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Retinal Implants: Improvements based on Micro-Nano Technologies and Optimization toward Localized Stimulation.

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Abstract

Several interdisciplinary research teams worldwide are currently developing promising retinal implants to offer new solutions to restore functions in patients with severe retinal degenerations leading to visual impairment. Together progress in neurosciences, especially related to visual pathologies understanding, and micro nano technologies improvements created the proper conditions to obtain the first retinal prosthetic (Argus I & II, Retina Implant AG, …). Our research work is dedicated to performances improvement, mainly long term bio-stability and life time, and high resolution. One way consists on using more resistant and biocompatible materials like diamond, together with three dimensional geometries for long term intimate connection between electrodes and tissues. A second way relies on electrical stimulation focalization related to better resolution. Electrochemical and electro-physiological in-vitro characterizations of our new implants were performed followed by several in-vivo experimentations.

Key words

Retinal implants, neuronal prosthetics, micro-technology, micro-electrode-arrays (MEAs), diamond electrodes, electrical stimulation, biocompatibility.

1. Introduction

A. General context of the study

Blindness comes from different retinal pathologies, leading either to degeneration then irreversible loss of photoreceptors (cones and rods), or progressive loss of ganglion retinal cells suppressing the link between the retina and the brain. As photoreceptors disappear, the two main neuronal retinal cell layers, i.e. bipolar and ganglion cells, may survive and specific retinal prosthetics allow some partial vision rehabilitation. Electrical stimulation of the visual system required in rehabilitation strategies can be obtained through different positions along the sensory pathway. Indeed, it is possible to directly stimulate the retinal cells (Sekirnjak, 2006), the optical nerve (Veraart, 2003; Brelén, 2010), or the visual cortex (Brindley, 1968) in case of total degeneration of the sensory organs like the retina and the optical nerve, Figure 1.

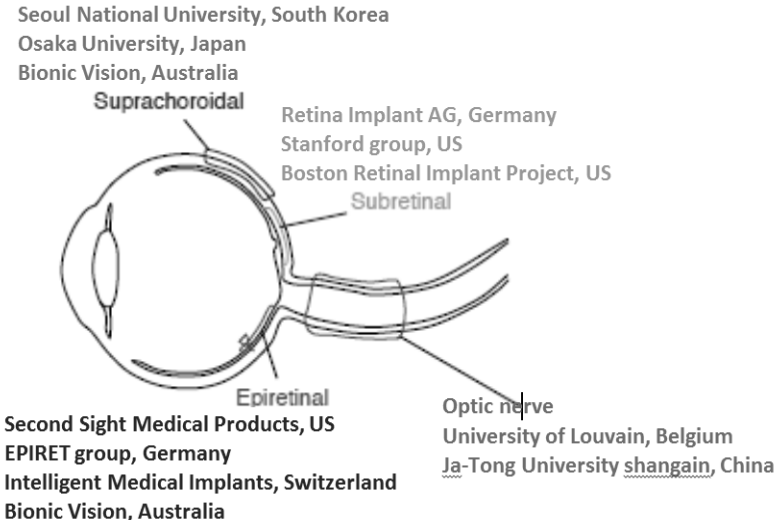


Figure 1. Illustration of visual rehabilitation strategies (Lorach, 2012)

In case of direct retina stimulation, two options are usually studied: (i) sub-retinal position, where the implant is carefully positioned at the photoreceptors layer, stimulating remaining active bipolar cells, although surgery is challenging with a risk of poor re-attachment of the retina; (ii) epi-retinal position, where the implant is positioned in contact with the ganglion cells linked to the visual cortex, Figure 2. Considering retinal diseases like pigmentary retinopathy or age related macular degeneration, the whole visual prosthetic system is composed of several components: a miniature camera located on glasses, an acquisition and digitization stage with

embedded signal processing providing the conversion of the light signal into electrical stimulation, a micro-electrode array in contact with the tissues of interest, Figure 3.

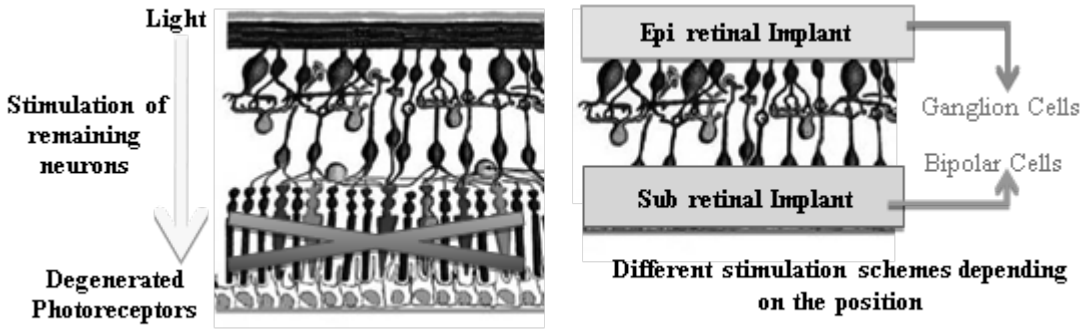


Figure 2. Stimulation principle, with epi or sub retinal positions.

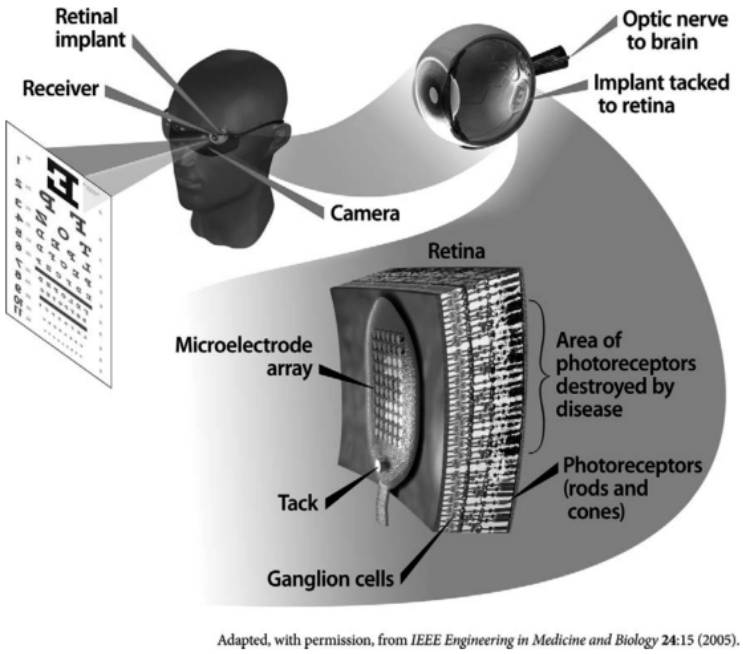


Figure 3. Principal components of a visual prosthesis: microelectrode array, visual and signal processing interface.

The signal processing unit required to convert the recorded image from the micro-camera into an efficient electrical stimulation signal is highly dependent on the choice of the implants, i.e. epi-retinal, sub-retinal, based on optical nerve or visual cortex direct stimulation. Although neuronal plasticity of the brain has been assessed, the best strategy appears to stimulate the retinal cells because the training phases after implant surgery will always be improved (Fregnac, 1988; Bavelier , 2002; Merabet, 2005). Our retinal prostheses are therefore based on the remaining retinal cells stimulation allowing neuro-bio signals to transmit through the optical nerve to the

brain to create a virtual image. At present time, several competitive companies developing retinal implants worldwide are ready or close to proceed to clinical tests: (i) Second Sight, United States, the pioneer in the field, has launched Argus I in 2002 and Argus II in 2007, obtaining later the FDA approval and EC label (Chader, 2009); (ii) Retina Implant AG, Germany, is working since 2005 on implants with 1500 micro-photodiodes (Zrenner, 2011); (iii) Intelligent Medical Implants, Switzerland, in 2009 (Hornig, 2007); Pixium Vision, France, develops since 2012 Iris 1 implants with clinical trials operated on 2013.

B. Study positioning

We have chosen the sub-retinal position to benefit from the best contact between the electrodes and the bipolar cells still active after the photoreceptor degeneration. We tested different geometries, including planar and tridimensional structures, as some preliminary studies in our group were suggesting a good integration of the bipolar cells into 3D topologies (Palanker, 2004). Moreover it was demonstrated that the stimulation thresholds should be lower in sub-retinal position because of the closer contact between electrodes and tissues (Jensen, 2007). In this paper, we will present the main issues of such visual neuro-prosthetics and the related non solved problems. We will propose our solution to optimize the implants versus a localized stimulation of the bipolar cells criteria. We will describe the technological developments concerning the micro-electrodes constituting the implant, which are highly based on micro and nano technology improvements as well as on the introduction of new biocompatible materials. We will continue with in-vitro and in-vivo characterization description and results.

2. Main issues

Psychophysical studies (Sommerhalder, 2004) have demonstrated that a minimum of 600 micro-electrodes was required to obtain 600 pixels images necessary to execute quite basic daily life tasks like visage recognition, text reading, or autonomous displacement into new environments. The visual acuity improvement depends on the effective stimulated surface of the retina. A first improvement consists on increasing the number of micro-electrodes together with high density arrays, leading to the individual electrode size reduction. But a very high electrode density is not efficient if these micro-electrodes are not independent from each other. Indeed, during electrical stimulation, a current is flowing from the active electrode representing one pixel to a counter electrode or to a ground electrode, while the retina is equivalent to an non homogenous conductive material. Depending on the implant geometry, the electrical field can

diffuse largely over the activated electrode (Joucla, 2009) and induced associated electrical coupling will limit the real resolution.

In parallel, it is mandatory to better control the bio-electrical interface existing between the electrodes and the retinal tissues, remembering that two different types of stimulation current can appear: (i) faradic currents where redox reactions may occur and (ii) capacitive currents corresponding to the accumulation of mobile charges. In the case of neuro-prosthetics, capacitive currents are preferred as they do not induce surface modification of the electrodes neither pH variations in the tissues (Cogan, 2008). The charge density also increases significantly when the size of the electrodes decreases, opening the way to cells degradation in the vicinity of these electrodes. Another improvement option is to work with new biocompatible materials allowing higher charge injection limits than traditional materials used in neural implants, like platinum with 0,35mC/cm², titanium nitride with 1mC/cm², or iridium oxide with 4mC/cm² (Robblee, 1980). We oriented our studies to use boron doped synthetic diamond to fabricate some categories of implants (Bongrain, 2011; Kiran, 2012), as it will be detailed in the next section.

3. Technological developments

A. Technological choices

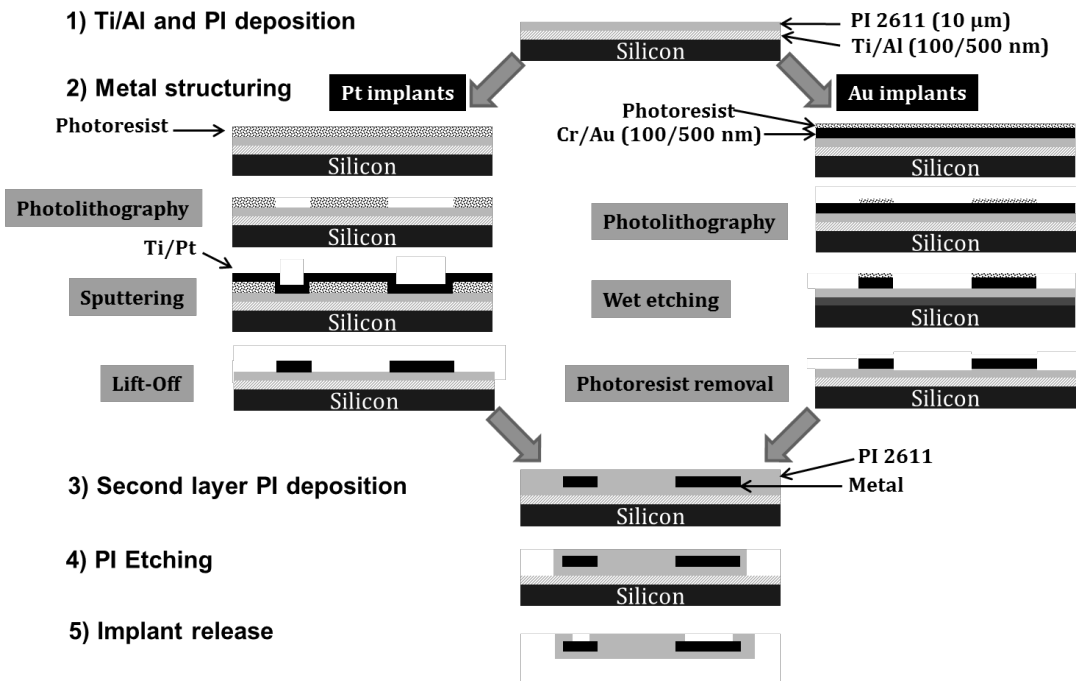
Different improvement approaches exist to solve partly issues previously introduced but we have chosen to focus our research on two points: (i) enhancement of focal electrical stimulation by tuning parameters such as size and configuration of micro-electrode arrays with a ground grid (Joucla, 2009) and the use of tridimensional electrodes; (ii) increase of charge injection limit with synthetic doped diamond as electrode active material.

Tridimensional diamond electrodes interest lies in the possibility to avoid glial cells layer (~20-µm thickness) which appears inevitably at the electrode surface after implantation and which constitutes an insulating layer between neurons to be stimulated and the electrodes. Bringing closer neurons to electrodes allows reducing injected current for a same visual acuity aimed. Moreover, we have demonstrated that the selected 3D well-shaped configuration enables to localize bipolar cells into these wells precisely where stimulating electrical field is confined (Djilas, 2011). Diamond interest results mainly from its properties: (i) wide electrochemical potential window (~3V) promoting stimulation by capacitive currents, (ii) carbon-terminated giving biocompatibility properties (Bendali, 2013), (iii) high corrosion resistance allowing long implants lifespan without degradation of the material (no fouling phenomenon of the electrodes).

Therefore, we have produced several successive series of implants: planar with a grid ground, with or without diamond to check the material influence (biocompatibility), tridimensional with different materials to validate on one hand, the confinement of bipolar cells within well shaped structures and on the other hand, the technological feasibility of such geometries.

B. Planar retinal implants

We have developed test structures of 4 electrodes in order to validate the whole steps: microfabrication (process optimization and comparison of materials), surgery (on P23H rat, model of retinitis pigmentosa) and in-vivo impedance measurements for the post-surgery follow-up. We also have developed implants with 16, 32 and 64 electrodes to validate the integration of higher electrode density arrays. Planar retinal implants in platinum or in gold were realized on flexible substrates in polyimide, material proved biocompatible (Seo, 2004; Rousche, 2003). There are composed of a metal layer (electrodes, leads and contact pads) between 2 polyimide layers, Figure 4.



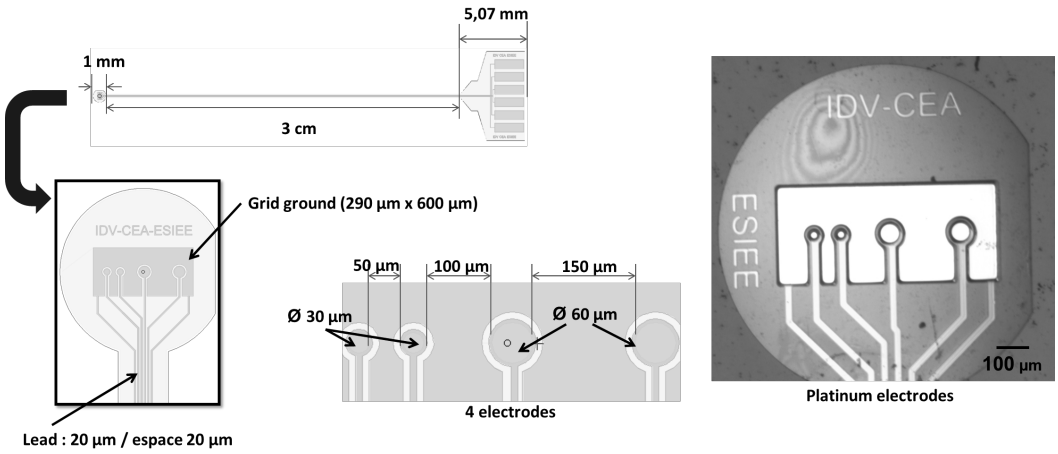


Figure 4. Fabrication process of planar retinal implants (top) ; design of test structures of 4 electrodes and example of achievement (bottom)

The fabrication process of planar retinal implants in diamond is original because it combines two materials, polyimide and diamond, which seem incompatible due to the high temperature growth of the diamond that polyimide would not stand. We have developed and patented (Bergonzo, 2011) an alternative solution allowing to have flexible implants with diamond doped micro-electrodes, Figure 5.

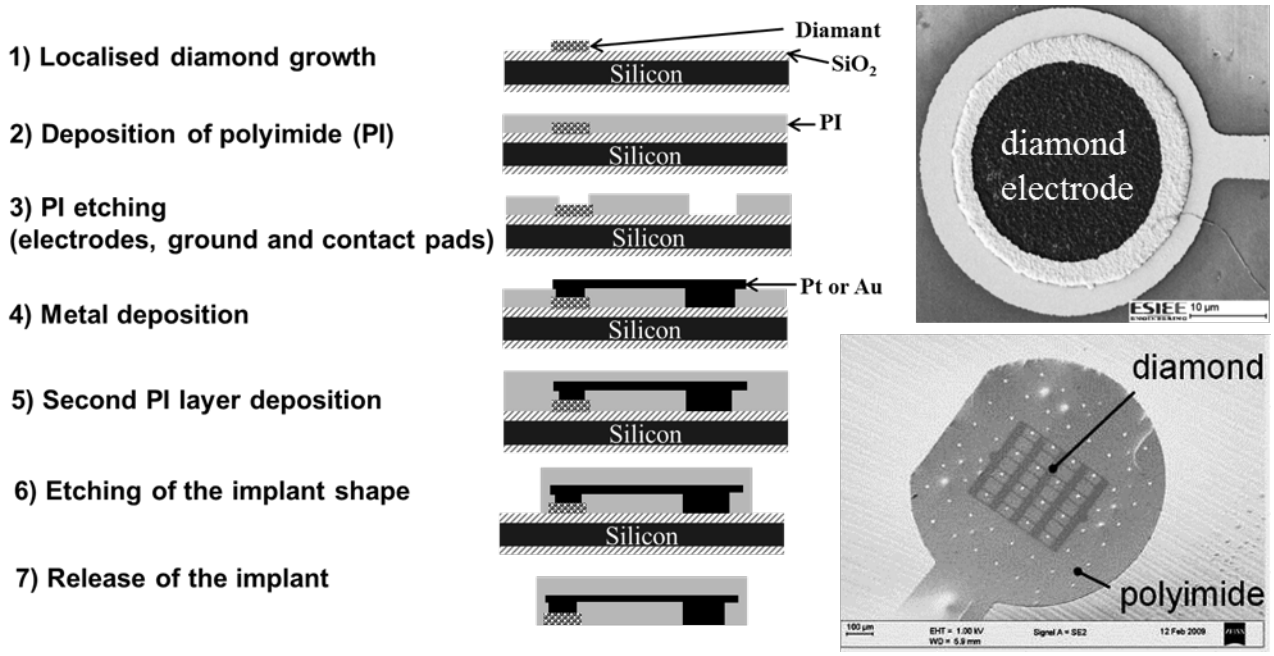


Figure 5. Fabrication process of planar retinal implant in diamond (left) ; achievement of an implant and zoom on an electrode (right)

C. Tridimensional retinal implants

In order to approve the 3D geometry, we successively have fabricated implants in polyimide then with platinum or diamond doped electrodes. The fabrication process, Figure 6, is

more complex than the planar process because a silicon mold is needed to define the shape and the depth of the wells (30 µm). This step is achieved with an isotropic wet etching (in a potassium hydroxide solution) followed by an oxidation of the surface.

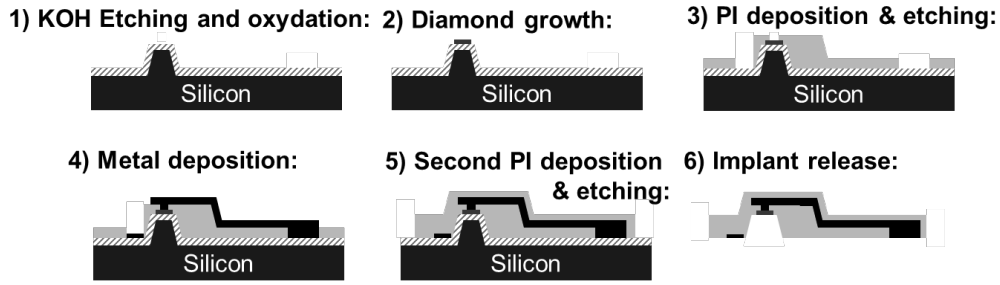


Figure 6. Fabrication process of 3D implants with diamond electrodes

We have then developed a solution which diamond growth is selective and localized with a thickness of about 300 nm. Platinum is however deposited by a standard technique of lift-off. A 10-µm layer of polyimide (PI2611) is then obtained by spin-coating followed by a bake under nitrogen atmosphere according to a temperature cycle of 6 hours that could reach 350°C. A specific step of photolithography is required to define the shape of the implant. Implants are finally released by etching the sacrificial oxide layer. Examples of achievements proposed on figure 7, show initial silicon molds used to define the geometry of wells originate from numerical modeling (Djilas, 2011), a complete implant with its connection and the corresponding zoom and an implant obtained in full polyimide.

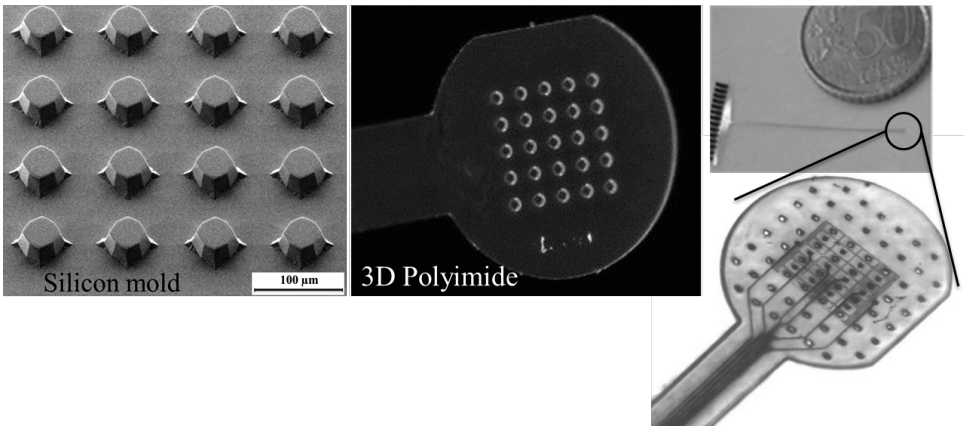


Figure 7. Examples of achievements

4. In-vitro and in-vivo characterizations

A. In-vitro characterizations

We have performed several characterization experiments: (i) electrochemical measurements to check interface properties of the micro-electrodes, (ii) impedance measurements depending on

frequency to check electrical properties of the micro-electrodes, (iii) histological measurements to check the subretinal positioning and the biocompatibility, (iv) electrical activation tests of the retina with our manufactured implants.

Electrochemical measurements are used to study electrode/electrolyte interface properties (before implantation) then electrode/tissue interface (after and during implantation). They were performed with a potentiostat (Autolab PGSTAT12) equipped with a three-electrode set-up (counter-electrode, Ag/AgCl reference electrode and working electrode) which measures the potential between the working and the counter electrode. Measurements are performed in a physiological solution, PBS (Phosphate Buffer Saline), with a pH of 7.2, close to extracellular medium. Cyclic voltammetry graphs performed in PBS give access to the potential window of the material, figure 8, and show that diamond presents a wider potential window (3V) than platinum (2V). Moreover, diamond exhibits a lower background current compared to platinum. For preliminary impedance measurements, we also used the NanoZ from MultiChannelSystem interfaced with Matlab for its low current test allowing fast impedance measurements of several sites for a chosen frequency of 1 kHz corresponding to neural signals.

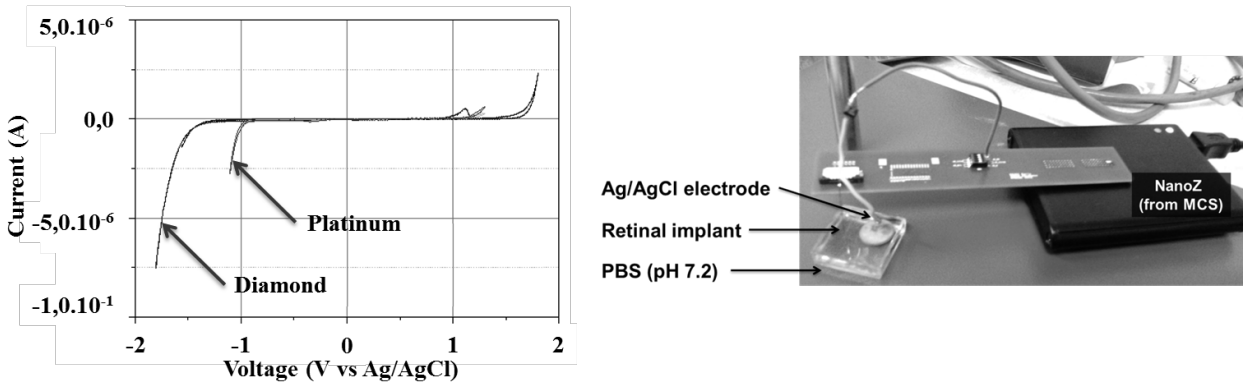


Figure 8. Cyclic voltammetry curve in PBS (sweep rate of 0,1 V/s) of two different electrode materials (left) and measurement set-up with the NanoZ of retinal implants in PBS (right).

Moreover, in order to test the ability of these implants to activate physiologically retinal tissues, we have carried out in-vitro experiments where ganglion cells activity (action potentials) is measured by an array of 252 electrodes (Marre, 2012) while retinal implant is on the other side of the tissue, in contact with bipolar cells (subretinal stimulation, left board). Stimulating electrically bipolar cells with an implant can determine if this one is able to trigger a physiological response within the retina (figure 9). Thus these tests reveal our ability to activate biological tissues so that the brain receives information sent by the implant, figure 9. We have demonstrated that, further to an electrical stimulation by the electrodes of the implant, we

observed an increase of an average discharge rate from the ganglion cells (right board) which means that ganglion cells notify this stimulation by action potentials sent to the brain.

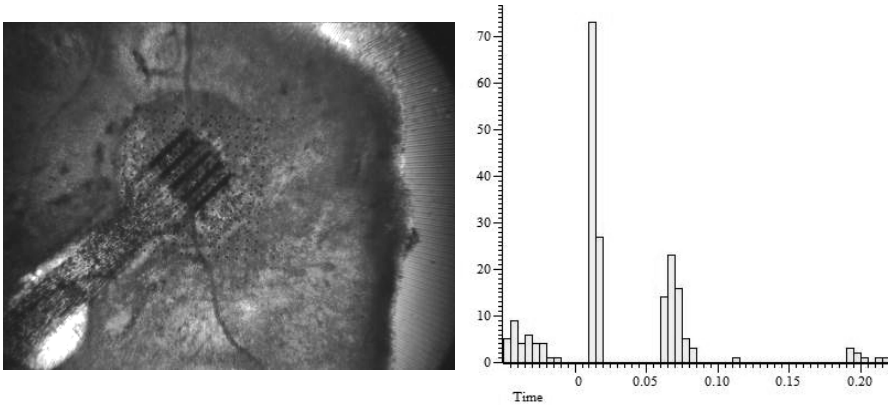


Figure 9. Left : exemple of experiment where the retina is recorded by an array of electrodes and stimulated by an implant. Right: Average discharge rate (%) of a cell depending on time with time 0 corresponding to the electrical stimulation by the implant.

B. In-vivo characterizations

In-vivo analysis has been carried out with P23H rats considered as model for retinitis pigmentosa. Indeed, they exhibit a slow degeneration of rods photoreceptors, followed by a gradual loss of other retinal neurons, close to clinical cases assessed on patients (Machida, 2000). We have tested to increase electrode density from 16 electrodes to 64 electrodes, figure 10. It appears that the size of the 64 electrodes implants becomes too large compared with the place available in the eye of the rat. We have thereafter lead in-vivo studies with our simple structure of 4 electrodes (similar to the ones on figure 4) allowing us to follow impedance evolutions of the 4 electrodes before and after implantation during several weeks as illustrated in figure 11.

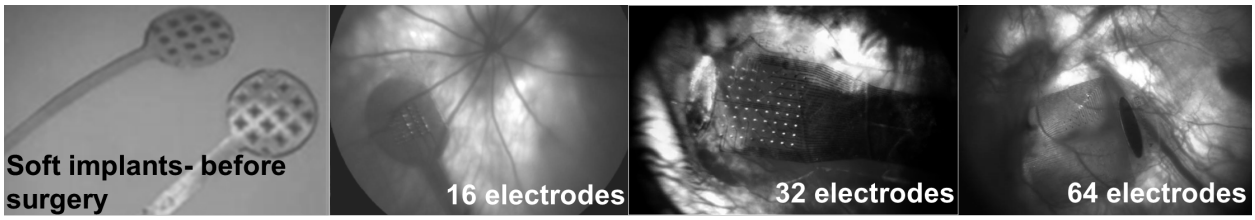


Figure 10. Pictures of flexible implants before implantation then 16 to 64 electrodes implants placed in subretinal position in rat P23H eye.

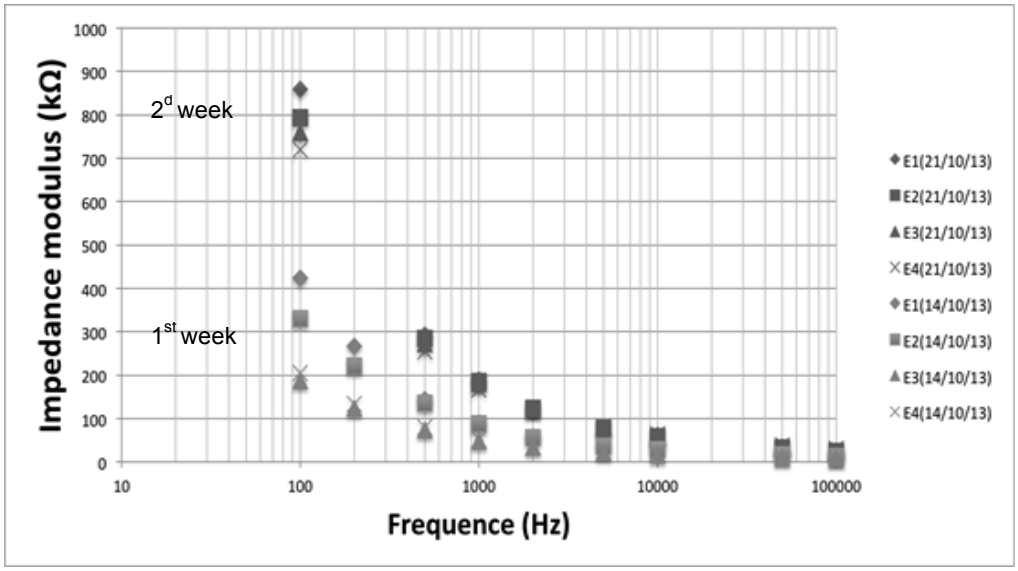


Figure 11. In-vivo recordings (here with the NanoZ) and impedance measurements (with the LCRmeter HP4284) after implantation, at one week interval.

We have finally in parallel validated the advantage of tridimensional structures in which retinal bipolar cells fit very well in the 3D topology. We have noticed a better stability of the interface implant/tissue with 3D structures compared to planar structures. A few measures by cell counting allow evaluating the ratio of the bipolar cell number among the total number of cells inside the wells comprised between 44% and 66%, figure 12.

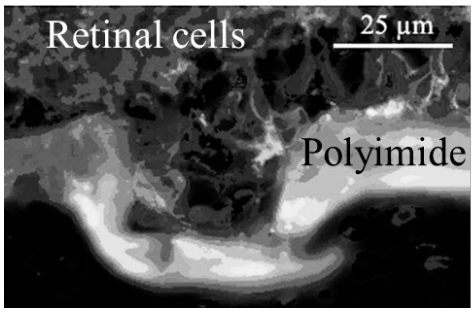


Figure 12. Illustration of the retinal neurons integration into 30-µm wells.

5. Conclusions and perspectives

We have here shown a certain number of issues related to visual neuro-prosthesis insisting on the contribution of micro and nano-technologies to solve some raised problems, especially the localization of stimulations. We have presented our results on several types of implants designed to be inserted in subretinal position so that the best contact between electrodes and bipolar cells can be achieved. Different geometries have been tested and characterized in particular with planar or tridimensional implants. This study confirms the interest of well-shaped structures to improve visual resolution. Indeed, this approach should thus allow stimulating independently neurons

isolated within each well corresponding then to independent pixels. A comparative analysis between planar and 3D implants remains to be done on more experiments to validate the focalization of the stimulation. We have also noticed that the use of synthetic doped diamond appears to be a material of choice for the electrodes of these new neuro-prostheses. About twenty implants with planar electrodes in platinum or diamond, as well as tridimensional implants have been implanted in rats carrying the P23H mutation where the retina expresses a degeneration of photoreceptors. These experiments on our implants allowed the stabilization of surgery steps and the initial in-vivo characterizations procedure validation.

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