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MICROSENSORS AND MICROBIOSENSORS FOR RETINAL IMPLANTS

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1. ABSTRACT

This paper concentrates on recent developments in microsensors and microbiosensors for the possible applications in visual prostheses, especially retinal prosthetic devices. A brief introduction on the developments of visual prosthesis will be presented. The importance for *in-vivo* pH measurements as well as the need for an implantable pH sensor will be demonstrated. Electrochemical biosensors developed for sensitive measurements of glucose and L-glutamate, a known neurotransmitter in the retina and brain will be reviewed. Novel electrode materials such as chemically modified thin-film diamond in applications for implantable biosensors will be shown. The challenges in the development of chronic implantable sensor systems, especially using MEMS technology for medical implants, will be discussed.

2. INTRODUCTION

Medical implants have been used widely for many decades and now play a major role in replacing or improving the function of every major body system to maintain a good quality of life. Some common implants include cardiac implants such as pacemakers and defibrillators (1), neural prostheses such as spinal cord stimulators (2), deep brain stimulators (3) and cochlear implants (4).

Inspired by the success of cochlear implants, which restore hearing for the deaf, research efforts worldwide are developing visual prostheses aimed at restorations of vision for the blind (5-7). Several recent developments from research teams and industrial developers working on visual prostheses have raised hopes as to the possibility of creating retinal implants and other strategies for restoring vision to blind individuals (5-11).

The impact of blindness is devastating to those who suffer from it as well as their families and loved ones. Millions of people with blindness or visual impairment face this challenge every day. Beyond that, the impact of blindness also has staggering costs associated with it. In the United States retinal blindness alone costs $4 billion annually in lost benefits and taxable income to the government.

3. VISUAL PROSTHESIS

3.1. Development of visual stimulation implants

The possibility to restore vision to blind patients using electricity began with the discovery that an electric charge delivered to a blind eye produces a sensation of light. This discovery was made by LeRoy in 1755 (12). LeRoy passed the discharge of a Leyden jar through the orbit of a man who was blind from a cataract and the patient saw "flames passing rapidly downwards". However, it was not until 1966 that the first human experiments in this field began with Brindley and Lewin's experiments with electrical stimulation of visual cortex (13). They used 80 cortical surface electrodes in a patient who was able to perceive spots of light called "phosphenes". Approximately 32 independent visual percepts were obtained. Another subject received a second 80-channel implant in 1972 (14-15). Of the 80 implanted electrodes and stimulators, 79 of them produced visual percepts of varied size and shape.

Since these early experiments, efforts have been underway to produce penetrating arrays of electrodes that offer the possibility of more closely spaced electrodes and therefore higher resolution cortical devices (16-19). Philip Troyk *et al* reported in a recent paper (20) the use of an animal model for cortical visual prosthesis research. They made extensive use of trained monkeys to investigate stimulation strategies in developing a multichannel sensory cortical interface.

While the cortical stimulation approaches have made progress, it has been hampered by physiology (5). The processing that has occurred by the time the neural signals have reached the cortex is greater than the more distal sites such as the retina. This results in more complex phosphenes being perceived by the patients. Cortical prostheses provide additional risks such as intracranial hemorrhage and infection to a blind patient who has an otherwise normal brain. These factors, and the lack of availability of implantable electronics have limited the clinical application of these devices.

The limitations of the cortical approach encouraged several groups worldwide over the past 20 years, to explore the possibility of producing vision in patients with an intact optic nerve and damaged photoreceptors by stimulating the retina (21-32).

A group led by Claude Veraart at the Neural Rehabilitation Engineering Laboratory in Brussels, Belgium has implanted a nerve cuff electrode with four electrodes around the optic nerve of a blind patient. That patient is able to reliably identify which quadrant she sees a phosphene and may be able to differentiate other phosphenes as well (33-34).

3.2. Epiretinal and subretinal stimulation

In retinal diseases like retinitis pigmentosa, blindness is caused by a loss of photoreceptors. In spite of nearly complete degeneration of the retinal architecture there is relative preservation of the inner retinal neurons (21-22). The approach of retinal stimulation by an intraocular prosthesis is to electrically stimulate the remaining retinal cells. There have emerged two major approaches to retinal stimulation: epiretinal and subretinal.

In the epiretinal approach, electrodes are placed on top of the retina to produce phosphenes. In subretinal approach, photodiodes are implanted underneath the retina and used to generate currents that stimulate the retina (9).

The epiretinal approach has been pursued by a team originally at the Johns Hopkins University (now at the University of Southern California, Los Angeles, CA) led by Eugene de Juan and Mark Humayun (31-32) and another at Harvard/MIT centers led by Joseph Rizzo and John Wyatt (23-24). Second Sight Medical Products, a privately held company formed in 1999 in Sylmar, CA is developing a chronically implantable epiretinal prosthesis (5).

The Chow brothers, an ophthalmologist and an engineer, who have pursued the subretinal approach, have formed a company, called Optobionics (Chicago, IL) (25-27). The artificial silicon retina (ASR) microchip they developed is a 2-mm-diameter silicon-based device that contains approximately 5000 microelectrode-tipped microphotodiodes and is powered by incident light. They believe this device excites the release of neurotrophic factors although animal experiments funded by them indicate no difference from sham implants.

In Germany two projects led by Eberhart Zrenner (28-30) and Rolf Echmiller (31), respectively are being sponsored by the German government to develop subretinal and epiretinal implants. There is also a new group in Japan led by Tano that is focusing on transretinal electrical stimulation i.e. stimulation outside the eye (32).

3.3 Development of first generation of intraocular retinal implant

Shown in Figure 1 is a diagram of one of the very first patient tests conducted 15 years ago at Johns Hopkins led by Dr. Mark Humayun and Dr. Eugene de Juan (21). A single electrode was placed onto the retina surface, no devices were implanted. Prior to the introduction of the array, a majority of the vitreous gel was removed. A stimulus was transmitted to the retina through the electrode and a perception as a bright spot was formed in the patient's eye. This test demonstrated the electrical stimulation could restore visual perception of dots and possibly more complex shapes. This type of acute testing led to the design of the chronic retinal implant.

Dr. de Juan and Dr. Humayun's group moved to University of Southern California (USC), CA from Johns Hopkins three years ago. Second Sight and the group at USC have been continuously developing the intraocular retinal prosthesis (11, 36-38). A large portion of this research and development was done with collaboration from many universities funded by National Eye Institute (NEI) and several national labs funded by Department of Energy (DOE). The DOE artificial retina project is a collaborative effort that exploits the unique

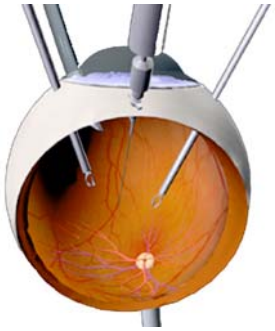

Figure 1. The configuration of one of the very first patient tests conducted 15 years ago at Johns Hopkins led by Drs Eugene de Juan and Mark Humayun (21).

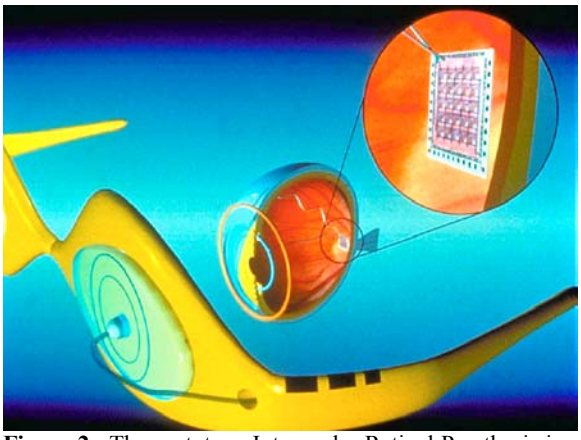

Figure 2. The prototype Intraocular Retinal Prosthesis is a wearable and implantable device. Visual signals from a camera are sent to an implanted receiver, and a visual image is then created by stimulating the appropriate electrodes on the surface of the retina (11).

Table 1. The comparison of concentrations of some chemicals in the eye (41-42)

Chemicals	In Plasma	In Vitreous
Na	146	144
Cl	109	114
Potassium		7.7
HCO_3^-	28	20-30
Ascorbate	0.04	2.21
Lactate	10.3	7.78
Glucose	6	3.44
Pyruvate		0.81
Collagen		286 microgram/ml
L-Glutamate		~ 0.1 – 10 microM

multidisciplinary resources of the DOE national laboratories in materials sciences, microfabrication, microelectrode construction, photochemistry, and computer modeling.

Similar to the concept of Star Trek's Geordi, the prototype Intraocular Retinal Prosthesis is a wearable and implantable device (see Figure 2). In this design, a small camera is housed in a pair of glasses which captures images such as letter "E", and then wirelessly transmits this data to an implantable electronic system. The implantable electronic system then stimulates the remaining nerve cells of the eye of a blind patient. The retinal neurons convert the light image into tiny neuro-chemical impulses that begin a cascade of neural activity which is transmitted via the optical nerve to the brain.

3.4. Candidate Retina Diseases for the Retinal Implants

Likely candidate diseases are retinitis pigmentosa (RP) or age related macular degeneration (AMD). It is difficult to determine exactly how many patients are blinded by these diseases since patients often stop seeing their ophthalmologist after being told there is nothing that can be done. However, estimates of legal blindness in the western world run as high as 300,000 people with RP and 3 million people with AMD. Nearly 1.2 million people are affected (but not yet blind) with RP worldwide and 10 million people are afflicted with AMD in the US alone (39).

Age-related macular degeneration (AMD) is estimated to cause severe visual impairment in about 5% of the elderly population. In the year 2030, the number of people developing macular degeneration is projected to significantly increase to 20% of the US population. Macular degeneration results in legal blindness. In practical terms, this means vision of less than 20/200 or visual loss which results in the inability to watch TV, recognize faces, drive or read.

Retinitis pigmentosa (RP) is another disease causing retinal blindness that a retinal prosthesis may help. Retinitis pigmentosa is a group of hereditary degenerative diseases in which the photoreceptors are lost. The incidence of RP is approximately in 1 in every 4000 live births. The visual handicap in retinitis pigmentosa typically starts with night blindness followed by peripheral vision loss and ultimately visual loss in the center of the patient's vision, rendering the patient completely blind. Unfortunately, many of the people who have RP tragically lose their vision before the age of 40.

3.5. Eye Chemistry and Charge transfer processes during stimulation

The eyeball is slightly ellipsoidal and has a volume of about 10 cm^3 for an adult of age of 18-30 years (40). Space inside the eye has a volume of about 4-6.5 ml and is filled with clear vitreous humor. The retina which lines the back of the eye is approximately 0.1 to 0.5 mm thick and resembles a thin wet tissue paper in strength. The human retina is a delicate organization of neurons, cells and nourishing blood vessels. A circular field of approximately 6 mm around the fovea is considered the central retina which is thicker than the peripheral retina due to increased packing density of photoreceptors. This central retina area is a preferred site for a retinal implant.

Table 1 lists the concentrations of some chemicals in the vitreous humor (41-42). The vitreous humor is a gel that consists of a network of collagen fibers bound by hyaluroinc acid. Approximately 98% of this gel is water; diffusion of low molecular-weight solutes such as inorganic ions, glucose and amino acids is unimpeded through the vitreous. Oxygen is largely supplied by the atmosphere. The major substrate for respiration in the

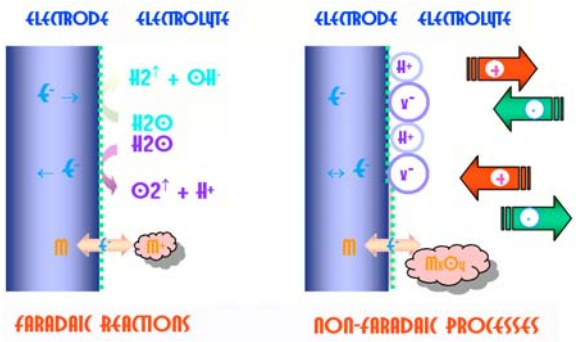

Figure 3. Some typical charge transfer mechanisms occur on the electrode/electrolyte interface during electrical stimulation (Unpublished data).

Figure 4. The Utah array is a 4.2 mm square grid with 100 silicon micro-electrodes, 1.0 mm long and a spacing of 0.4 mm (16).

Table 2. Some typical reactions involved in the Pt electrode charge injection process (43)

Reactions	Chemical Formulas
H-atom absorption	$Pt + H_2O + e^- \Leftrightarrow Pt–H + OH^-$
Hydrogen evolution	$2H_2O + 2e^- \Rightarrow H_2\uparrow + 2OH^-$
Oxygen reduction	$O_2\uparrow + 2H_2O + 4e^- \Rightarrow 4OH^-$
Oxygen evolution	$2H_2O \Rightarrow O_2\uparrow + 4H^+ + 4e^-$
Oxide formation and reduction	$Pt + H_2O \Leftrightarrow PtO + 2H^+ + 2e^-$
Corrosion of the electrode	$Pt + 4Cl^- \Rightarrow [PtCl_4]^{2-} + 2e^-$
Oxidation of chloride ion	$2Cl^- \Rightarrow Cl_2\uparrow + 2e^-$

retina is glucose. Most of the glucose (~70%) utilized by the retina is converted to lactate. Glutamate, one of many neuro-active amino acids has been found in higher concentration in the retina. The glutamate is actively metabolized by normal retina tissue.

Electrical stimulation of neural tissue requires charge injection into the biological environment. This is achieved through both Faradaic and non-Faradaic reactions at the interface of electrode/tissue surface. Figure 3 shows some typical charge transfer mechanisms which occur in the electrode/electrolyte interface during electrical stimulation. Some Faradaic reactions have the potential to dramatically alter pH levels, leading to tissue damage (43). Table 2 lists some typical reactions involved in the Pt electrode charge injection process. It is clear that most of these reactions involve pH changes in the electrode/electrolyte interface.

3.6. Micro stimulating electrodes for visual implants

One of the key components for visual implants is the stimulating electrode array. The electrode array in contact with the living tissue forms an interface for the electronic device and the biological tissues (44). As these micro stimulating arrays will serve as platforms for the incorporation of microsensors and microbiosensors, some typical electrodes used for visual implants are briefly reviewed here. There are mainly two types of electrode arrays used in the visual implants; 3D needle type or planar type (16-20, 45-48). For cortical stimulation, needle type electrode arrays are mostly being used today, although Dobelle (10, 46) has implanted surface arrays in patients in Portugal. A typical example for this type of electrode is the Utah array (Figure 4). The Utah array is a 4.2 mm square grid with 100 silicon micro-electrodes, 1.0 mm long and a spacing of 0.4 mm (16).

The needle type electrode array developed by Huntington Medical Research Institute, Pasadena, CA (HMRI) has been successfully used for implantation in cortical stimulation studies (20). Figure 5a shows the configuration of the HMRI array. The long stabilizer pins help to maintain the position of the array in the cortex. Figure 5b shows the scanning electron micrograph of a typical microelectrode tip of the HMRI array showing the Parylene insulation and the exposed iridium tip.

Planar electrode arrays are usually made from flexible polymers such as silicone and polyimide. An example of flexible polyimide electrode array is shown in Figure 6. The microelectrode film was composed of eight squares of platinum arranged in two rows of four electrodes embedded in polyimide. The size of each electrode was 0.1x0.1 mm; the center to center distance was 0.33 mm. A flat wire of 80 mm length connected the electrodes and the micro-connectors (47-48).

The electrode arrays used in the early clinical studies by Humayan's group are mainly silicone based flexible arrays (11, 38). Figure 7 shows a fundus photograph of an implanted silicone electrode array developed by Second Sight. The electrode array was composed of 16 platinum disks arranged in a 4x4 square array. A single 25 μm diameter platinum wire was attached to each disk. The disks and wire were encapsulated in medical grade silicone, except for the surface of the platinum disks juxtaposed against the retina, which was not enclosed by the silicone.

The exposed surface of the platinum disks formed an array of planar, stimulating electrodes in a silicone matrix. The disks were 400 μm in diameter and mounted on 600 μm centers. The side of the implant that was placed next to the retina measured 3x5 mm and was curved to match the retina. The implant was less than 1mm thick. The 16 wires from the disks formed a cable, extending from the electrode array. Each wire was individually insulated. The cable was about 10 cm long, a

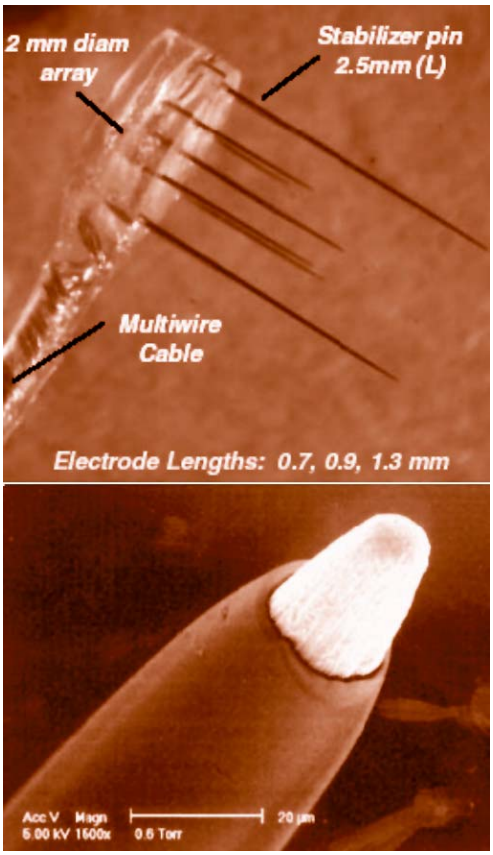

Figure 5. (a) Upper: Configuration of HMRI (Huntington Medical Research Institute, Pasadena, CA) arrays used for implantation. The long stabilizer pins help to maintain the position of the array in the cortex. Figure (b) Bottom: Scanning electron micrograph of a typical microelectrode tip showing the parylene insulation and the exposed iridium tip (20).

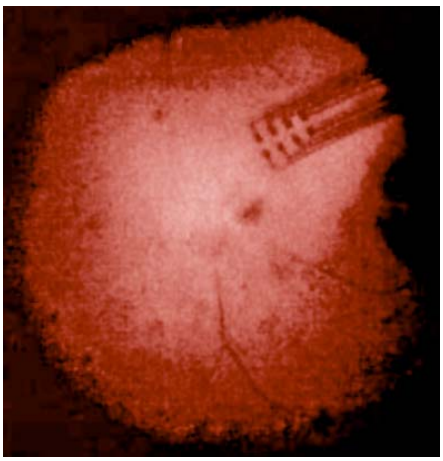

Figure 6. Intraoperative fundus photograph of an implanted microfilm electrode (2x4 array) that was positioned parafoveolar without additional adhesive due to the tension of the curved microfilm. The flat platinum microelectrodes embedded in thin polyimide film were developed for epiretinal stimulation (47-48).

sufficient length to allow the cable to exit the eye through the sclerotomy and is sutured to the sclera, in the superotemporal quadrant, under the conjunctiva.

4. MICROSENSORS AND MICROBIOSENSORS FOR VISUAL IMPLANTS

4.1. Integration of sensors with visual implants

The first generation of the retinal implant is only used for the stimulation and no sensor feedback control functions are implemented. By integration of sensors with those medical implants, the device performance *in vivo* can be monitored. The information gathered by these sensing electrodes may be used for the microchip's decision making process to adjust the stimulation current accordingly. The retinal implant with integrated sensor systems permit early corrective therapy or provide the feedback in order to control the devices to form so called "smart" closed-loop controlled medical implants. A similar approach is a glucose sensor controlled insulin pump for improving diabetes management. Information gained from such monitoring also provides insight into the strengths and weaknesses of the design of the device and enables improvement in future product design.

Use of these sensing electrodes is more valuable during clinical trials of the stimulation system. These sensors will make the "blind' researcher to "see" how the retinal implant and the electrode array works while the blind patients describe the percepts they observe.

Some examples of sensors needed for the visual implants are temperature sensor, pressure sensor, chemical sensors such as pH sensors and ion selective electrodes, some gas sensors for oxygen, hydrogen and chlorine, impedance/voltage sensors, and biosensors, such as glucose, ascorbate, lactate and glutamate sensors.

4.2. Microsensors for visual implants

4.2.1. Micro-pH sensor

The importance of monitoring pH changes during neural stimulation has been demonstrated in a study for cochlear implants (43). Figure 8 shows typical stimulus induced pH changes recorded in saline and measured using pH electrodes approximately 0.2 mm from two Pt ball electrodes. The pH was measured before, during, and following 5 min of bipolar stimulation with 340 µC/phase at 1000 Hz. For these Figures a negative pH shift corresponds to an increased acidity, while a positive pH shift reflects increased alkalinity.

The changes in pH can have a significant effect on the electrode/electrolyte interface. Shifted pH will change the electrode's corrosion potential and cause electrode materials to dissolve. Large pH changes also affect cell function, altering the structure and activity of proteins, ionic conductance of the neural membrane, neuronal excitability and even causing tissue damage.

A similar study has been carried out to establish a test system to monitor pH changes in retinal implants under pulse stimulation in our group (unpublished data). The

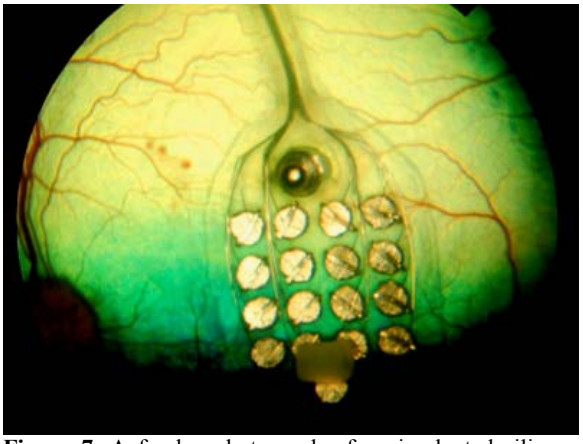

Figure 7. A fundus photograph of an implanted silicone electrode array developed by Second Sight. The electrode array was composed of 16 platinum disks arranged in a 4x4 square array (11, 38).

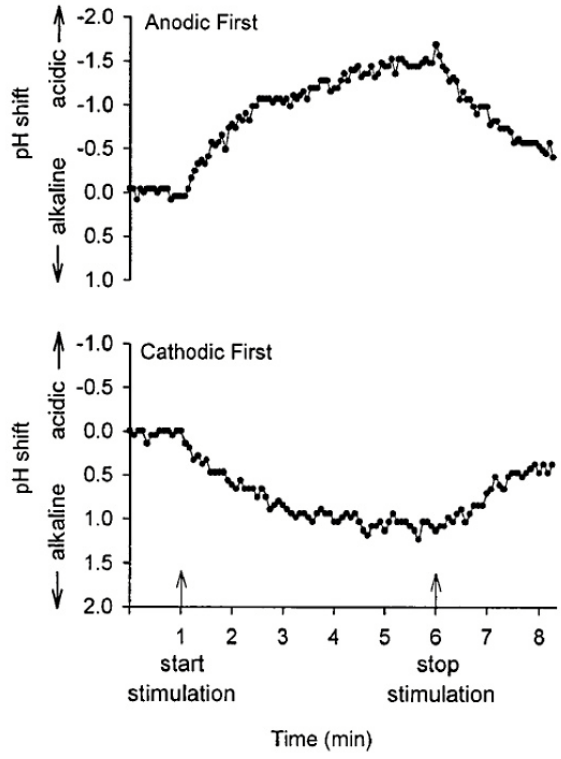

Figure 8. Typical stimulus induced pH changes of bipolar stimulation recorded in saline and measured using pH electrodes approximately 0.2 mm from two Pt ball electrodes. A negative pH shift corresponds to an increased acidity, while a positive pH shift reflects increased alkalinity (43).

results provide an insight to the electrochemical mechanisms at the interface of the electrolyte medium and retinal stimulation electrodes. It also provides information for the safety margin of the stimulation parameters. A needle type commercial micro-pH electrode made by WPI (*www.wpiinc.com*) with a tip diameter of 100 μm was used for the study.

While these pH shifts are measured using a pH microelectrode at close proximity, the pH level at the surface of the electrode may have great changes. When a retinal implant is placed in contact with retinal tissue, the pH effects on the tissue may be dramatically enhanced. To minimize such possible effects, a biphasic charge balanced pulse should be used for stimulation in a buffered medium.

A wireless pH sensor has been reported to record salivary pH continuously (49). The sensor system transmits pH data via a telemetry system for about 19 hours with a 3V lithium battery (190 mAh). The error of transmitted pH data value was less than 0.15 pH in the range of pH 5.0 to 9.0.

4.2.2. Diamond based electrochemical sensors

Diamond is one of nature's best insulators; but when doped with boron or nitrogen, the material possesses semimetal electronic properties, making it useful for electrochemical measurements (50).

Researchers at Argonne National Lab developed a novel material Ultrananocrystalline Diamond (UNCD) thin-films as a substrate for biosensors (53-54). They studied the stability of covalently bonded DNA/diamond surface and compared it with other alternative electrode surfaces, such as Au or doped Si. They concluded that diamond surface is more stable than any other microelectronics-compatible materials**.**

The electrochemical properties of thin-film doped diamond provide a wide range of applications due to the wide electrochemical windows (>3V) at the surface, before hydrogen forms at the cathode and oxygen at the anode (51-53). This is also an attractive advantage for an electrochemical sensor in the visual implants. It not only reduces the background noise of the sensors, but also minimizes the possibility of the retina damage due to side electrochemical reactions. The chemical inertness of the diamond is another key factor offering the opportunity to use such electrodes (anodes as well as cathodes) in very aggressive media like the vitreous humor inside the eye, thus increasing drastically their lifetime when incorporated with chronic visual implants.

4.3. Microbiosensors

Biosensors, especially glucose biosensors, comprise the most extensively studied class of enzyme biosensors because of the relatively high durability of the enzyme, typically glucose oxidase, and the high practical relevance of glucose determinations. In the past decade, numerous publications have appeared about different biosensors. However, a survey of this literature shows that only a limited number of these devices have been applied to real samples, and very few are commercially available (55-60).

In-vivo chemical sensing would benefit diagnosis and treatment of serious clinical problems and monitor vital

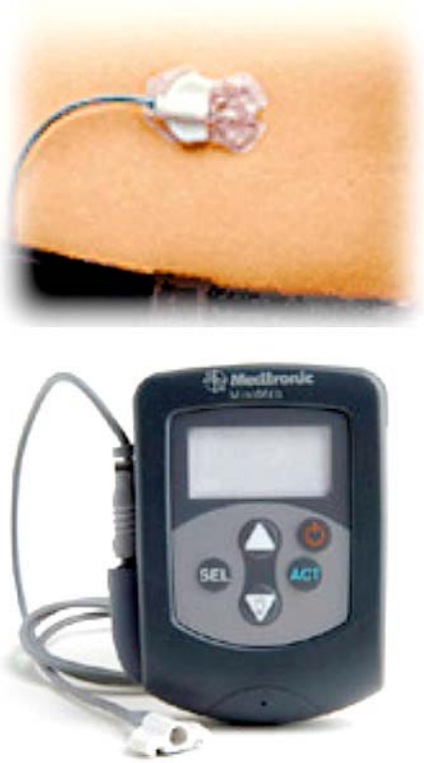

Figure 9. (Upper): Medtronic/MiniMed continuous Subcutaneous Glucose Sensor and (bottom): a continuous glucose monitoring system – CGMS (61).

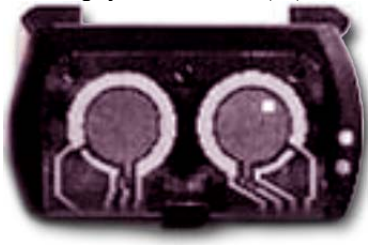

Figure 10. The extracted glucose is measured by an amperometric biosensor (AutoSensor) using detection of H_2O_2 generated by the glucose/glucose oxidase reaction (63-65).

functions in the intensive care unit or operating theatre to monitor the surgical intervention. The biosensors developed for the retinal prosthesis should be preferable in micro or sub-micro scales due to the limited space available on implantable electrode arrays and should be suitable for acute or chronic implantation.

Most commercial biosensors and implantable biosensors are for the detection of glucose. Some of such glucose biosensors are reviewed in this paper. The strategies and techniques successfully utilized in these sensors as well as some limitations or drawbacks are of great interests in the development of microbiosensors for visual implants.

Glucose detection inside the eye is potentially important for the development of retinal implants. The nutrition supplies including glucose for retina are provided by both choroidal and the retinal circulation. An exceptional high rate of glucose metabolism inside the retina was reported (42) and this may be the cause for lower glucose concentration in the vitreous humor than that in plasma (see Table 2). A clinical study suggested that chronic implantation of retinal arrays likely obstructed the nourishment to the retina and caused the both inner and outer retina damage (25). Closely monitoring the glucose concentration changes during retinal stimulation and array implantation will reveal such blockage of nourishment.

4.3.1. Subcutaneous continuous glucose sensors

The measurement of glucose in diabetic patients by means of *in-vivo* biosensors could be important in optimizing insulin therapy thus avoiding or at least delaying diabetic complications. Continuous glucose monitoring makes it possible to detect a greater number of hypoglycemic events.

While blood glucose has traditionally been the analyte of choice in defining and managing diabetes, other measures may in fact have even more clinical importance. Subcutaneous sensors measure the interstitial glucose concentration rather than that of blood (58-59).

In 1999 the Food and Drug Administration (FDA) approved a continuous glucose sensor (continuous glucose monitoring system (CGMS)), made by Medtronic MiniMed (Northridge, CA), which utilizes a subcutaneous needle electrode and measures glucose by an amperometric method (61-62). Figure 9 shows the needle type subcutaneous sensor and a pager-sized controller (*www.minimed.com*). The sensor is attached to a sterile 22-gauge needle that is removed after sensor insertion in the subcutaneous tissue. The biosensor is based on the glucose oxidase and hydrogen peroxide system. Interstitial glucose is converted by the glucose oxidase to produce hydrogen peroxide, which is oxidized on a platinum electrode to generate an amperometric response. The sensor has a lifetime of 2-3 days and measures interstitial glucose every 10 s. This signal is reported as an average glucose concentration every 5 minutes (total of 288 readings/day). Such a small sensor could be used to monitor glucose in the eye.

4.3.2. Glucose sensor based on reverse iontophoresis

In 2002 the FDA approved, for use in children, the GlucoWatch Biographer, manufactured by Cygnus (Redwood City, CA), which also measures interstitial glucose concentration sampled through a process called reverse iontophoresis (63-65). Iontophoresis is a technique whereby a low-level electric current (0.3 mA/cm^2 in these studies) is passed through the skin between an anode and a cathode (Figure 10). The current is carried primarily by the migration of sodium ions toward the cathode. Uncharged molecules (e.g., glucose) are carried along by convective transport (electroosmosis).

The GlucoWatch provides frequent, automatic, and noninvasive glucose measurements up to three readings per hour for as long as 12 h after a blood glucose

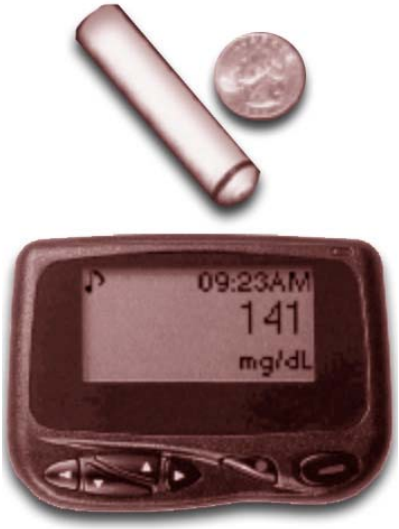

Figure 11. Upper: Implantable glucose sensor the size of a AA battery. Bottom: The receiver is an externally worn pager-sized device. Sensor glucose data are transmitted wirelessly from the sensor to the receiver (66).

measurement for calibration. The amount of glucose extracted at the cathode has been demonstrated to correlate with blood glucose in diabetic patients. In the biographer, the extracted glucose is measured by an amperometric biosensor using detection of H_2O_2 generated by the glucose/glucose oxidase reaction. With some modifications, such biosensors may be used to measure glucose inside the eye or through a flow cell.

4.3.3. Chronic implantable biosensor

A long-term implantable glucose sensor providing continuous real-time data has been developed by DexCom (San Diego, CA). It was implanted for six months just under the skin in the abdomen of 15 adults with type 1 diabetes (66). The study describes the impact of presenting continuous real-time glucose data at home to 15 adult patients with type 1 diabetes.

The sensor is about the size and shape of an AA battery. The control unit is an externally-worn, pager-sized receiver that received wireless-transmitted information (see Figure 11). The sensor was implanted in the subcutaneous tissue of the abdomen in 15 patients with type 1 diabetes in an outpatient procedure under local anesthesia.

After the sensor start-up period and calibration, the receiver calculates glucose measurements in mg/dl or mmol/l every 5 min. The data can be displayed to the patient in real time on the receiver as a number (in mg/dl or mmol/l) and as glucose trend graphs. The sensor device was not only capable of providing study participants continuous glucose level data, but it could also provide auditory or vibratory alerts when glucose levels were high (more than 11.1 mmol/l; 200 mg/dl), or low (below 5.6 mmol/l; 100 mg/dl).

Although it is a totally implantable sensor system, a disadvantage of this sensor is that, with its implantable electronics and battery, it is much larger than the percutaneous needle type sensor. The bulk size of the sensor may prevent ready adaptation to visual implants, especially retinal implants due to the limited space inside eye.

4.3.4. Glutamate sensor

Glutamate is the main excitatory neurotransmitter in the central nervous system, and plays an important role in neurodevelopment and neurodegenerative disorders during aging. Studies on neural stimulation have established the link between the activation of neurons and the release of L-glutamate, a neurotransmitter in the retina (41-42, 67-68).

The key to adequate neuronal stimulation requires control over spatial and temporal neurotransmitter delivery. A group of researchers from Wayne State University have been employing caged, phototriggered neurotransmitters in this role (68-70). The macro-molecules caged glutamate are activated within nanoseconds by light, delivered via a microfluidic/optical neurotransmitter delivery system. However, high levels of glutamate have demonstrated toxicity, in some instances (41-42). A group at Stanford has been pursuing this approach (71).

Most amperometric glutamate biosensors are operated in a similar way as the popular glucose biosensors (56). The glutamate electrode measures dissolved glutamate. L-glutamate oxidase is chemically immobilized in a membrane which is in contact on one side with the sample solution and on the other with the amperometric electrode. L-glutamate diffuses into the enzyme layer where it is oxidized by the enzyme according to the reaction:

$$\text{L-glutamate} + O_2 + H_2O \rightarrow \alpha\text{-ketoglutarate} + H_2O_2 + NH_3$$

Both the decrease in oxygen and increase in hydrogen peroxide at the electrode surface are directly proportional to the L-glutamate concentration in the sample solution.

A research group in Japan is using a micromachining technique to fabricate a miniaturized glutamate sensor that performs *in-vitro* glutamate monitoring (72). Figure 12 shows the structure of a dual channel microfabricated glutamate sensor for the direct assay of L-glutamate in a flow injection system working at a potential of -50 mV vs. Ag/AgCl. Electrode characteristics were obtained and compared with carbon paste electrodes based on different peroxidases [horseradish peroxidase (HRP) or fungal peroxidase (ARP)], co-immobilized with L-glutamate oxidase (GlOx) using different immobilization techniques.

The neurotransmitter released from cultured rat cortex neurons by stimulating the cells with 100 mM of KCl solution was measured and shown in Figure 13. By comparing two currents at a dual electrode cell

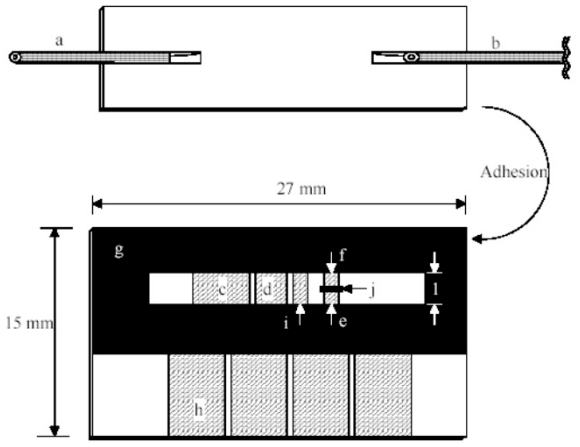

Figure 12. Structure of a dual channel glutamate sensor. (a)(b) sampling or outlet capillary; (c) counter electrode; (d) reference electrode; (e) working electrode 1; (f) working electrode 2; (g) THB photoresist film (20 μm thick); (h) 4 pads to potentiostats; (i) photoresist; (j) microseparator (72).

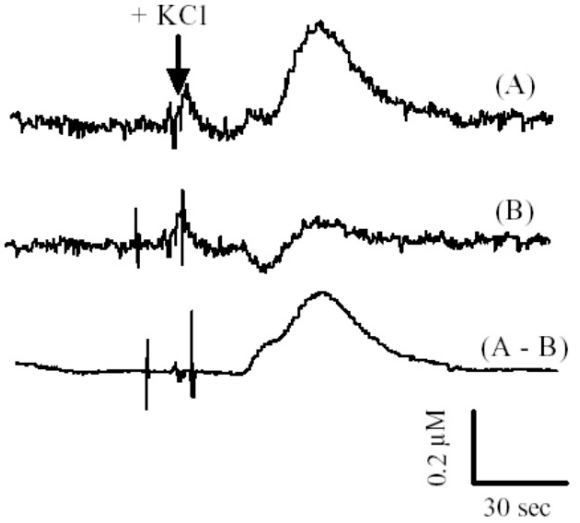

Figure 13. Variations in the glutamate concentration (72) from cultured rat cortex neurons stimulated by KCl obtained using the dual microfabricated glutamate sensor. (A) shows the current at the working electrode modified with BSA-GluOx/Os-gel-HRP bilayer films. (B) shows the current at the working electrode modified with BSA/Os-gel-HRP without containing GluOx. (A-B) shows the trace when trace (B) was deducted from trace (A).

modified/unmodified with glutamate oxidase, they were able to eliminate the baseline fluctuation and pumping noise, and observe the transient glutamate release from rat nerve cells. They demonstrated the ability to continuously measure glutamate with high selectivity and reliability in a differential measurement mode using their dual working electrode microfabricated biosensors.

Other miniature or micro-biosensors such as the wire type or needle type electrodes for glutamate have been reported. O'Neill *et al* recently described glutamate biosensors based on noble metals Pt, Au wires (1.6 mm in diameter) and Pd, glass carbon disks (3.0 mm in diameter) to detect H_2O_2 (73). The electrode response is compared for two different surface modification configuration: glutamate oxidase cross-linked onto poly(o-phenylenediamine) and GluOx/horseradish peroxidase/redox polymer.

A research group from University of Kentucky reported a micro-fabricated multi-site electrode for *in vivo* measurements of glutamate (74-75). The ceramic-based microelectrodes are triangular in shape, 1 cm in length and taper from 1 mm to a 2–5 μm tip. The biosensor presented a low detection limit for glutamate to ~0.5 μM. The microelectrodes have been used to study glutamate uptake and release in rat prefrontal cortex, cortex, cerebellum and striatum.

4.3.5. Multi-analyte biosensors

In addition to the dual sensors used in differential measurement mode, multi-analyte biosensors may also need to be integrated with visual implants to detect simultaneously multi-analyte changes such as glucose metabolization processes to form lactate in the retina (42).

Wang and Zhang described a miniature needle-type *in-vivo* sensor suitable for the simultaneous amperometric monitoring of glucose and insulin (76). Such a sensor system has the capability of simultaneous monitoring of both glucose and insulin which may be used to improve management of diabetes. Figure 14 shows the integrated microsensor consists of dual (biologically and chemically) modified carbon-paste working electrodes inserted into a 14-guage needle. The glucose probe is based on the biocatalytic action of glucose oxidase, and the insulin one relies on the electrocatalytic activity of ruthenium oxide. They have demonstrated that largely differing levels of insulin and glucose can be monitored simultaneously using the needle-type combination microsensor. The multiple-analyte sensing approach can be extended to the integration of additional chemical sensors and biosensors.

4.4. Challenges in the development of implantable sensors

While many challenges exist in the development of implantable sensors (58-60), three of them will be discussed and they are biocompatibility and implant package, chronic stability of sensors and *in-vivo* calibration of implantable sensors.

4.4.1. Biocompatibility and implant package

Biomaterials are used in contact with living tissue, resulting in an interface between living and nonliving substances (77-78). All medical implants are coated or packed by biomaterials which are inert substances designed for implantation or incorporation within the human body. However, not all of the materials used inside the medical implants are biocompatible. The materials used to make medical implants such as electronic components, active metals or alloys are not biocompatible. For a reliable medical implant, especially an active device such as retinal implant, the package or coating should have

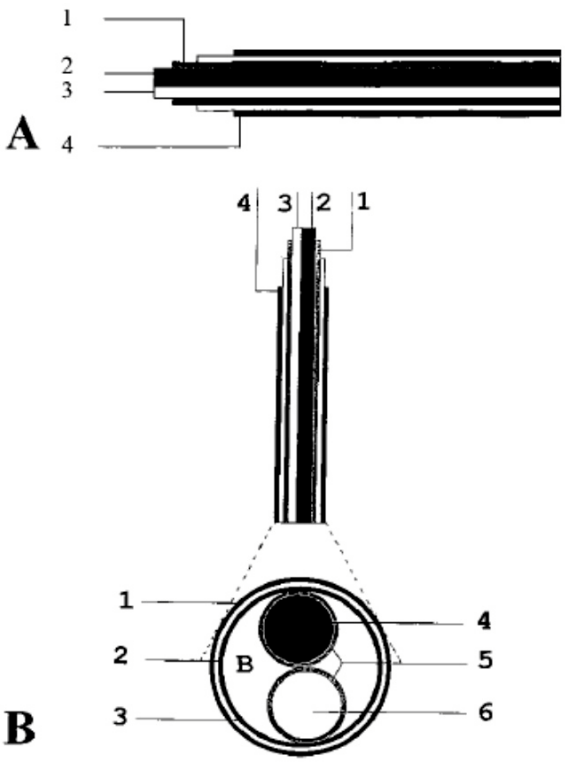

Figure 14. Schematic drawing of the integrated needle-type glucose/insulin microsensor. (A) 1, stainless steel needle body (reference electrode for glucose); 2, glucose sensor; 3, insulin sensor; 4, Ag/AgCl (reference electrode for insulin). (B) Cross-sectional view of the sensor tip: 1, Ag/AgCl; 2, insulating layer; 3, stainless steel; 4, glucose sensor; 5, Teflon tubing wall; 6, insulin sensor (76).

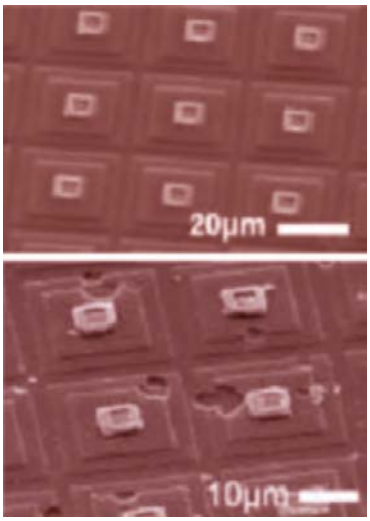

Figure 15. Micrograph comparing surfaces of the microphotodiode arrays before and after 10 months of implantation in the rabbit eye. The microscopic damage of the silicon oxide passivation layer and pitting corrosion of the underlying silicon is clearly visible. The microphotodiode arrays are manufactured on a silicon wafer using CMOS process technology (47-48).

a life time of 40 years. Should this coating or package fail during the implantation, toxic materials may leach out and cause possible tissue or neural damage. The leakage may also cause the failure of electronic devices inside the implants.

The technology of microelectromechanical systems (MEMS) has resulted from combining the microfabrication processes (integrated circuit manufacturing processes) with innovations in micromachining techniques, such as wet and dry etching processes. This combination has provided a powerful set of tools for batch processing and miniaturization of micro-systems. Silicon based MEMS technology has been used to fabricate micro-electronic devices and micro-electrode arrays of medical implants. Coating of MEMS devices to protect them from corrosive saline's attack is a difficult task for a medical implant. The package must enable these devices to withstand the body's harsh environment.

In vivo experiments (47-48) revealed a decay of the passivation layer of the device when implanted for less than a year. Figure 15 shows the micrograph comparing surfaces of the Microphotodiode arrays before and after 10 months of implantation in the rabbit eye. Microscopic damage of the silicon oxide passivation layer and pitting corrosion of the underlying silicon is clearly visible. These microphotodiode arrays are manufactured on a silicon wafer using CMOS process technology similar to those made by Optobionics (25-27).

4.4.2. Long-term stability of implantable sensors

The second challenge for the implantable sensors is long-term stability. In contrast to the excellent stability and sensitivity of most sensors' function *in-vitro*, a reduction in sensitivity occurs after implantation, with a resulting rapid decrease *in-vivo* signal followed by complete sensor failure within hours or days. The membrane material or enzyme used for sensor preparation seems to be a major cause for the loss of sensitivity, resulting finally in a loss of sensor function (79).

If long-term sensing is to be achieved, a sufficient reserve of bio-components must be contained in a sensor or ready to be replenished. The stability of such stored bio-components also greatly affects the life-time of the implantable biosensors.

4.4.3. Variation of analyte and *in-vivo* calibration of implantable sensors

The perturbation of the co-substrate or analyte concentration is another hurdle for the implantable sensors in continuous biosensor operation. A typical example is the oxygen concentration (as a co-substrate or as an analyte) variation in glucose oxidase based glucose biosensors. To overcome such problems, one approach is to use a glucose dehydrogenase (GDH) enzyme for conversion of glucose to measurable redox equivalents. This enzyme has some advantages in electrochemical systems over the more widely used glucose oxidase, which is sensitive to the variation in oxygen content of the blood sample. In fact, the GDH has been used in TheraSense's FreeStyle® blood glucose monitoring system and Roche's Accu-Check Advantage® glucose system (80).

For an implantable sensor, the background current *in-vivo* is likely to be higher than *in-vitro* due to current produced by electrochemical interferents. When the bioagents's activity is lowered, the sensor's response slope will be changed too. How to initially calibrate the device, and when and how to recalibrate it *in vivo* become one significant problem in using an implantable sensor.

In practice, a one point or two-point calibration process is used to "update" the sensor's calibration curve. The implantable biosensor, made by DexCom Inc has used a commercial glucose system to calibrate the implanted sensor (66). After sensor implantation, patients were asked to take a minimum of two self-monitored blood glucose (SMBG) values per day using a commercial glucose meter. The SMBG blood glucose data are electronically uploaded to the receiver and used to calibrate the transmitted sensor glucose signal. A similar approach is used for the glucose sensor of GlucoWatch by Cygnus (63-65).

When using SMBG blood glucose data to recalibrate the implantable sensors, the relationship between glucose level in the blood and in the tissue needs to be understood. A study was conducted to establish the time-lag for the glucose values in blood and in tissue. It was found that a consistent time difference of 4-10 min between blood glucose and interstitial glucose in subcutaneous abdominal tissue (62). Such time differences should be taken into account in the recalibration of sensors and in the design of a closed-loop system. The report suggested that the time difference may not be due to a "lag time" as defined by physiological processes, but rather the response characteristics of the glucose sensor system. MiniMed's CGMS sensor systems and a YSI glucose analyzer (YSI 2700 Select; YSI, Yellow Springs, OH) were used in the study.

5. PERSPECTIVE

Microsensors and microbiosensors reviewed in this paper are not specifically designed for visual implants. One of the authors' motives to introduce the visual implants to researchers in the sensor development fields is to encourage them to develop suitable sensors which will be ready for the incorporation with the visual implants or be suitable for the *in-vitro* and *in-vivo* tests during the development of such medical implants.

Smaller and thinner electrode arrays with flexible polymer substrates to follow the curvature of the retina are the main trends in the development of micro-stimulating electrodes for retinal implants. Planar array configurations with a 3D micro-electrode structure to increase the charge injection capability will be one of the main focuses for many researchers in this field. Using novel nanotechnology combining with well established MEMS methods will produce batch fabricated, low cost electrodes for neural stimulation and for real-time or instantaneous measurement of chemicals and biochemicals inside the eye.

A Food and Drug Administration (FDA) approved prototype Intraocular retinal implant developed by Second Sight (Sylmar, CA) has been chronically implanted in 6 patients over the past two years as part of a limited clinical trial conducted by Doheny Eye Institute (University of Southern California, Los Angeles). The prototype device currently implanted in the patients has a 16 electrode array. Higher density devices are needed for higher precision activities. We expect to implant this type of high density electrode device in patients within five years.

Newer models of retinal implants will have higher resolution and someday may allow patients to read, watch television, and recognize faces. Ultimately, a sensor system with implantable microsensors and microbiosensors will be integrated into the device to form a closed-loop "smart" retinal implant.

6. ACKNOWLEDGEMENT

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