual needle sensors is limited to up to 14 days. The only available implantable glucose sensor, which can operate for 3 to 6 months, needs to be calibrated 2x a day to provide accurate results and has other disadvantages Sencell can theoretically operate for very long time periods and may only require one calibration at the time of start of use. The ultra-small size and the anticipated longevity of the Sencell sensor (> 6 months), as well as the minimal impact of the device on the environment in comparison to the other existing sensor solutions, makes Sencell an attractive device for people with type 1 and type 2 diabetes and their health care providers

Mode of Operation of the SENCELL Device

As shown above, the osmotic pressure in the chamber depends strongly on the competitive affinity of glucose and a competing ligand to an affinity receptor molecule inside the chamber. The miniaturized SENCELL osmometric affinity sensor consists of a very sensitive pressure sensor based on a 3-D printing technology on the nanoscale (Nano3DSense) to determine the osmotic pressure and a chamber closed on one side by a semipermeable membrane to hold the sensitive fluid. The sensor is located at the basis of a second membrane, which moves with increasing or decreasing osmotic

pressure in the chamber. The semipermeable membrane on the other side is a ceramic size exclusion membrane with a mean pore diameter of 5 NM. Both the GBM and the GL have a size that Is more than ten times larger and cannot pass through the pores. In contrast, glucose and water molecules can freely flow between the compartments. A sketch of the set-up of the glucose sensor is shown in Figure 6.

Figure 6.: Sketch plot of the SENCELL glucose sensor at low glucose concentrations

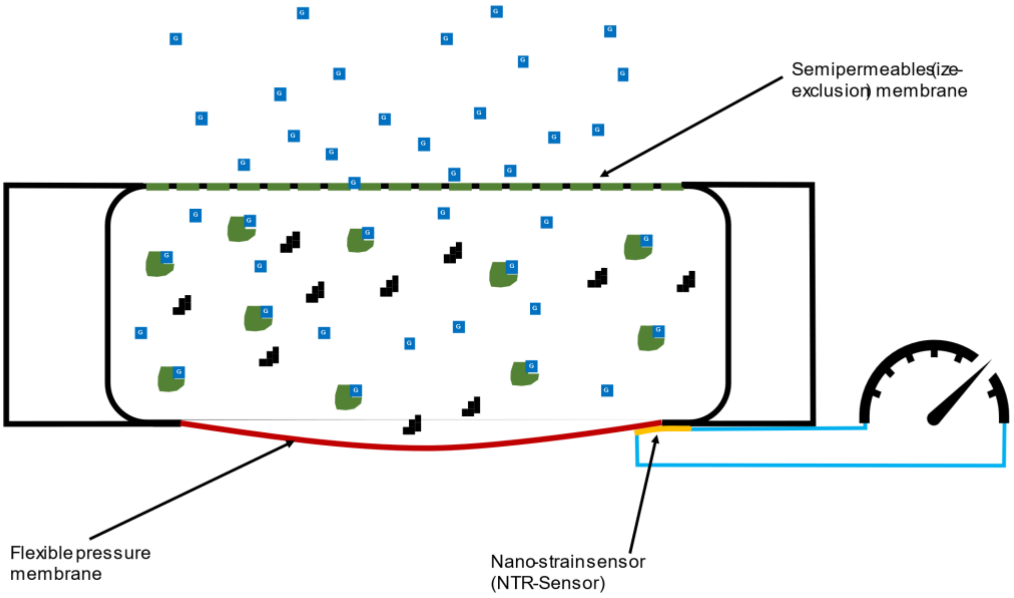


When the glucose concentration increases in the interstitial fluid, glucose molecules will be penetrating through the semipermeable membrane into the osmotic pressure chamber in the attempt to equilibrate the glucose concentration differences. This will happen until the glucose concentration in the inside is similar to the glucose concentration in the outside. Through the increasing number of glucose molecules inside the chamber competing with dextrane for the GBM binding site, dextrane will finally be forced out of the complexes, and glucose will take the binding places. This reaction increases the amount of osmotically active particles inside the chamber, which cannot pass the membrane. In consequence, water molecules will diffuse into the chamber in the attempt to equalize the difference in the concentration of the osmotically

active particles. This event increases the osmotic pressure inside the chamber and the pressure membrane will react with a corresponding movement.

A nanosensor is located at the basis where the pressure membrane is fixed. It is spanning over the fixed and movable part of the pressure membrane. Membrane movement induced by pressure changes will result in an electronic signal, which can be captured and displayed by means of appropriate electronic measurement equipment. This state is displayed in Figure 7.

Figure 7.: SENCELL sensor response to increasing glucose concentrations in the interstitial fluid



One of the key features of the SENCELL device is the combination of the osmotic pressure sensing technology, which is based on the completely reversible affinity binding competition between glucose and GL for the GBM binding site with a unique nanosensing technology, which enables a substantial miniaturization of the overall sensor design.

As shown in preclinical experiments, the SENCELL osmotic-pressure sensor developed by Lifecare AS has sufficient sensitivity and shows a linear signal response in the required pressure range for measuring of tissue glucose concentrations.