

Grade 1,3

Protocol for the experiment

**Diffusion Kinetics of Polymers Applied for Implantable
Devices**

Master Biomedical Technologies, Labwork Implantology

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1 Introduction

Glucose transport is an important component of the energy metabolism. Since cell membranes are impermeable to glucose, the transport of the molecule is carried out by transport proteins. A distinction is made between secondary active transport in the intestine and kidney, in which glucose transport is coupled to the transport of sodium ions, and facilitated transport in all other cells, for which proteins of the GLUT family are responsible. One representative of this family is the GLUT2 protein, which is predominantly involved in glucose homeostasis, including the sensing of glucose in pancreatic β -cells [1]. When the circulating glucose concentration increases, the transport of glucose into the β -cells triggers a cascade, at the end of which Ca^{2+} channels are activated and Ca^{2+} ions enter the cell. Due to the increase of positive charges, exocytosis of insulin secretory granules takes place, releasing insulin from the β -cell to initiate the uptake of glucose into cells [2]. If the insulin production is insufficient due to destruction of the β -cells, the disease is referred to as chronic type 1 diabetes mellitus. A promising treatment could be the transplantation of islets, in which new β -cells are introduced into the body in a minimally invasive way. However, since these foreign cells are often attacked by host immune cells, encapsulation of biomaterials in which the cells are embedded prior to implantation is necessary for protection. At the same time, the encapsulation must be permeable to insulin and glucose so that glucose can reach and enter the β -cell to trigger the production of insulin and that the insulin can be released into the body. In addition, the supply of oxygen, hormones, growth factors, and nutrients to the implanted cells must be ensured, which is why a suitable semipermeable membrane that allows the aforementioned substances to pass is necessary [3].

In this experiment, the diffusion of glucose and insulin through different semipermeable polymer membranes is investigated. The aim is to identify the membrane that is permeable to glucose and insulin and thus suitable for encapsulating implanted islets.

2 Methods

Prior to the laboratory work, the samples were prepared. For this purpose, a glucose and insulin standard solution with concentrations of 40 μM and 200 $\mu\text{U/ml}$ were added to the two chambers of reactors separated by polymer membranes. Subsequently, the experimental set-up was left for 60 minutes and samples were taken from both chambers at eight time points (see table 1). For the glucose and insulin concentration measurement,

samples were taken from the respective opposite chambers where the substance was added initially.

Table 1: Time points of sampling from both chambers

Sample	1	2	3	4	5	6	7	8
Timepoint [min]	0	1	2	5	10	20	30	60

In the first part of the laboratory work, a Glucose-Glo assay was performed for the detection of glucose. For this purpose, triplicates of the eight collected samples and the standards were filled into wells of a 96-well plate and the same amount of detection reagent was added to each. The reagent was previously mixed from a luciferin detection solution, reductase, reductase substrate, glucose dehydrogenase and nicotinamide adenine dinucleotide (NAD). During the following incubation time of 60 minutes, the oxidation of D-glucose contained in the samples to D-glucono-1,5-lactone was catalysed by glucose dehydrogenase, with NAD being reduced to NADH in a side reaction. In the presence of NADH, pro-luciferin was reduced to luciferin by reductase. After the incubation period, the amount of luciferin, which is proportional to the glucose concentration, was measured by placing the 96-well plate in a luminometer. This measured the luminescence emitted by luciferin in the presence of ATP and rLuciferase. The luminescence values were stored in tabular form [4]

In the second part, a sandwich insulin enzyme-linked immunosorbent assay (ELISA) was performed, which was used to detect insulin in the other eight samples. To do this, a microtiter plate with 96 wells, which were coated with anti-insulin antibodies at the bottom, was filled with triplicates of the samples and six standard solutions. The antibodies bound to the insulin contained in the samples and thus fixed it to the bottom of the wells. Subsequently, Enzyme Conjugate (ENZ) containing biotin conjugated anti-insulin antibodies was added into the wells. During the incubation period of 30 minutes, binding was performed between the antibodies and the fixed insulin. Afterwards, unbound antibodies were removed by washing the samples three times so that the subsequently added streptavidin peroxidase enzyme complex could bind only to the remaining biotinylated anti-insulin antibodies during the following incubation period of 30 minutes. Unbound enzyme complexes were removed by washing, the substrate tetramethylbenzidine (TMB) was added, and the samples and standards were incubated for 15 minutes so that oxidation of TMB could be catalysed by the bound enzyme complexes. This leads to a colour development of the samples, which was proportional to the insulin concentration. The en-

zyme complexes were inactivated by a stop solution containing H₂SO₄ stopping the conversion of TMB. The optical density of the samples and standards was measured using a plate reader with light of wavelength 450 nm. An additional measurement was performed with light of wavelength 620 nm, which allowed the absorption values of the background to be recorded. Both tables were saved for evaluation [5].

3 Results

The recorded luminescence values for the determination of the glucose concentration, as well as the absorbance values for the determination of the insulin concentration were evaluated with Excel and GraphPad Prism.

To determine the glucose concentration, a calibration curve was created. For this purpose, the average of the luminescence values of each standard triplicate was calculated and the resulting means were plotted on a graph as a function of the corresponding glucose concentrations (see figure 1A). Subsequently, a linear regression was performed so that the dependence between concentration and luminescence could be determined on the basis of the slope of the regression line and the y-axis intercept. The concentrations C of all samples were calculated using formula 1:

$$C = \frac{L - b}{m} = \frac{L + 10364}{59035} \quad (1)$$

Where L is the luminescence, b is the determined y-axis intercept, and m is the determined slope. Next, the mean and standard deviation of each triplet was calculated and the mean values of both conditions were plotted as a function of the diffusion time (see figure 1B).

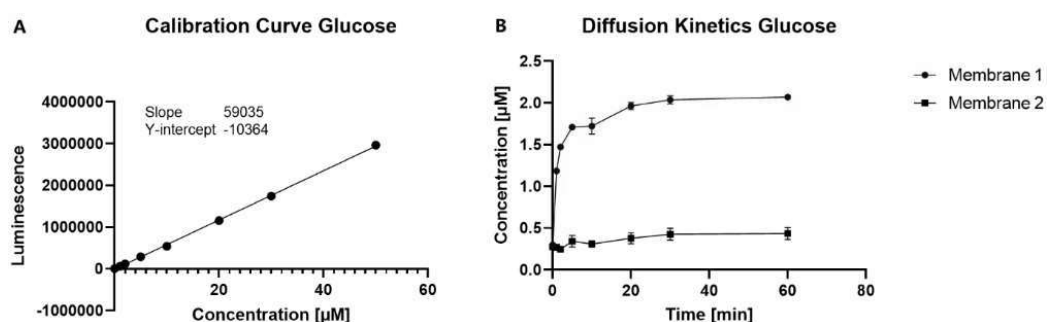


Figure 1: Calibration curve of the glucose measurement (A) and glucose concentration over diffusion time (B)

In the graph, it can be seen that both curves did start at an initial concentration of 0.3 μM. Using membrane 1, there is concentration saturation at approximately 2 μM glucose concentration and after about 5 minutes 80 % of this final glucose concentration was reached. In the case of membrane 2, this value was reached after about 5-10 minutes.

Using that membrane, a minimal increase in glucose can be noted, with stagnation occurring at a concentration of approximately 0.4 μM .

The procedure for determining the amount of diffused insulin was similar to the one used for glucose concentration determination. However, first the absorbance values measured with light having a wavelength of 620 nm were subtracted from the respective ones measured with light of a wavelength 450 nm. Thus, the absorbance of the background was subtracted from the absorbance values of the individual wells so that the background would not distort the calculation of the insulin concentration. Subsequently, the absorbance values of the standards were plotted as a function of the corresponding concentration values, a linear regression was performed and the relationship between insulin concentration and absorbance was determined using the slope and y-axis intercept of the resulting calibration curve (see figure 2A). The insulin concentration C for each well was calculated using following formula 2:

$$C = \frac{A - b}{m} = \frac{A + 0.05435}{0.02567} \quad (2)$$

Here, A is the luminescence, b is the determined y-axis intercept, and m is the determined slope. Afterwards, the mean and standard deviation of all triplicates of the concentrations were calculated and the resulting mean values of the two conditions were plotted in a graph as a function of diffusion time (see figure 2B).

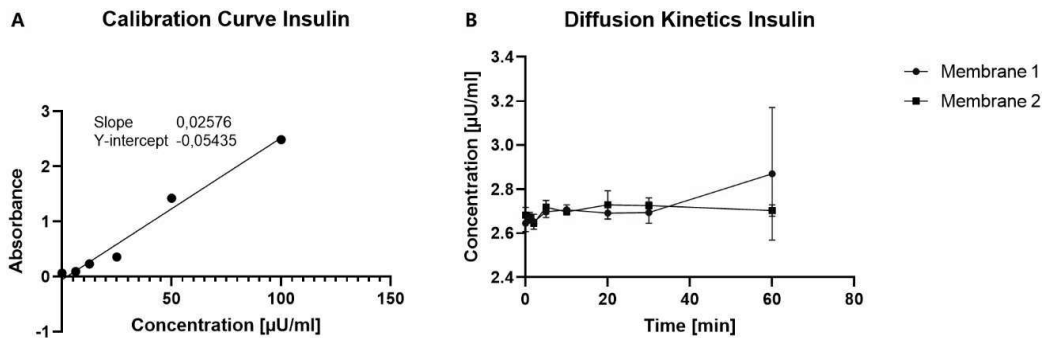


Figure 2: Calibration curve of the insulin measurement (A) and insulin concentration over the diffusion time (B)

In the graph, the initial concentration of both curves lies around 2.65 $\mu\text{U/ml}$. For both membranes, the concentration values fluctuate approximately around this value, but no increase in insulin concentration and thus no diffusion of insulin through the membrane was observable. Due to the constant concentrations, no time point could be determined for either membrane at which 80 % of the final insulin concentration was reached. In the curve of membrane 1, the last measured value at 60 minutes with 2.85 $\mu\text{U/ml}$ is slightly above the other values. Since the standard deviation is relatively high for this measured value, it is assumed to be an outlier.

4 Discussion

The results showed that membrane 1 and 2 were permeable to glucose, but only low concentrations of 2 μM and 0.4 μM could be detected on the other side of the membrane. Of these, the largest glucose fraction of 80 % diffused through the membrane relatively quickly within 5-10 minutes. In β -cells, the low level of diffused glucose could lead to low insulin production, which might not be sufficient to efficiently reduce the circulating glucose amount. The constant insulin concentration of both membranes suggests that they were not permeable to insulin. Thus, the material would not be suitable for encapsulation of β -cell implants, which must be permeable to both glucose and insulin. In the case of these membranes, the glucose would diffuse through the membrane and trigger the production of insulin, but the insulin could not leave the encapsulation.

The measurement results of glucose and insulin showed similar offset values of the initial concentration for both membranes, although at this point no diffusion of these substances should have taken place. Due to this similarity, it is assumed that both calibration curves were improper. In addition, the diffusion kinetics curves contained kinks and sometimes high standard deviations. The aforementioned deviations could have been caused by measurement errors during the laboratory work. For example, it is possible that the prescribed time points were not adhered to in advance when preparing and taking the samples and that incorrect amounts of the detection substances were added during the measurement using the Glucose-Glo Assay and Insulin ELISA. Additionally, it cannot be excluded that the samples were not exposed to direct sunlight, which could have an influence on the chemical reactions of both measurement methods. Furthermore, it can be assumed that deviating reaction and incubation times led to inaccuracies.

In addition to the analysis of the diffusion kinetics of glucose and insulin through the membranes, further tests should be performed to determine whether other substances such as oxygen, growth factors, hormones, and nutrients can also pass through the encapsulation so that the β -cell would be sufficiently supplied with the vital substances. Furthermore, it could be tested whether the membranes were non-permeable for the insulin or if it was adsorbed by them. This would require further tests in which the fluids from both chambers would be examined for glucose and insulin. If the concentration in the initial chamber was lower, although no increasing concentration could be detected in the second chamber, this would be a sign that the molecules were adsorbed by the membranes.

5 References

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