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Chapter 7

The Photosynthetic Pancreas: From Fantasy to Reality

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Additional information is available at the end of the chapter

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Abstract

Islets of Langerhans implantation is a viable method to treat type I diabetes. Unfortunately, during islets isolation their vascular system is disrupted, and they need external supply of oxygen and other nutrients. A photosynthetic bioartificial device was constructed to support the oxygen consumption of the islets and to treat type I diabetes. The bioartificial device is built in layers where the core is an illumination module composed of a LED array and a light guide. The next layer is immobilized photosynthetic organism (*Synechococcus lividus*). An oxygen-permeable silicon/Teflon membrane separates the photosynthetic layer from the islets of Langerhans layer. This layer is protected from the immune system of the body by a porous Teflon membrane. The device is powered by batteries that supply electricity to a LED array. The oxygen produced by *S. lividus* is consumed by implanted islets of Langerhans that produce insulin and allow the reversal of diabetes in the patient. In this chapter, we demonstrate the ability of *S. lividus* to produce oxygen after being implanted for prolonged periods and eventually the ability of the device containing *S. lividus* and the islets of Langerhans to reverse diabetes for 10 days. To achieve this task, we developed improved media to grow cyanobacteria and, inter alia, developed a method to disperse light uniformly and in very short distances.

Keywords: Bioartificial device, Cyanobacteria, Diabetes, Implanted islets of Langerhans, Synechococcus *lividus*

1. Introduction

Type I diabetes (T1D) is a severe disease resulting from the destruction of the beta cells in the islets of Langerhans (islets) residing in the pancreas. The loss of beta cells leads to lack of insulin and increased glucose levels in the blood (hyperglycemia). If untreated, T1D 1 leads to permanent damage to small blood vessels which in turn causes blindness, kidney failure, heart failure and eventually premature death [1,2]. Even when treated with external insulin, blood glucose levels are not stable and fluctuate according to eating habits and insulin administration. On the other hand, too much insulin can cause a dangerous drop in the blood glucose level (hypoglycaemia), which leads to confusion, loss of conscience and, if not immediately treated, sudden death.

Great efforts are made to develop a "closed loop" insulin pump that will secrete insulin when needed (after a meal) and sense the glucose level in order to stop secretion when glucose levels drop to normal levels. Islet implantation is another successful approach that utilizes donor's islets isolated from the pancreas to replace the missing islets of the patient. This approach, although promising, faces several obstacles that decrease its feasibility.

1. Harvesting of the islets from the donor pancreas is performed by enzymatic process, completely disrupting the blood vessels to the islets. As a result, all nutrients supply and insulin secretion from the islet rely on slow diffusion mechanism. The solubility of oxygen in blood fluid is low and its consumption is high. Therefore, it is the first nutrient becoming the limiting factor and it must be supplied [3].
2. Donor islets will be rejected by the immune system of the host, and therefore, constant harmful immune suppression drugs are needed [4,5].

To approach these two fundamental problems, Beta-O2 Technologies had designed implantable flat geometry device containing islets that were supplied with oxygen by immobilized and illuminated cyanobacteria. The implanted islets are partly protected from the attack of the immune system by a porous Teflon membrane. **Photosynthesis** was the source of the supply of oxygen, which is the focus of this chapter . Indeed, the designed device termed bioartificial pancreas contains battery-powered illumination module, photosynthetic organisms that produce oxygen and islets that produce insulin upon demand.

To create this "science fiction" bio-artificial pancreas device, a multidisciplinary approach was needed involving optics, material science, diffusion models, plant and islet biochemistry and other fields.

2. The construction of the photosynthetic bioartificial pancreas

A. To construct such a complex device, several requirements had to be set:

1. The device must be as compact as possible to reduce the trauma after implantation.

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2. The batteries driving the operation of the device should last at least one month for the proof of concept.
3. The power unit (batteries) must be separated from the bioreactor to decrease the immune response against foreign body, which depends upon size.
4. The light distribution must be even in all the active areas to ensure uniform oxygen production.
5. All materials exposed to body fluids must be biocompatible to provoke minimum response of the immune system.
6. The photosynthetic organism must be functional in body temperatures and withstand immobilization in the device and implantation conditions. It must also be spread even so that oxygen production will be uniform, constant and in direct contact with the islets.
7. There should be no hindrance in the diffusion of the oxygen from the oxygen producers to the oxygen consumers — the islets.
8. The islets must be active insulin producers and must be spread and immobilized evenly in the device to maximize diffusion quality.
9. The device must be protected from the body's immune system.
10. The electrical parts of the device must be completely insulated from fluid penetration.

B. The selection of the photosynthetic organism

The constraints: The aim of the photosynthetic organism in this system is producing oxygen for the islets**.** Body temperature is too high for most photosynthetic organisms. The organism has to withstand temperatures between 37 and 42°C. Since the organism has to be embedded in aqueous matrix or hydrogel, microalgae were the organism of choice. Indeed, the first work showing the beneficial effect of photosynthetic oxygen production on insulin secretion was conducted with the green algae *Chlorella* [6], but it was an in vitro study. Very few green algae can survive and be active at 37°C, and fewer still can withstand the much higher temperatures needed for immobilization in agarose. Therefore, we chose to introduce thermophilic cyanobacteria. Cyanobacteria are the first photosynthetic oxygen-evolving organisms on earth, and during the 3.5 billion years of their existence they adapted to any environment containing light, humidity and few minerals. Many cyanobacteria species grow at relatively low illumination, can survive hard conditions and still grow and produce oxygen. Several members of this large group of organisms live in proximity to geysers at temperatures reaching 70°C [7]. We chose to use *Synechococcus lividus* (*S. lividus*), a unicellular cyanobacteria found in hot springs in Yellowstone National Park, USA [8]. This strain was obtained from the Pasteur culture collection (PCC6717). PCC 6717 has an optimum growth at 52–57°C but grows well also Between 37°C and 42°C. To acclimate the *S. lividus* to body temperatures, it was grown for 10 generations in liquid culture at 40°C before being taken for immobilization in the device. Its oxygen production characteristics were checked prior to immobilization and

were shown to be stable. In nature (**Figure 1**), *S. lividus* creates bacterial mats with other organisms. Therefore, it is used to survive and function in the immobilized state. Occasionally, *S. lividus* will be termed algae for convenience, especially with relation to the device.

Figure 1. Bacterial mat containing thermophile cyanobacteria. A slice of bacterial mat (right) from Octopus spring (left) in Yellowstone National Park, USA. On top there is a layer of (mostly) cyanobacteria. Right panel is a picture by Prof. David M. Ward (October 13, 2008, by Charles Fergus, Penn State News).

C. The choice of growth medium for *S. lividus*: How much is good enough?

The cyanobacteria are enclosed within a silicon box to be protected from the host's immune system. All gas exchange (uptake of CO_2 and secretion of O_2) are performed via the silicon membrane. Therefore, all nutrients must be supplied within the silicon box for the period of implantation. This poses two problems:1. The osmolality of the BG-11 medium which is the standard medium for the growth of cyanobacteria is about 40 mOsmol/l of water, compared with 275–295 mOsmol/l of body liquid. To equilibrate the osmolalities, water molecules as vapor will escape the algae medium, leading to drying of the algae compartment. 2. BG 11 medium can support cyanobacterial growth when the medium is changed frequently, but cannot support long-term growth or photosynthesis without frequent changes of the medium. In the design of the bioartificial pancreas, the first milestone was to show treatment of diabetes for a month supported by oxygen produced by the cyanobacteria. It was difficult to obtain such a performance of the cyanobacteria based on BG-11, so efforts were made to improve the medium in order to extend its ability to support the photosynthetic activity of *S. lividus*. After several futile efforts of elevating individual components of the medium, the overall BG-11 concentration was increased. Surprisingly, increasing the BG-11 medium concentration 5 to 7-fold resulted in a 3-fold increase of the *S. lividus* cell density (**Fig. 2**B). This result indicates that BG-11x5-7 is a superior medium for the growth of *S. lividus* in solution, compared to BG-11. Also, since the osmolarity was increased it resembles that of body fluids, so no water loss from the algae compartment is anticipated. These findings enabled the use of medium that can support a long-term oxygen production by the *S. lividus* under the conditions set in the bio-artificial pancreas. It was later shown that indeed, *S. lividus* immobilized and im-

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planted with BG-11x5 developed much better than *S. lividus* implanted in the original BG-11, produced higher oxygen amounts and maintained its volume under immobilized conditions.

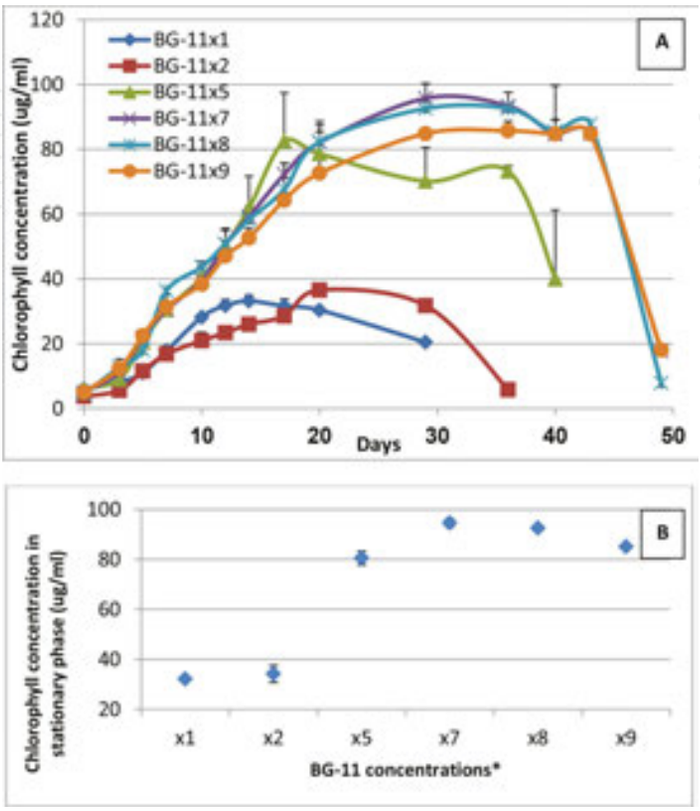

Figure 2. Dependence of bacterial growth on BG-11 concentration. Panel A: Dependence of *S. lividus* culture growth on the BG-11 concentration over time. Panel B illustrates the maximum concentration each culture has reached. Each result is the average of at least three experiments, apart of BG-11×2 that was tried only once.

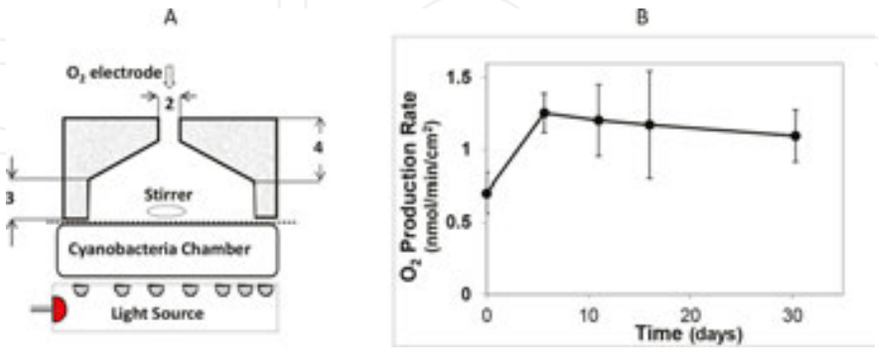

Figure 3. The oxygen measurement setup of the bioartificial pancreas. A device containing algae only (*S. lividus*) immobilized in agarose plus BG-11x5 was placed in a home-made oxygen measurement cell where a Clark-type oxygen microelectrode is inserted (Panel **A**). *S. lividus* is illuminated by a LED array as described later. The device was kept in vitro under identical conditions to the implanted device and tested periodically (Panel **B**) for oxygen production.

As shown in **Figure 3B**, the oxygen production did not diminish, but actually improved during the one month the device was tested. The BG-11 concentration in this device was BG-11×5.

The ability of the media with elevated BG-11 concentrations to support oxygen production in the device was shown both before and after implantation. It was shown that *S. lividus* alone was able to produce oxygen that will diffuse into the islets of Langerhans compartment and will allow the islets to function properly. As can be seen in **Figure 4**, an oxygen electrode is inserted into the islet part of the bioartificial pancreas. The deeper the electrode penetrates into the islet slab and gets closer to the *S. lividus* chamber, the higher is the oxygen concentration. The oxygen levels detected can support islet activity at any location and depth of the electrode.

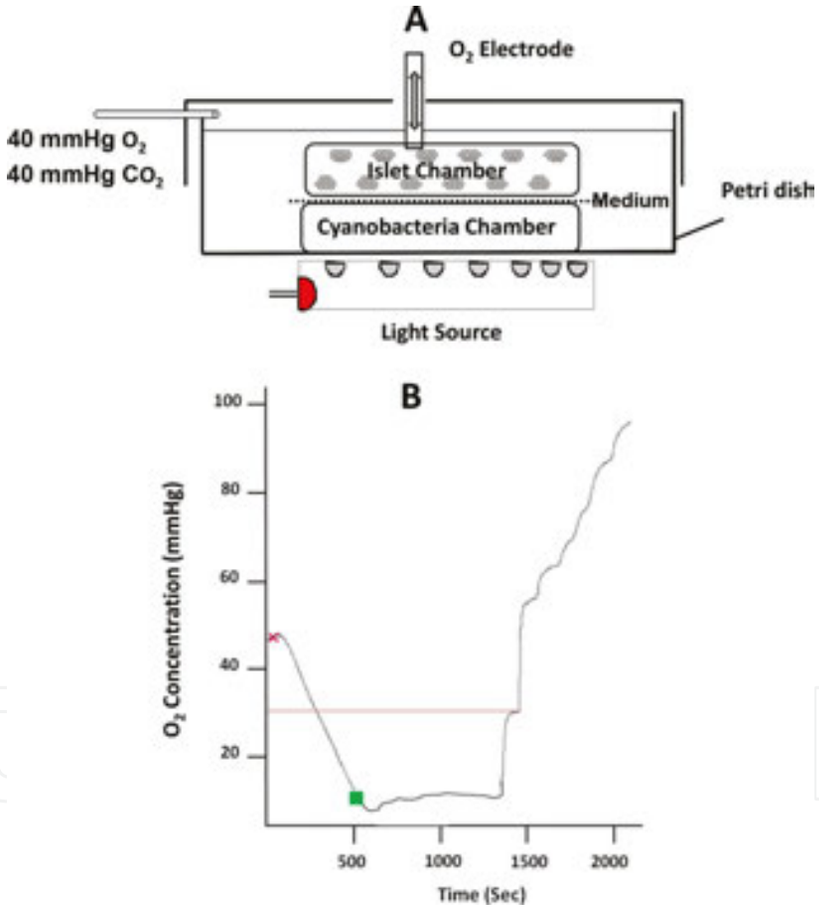

Figure 4. Measurement of the O_2 gradient in the islet slab in the device. Panel A: Device with oxygen electrode. Oxygen microelectrode was inserted by a micromanipulator at increments of 0.1mm into the islet slab with a thickness of 0.6 mm. Panel B: The O_2 concentration as a function of illumination and location of the oxygen electrode. At the start of the test, the electrode tip is located at the surface of the islet slab, the furthest point from the O_2 source. The light is turned off and the oxygen is consumed (concentration drops). The light is turned on leading to balanced O_2 concentration. Under illumination the electrode is inserted into the islet slab at 0.1mm increments, leading to a gradual increase in O_2 concentration The horizontal line indicates the O_2 concetration sufficient to support the islet's O_2 consumption.

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D. The illumination requirements for growth and oxygen production: the struggle for homogeneous light

The cyanobacteria were chosen for their ability to produce oxygen even at very low light intensities, below 1 μE/m²/s. Since the device operates on batteries, the longer the batteries Last, the longer the device would work. The milestone was achieving normoglycemia for one month in diabetic rats, and for that time span the batteries should hold. The light

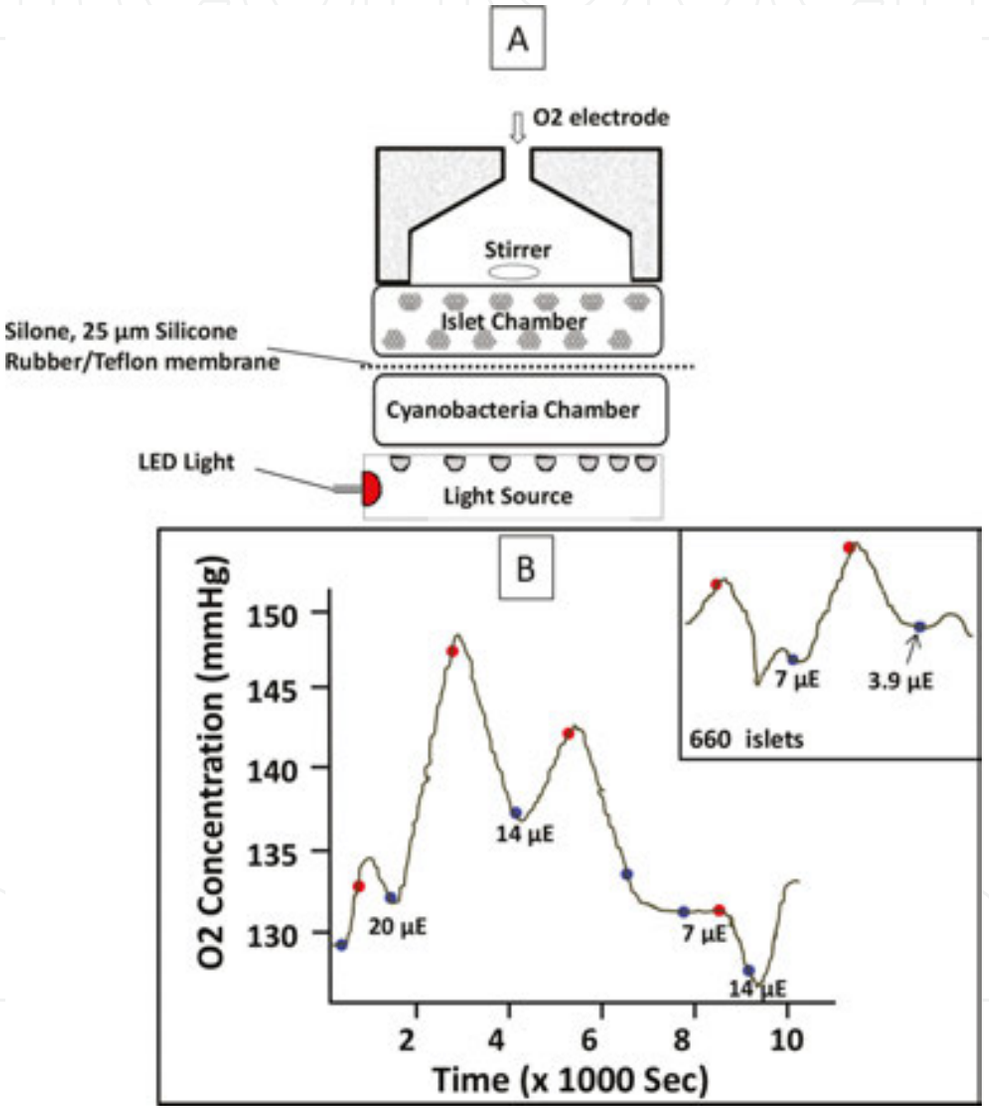

Figure 5. Compensation between oxygen production by *S. lividus* and oxygen consumption by the islets. Panel A. Schematic view of the oxygen measurement setup of the full device. Panel B. Measuring the oxygen production rate of *S. lividus* (cyanobacteria) plus the islets in order to determine the light intensity required for achieving the compensation point of the device. 1,200 islets of Langerhans, each consuming 2.5–3 pmole/min O_2, were embedded in alginate and placed on top of the *S. lividus* slab. Light intensity of 7μE/m²/s was needed to reach the compensation point. The insert (600 islets) demonstrates that compensation point is reached with light intensity (3.9μE/m²/s) which is half the light intensity needed for reaching the compensation point with 1,200 islets.

intensity needs to be such that it allows *S. lividus* to produce enough oxygen for the consumption of the islets. Therefore, the oxygen production should be equal to the oxygen consumption by the islets, what we termed **the compensation point** of the device (**Figure 5B**).

Higher intensities cause excess oxygen production that can lead to oxidative stress for both *S. lividus* and the implanted islets. Also, over-illumination causes loss of battery power and early termination of the trial.

Light quality: After checking several LED types with different wavelengths, 660nm wavelength was chosen since it is absorbed well by chlorophyll. It proved best both for oxygen evolution and also was electrically most efficient. ($E = hC/\lambda$, where E is the energy, h is Planck's constant, C is the speed of light and λ is the wavelength. The longer the wavelength, the lower is the energy of the photon, and the electric energy needed for its production.). The second, more formidable task was to disperse the light as much as possible when it enters the *S. lividus* chamber. The reasons for that are: the direct result of the illumination is oxygen production from *S. lividus*. We need the oxygen production to be uniform at all parts of the algae compartment because the oxygen diffuses directly to the islets compartment. The islets, too, need the oxygen in the most even manner, so as not to have parts with too little oxygen that will lead to islet necrosis. On the other hand, too high intensity will lead to oxidative stress, followed by bleaching of the cyanobacteria. At such short distance of less than a millimetre, it is extremely hard to disperse the LED light uniformly. Early attempts (**Figure 6**) show non-uniform growth of *S. lividus* as a result of exposure to non-uniform light.

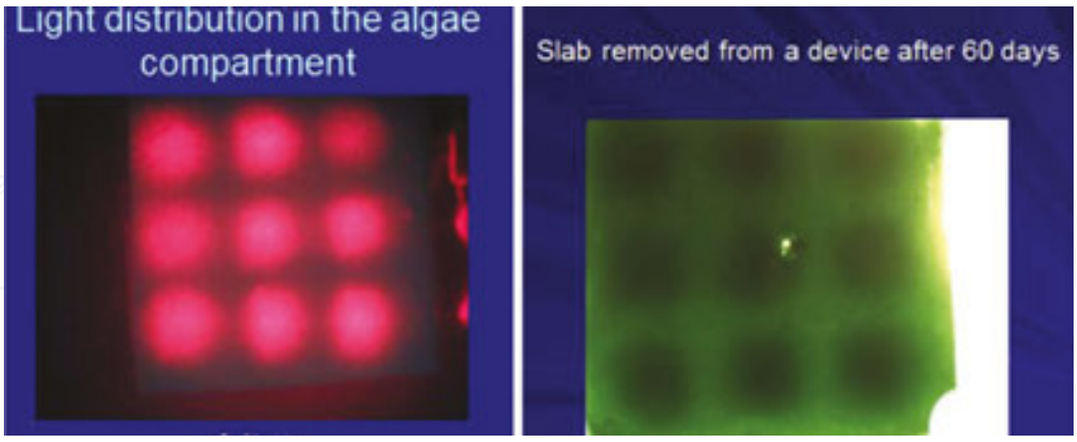

Figure 6. Correlation between light distribution in the device and *S. lividus* growth Left: The LED array on which the immobilized *S. lividus* was implanted Right: an agarose slab of *S. lividus* implanted in a rat for 60 days on a LED array.

To achieve uniform light source we constructed a LED light guide that allows light dispersion (patent no.) and uniform light to the *S. lividus* compartment (**Figure 7**).

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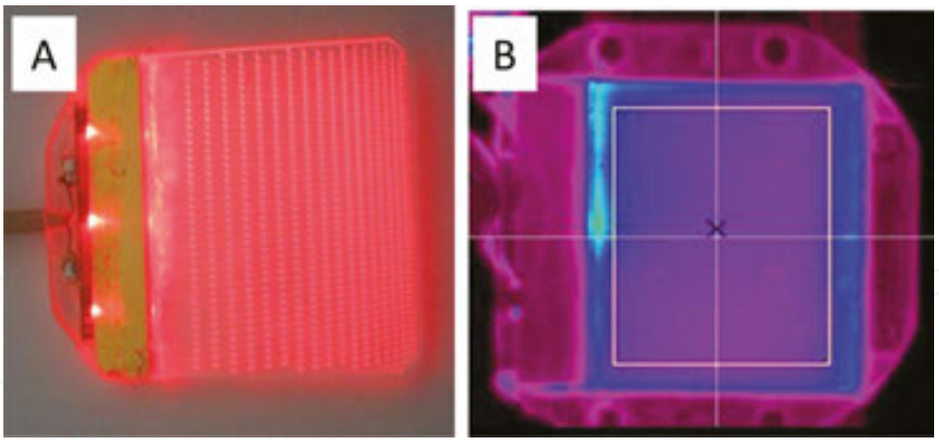

Figure 7. The bioreactor light guide Panel A. the LED operated light guide Panel B: *S. lividus* immobilized in agarose slab, illuminated by the light guide. The blue colour is an artefact of the camera.

E. The immobilization of the organism: several approaches

To avoid coalescence of both organisms, the islets and the cyanobacteria have to be separated from each other. We used hydrogels for immobilization. Two general methods were used to immobilize the unicellular *S. lividus* in the device. The first one was immobilization in alginate. Alginate is a polysaccharide extracted from brown algae (kelp). It provides flexibility and strength for the kelp to withstand the ocean currents and waves without breaking or tearing. It is provided as a viscous liquid similar to glycerol, which solidifies rapidly upon exposure to divalent ions, like calcium ions. We used it in the first devices, but stopped when we started to inject the algae with its immobilizer into a closed compartment. It was impossible to inject the alginate because of its high viscosity. Therefore, we decided to use agarose as the immobilizer. The fact that we use thermophile cyanobacteria helped to mix *S. lividus* with the liquid agarose brought to 50°C and inject it into the device under sterile conditions. For immobilization of the islets we used alginate, and cross-linked it with strontium.

F. The construction of the bioartificial pancreas

Physically, the best approach to supply oxygen to the islet by photosynthetic microorganisms is to mix them in the same compartment [6]. Attempts to combine the photosynthetic organisms with the islets in the same chamber and the same medium (DMED or RPMI) resulted in the rapid death of *S. lividus*. Attempts to construct mini balls of *S. lividus* isolated by a sol–gel coat or other protection failed as well. It was clear early on that the *S. lividus* chamber and the islet chamber must be separated by a membrane that allows the diffusion of only gases. This approach holds the risk of gas diffusion constraints from the algae chamber to the islets chamber and vice versa. To minimize this problem, a thin silicone/Teflon membrane is used between the algae and the islet chambers. A second membrane that allows insulin to diffuse out and nutrients in, but blocks the immune cells, was needed between the islets and the body.

Several approaches were tested, but eventually we embarked with a flat device illuminating both directions having the illumination panel at the core, coated with immobilized *S. lividus* and above it two islets chambers, again on both sides (**Figure 8**).

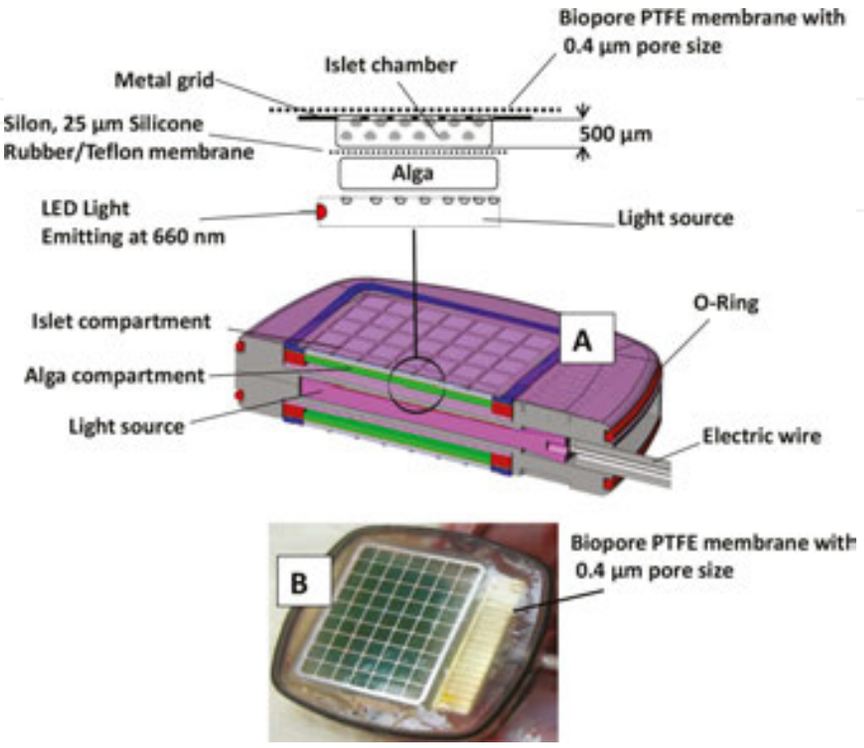

Figure 8. The photosynthetic bioartificial pancreas. The device has a core illumination panel directing the 660 nm light to both sides. The algae chamber is laid directly on the illumination panel and is separated from the islet chamber with gas-permeable thin silicone/Teflon membrane. The islet chamber is protected from the immune cells by porous biopore membrane. Panel A: Detailed cross-section of the device. **Panel B**: Actual view of the device.

G. The implantation of the device: reversing diabetes in rats

As stated before, the designed device was aimed to treat type 1 diabetic (T1D) rats as model animals. Initially, to learn the dose needed to cure STZ (Streptozotocine) diabetic rat, we implanted various islet doses under the kidney capsule, and found that about 2400 islets are sufficient to reverse diabetes. Therefore, we constructed our device with 2400 islets. To allow implantation into non-suffering animal, it was treated with slow-release insulin capsule (**Figure 9**, LPin). T1D animals were implanted with the device containing 2400 islets and the slow-release insulin capsule was removed (**Figure 9**, LPout). At this stage, the only entity that can produce insulin and reverse the diabetes is the implanted device (**Figure 9**).

This experiment, which is the culmination of efforts ranging from physics, optics, material engineering, islet biology and microbial photosynthesis, is a remarkable multidisciplinary project ending in success, although only partial.

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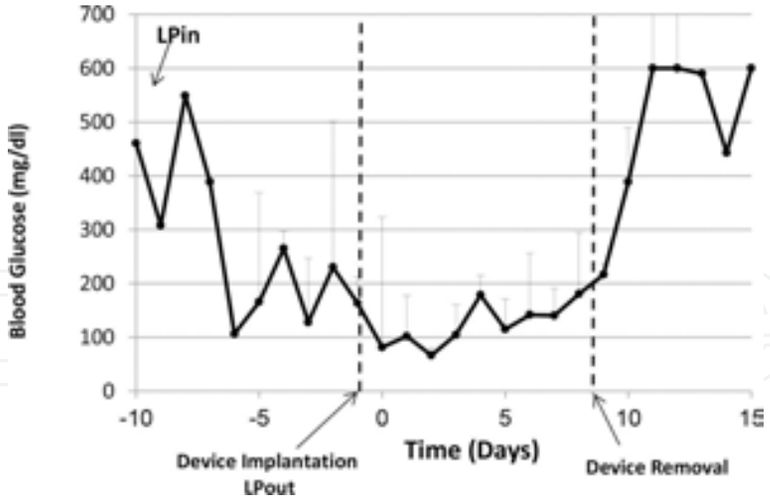

Figure 9. The effect of bioartificial pancreas implantation on the glycemic state of the implanted diabetic rat. The implanted device, driven by light and supplies oxygen by photosynthesis, allowed the implanted islets to function well and produce enough insulin to reverse diabetes in rats for 10 days (day 0 to day 9).

H. Conclusions and further possibilities

Several issues should be addressed when planning to continue toward a working device to treat lab animals and later, humans, with the bioartificial photosynthetic device.

1. The device can probably be active in the body for few months. However, the implanted batteries which power the light need to be recharged. Therefore, a recharging mechanism is needed.

2. Most of the oxygen is produced from photosynthesis during the logarithmic phase. The growth leads to self-shading that increasingly leads to light limitation. Overall produced oxygen will then eventually decrease. Example of that can be seen in **Figure 6**, where above the LEDs, *S. lividus* is much darker than the surroundings. A possible solution is developing a method of removing the excess cyanobacteria (see next paragraph).

3. Although great advances were made in improving *S. lividus* growth medium, eventually it will be exhausted, leading to a culture in the stationary phase and lack of oxygen production. A method must be found to exchange the medium periodically.

4. Scaling up the device to human size will require more islets packed at a higher density. To compensate for the increased islet density, more O_2 has to be produced. However, using this type of solid-state bioreactor, we reached the maximum O_2 production rate. Therefore, in order to supply O_2 to bioartificial pancreas with dimensions that are practical for implantation in human, the photosynthesis approach should be greatly improved.

To summarize, we showed the ability to combine cells of two organisms, one that produce O_2 and the other that consume it, into one implantable device, achieving normoglycemia in

T1D rat. In addition, we developed the effective means to disperse light in water and at very short distances.

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