Retina Implant Project
Chronic Implantation of Active Epiretinal Implants

Dissertation

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by

Eduardo Büchele Rodrigues

Department of Ophthalmology
Philipps-University Marburg

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Statement of originality

"I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma of this university or other institute of higher learning, except where due acknowledgement has been made in the text. The animals experiment that provided the data for the investigations in this dissertation were jointly conducted with PD Dr. Lutz Hesse and Dr. Thomas Schanze".

Eduardo Büchele Rodrigues

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1. Introduction

1.1 Normal Vision

Due to ethical reasons, it has not yet been possible to accomplish experiments of the retina implant project in human beings. The cat is known to be a reliable model to record cortically the electrical stimulation to the retina, and was therefore chosen for the experiments described in this dissertation. It is useful to clarify what features differentiate humans and cats as far as the structure and function of the visual system are concerned. The complex anatomy, histology and physiology of the eye as well as the visual system of humans and cats are briefly described in this chapter.

1.1.1 Eye and visual system

1.1.1.1 Structural anatomy of the eye

The antero-posterior diameter of the human eye globe averages between 22.1 mm and 24.1 mm. The cornea is the external layer and measures 11.7 mm horizontally and 10.6 mm vertically. The thickness of the cornea varies from 0.7 mm to 1.1 mm (Thomson, 1912). The anterior chamber contains aqueous humor and represents an area bordered anteriorly by the posterior surface of the cornea, posteriorly by the anterior surface of the iris and the pupil and the pupillary portion of the lens, and peripherally by the anterior chamber angle, in particular, the trabecular meshwork, scleral spur, ciliary body, and iris root. The anterior chamber contains aqueous humor, a crystal-clear fluid with a specific gravity around 1.0035. The lens lies behind the pupil, and the distance between the ciliary body and the equator of the lens is 0.5 mm. The lens axis measures about 4 mm in adult or young individuals. The vitreous body is a clear, transparent, gel-like substance that fills the entire eye posterior to the lens. It contains about 99% water, weights about 4 g, and has a volume of 4 cc (Hogan, 1963).

The retina is the inner layer of the posterior segment of the eye, which is attached to the choroid via the retinal pigment epithelium. The retinal layers measure 0.56 mm in thickness at the disk. Peripherally, the retina at the equator is 0.18 mm and at the ora serrata 0.1 mm in thickness. About 6 to 7 mm posterior to the corneoscleral junction sits the ora serrata, and is 2 mm wide on its temporal side and 0.8 mm wide on its nasal side. The fovea

or central retina is a small pit in the retina at the termination of the visual axis. The optic nerve head is just nasal and slightly superior to the fovea, at a distance of 3.42 mm from the foveola. The optic disc has an average diameter of 1.76 mm and 1.92 mm. A highly vascular tissue at the posterior extension of the ciliary body stroma is the choroid. Estimates of its thickness vary from 0.25 mm at the posterior pole to 0.15 mm at the ora serrata. Posterior to the choroid situates the sclera, consisted of dense fibrous tissue and containing openings and canals for the various vessels and nerves entering and exiting the globe. Externally, it is whitish in color. The posterior pole is the site where the sclera is at its thickest, measuring 1 to 1.35 mm in this area (Hogan et al., 1971).

1.1.1.2 Morphology of the retina

The retina consists of ten layers: the retinal pigment epithelium, rod and cone layer, outer limiting membrane, outer nuclear layer, outer plexiform layer, inner nuclear layer, inner plexiform layer, ganglion cell layer, nerve fiber layer, and internal limiting membrane (Figure 1). The retinal pigment epithelium, the most external layer of the retina, consists of 4.2 to 6.1 million cells in a uniform layer of single hexagonal in shape (Tso and Friedman, 1967). In the macula, these cells are more pigmented and measure 14 μm in diameter by 12 μm in height. At the ora serrata they measure 60 μm in diameter. Their projections are in close contact with the outer segments of the photoreceptors. The pigment epithelial cells have several functions, among which is their important role in the processing and recycling of vitamin A and the metabolism of the photoreceptors. The rod and cone layer lies internally to the retinal pigment epithelium, and there are approximately 110 to 125 million rods and 6.3 to 6.8 million cones in the human retina. The foveola is free of rods, and they first appear 130 μm from the center of the fovea. The fovea is composed of about 100,000 compactly arranged cones in an area of about 147,300 mm^2 (Yamada, 1969). The outer limiting membrane connects the cell membranes of the rods and cones to the Müller cells, and the outer nuclear layer contains the cell bodies of the rods and cones. Next in the histology comes the outer plexiform layer or fiber layer of Henle, which contains nerve fibers connecting the receptors and the first-order neurons. The inner nuclear layer contains horizontal cells, Müller cells, bipolar cells, and amacrine cells. While horizontal and bipolar cells synapse to pedicles and spherules in the outer plexiform layer, the amacrine and bipolar cells make synaptic contacts in the inner plexiform layer. As the bipolar cells are the first-order neurons for the photoreceptors, the Müller cells provide support and nutrition for the

retina. Dendritic processes of ganglion cells extend into the inner plexiform layer and axons form the retinal nerve fiber layer. The internal plexiform layer contains the axonal fibers traveling between the first-order neurons and the ganglion cells. This layer varies in thickness from 18 to 36 μm and is the site of many synapses between bipolar, amacrine, and ganglion cells.

The ganglion cell layer contains the cell bodies of the second-order neurons. The ganglion cells are generally rounded and have a diameter of 25 to 30 μm except in the macular area, where their diameter is about 12 μm. Ganglion cells are multipolar, with axons extending into the inner plexiform layer as well as laterally. There are 1.2 million ganglion cells in a normal adult human retina, and each of the axons from these cells merge to form the optic nerve. Throughout the retina, there is one ganglion cell for every 100 rods and for every four to six cones. However, in the macula, the ratio of ganglion cells to photoreceptors is higher, and the receptor field of each ganglion cell is smaller, thus resulting in greater image resolution. No ganglion cells exist at the center of the fovea. There are up to 18 forms of ganglion cells described in the human retina, and they share the basic function of transmitting a signal from the bipolar cell to the lateral geniculate body. Variations are seen in the size, degree of arborization, spread of dendritic processes into the bipolar synaptic field, and pattern of synaptic connections with amacrine cells. These anatomic differences among the ganglion cells have been shown to correlate with their electrophysiologic functional specificity, and make the stimulus of this layer so complex. Despite the seemingly independent rod and cone circuitry of the retina, only a single set of ganglion cells actually transmits retinal signals toward the brain. Thus, signals from both rod and cone photoreceptors ultimately must feed into the same ganglion cells. Transmission is from cones to cone bipolar cells to ganglion cells. The rod pathway is more circuitous as it does not synapse onto ganglion cells directly but only indirectly through the amacrine cells. Rods synapse onto bipolar cells and then onto amacrine cells that pass the signal to ganglion cells. The retina is rich in amacrine cells, and they serve as a switch that can disconnect the rod pathway from the cone pathway in bright light photopic vision, where the cone pathway must have exclusive access to the vision transmission to the cortex without interference from rod signals. The nerve fiber layer consists of the axons of the ganglion cells as they proceed from all areas of the retina toward the optic disc to form the optic nerve. These fibers are accompanied by the processes of glial and Müller cells. The most internal layer of the retina, the internal limiting membrane, is about 2.5 μm thick and constitutes the boundary between the retina and vitreous surface. The internal limiting membrane is constituted of the basement membrane produced by footplates of Müller cells, composed of collagen fibrils,

proteoglycans, the basement membrane and plasma membrane of the Müller cells (Rodrigues et al., 2003a; Schmidt et al., 2003a).

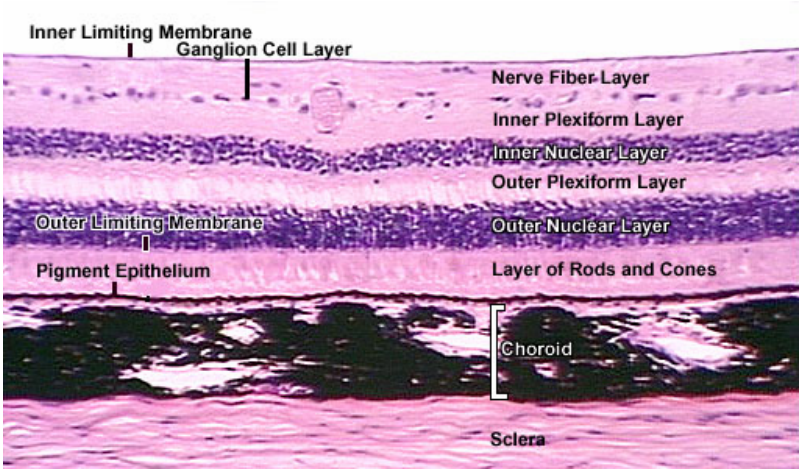

Figure 1: Ten layers of the retina. From outer layer, the retinal pigment epithelium, rod and cone layer, outer limiting membrane, outer nuclear layer, outer plexiform layer, inner nuclear layer, inner plexiform layer, ganglion cell layer, nerve fiber layer, and internal limiting membrane. Externally to the retina is the choroid.

1.1.1.3 Structure organization of the visual system

The human primary visual sensory system comprises the retina, optic nerves, optic chiasm, optic tracts, lateral geniculate nuclei, geniculostriate radiations, striate cortex, visual association areas, and related interhemispheral connections (Figure 2). The functional organization of the visual sensory system begins at the retina, where a considerable degree of retinal processing modifies neural signals before transmission to central structures. Ganglion cell subsets selectively encode specific aspects of visual information, such as contrast, color, image velocity and movement direction. In the human retina, there are four different percipient elements (rods and three types of cones), at least four types of bipolar cells, three types of amacrine cells, three types of horizontal cells, and no fewer than eighteen types of ganglion cells in addition to Müller cells. Visual function across the retina takes a pattern of concentric zones that increase in sensitivity toward the central retina area, the fovea, which subserves the highest sensitivity. Retinal ganglion cells have been classified between X, W, and Y cells (Enroth-Cugell et al., 1980). X cells subserve high-resolution pattern vision while Y cells subserve movement vision. From distinctions in soma size, dendritic morphology, alpha, beta, and gamma cells can be divided. Alpha cells correspond to Y cells, beta cells to X

cells, and gamma cells to W cells. Two different systems composed of 2 types of morphologically distinct cells (the magnocellular and parvocellular layers) appear to project to different divisions of the lateral geniculate nucleus, and this segregation continues throughout the visual pathway (Leventhal et al., 1981). The parvosystem is characterized by color opponency, high spatial resolution, and low contrast sensitivity. The magnosystem is characterized by color ignorance, low spatial resolution, fast temporal resolution, stereopsis, and high contrast sensitivity (Livingstone and Hubel, 1987).

The next step in the visual pathway the optic nerve. The optic disc is the collective exit site of all retinal ganglion cell axons. Behind the laminar cribrosa, mielinated cells first appear and oligodendrocytes constitute approximately two thirds of the interstitial cells. The optic nerve is considered analogous to white matter tracts of the brain rather than to peripheral nerves due to the presence of oligodendrocytes instead of Schwann cells. The optic chiasm derives from the merging of the two optic nerves. Most of the fibers in the optic tract terminate in the ipsilateral lateral geniculate nucleus. The optic tract containing retinofugal fibers terminates in the lateral geniculate nucleus. Neurons of the lateral geniculate nucleus contribute axons that form the geniculocalcarine radiations. Lateral geniculate nucleus is divided into a large dorsal and small ventral (pregeniculate) nucleus. In humans there are six gray layers of neurons discernible in the dorsal lateral geniculate nucleus. It is evident that crossing visual axons from the contralateral eye terminate in lamina 1, 4, and 6 and uncrossed ipsilateral axons end in lamina 2, 3, and 5. Each lamina receives input from one eye only. The macular fibers are also relatively confined to the dorsal section of the optic tract (Kupfer, 1962). Upper retinal quadrants are represented medially, and lower retinal projections terminate laterally. Thus, the retinotopic organization of the posterior optic tract appears to rotate 90° as it enters the lateral geniculate nucleus. Input to the lateral geniculate nucleus from cortical area 18 is relatively sparse and is found predominantly in the ventral magnocellular layer. Layers 1 (receiving contralateral retinal projections) and 2 (receiving ipsilateral retinal projections) contain larger neurons and are the magnocellular lateral geniculate nucleus layers. Layers 3 through 6 are parvocellular lateral geniculate nucleus layers. The geniculocalcarine fibers tract begins in the lateral geniculate nucleus and constitutes the segment of the visual pathway that projects to the primary visual cortex (area 17 or V1). The macular projection area extends 1 to 2 cm laterally onto the posterior surface, and is very large. Most cells in V1 respond only to stimuli in very restricted locations in the visual field and with very specific psychophysical properties. The functional architecture of the striate cortex can be best appreciated by analysis of its input from the lateral geniculate nucleus. The magnocellular system probably mediates low spatial resolution with high

contrast sensitivity, orientation, and movement sensitivity, directionality, and stereopsis. For the visual environment to be analyzed, recognized, and interpreted, afferent visual information must be transferred from the striate cortex to higher visual association areas 18 (V2 or parastriate cortex), area 19, and to other analytic locations termed V3, V4, and MT (Livingstone and Hubel, 1987). Visual cortex area V2 probably participates in sensorimotor eye movement coordination through frontooccipital pathways and is a site of origin of corticomesencephalic optomotor pathways concerned with the smooth pursuit of visual targets. Cortical area V3 (peristriate cortex) accounts for the major lateral expanse of the occipital lobe and extends into the posterior parietal as well as the temporal lobes. Visual information must be ultimately analyzed in the dominant parietal lobe, which is usually in the left hemisphere (Duke-Elder and Wybar, 1961).

Visual space is represented on the retina in a direct point-to-point relationship. Superior visual field is projected onto the inferior retina, and the nasal field is projected onto the temporal retina. This relationship maintains this way throughout the visual system. Most visual fibers in the optic nerves and optic chiasm are derived from the large population of cells described as midget ganglion cells. These cells are now termed P cells because they project to the parvocellular lateral geniculate nucleus. The P cells largely subserve macular vision where they outnumber M cells by about 60 to 1. The larger caliber of peripheral retina axons subserving extramacular visual space tends to be distributed toward the periphery of the optic nerve. This retinotopic order is tighter in the orbital optic nerve and becomes less accurate as the fibers approach the chiasm. The arrangement of the retinal ganglion cell axons becomes considerably more complex as they approach the lateral geniculate body. Because of these anatomic variations, no finite point-to-point retinotopic representation, such as that based on surface landmarks, can be consistently applied at the occipital lobe (Horton and Hoyt, 1991).

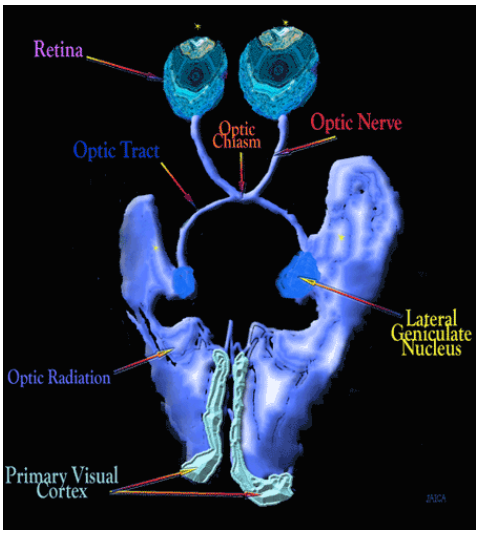

Figure 2: Visual pathway. Retina, optic nerve, optic chiasm, optic tract, lateral geniculate nucleus, optic radiation, primary visual cortex.

1.1.1.4 Eye morphology and visual system of cats

Anatomically, the globe diameters of cats are on average not so different from those of humans. Transverse and antero-posterior diameters of the globe are around 22.30 mm. The diameter of the eyeball in its highest point averages 21.55 mm. The diameter of the sclero-corneal junction is 16.2 mm. Whereas the true corneal thickness in cats measures 0.68 mm, its total dioptric power of the cornea is around 38.9 dioptries. Indices of the cornea and intraocular fluids are practically the same as in men, 1.376 and 1.336 respectively (Freeman and Lai, 1978). The transverse diameter of the cornea averages 16 mm. As the volume of the anterior chamber varies from 0.8 to 1.0 ml, the depth of the anterior chamber is very large, around 4.5 mm (Figure 3). The pupil size in schematic eye is around 10.00 in diameter. Thickness of the posterior wall (retina to sclera) of the eye in cats varies from 0.45 mm to 0.6 mm, and it sits around 8.10 mm from the posterior surface of the lens. The intraocular pressure in cats averages 17.4 mmHg, slightly higher than humans (Studer et al., 2000).

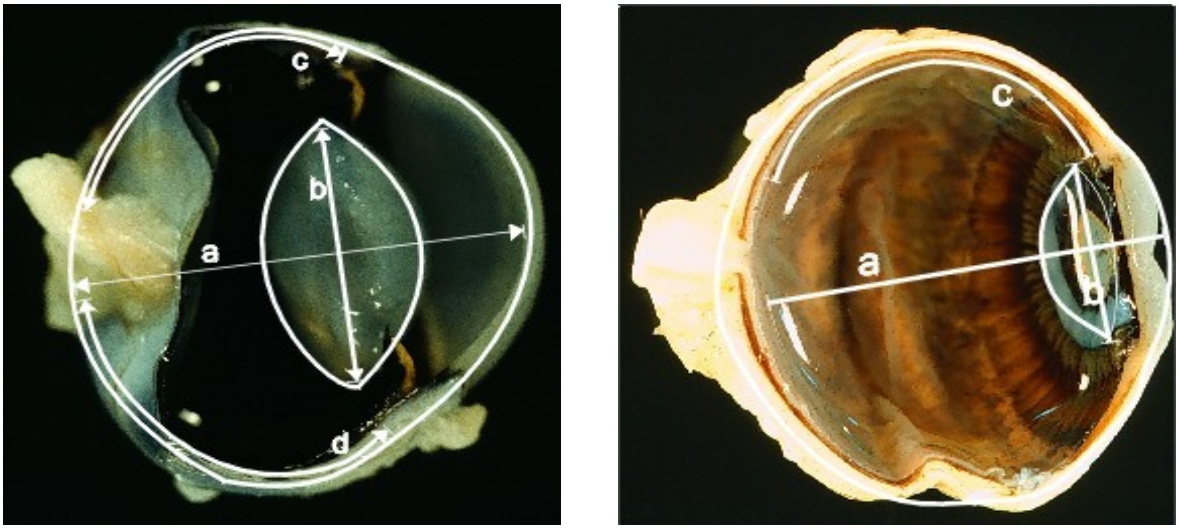

Figure 3: Left. Esquematic representation of a cat's eye. The large lens is situated very posteriorly. The cornea is large and the anterior chamber deep. Right. Esquematic representation of a human's eye. The lens is small is situated anteriorly. The vitreous cavity comprises over 90% of the eye volume.

Thanks to its large, prominent curved cornea, cats have an angle of vision of 200 degrees. The biological advantage of a large cornea is not only the larger field of vision; it also enables the lens to be larger in diameter with a larger maximal size of the pupil. The result is a large light-gathering capacity, good for nocturnal vision. A large cornea is, however, a weak dioptric power cornea, so that the crystalline lens has to be relatively more powerful. Therefore, the lens must be larger and very posteriorly located, resulting in a system that is limited as far as the amplitude of accommodation is concerned. The lens in cats is only 8.1 mm before the retinal photoreceptors, and the posterior surface of the lens is located 13.7 mm far from the cornea. Most of the anterior surface of the lens is located 5.20 mm behind the anterior corneal surface. While the thickness of the lens in cats is 8.50 mm and diameter is around 12.4 mm, the total dioptric power of the lens is 52.9. The nodal point in cats is also situated more posteriorly, resulting in a smaller but brighter image. The iris in cats is slightly posteriorly located to the corneo-scleral junction. As the position of the equator measured from the posterior pole averages 45 % of the thickness of the lens, the pars plana in cats is around 5 to 6 mm behind the limbus. In the cats, 1 angle of vision subtends 0.218 mm of retina, whereas in humans 1 angle represents 0.294 mm. The

refractive state in a cat's eye is +1.50 dioptries, and in a fully accommodated state eye it is about +2.50 dioptries (Freeman and Lai, 1978).

At histological sections the retina in cats can be divided into outer segments, inner segments, outer nuclear layer, outer plexiform layer, inner nuclear layer, and ganglion cells. Cats have no distinguished fovea, but only a central area definitely used for fixation and binocular stereoscopic vision. This area centralis is characterized in histological preparations by a double layer of ganglion cells. In the rest of the retina the ganglion cells layer is composed of one layer, which covers the inner surface of the retina, and measures from about 8 μm to about 35 μm. The inner nuclear layer is the nuclei of the horizontal cells. The inner nuclear layer is formed of small cells, and it varies from about five cells centrally to about two cells peripherally. The outer nuclear layer forms a denser layer towards the periphery. The photoreceptors form a dense central layer that thins towards the periphery. The photoreceptor cells in cats are clearly differentiated in an inner and an outer segment, forming the bacillary layer term. This layer is about 35-40 μm thick centrally thinning in the peripheral regions to about 20 μm. In cats, no cones can be found in the bacillary layer or assumed on the basis of the cone nuclei in the outer nuclear layer. Hence, it is necessary to assume that they are color-blind (Kolb and Famiglietti, 1976). The retina of cats is rich in amacrine cells; cats and rabbits are known to have 30 to 40 types. Ten different types of ganglion cells were isolated in cats (Figure 4). Ganglion cells in the feline retina form a single layer on the inner surface of the retina. They vary from 8 μm to 35 μm. Area centralis in cats lies temporally to the optic nerve and is composed of a double layer of small ganglion cells. Histologically, a dense network of capillaries exists in the retina of cats. It consists of two layers which are interconnected and which surround the inner nuclear ganglions layer, the meshes being finer in the deeper part. Mural cells are present at the wall of the capillaries. As the blood vessels pass through the margin of the papilla, they are divided into three branches (Shkolnik-Yarros, 1971).

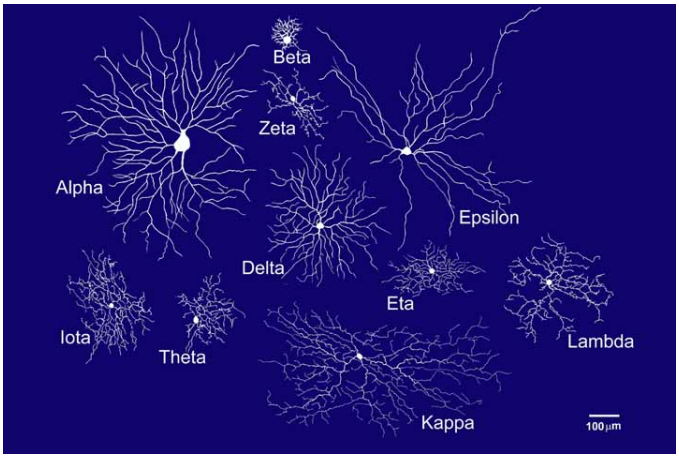

Figure 4: The ten types of ganglion cells in the cats.

The tapetum lucidum is the structure responsible for the eyeshine in cats. It covers the greater portion of the upper half of the posterior pole of the eye. In cross-section, it comprises as many as 20 layers of rectangular cells staining a light brown color with a Masson‘ s trichrome, and with a marked fibrous appearance, due to slender rods packed together in discrete groups. These groups are arranged in a regular manner with their long axes parallel to the plane of the retina. The tapetum lucidum is traversed by small capillaries, branches of the choroidal vessels, which form an extensive anastomosing network between the tapetum and pigment epithelium. The retinal pigment epithelium lacks pigment granules in the tapetal areas. The tapetum is seen to lie between the subretinal choriocapillaris internally and choroid externally. The tapetal cells are rectangular in shape. The optic disk in cats is small, non-myelinated, circular, slightly depressed, generally surrounded by tapetum and may be slightly melanotic. The papilla itself presents on the same level as the surrounding retina.

Recent studies have attempted to find a correlation between anatomic and functional classes of cats' and primates' ganglion cells. In a cat's and a primate's retina, two functional classes of ganglion cells have been identified. The Y cell in cats, which is analogous to the M cell in primates, projects to the magnocellular layers in the lateral geniculate body. This cell is a phasic cell with high contrast sensitivity but no color sensitivity. It constitutes approximately 10% of the primate's ganglion cells (Ranck, 1975). In contrast, the X cell in cats, which behaves like the P cell in primates, projects to the parvocellular layers in the lateral geniculate body. This cell type has a tonic response to stimuli and is color-sensitive. It is estimated to constitute 90% of the primate ganglion cells. In the eye of the cat, almost as many fibers remain on the same side as those that cross through the optic chiasm. Their

reorganization of the visual system attained a level indistinguishable from that found in monkeys or in humans. This finding makes the cat a very good model for the study of its visual field responses to retina stimuli. The central overlapping of the two fields of view is also larger in cats. A further feature that remains common to all mammalian species is the termination of the optic nerve fibers in both the ventral and the dorsal divisions of the lateral geniculate nucleus (Shkolnik-Yarros, 1971).

1.2 Abnormal vision

Blindness can be caused by damage at any part of the visual system, from the retina, optic nerve, up to the visual cortex. There are several hundreds causes of blindness, either treatable or not. Media opacities blindness such as corneal dystrophy or cataract can be treated successfully by either penetrating keratoplasty or cataract surgery. About 50% of all blindness is caused by damage to the retina (Krumpazsky and Klauss, 1996). Age-related macular degeneration and diabetic vitreo-retinopathy are the leading causes of blindness in the western world. For retinal and vitreous diseases such as vitreous hemorrhage or retinal detachment, efficient surgical therapies have aroused in the last 30 years. Scleral buckling surgery and pars plana vitrectomy are successful procedures in about 95% of the cases of retinal detachment disease. Vitreous hemorrhage can be totally removed with a pars plana surgery, and visual acuity is recovered in the majority of cases. However, some causes of blindness caused by degenerative damage in the outer retinal layer, such as retinitis pigmentosa or age-related macular degeneration have disappointing treatment modalities for those patients with end-stage disease. In those diseases, the retina becomes damaged or compromised by degenerative changes in the outer retinal layer leading to functional impairment of the visual pathway.

1.2.1 Blindness at the level of optic nerve and visual cortex

Several diseases may cause blindness at the level of the optic nerve or visual cortex. Glaucoma, an ocular disease currently considered to be a neuropathy, is the third non-treatable cause of blindness in the western world, following diabetic vitreo-retinopathy and age-related macular degeneration (Krumpazsky and Klauss, 1996). Optic nerve neuropathy, optic neuropathy, optic nerve inflammations are other causes of blindness at the optic nerve.

Patients may be blind by cerebrally blindness because of several causes, although not so often as those caused by optic nerve or retina damage. Stroke, hypotension, meningitis, multiple sclerosis, brain tumor, and head trauma are some examples of cortical blindness. Cerebrally blind patients have usually visual field impairment, normal fundi, and normal pupillary reactions.

1.2.2 Retinal diseases leading to blindness

1.2.3 Retinal diseases leading to blindness by damage to the photoreceptors and retinal pigment epithelium

1.2.3.1 Retinitis pigmentosa

Retinitis pigmentosa is a group of inherited disorders of the retina characterized by progressive visual dysfunction involving photoreceptors and subsequently other cell layers. Incidence varies around 1:3000 (Berk et al., 1994). Worldwide, about 1.5 million people are affected (Sharma and Ehinger, 1999). The age of onset of visual impairment in the different types of retinitis pigmentosa ranges from infancy to older years. The ideal classification system is based on the genetic factor. The impaired retinal sensitivity in retinitis pigmentosa is believed to be from loss of quantum caught by the reductions in rhodopsin levels in the retina. The eletrooculogram is altered in the majority of the cases. Night blindness and progressive loss of visual field are common clinical findings in retinitis pigmentosa (Tanino and Ohba, 1976). The visual fields may change dramatically over several years or, rarely, over a few months (Deutman, 1977). The fundus appearance of retinitis pigmentosa includes attenuated retinal vessels, mottling and granularity of the retinal pigment epithelium, migration of pigment from disrupted pigment epithelium into the retina to form clumps of various sizes or bone-spicule formations. Atrophy of the retinal pigment epithelium and choriocapillaris eventually occur so that larger choroidal vessels become prominent. Fluorescein angiography shows transmission defects of the retinal pigment epithelium with later diffuse leakage. Cataracts are the most frequent of anterior-segment complications that can be seen with retinitis pigmentosa, affecting 39% to 72% of the patients (Krumpazsky and Klauss, 1996). Keratoconus, high myopia and astigmatism can also occur. Most patients with advanced retinitis pigmentosa will have with electroretinogram examination undetectable responses to single-flash, typically less than 10 mV. Retinitis pigmentosa causes blindness primarily due to degeneration to the photoreceptors. Many patients at end-stage of the disease have visual acuity of no light perception, light perception, or object movements.

No treatment stops or reverses the loss of photoreceptors in retinitis pigmentosa. Over the years, many treatments have been tried with no benefits: minerals, vasodilators, cortisone, transfer factor, dimethyl sufoxide, ozone, and muscle transplants (Berson, 1996). High doses of vitamin A have been suggested to slow the rate of progression of the most common form of the disease (Berson et al., 1993). Efforts to alter the expression of the

mutated gene or to introduce a normal gene into the genome are in their infancy, but the results are encouraging (Acland et al., 2001). Another young research area with a potential for therapeutic application is the replacement of the retinal pigment epithelium of the degenerated neural retina by transplantation of the respective cell types. Clinical trials are being conducted both with retinal pigment epithelium and neuroretinal transplants, but their usefulness is yet to be established (Berger et al., 2003).

1.2.3.2 Age-related macular degeneration

Age-related macular degeneration is the most common cause of blindness in patients older than 65 years in developed countries, and it ranks second after diabetic vitreo-retinopathy as the leading cause of blindness in patients from 45-64 years (Leibowitz et al., 1980). Nearly 30% of persons older than 75 years are affected by age-related macular degeneration (Ferris, 1983). Several risk factors have been suggested for age-related macular degeneration, including heredity, sex (more women are affected than men), light ocular pigmentation, hypertension and cardiovascular disease, dermal elastic degeneration in sun-protected skin, smoking, and phototoxicity (Blumenkranz et al., 1986; West et al., 1989). Blindness associated with age-related macular degeneration is caused by degenerative changes in the retinal pigment epithelium and later the photoreceptors. The disease may take the form of a localized degeneration without the complications of vascular invasion or neovascularization. Degeneration of the retinal pigment epithelium precedes or accompanies death of the associated rods and cones (Bressler et al., 1988). The earliest clinical sign of age-related macular degeneration is the development of drusen, which are extracellular deposits that lie between the basement membrane of the retinal pigment epithelium and the inner collagenous zone of Bruch's membrane. (Smiddy and Fine, 1984). Although some patients retain good visual acuity levels, in advanced cases visual acuity levels worse than 20/800 are observed.

There are two manifestations of age-related macular degeneration, the dry and wet form. The atrophic or dry form of age-related macular degeneration has been called geographic because the areas of retina pigment epithelium atrophy tend to form well-demarcated borders that do not relate to specific anatomical structures (Gass, 1977; Young, 1987). Atrophic age-related macular degeneration leads to significant visual loss in almost all cases. Initially, these areas form discrete patches of atrophy that coalesce with time, then evolving the whole macular region. In areas of geographic atrophy, the outer nuclear layer

rests directly on the basal lamina. The outer plexiform layer is thinned and vacuolated, but the inner nuclear layer and ganglion cells are little affected. The second type of age-related macular degeneration is the wet form or choroidal neovascularization. This term used to describe the growth of abnormal new vessels beneath the sensory retina or retinal pigment epithelium. Choroidal neovascularization is an abnormality found in many diseases in which the integrity of the retinal pigment epithelium, Bruch's membrane, and choriocapillaris have been compromised (Campochiaro and Glaser, 1986). Choroidal neovascularization may appear as a dirty-gray discoloration beneath the retina and may be accompanied by an overlying sensory retinal detachment and cystoid edema. Choroidal neovascularization can be responsible for a turbid sensory retinal, retinal pigment epithelium detachment, or hemorrhage. Fluorescein and indocyanine green angiography confirm the presence and location of choroidal neovascularization (Ávila, 1997a). The early angiogram of a classic choroidal neovascularization may reveal a discrete arborized plexus of vessels with a nodular border. Later in the angiographic study there is intense hyperfluorescent fuzziness to the border of the membrane, a result of dye leakage into the subretinal space. Choroidal neovascularization arises from the choroid and passes through Bruch's membrane to invade the subpigment epithelial and subsensory retinal space (Heriot et al., 1984). The choroidal neovascularization proliferates under the retinal pigment epithelium and destroys it. New vessel growth is accompanied by fibrous tissue, which ultimately becomes the dominant pathologic change and results in a disciform scar involving the choroid, retinal pigment epithelium, and outer sensory retina.

A number of treatments are available or under investigation for neovascular age-related macular degeneration (Farah et al., 2001). In eyes with intermediate age-related macular degeneration, prophylactic oral supplementation with antioxidants and zinc is the only treatment proven to reduce the risk of vision loss resulting from progression to advanced age-related macular degeneration. Choroidal neovascularization can be treated with laser photocoagulation, which may preserve the central vision in some eyes. The *Macular Photocoagulation Study* has shown that intense argon blue-green laser is beneficial in eyes having discrete choroidal neovascularization (200 μm) from the edge of the center of the foveal avascular zone. The risk of severe visual loss was reduced from 60% in untreated eyes to 25% in treated eyes 18 months after laser therapy (Macular Photocoagulation Study Group, 1986). Photodynamic therapy with verteporfirin has been shown to be a safe form of therapy for the treatment of predominantly classic forms choroidal neovascularization associated with age-related macular degeneration (Photodynamic Therapy Study Group, 1999). Photodynamic therapy selectively targets choroidal neovascularization without causing

immediate damage to central vision (Bressler, 2001). Some controversy still exists whether it will have a good outcome after a few years of observation. Macula translocation surgery rotates the retina so that the central retina is moved away from the choroidal neovascularization. Macular translocation constitutes an alternative treatment in some cases of choroidal neovascularization, especially for those cases in which the fellow eye is already severely damaged, and the visual acuity has recently dropped. Transpupillary thermotherapy offers a potentially selective treatment for choroidal neovascularization (Nehemy et al., 2001). It uses a relatively large spot size, low irradiance, and long exposure times with an infrared laser to deliver hyperthermia to the choroid and retinal pigment epithelium, theoretically causing a targeted choroidal neovascular lesion to evolute. More research is still necessary for confirmation of the transpupillary thermotherapy efficacy. A variety of anti-angiogenic drugs are currently being investigated, but none is yet available for clinical use. For those patients with old and advanced age-related macular degeneration, there is no currently effective treatment available.

1.2.3.3 Other degenerative retinal diseases

The association of retinitis pigmentosa and partial or complete congenital deafness is exemplified by the Usher's syndrome. The disease is the result of the action of an autosomal recessive, and possibly a pleiotrophic gene, because there can be dissociation of the two conditions in the same family. Incidence of Usher's syndrome is around three cases per 100,000. It is the most frequent cause of deaf-blindness in adults (Bateman et al., 1980).

Another retinal degeneration causing blindness is choroideremia, which is an X-linked, hereditary retinal dystrophy characterized by bilateral, symmetric nyctalopia, peripheral visual field constriction, and late central visual loss in affected males and, rarely, carrier females. Histopathologic examination of eyes with advanced choroideremia reveals severe choroidal atrophy across the fundus with absence of retinal pigment epithelium, choroidal vessels, and outer retina with direct contact between the sclera and retinal remnants (Grutzner and Vogel, 1973). Pigmentary stippling initially occurs and fine atrophy of the retinal pigment epithelium in the equatorial and posterior part of the fundus is noted. At late stages there is widespread chorioretinal atrophy, and it is often possible to see through to yellow-white sclera.

1.3 Vitreous body and lens surgery – the approach for the epiretinal array implant

When the first pars plana vitrectomy was performed by Machemer in 1972, a small instrument capable of excising vitreous while maintaining intraocular pressure by a simultaneous infusion was created (Machemer et al., 1972). Later on the intraocular fiberoptics light pipe was introduced to the vitreoretinal surgery. Since then instrumentation and technique have been largely improved. Less toxic irrigating solutions, improved contact lenses, safe intraocular diathermy, and endolaser photocoagulation were further improvements (Ávila et al., 1997b). The introduction of motorized scissors, delamination techniques, intraocular photocoagulation, and intraocular diathermy has markedly improved the success rate in pars plana vitrectomy. The cannulated extrusion needle allows drainage through a preexisting hole obviating a posterior retinotomy. Another advance in vitreo-retinal surgery was heavier-than-water perfluorocarbon liquids (Chang et al., 1988). Slow infusion of perfluorocarbon liquid pushes the subretinal fluid anteriorly, gradually flattening the retina in the liberation of subretinal fluid to treat retinal detachment.

The technique of pars plana vitrectomy is briefly summarized: the entry scleral incisions are 4 mm from the limbus. The first incision is for the infusion cannula usually placed in the inferotemporal quadrant. The second and third sclerotomies are performed. The vitreous is removed using the vitreous cutter. The vitrectomy instrument is used to remove as much formed vitreous tissue possible and to cut transvitreal membranes, releasing vitreous traction. Cryotherapy or retinal photocoagulation is made to create retino-choroidal adhesion when retinal breaks are present. To prevent intraoperative bleeding, fibrovascular membranes should be treated with diathermy before they are cut. Technique of air-fluid exchange usually follows. Long-lasting gases, air, or silicone oil can be used as vitreous substitutes. After the end of the procedure, the scleral and conjunctival incisions are closed to avoid wound leakage (Rodrigues et al., 2003b). Drugs such as steroids or enzymes may be used as adjuvants for the pars plana vitrectomy (Salum and Belfort, 1997; Hesse et al., 2000a).

Nonclearing vitreous hemorrhage caused by proliferative diabetic retinopathy is the most common indication for pars plana vitrectomy (Figure 5). Pars plana vitrectomy for proliferative diabetic vitreo-retinopathy is necessary to release all of the vitreous traction, to both remove the intravitreal blood and treat or prevent a traction retinal detachment (Kroll et al., 1986; Kroll et al., 1987). Other indications for pars plana vitrectomy include retinal detachment, rhegmatogenous retinal detachment with proliferative vitreoretinopathy, macular diseases such as macular hole, and the exudative form of age-related macular

degeneration (Rodrigues et al., 2003a; Schmidt et al., 2003b). With current surgical techniques greater than 95% of all retinal detachments can be successfully repaired. In cases of nonmagnetic intraocular foreign body, or in cases in which the retina cannot be examined because of a dense vitreous hemorrhage or a cataract after eye injury, pars plana vitrectomy is required. Vitrectomy instruments can safely and successfully remove vitreous that is opaque as a result of hemorrhage, amyloid, or inflammatory cells. Pars plana vitrectomy has been advocated as an adjunct to the antibiotic treatment of endophthalmitis, and to remove chronic vitreous opacities secondary to uveitis. Safe retrieval of posteriorly dislocated intraocular lenses and crystalline demands a vitrectomy (Moreira Jr. et al., 1995; Lavinsky et al., 2002).

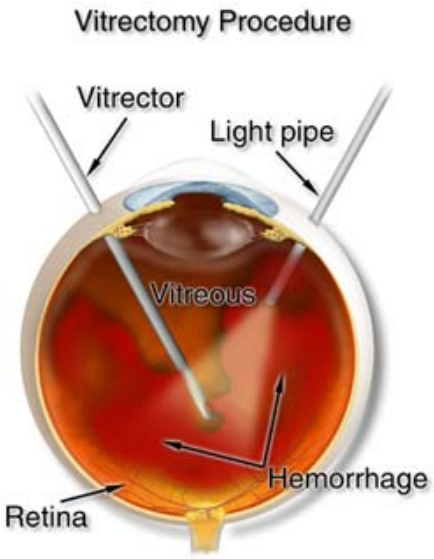

Figure 5: Set-up for pars plana vitrectomy in vitreous hemorrhage.

1.4 Compensation of blindness by microelectronic devices – visual prosthesis

A visual prosthesis is a new treatment under research to restore vision in patients who are blind due to retinal or visual pathway diseases (Ross, 1998).

1.4.1 Historical review of the initial experiments of visual stimulation in blind patients

The discovery of electricity in the 18th Century allowed some experimental tests in animals without therapeutic interest (Watson, 1751). The French chemist and physicist Le Roy was the first to report generation of phosphenes after electrical stimulation as an attempt to restore vision in a blind human (Le Roy, 1755). Phosphenes are defined as perception of spots of lights after electrical or magnetic stimulation of the visual pathway. Meyer-Schwickerath and Magnun were the first to establish a correlation between the frequency and power of electrical stimulation to the characteristics of phosphenes (Meyer-Schwickerath and Magnun, 1951). Later, Potts and Inoue stimulated the cornea with electrodes and obtained visual field correspondence after cortical recordings, and those results were later confirmed by Hirose et al. (Potts and Inoue, 1969, Potts and Inoue, 1970; Hirose et al., 1977).

In the late 19th Century, D´Arsonval and also Thompson registered phosphenes after experiments with electromagnetic fields (D´Arsonval, 1896; Thompson, 1910). Magnusson and Stevens established changes in phophenes intensity after increasing electromagnetic stimulus instead of electric impulses (Magnusson and Stevens, 1914) (Figure 6). Although new attempts to create magnetic vision sensation were performed in the 20th century, no defined image was obtained, and this method was not further researched (Marg, 1991; Marg and Rudiak, 1994).

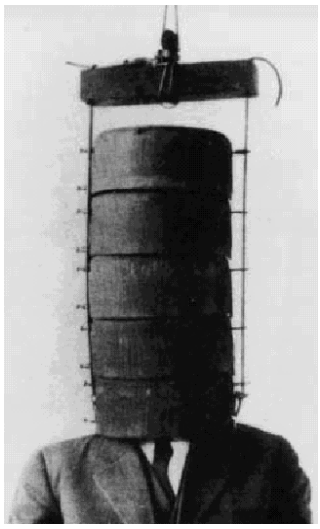

Figure 6: Magnetic pulses stimulated by Magnusson and Stevens, 1914.

Available at: www.tandf.co.uk/journals

1.4.2 The use of electrical energy to create a visual prosthesis

Recent advances in microtechnology, biotechnology, computer electronics, and vitreoretinal surgery in the last decades have encouraged the development of a visual prosthesis by microelectronic means for visual rehabilitation. Two german consortia (MPD-array and Epi-Ret) received the first government-sponsored grants in 1995 to develop research programs, divided in the subretinal or epiretinal approach. In 2000 the Retina Implant Project was reorganized, and divided into epiretinal, subretinal, physiology, and biocompatiblity approaches (Epi-Ret, Sub-Ret, Biocompatibility, and Physiology). Three groups in the United States are working on a retinal prosthesis coordinated by Rizzo, Humayun, and Chow. A belgian team has begun an optic nerve-based prosthesis program. Cortical prosthesis are being pursued by 3 groups in the United States. A japanese group is investigating the development of an hybrid retina implant. Table 1 describes the worldwide research groups working on the development of a visual prosthesis.

There are several situations where electrical devices can improve functioning of an impaired tissue, such as cochlear implants of pacemakers. Cochlear implants have allowed some deaf patients to have telephone conversations. However, as far as the eye is concerned,

complexity is much higher. 130 million photoreceptors send detailed information through the retina up to 1.2 million highly specialized ganglion cells, and these cells transmit processed electrical information to the visual cortex.

One important issue for a successful visual prosthesis is whether an electrical stimulation will create light perception. Several research groups have proved that electrical stimulation of the visual system has resulted in perception of phosphenes (Foerster, 1929; Brindley and Lewin, 1968; Button and Putnam, 1961; Dobelle et al., 1974; Dawson and Radtke, 1977; Cha et al., 1992a; Rizzo and Wyatt, 1997; Humayun et al., 1999a; Shimazu et al., 1999). Another interesting factor should be if a visual image can be created by stimulation of small areas of neuronal tissue (pixels), and how many pixels are necessary for image recognition. Furthermore, researchers must find out what are the electrical parameters for one pixel to be safe and effective. Studies have shown that 625 pixels may be the minimal stimulation for the creation of simple vision (Cha et al., 1992b). The efficacy of an electrical stimulation on tissues may be influenced by several other factors: the electrical properties of the target cell; the distance from the electrodes to the target cell; impedance of target tissues; relationship between the threshold current and duration of the neuronal activation; and the influence of repetition of stimuli in the visual pathway (Rattay, 1998; McCreery et al., 1990; Schmidt et al., 1996; Eckmiller, 1997)

Retinal prosthesis should benefit those patients with blindness due to retinal pigment epithelium or photoreceptor diseases. For instance, it would not be helpful for patients suffering from diabetic vitreo-retinopathy because those diseases damage nerve fibers in the retina that lead visual stimulus to the brain. Optic nerve prostheses may also need an intact retinal ganglion cells layer to creating visual perception. Cortical prosthesis may be valuable for blindness at the level of the retina, optic nerve, and some cases of cortical blindness.

Table 1

International Working Groups for a Visual Prosthesis

Epiretinal Stimulation	Epi-Ret Group, Germany Humayun et al., USA Rizzo, Wyatt et al., USA Hybrid implant group. Japan
Subretinal Stimulation	Sub-Ret group, Zrenner et al., Germany Chow et al., USA
Optic Nerve Stimulation	Veraart et al., Belgien
Cortical Stimulation	Dobelle et al., USA Normann et al., USA Schmidt et al., USA

1.4.2.1 Correcting blindness with electronic devices at the level of the visual cortex and the optic nerve

1.4.2.1.1 Visual cortex prosthesis

Once blindness has occurred due to optic nerve damage, the approach is restricted to the cortical or thalamic level. A cortical prosthesis bypasses all diseased neurons distal to the primary visual cortex (Karny, 1975), therefore, it has the potential to restore vision to a larger number of blind patients. However, spatial organization is very complex at the cortical level, and two adjacent cortical loci do not correspond to two adjacent areas in space, in a way that patterned electrical stimulation may not produce similar perception. Every small area of the

cortex is highly specialized for color, motion, eye preference, and other parameters of visual stimuli. Then, it should be difficult to obtain simple perceptions when stimulating few hundreds of neurons in the case of intracortical microstimulation. More importantly, surgical complications may have serious complications on usually healthy subjects, including death. Pain after meningeal stimulation and focal epileptic reactions have already been observed in experiments of visual prosthesis (Margalit et al., 2002; Dobelle, 2000).

A cortical prosthesis can be placed intracortical or on the cortex surface. The lower current threshold of the intracortical microstimulation, the predictable forms of generated phosphenes, the opportunity to increase the number of electrodes, and the reduced power requirement and current per microelectrode are advantages of the intracortical microstimulation in comparison to surface cortical stimulation. Protection of the skull for the electronics and the electrode array is an advantage of a visual prosthesis on the cortex surface. As disadvantage, the cortex surface implant may create a communication between the epidural and the subaracnoid space, and infection may ensue through that communication.

Electrical stimulation of the visual cortex was first reported by Foerster, causing his subject to perceive phosphenes (Foerster, 1929). Later, a visual prosthesis system based on electrodes to be implanted on the cortical surface was created by Brindley and Lewin (1968) and later tested by Brindley and Rushton (1974). Electrode arrays were implanted over the occipital cortex in several blind volunteers. Wires connected each electrode to a radio receiver screwed to the outer bony surface of the skull. With this system, a patient was able to see phosphenes at different positions of the visual field, demonstrating that many of the implanted electrodes were functional.

Later, Dobelle et al. (1974) conducted experiments in non-blind patients, and testing color induction and changes in the intensity of the phosphenes in response to different electrical impulses were obtained. In further experiments they utilized 64 channel platinum disk electrode arrays on the surface of the occipital cortex of blind patients. These electrodes were connected with a camera consisting of a 100x100 charge-coupled phototransistor array (Figure 7). Running letters were used to stimulate the camera. The prosthesis allowed these blind patients to recognize 6-inch characters at 5 feet (approximately 20/1200 visual acuity). Recently, Dobelle (2000) has reported long-term results of a portable artificial vision system based on cortical electrical stimulation with 64 sub-dural electrodes. One volunteer perceived localized phosphenes even though he had been blind for more than 20 years. He was able to scan visual objects and could even count on the fingers of one hand. Others also reported

visual sensations produced by similar intracortical microstimulation of the human occipital cortex (Bak et al., 1990).

A new concept of a microelectrode stimulation array was proposed by Normann et al., (1996 and 1999). They implanted intracortically 100 electrodes of 1.5 mm high in a distance of 400 μm from each other (Figure 8). Phosphenes were created with a 1.9 μA power of amplitude. They minimized the traumatic risks of a cortical prosthesis by injecting the electrode arrays pneumatically with an 8.3 m/s speed. Due to the intracortical location, low amplitude and consequently high definition stimulation were achieved.

Schmidt et al. (1996) were the first to utilize penetrating electrodes in order to refine spatial resolution that was five times more accurate than that with surface stimulation. They implanted 38 intracortical electrodes with a diameter of 37.5 μm and 2 μm high in a patient blind secondary to glaucoma for 22 years. The phosphenes were percepted yellow, blue, red, and white. The perceptions were stable and free of interference, and the resulted phosphenes correlated with electrode stimulation in the cortex.

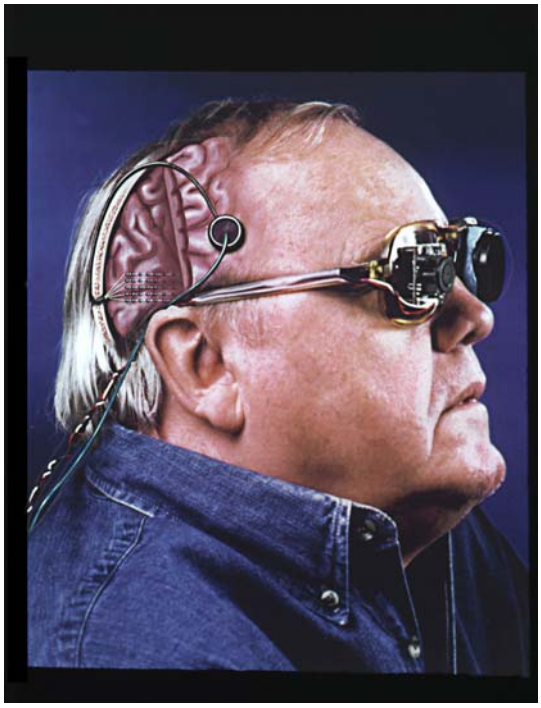

Figure 7: The miniature television camera, mounted on the glasses feeds its' signal to a "frame grabber" on the belt. This information is processed by a computer and transmitted to the stimulator, also located on the belt. Signals are then transmitted to electrodes on the visual cortex through a percutaneous pedestal. Courtesy of Dobelle.

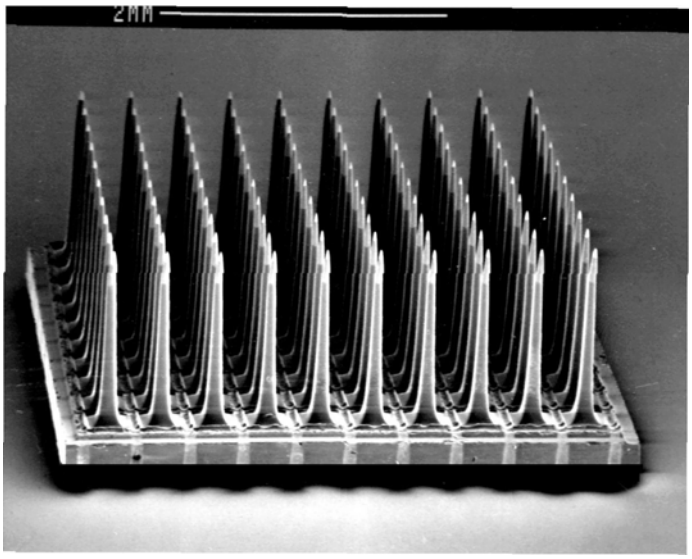

Figure 8: 100 x 100 electrodes for the intracortical prosthesis. Courtesy of Normann.

1.4.2.1.2 Optic nerve prosthesis

An optic nerve structure for the implantation of a stimulating electrode array is being researched. However, 1.2 millions axons run in high density in a diameter of 2 mm, what makes focal stimulation at the optic nerve technically difficult to create detailed perceptions. Besides, to contact the optic nerve fibers the dura-mater has to be dissected, which brings risk of central nervous system infection or impaired optic nerve blood flow.

Two groups, Shandurina and Lyskow (1986) and also Veraart et al. (1998) have reported the creation of phosphenes after optic nerve stimulation. The group in Belgium coordinated by Veraart developed a self-sizing spiral cuff electrode with four electrical contacts, which was implanted around the optic nerve in a 59 years old blind patient with end-stage retinitis pigmentosa (Veraart et al., 1998)(Figure 9). Electrical stimuli on the optic nerve produced localized and color phosphenes that were broadly distributed throughout the visual field. The results were reliably reinduced after 118 days. In addition, no postoperative complications were reported.

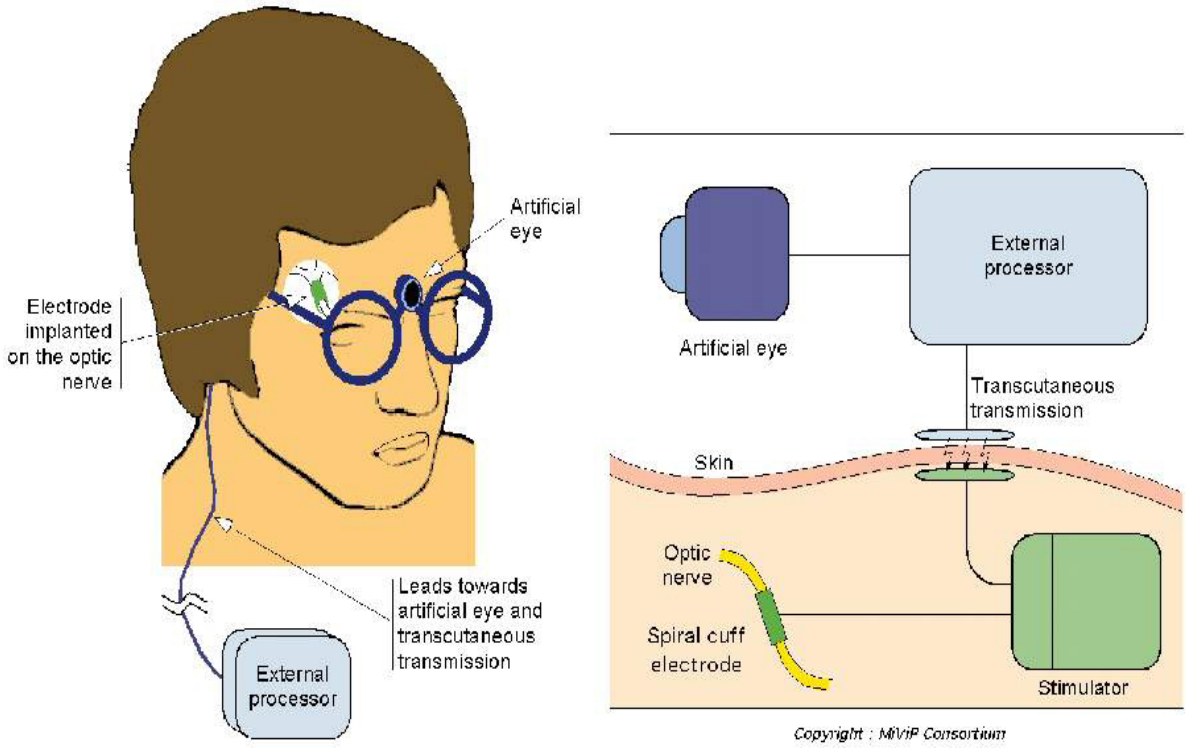

Figure 9: Set-up for an optic nerve prosthesis. The left part of the drawing represents the basic concept whereby an image captured by an artificial eye (a dedicated camera) is transformed by a wearable micro-computer (the external processor) in suitable signal that are transferred through the skin by means of a pair of antennae. Next, an internal stimulator will decode the signals and send the appropriate electrical current through the optic nerve in order to obtain corresponding visual perceptions. The left part of the figure shows the same components in a more realistic view and appropriate location on the volunteer. As can be seen from the figure, the implanted parts are limited to a cuff around the pre-chiasmatic (intra-cranial) optic nerve, a stimulator box (about 25 x 20 x 6 mm) slightly engraved in the parietal skull surface below the scalp and a subcutaneous antenna (25 mm diameter disc of 3 mm thickness) located behind the ear. The system receives the stimulation data as well as the power supply through the antenna. So there is no implanted battery. Courtesy of Dr. Delbeke and Dr. Veraart.

1.4.2.2 Restoring blindness with retinal prosthesis

Retina implants are being researched to provide useful visual perception in patients with blindness caused by some retinal diseases affecting the photoreceptors, such as retinitis pigmentosa and age-related macular degeneration (Eckmiller, 1997; Zrenner et al., 1997; Schanze et al., 1998; Chow and Peachey, 1998; Humayun et al., 1999a; Walter et al., 1999; Zrenner et al., 1999; Hesse et al., 1999; Yagi and Hayashida, 1999; Hesse et al., 2000b; Monroe, 2000; Grumet et al., 2000; Walter and Heimann, 2000; Schwahn et al., 2001; Zrenner et al., 2001; Schanze et al., 2002; Zrenner, 2002; Schanze et al., 2003). Retina Implants consist a prosthesis that can bypass degenerated photoreceptors and directly stimulate neuronal components of the visual system, ganglion cells. Patients eligible to this implant must have an intact visual system, from the optic nerve up to the brain. Patients with retinitis pigmentosa are the first candidates for a retinal prosthesis because in this disease devastating visual loss occurs frequently, and any significant improvement in vision produced by a prosthesis would be useful. A second group of patients with certain forms of macular degeneration, including the age-related macular degeneration, may benefit from retinal prostheses at advanced stages of disease. As age-related macular degeneration causes rarely visual acuity worse than 20/800, they would benefit only by further, more accurate retinal prosthesis, perhaps in conjunction with other forms of current treatment under research such as gene therapy (Acland et al., 2001). Nevertheless, studies are needed to ensure that the peripheral vision present in patients with age-related macular degeneration will not be damaged by surgical procedures and the effects of the retinal implants themselves.

The first evidence that blind humans electrically perceive phosphenes in response to ocular stimulation occurred in the late sixties (Potts and Inoue, 1969; Potts and Inoue, 1970). Further tests proved the elicitation of electrical-evoked responses after retinal stimulation (Knighton, 1975). Others showed that blind patients with retinitis pigmentosa had visual perception with spatial resolution after local electric stimulation of the inner retinal surface. One of the probands had a vision of no light perception and achieved resolution of 1.75 degrees (4/200)(Humayun et al., 1994; Humayun et al., 1996). There is however concern that most patients have required a level of stimulating current that approached or exceeded accepted safety limits for long-term use. Acute epiretinal stimulation with a duration of 6 to 8 hours was also accomplished in primates to study the effects of electric stimulation, and a safe retinal stimulation was achieved (Gerding et al., 2001).

Two types of retinal implants are under research: epiretinal and subretinal (Figure 10). There are mechanical or biologic advantages to either subretinal or epiretinal approaches.

Both epi- and sub-retinal approaches are faced with the challenges of developing efficient, low-power sophisticated stimulator electronics. The epiretinal implant generate a stimulus by a camera and a processing unit (Figure 11). After receiving a visual message from the processing unit, an image is built in the epiretinal implant, which stimulates the ganglion cells and the further visual system. In the subretinal approach, light-sensitive microphotodiodes with microelectrodes are implanted between the retinal pigment epithelium and outer retinal layer. The light stimulus on the retina generates currents in the photodiodes connected to microelectrodes. The microelectrodes stimulate the retinal sensory neurons, and then the normal retinal visual pathway is activated.

Microelectronic technology should not be the barriers to development of a clinically useful prosthesis. Concerning the retinal approach, scientists must figure out the best way to "communicate" an electronic device to the neural retinal tissue. Further, a chronically biocompatible retinal-electronic interface must be achieved, including a demonstration that the charge levels required for neural activation are within acceptable standards for long-term stimulation. Moreover, a safe surgical technique to implant microelectronic devices must be pursued. For the last 30 years, vitreoretinal and lens surgery have been utilized in clinical practice, and many diseases deemed untreatable in the past may now benefit from those approaches. Now, researchers must use these previous and current surgical experiences and techniques to implant a retinal device inside an eye, in a highly atraumatic way, with few complications.

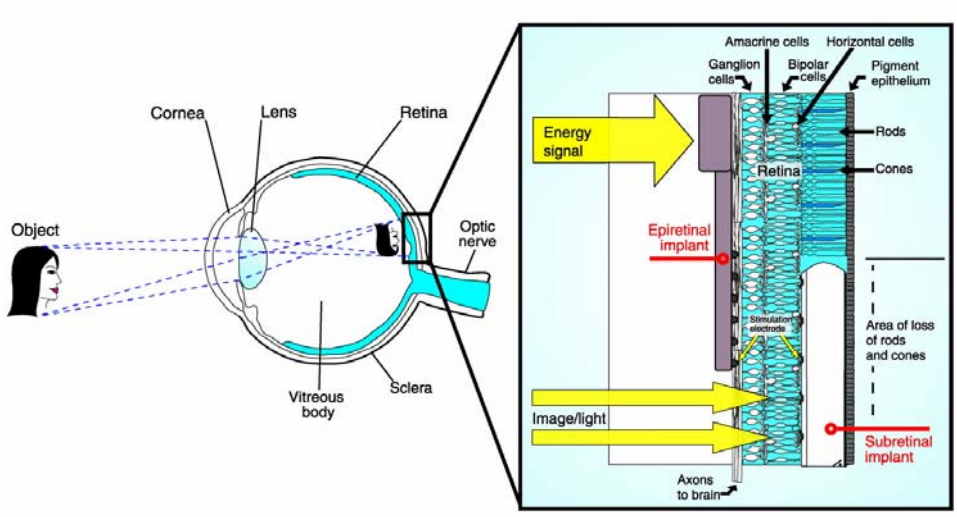

Figure 10: An object (in this case a face) is projected by the cornea and lens onto the retina in an upside-down manner and is transformed into an electrical image by the photoreceptor cells (rods and cones) of the outer retina. With a subretinal implant, the rods and cones are replaced by a silicon plate carrying thousands of light-sensitive microphotodiodes, each equipped with a stimulation electrode. Light from the image directly modulates the microphotodiodes, and the electrodes inject tiny currents into the remaining neural cells (horizontal cells, bipolar cells, amacrine cells, and ganglion cells) of the retinal inner layer. The epiretinal implant has no light-sensitive areas but receives electrical signals from a distant camera and processing unit outside of the body. Electrodes in the epiretinal implant (small black knobs) directly stimulate the axons of the inner-layer ganglion cells that form the optic nerve. Reprinted from Zrenner, 2002.

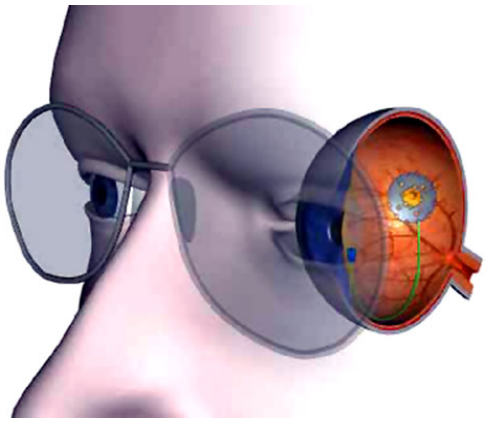

Figure 11: Esquematic representation on the position of the epiretinal implant.
Available at: http://www.iip-tec.com/iip/pix/impl_auge.jpg

1.4.2.2.1 Epiretinal implants

Stone et al. demonstrated that a considerable amount of healthy ganglion cells presented in advanced cases of retinitis pigmentosa (Stone et al., 1992). Analysis of the retina of retinitis pigmentosa patients with advanced disease revealed that inner nuclear layer cells, mainly bipolar cells (78.4%), and ganglion cells (29.7%) are relatively retained in comparison the low number of outer nuclear layer, photoreceptors (4.9%)(Santos et al., 1997; Humayun et al., 1999b). As far as age-related macular degeneration is concerned, the nuclei of the outer nuclear layer were also significantly attenuated in eyes with disciform age-related macular degeneration, whereas the ganglion cell and inner nuclear layers were relatively preserved (Kim et al., 2002).

The presence of partially intact inner retinal layers makes the concept of an epiretinal approach feasible. It is not yet known which are the preferred cells for the stimulation. In acute human testing, the inner nuclear cells were most likely to be activated by hand-held electrical stimulating devices (Weiland et al., 1999). Retinitis pigmentosa simulation was carried out by aspartate mediated block of photoreceptor bipolar synaptic transmission. Retina stimulation with epiretinal implants was following performed. Electrically evoked potentials and not visually evoked potentials were obtained (Walter and Heimann, 2000). The cell body of the ganglion cells are mapped in the retina in a relatively correlation to the contralateral visual field, and visual perception after their stimulation is expected to be focal points. Rather, the axons of those cells run on the individual ganglion cells, and their stimulation causes the appearance of an arc.

There are other problems in the decodification of the retina-chip contact. Epiretinal implants may stimulate one or more cells of the inner retinal layer (ganglion cells, fiber layers, or inner nuclear cells). The ganglion cells lie just beneath the inner retinal surface, the internal limiting membrane, and they are densely packed into a single cell layer. Up to 18 types of ganglion cells have been found in humans, and their understanding may facilitate the location of exact parameters of a visual prosthesis. The topographic relationship of the ganglion cells to the underlying photoreceptors, and hence to visual field coordinates, is reasonably straightforward over much of the retina, and it becomes an advantage to the epiretinal approach. Epiretinal implants have to stimulate the inner retina in a spatiotemporal pattern understood by the visual cortex. In cats, epiretinal stimulation provided a cortical resolution of 1 degree visual angle (Eckhorn et al., 2001). Electrical stimulation has also been constantly recorded in the cortex of cats, pigs and rabbits (Hesse et al., 2000b; Schwahn et al., 2001; Eckhorn et al., 2001; Schanze et al., 2002). Findings suggest that a pulse duration of

> 0.5 msec activates deeper cell bodies (bipolar cells) (Margalit et al., 2002). It is yet to be found if, with lower energy, the parvocellular or magnocellular pathway will be activated; and if grouped or isolated impulses are more effective (Eckhorn et al., 2001). After stimulating the retina of cats with electrode fibers and polyimide array, Schanze and co-workers found defined parameters and electrical stimulus for an epiretinal implant concept (Schanze et al., 1998; Schanze et al., 2002).

Interfacing the inner retinal surface poses a couple of problems. Two different forces, arising from angular acceleration during ocular rotation, may shear the implant from the retinal surface: 1. The inertial force from the mass of the implant and 2. Fluid dragged from intraocular fluids. The implant must be small and thin enough to remain attached to the retina without stressing it excessively. Technically speaking, fixing the epiretinal implant on the internal limiting membrane is very difficult. The distance from the implant position to the retina influences how much electrical power has to be used for stimulation. An inner retinal approach also carries the potential risk of inciting destructive reactions of Müller cells, which could form, among other things, epiretinal membranes or cellular proliferation with consequent proliferative vitreoretinopathy, posing the eye to increased risk of loss. Besides, they might act as a barrier of high electrical resistance beneath the stimulating electrodes. In the present stage of research, most of the information concerning retina implants can be obtained not from humans, but from animals.

Several different epiretinal implants have been tested in animal models. The testing of epiretinal implants was earlier done with one-piece implants. The goals of those studies were to verify biocompatibility of an inactive epiretinal implants fixated on the retina (Walter et al., 1999, Majii et al., 1999, Walter and Heimann, 2000). Silicone rubber, polyimide, and platinum were the composition of those implants. There is a further, much more complex prototype of epiretinal implant, which is composed of three pieces: a silicone intraocular lens containing electronic components for wireless signal and energy transfer of the microchip device (electronic intraocular lens), a flexible microcable, and a thin microelectrode array in contact with the retina tissue. Studies with those complex epiretinal implant prototypes were done to evaluate either active stimulation by epiretinal implant, or to test a surgical technique to introduce the epiretinal implants (Vobig et al., 2000; Gerding et al., 2001; Kerdraon et al., 2002; Schanze et al., 2003). Others performed electrophysiologic testing only with the flexible microcable and the microelectrode array (Hesse et al., 2000b; Schanze et al., 2002). Those complex epiretinal implants were composed of silicone rubber, polyimide, platinum, ceramic, or titanium nitride. The reason for doing a more complex design with an electronic intraocular lens is that with this prototype, the electronics are maintained far from the retina

(in the anterior part of the vitreous cavity or in the capsular bag). After removal of the vitreous gel, the vitreous cavity will be filled with liquid. The liquid works as a heat sink, which allows the heat generated by the electronics to be greatly dissipated (Piyathaisere et al., 2001). Table 2 describes the characteristics of the epiretinal implants tested so far.

The retinal prosthesis of the german Epi-Ret group is projected to have three components. A first part is a very tiny sensor array, like a camera, positioned outside the eye, in order to take the visual images. The retina encoder is the second component, which is a learning neural computer that transforms a visual scene received from the camera into nerve signals, just like the retina does. The retina encoder calculates time and amplitude functions of pulse trains, which are necessary to stimulate the respective ganglion cells adequately. The retina encoder together with a photosensor array for pattern reception and a transmitter for the transmission of signals and energy are housed in glasses. The complex visuotopic organization of visual cortex may require that a spatial translator be interposed between a video camera and the stimulation pattern delivered to an array of electrodes implanted in the visual cortex of a blind patient. The signals are transmitted wireless to the implanted retina implant. The retina implant is a flexible microcontact foil consisting of a receiver for the signals produced by the retina encoder and stimulation microcontacts with their corresponding controlling devices to stimulate the retinal ganglion cells. The epiretinal implant does not have light sensitive elements. People affected by retinal degeneration are supposed to gain a significant lasting improvement in their quality of life. The person wearing the retina implant may be able to independently orientate himself again, move freely in an unfamiliar environment and identify and perceive the form and location of larger objects (Figure 12).

A new concept of epiretinal stimulation has arisen. In this approach, the release of a neurotransmitter is reported as an alternative for creating electrical impulses at the vitreoretinal interface. Inactive forms of neurotransmitter may be implanted over the retina. Under light stimulation, the active form should be generated and electrical stimulus created (Lezzi and Fishman, 2002) (Figure 13).

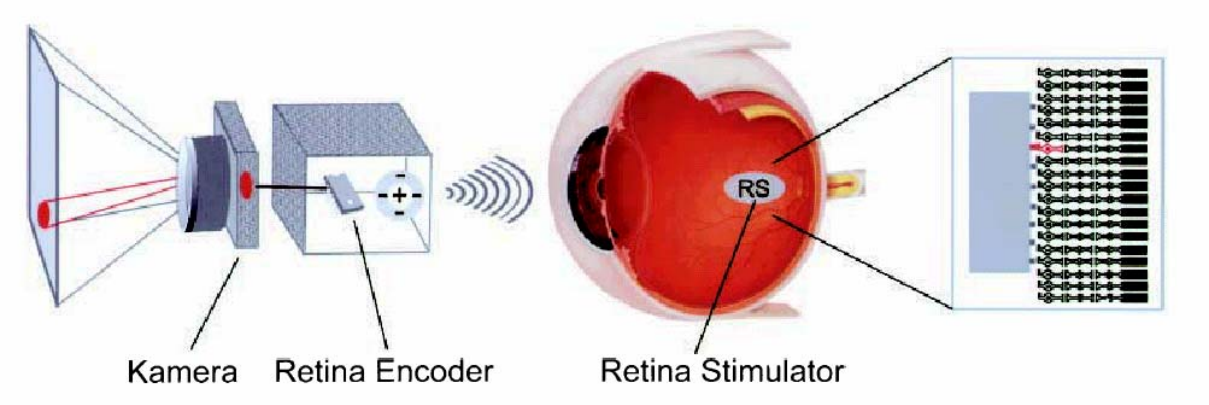

Figure 12: Esquematic concept of the Epi-Ret group prosthesis.

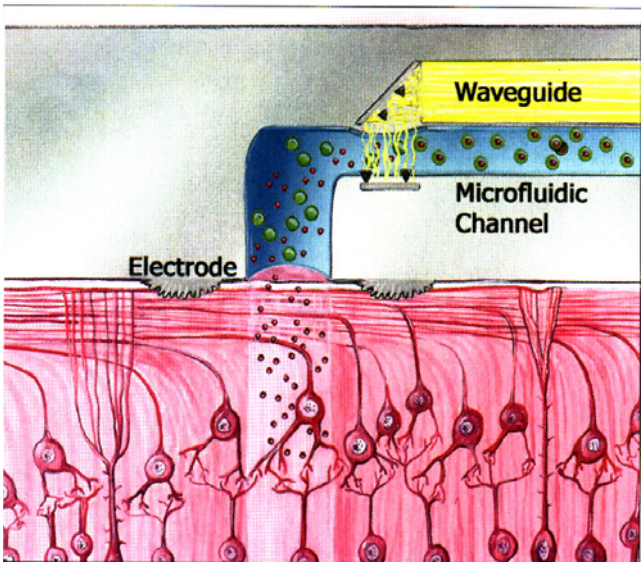

Figure 13: A new concept of epiretinal stimulation is formulated by Lezzi and Fishman, 2002. The electrical stimulation works in the context of a neural stimulation by neurotransmitter.

1.4.2.2.2 Subretinal implants

There are several teams exploring the subretinal approach to develop a retina implant (Chow and Peachey, 1998; Zrenner et al., 1999). The subretinal implant comprises thousands of light-sensitive microphotodiodes with microelectrodes, which should be implanted between the retinal pigment epithelium layer and the outer layer of the retina (Figure 10). The subretinal approach seems to be the most physiological because the device directly substitutes photoreceptor cells. Subretinal implants also have the advantage of ease of

surgical positioning of the microphotodiodes (the pigment epithelial cells of the retina pump out the sub-retinal space in such a way that the implant is sequestered in the subretinal space)(Figure 14). Regarding the device for the subretinal implant no external camera is needed, and eye movements may be used still to locate objects. Works demonstrate that there are basically two surgical approaches for the introduction of the subretinal implant, either the access to the retina through the vitreous humor or directly to the subretinal space through a scleral incision (Peyman et al., 1998; Taneri et al., 1999; Schwann et al., 2001; Zrenner et al., 2001).

Subretinal implants also poses potential disadvantages. Experiments in-vivo have shown that the light from the environment may not be sufficient to stimulate the microphotodiodes, and an external aid device should be developed (Zrenner, 2002). A subretinal device might impair diffusion of nourishment from the retinal pigment epithelium layer caused by the presence of a subretinal prosthesis. A mechanical barrier between the retina and the choroid may cause fibrosis after chronic subretinal implantation. This might also lead to proliferation and migration of the retinal pigment epithelium cells and alterations in Müller cells, both of which could destroy the retina. Furthermore, the extremely long horizontal extensions of the horizontal cells might compromise transfer of spatially detailed visual information. Some histological studies of the retina with subretinal implants have demonstrated smaller inner nuclear and ganglion cell layer densities, but no inflammatory responses (Peyman et al., 1998; Kohler et al., 2001). Others have reported irregular glial proliferation above the array. Porous electrode arrays are made to avoid this barrier effect (Schubert et al., 1999). The subretinal implants have also the disadvantage that due to the small subretinal space only small implants may be inserted surgically.

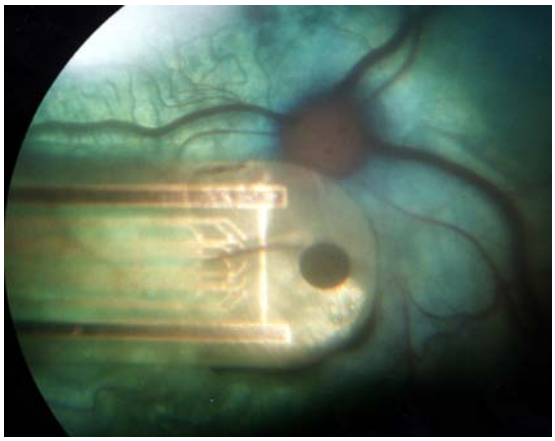

Figure 14: Four months post-operative picture of a subretinal implant in cats. The implant is positioned in the subretinal space of the central retinal region, and there is no fibrovascular response surrounding it.

1.5 This work

This work is motivated by the challenge to assess the potential benefits for a blind patient equipped with an epiretinal prosthesis. This thesis was carried out as part of the BMBF-funded (German Ministry of Education and Research) development of an epiretinal prosthesis. The retina implant project is a multi-center project with the participation of several universities in Germany. This is a very and complex project, as engineers, ophthalmologists, neurophysics, and others participate in the project. The co-operation of those multi-disciplinary team makes possible the development of the retina implant project. All experiments in this work were part of the Epi-Ret implant group. This thesis had the active participation of engineers of the Fraunhofer Institute for Biomedical Engineering, St. Ingbert (development and construction of the epiretinal implant); the physics Dr. Thomas Schanze and Andreas Rentzos from the department of Neurophysics, Philipps-University Marburg (electrophysiological testing of the epiretinal implants); and the ophthalmologist Dr. Lutz Hesse (the surgeries for implantation). It was not the goal of this work to evaluate the several multi-disciplinary aspects of the development of epiretinal implants, but only some specific goals related to ophthalmology, as described in section 2. I participated in this investigation by assisting the surgeries for the implantation, by giving medical support in the experiments for electrophysiology proving, and by examining the cats in the whole follow-up period.

The research group of the University of Marburg has a new epiretinal prosthesis to be tested. This device is comprised of three parts: an electronic intraocular lens, a flexible microcable, and the microelectrode array. In order for a potential visual implant to be useful to the blind patient, a successful surgical technique for the implant must be achieved in animals. Cats were chosen for several reasons, including the well know similarity to the primate visual system, the previous experience of the working team with the surgical techniques and electrophysiological testing, as well as the already proved retinotopic cortex activation (Hesse et al., 2000b, Schanze et al., 2002).

Toward achievement of an functioning retinal prosthesis, the biocompatibility of the surgery and chronic implantation of the prosthesis must be tested. Long-term implantation and electrical stimulation of an intraocular prosthesis has to be safe and efficient. Intraocular stimulation with an active epiretinal implant has not yet been tested chronically in the eye, neither in animals nor in humans. Knowledge of the reaction of the animal's eye is influential on the design of the implant. Phacoemulsification, vitrectomy, and implantation of the epiretinal implant into the eye of cats were performed. The cats were followed by close

clinical observation for evaluation of the reaction of the eye to a working electronic device. Electrical stimulation tests were performed and data were recorded. The following issues are addressed: the technique for surgical implantation of the electrode array on the retina; the biocompatibility and functionality of the implanted epiretinal implant; and the mechanical effects of the electrode array on the ocular tissues.

2. Objectives

1. To evaluate the surgical technique to insert and fixate a new generation of epiretinal implants.

2. To evaluate the biological reaction of the intraocular tissues under the presence of an epiretinal implant.

3. To observe the long-term stability of the epiretinal implant in the cat's eye.

4. To verify the epiretinal implant function after the electric retinal stimulation by performing electrophysiological tests and cortical recordings.

5. To review the current status of the researches to developing a visual prosthesis for the blind.

3. Materials, Method and Procedures

3.1 Animal Model

Surgical and electrophysiological procedures were performed in anesthetized cats (*n= 6, 3–5 kg*). The procedures were in accordance with the guidelines of the European Communities Council Directive (86/609/EEC) and were approved by an official German Animal Care and Use Committee. In addition, the NIH Principles of Laboratory Animal Care (Publication No.85-23, revised 1985), the OPRR Public Health Service Policy on the Human and Use of Laboratory Animals (revised 1986), the US Animal Welfare Act, and the ARVO guidelines were followed.

3.2 Epiretinal implant specifications

The epiretinal implant is part of a whole retinal prosthesis system (Figure 15). The concept of this epiretinal implant itself is to generate small electric currents by an electronic device connected with the electrodes after fixation of the implant on the retina. The implant activation is achieved by an external device called retina encoder. The retina encoder processes the data of visual scences recorded by a camera.

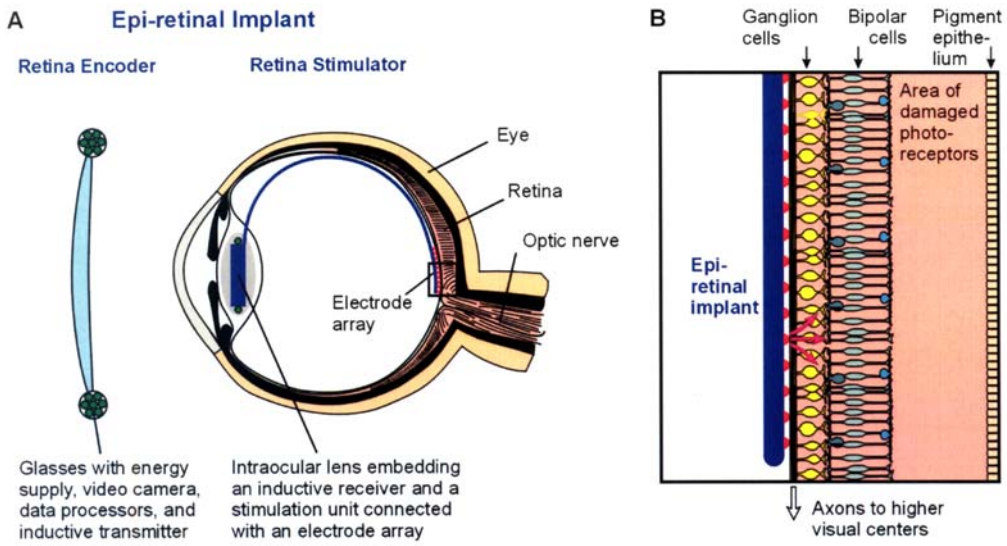

Figure 15: Structure and system of a complex epiretinal prosthesis.

The retina stimulator or retina implant itself consists of three major components (Figure 16). The first component contains the electronics required to receiving energy and signals for retina stimulation. This unit will be implanted in the eye by replacing the lens, the same position as for the intraocular lens implanted in the cataract surgery. It is then denominated electronic intraocular lens. This unit is formed based in silicone photodiodes. Four of them were connected in series on the polyimide substrate to reach a voltage high enough to generate a current for stimulating retinal ganglion cells with the electrodes positioned on the inner retinal surface. The layout of the stimulator substrate consisted of three rings arranged concentrically and connected via s-shaped interconnects that allow a three dimensional adaptation of the rings to the spherical shape of the ocular bulbus. The

second component is a microflexible folie that makes the electrical connection between the electronic intraocular lens and the third unit.

The third unit is formed by the electrode arrays of the epiretinal ganglion cell stimulation. The electrodes were protected with a surface made of aluminum oxide ceramic plate. The implants were mounted on a stack and a layer of 20 μm parylene C deposited in a process chamber. Parylene, the generic name of poly-paraxylenes, has the property of being an excellent encapsulation and insulation chemical. The electrode was made of an one channel retina stimulator made of polyimide substrate with integrated platinum electrodes. A combined parylene/silicone encapsulation of electronic components was used to protect the electronics against moisture, ions, and potential mechanical stress during implantation (Figure 17).

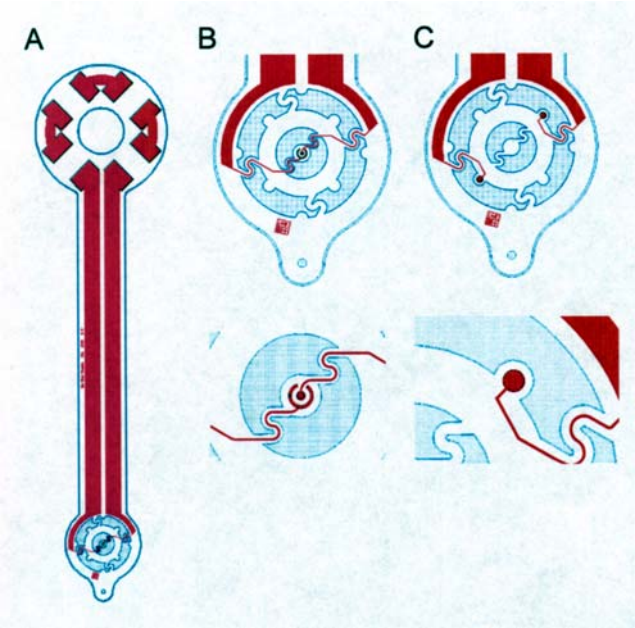

Figure 16: The epiretinal implant is composed of three units. The two different configurations are illustrated: concentric and bilateral platinum-polyimide eletrodes.

Finally the epiretinal implant was encapsulated with silicone to protecting the electronics and to allowing the stimulator's implantation in the eye. Polydimethylsiloxane was the material chosen for encapsulation, as it has the characteristics of being flexible, biocompatible, inert and transparent.

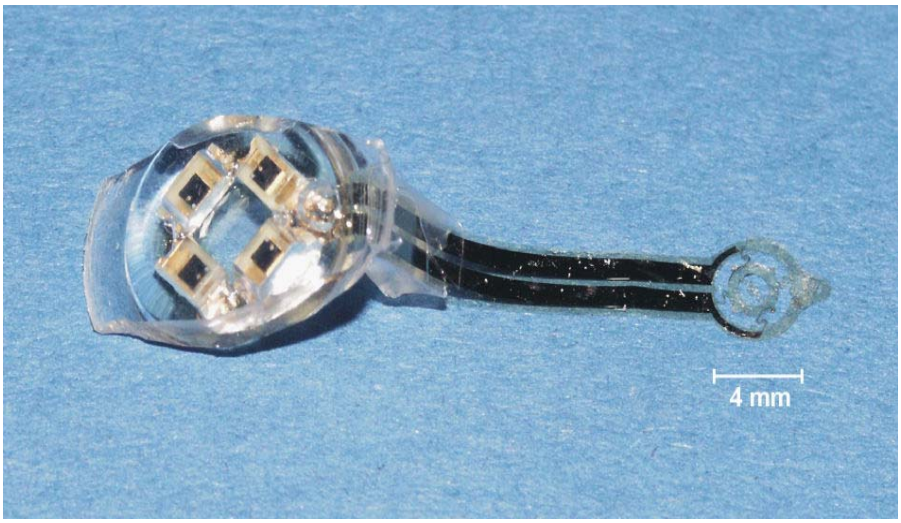

Figure 17: The epiretinal implant is composed of three parts. The electronic intraocular lens is the large round piece and sits on the left. Between the electronic intraocular lens and the microelectrode array one can observe the flexible microfolie, the black cable carrying the impulses from the electronic intraocular lens to the microelectrode array. The microelectrode array is the third piece in a round form on the right side of the picture, and measures 4 mm diameter. Polydimethylsiloxane served as final encapsulation material.

Table 2
Different epiretinal implants

	Size of implant (mm)	Parts	EI Generation	Material	Electrically Active or Inactive	Tack material	Position implanted
Walter et al 1999	5 x 3 x 0.1	1	1st	silicone rubber (polydimethylsiloxane)	Inactive	Yes titanium	vitreous
	10 x 2 x 0.02	1	1st	polyimide + platinum	Inactive	Yes titanium	
Majii et al 1999	3 x 5 x 1	1	1st	platinum + silicone	Inactive	Yes	vitreous
Hesse et al 2000	0.1 x 0.1	1	2nd	polyimide + platinum	Active	No	vitreous
Walter et al 2000	plane 0.1, plane 0.05 †	1	1st	polyimide + platinum silicone TiN	Active	No	vitreous
Vobig et al 2000	not informed	3	2nd	silicone IOL + PDMS platinum	Inactive	Yes ND	capsular bag + vitreous
Kerdraon et al 2002	19 x 5	2	2nd	biocompatible ceramic+ platinum wires+ silicone	Inactive	No	anterior segment + vitreous
Schanze et al 2002	ND	3	2nd	polyimide platinum	Active	No	vitreous
Schanze et al 2003	ND	3	2nd	polyimide-platinum	Active	No	capsular bag + vitreous
This work	29 mm 7 mm	3	2nd	platinum-polyimide	Active	No	capsular bag + vitreous

† array was connected to the external stimulator with a wire
ND: no data; EI: epiretinal implant; IOL: intraocular lens
TiN: Titanium nitride; PDMS: Polydimethylsiloxane

3.3 In-vitro testing of electrical stimulation under intraocular liquids

The eye is filled by the anterior chamber liquid in the anterior segment and by the vitreous gel in its posterior part. After implantation of the epiretinal prosthesis in the eye, the eye may be filled by several liquids, which are routinely used in ophthalmic surgery. The goal of the following tests described in this section is to test the interaction of the most common intraocular fluids with electrodes and their electrical conduction capacity.

In the first experimental set-up, electric conduction capacity was tested by placing two platinum-polyimide electrodes of a simplified electrical equivalent circuit of the retina stimulator in contact with five different intraocular fluids, ringer-lactate, perfluorocarbon liquid (F-Octane, Neu-Ulm, Germany), silicone oil (AcriSilOl 5000, Acritec, Berlin, Germany), viscoelastic (Healon, Pharmacia, Erlangen, Germany), and glucose 5% (G5, Alcon, Forth Worth, USA). The goal was to figure how much electric current each of those fluids can conduct.

Second, electrical conduction of the active epiretinal array was tested by placing it under the five different liquids separately: ringer lactate, perfluorocarbon liquids, silicone oil, viscoelastic, and glucose 5 %. Electric stimulation was constantly performed on sinusoidal waves of 100 mV/1 KHz (Figure 18). After testing the electric conduction, the cleaning of those liquids out of the microelectrode array was observed under a high magnification microscope (Zeiss, Germany).

Third, fresh enucleated pigs' eyes (n = 2) were fixed in a mounting plate. After opening the eye at a 2 mm limbus incision, and a 360 degrees limbus opening was accomplished. The cornea and limbus were extracted, followed by open-sky manual lens and vitreous removal. While the retinal tissue was exposed, the epiretinal array was placed on the retina, and the zero measurements were obtained. Then, four different intraocular liquids were independently injected and the microelectrode array electronic stimulated, as following:

1) Electronic testing under perfluorocarbon liquids: 3 ml of perfluorocarbon liquids were injected into the eye, and microelectrode array functioning was tested. Electric conduction was observed for 15 minutes. Following 1 ml of ringer lactate solution was injected. Three hours later electrical measurements were carried out again. The perfluorocarbon and ringer lactate solutions were removed. Vitreous remnants were then carefully removed, and a new zero measurement obtained.

2) Testing under silicone oil was observed: The epiretinal implant was again positioned on the retinal surface. 3 ml of silicone oil were infused to the eye. Electric conduction was followed for 1 hour.

The microelectrode array was cleaned and a new zero measurement obtained. Microelectrode array was positioned on the retina, and new electric conduction values obtained.

3) Electronic testing under Ringer-lactate: 3 ml of ringer lactate were infused. Electric conduction testing lasted 3 minutes.

4) Electronic testing under viscoelastic: 2 ml of viscoelastic were infused following silicone oil and microelectrode array positioning.

Positioning and movements of the microelectrode array under silicone oil, perfluorocarbon liquids, perfluorocarbon plus ringer lactate were then evaluated.

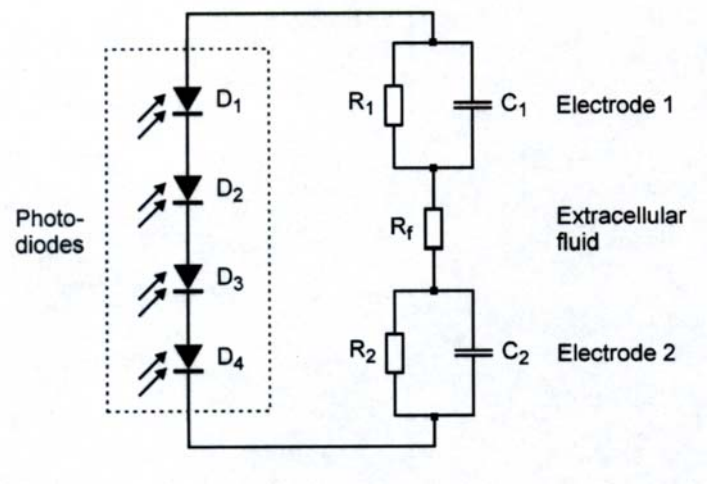

Figure 18: Simplified electrical equivalent circuit of the retina stimulator. Four photodiodes are connected in series. Electrode properties are modeled by a resistor and a capacitor connected in parallel. The electrical behavior of the extra-cellular fluid between the two electrodes is approximated by a resistor.

3.4 Anesthesic and implantation surgical procedure

The whole surgical procedure was carried out in the dorsal decubitus position, with the head adjusted to obtain a vertical optical axis. Pre- and post-operative veterinary and eye care were performed. Prior to any surgical procedure the cats received atropine sulfate (0.03 mg/kg) to reduce salivation. Anesthesia was started by intramuscular injection of a mixture of ketamine hydrochloride (Ketanest, 10-15 mg/kg) and xylazine hydrochloride (Rompun, 1 mg/kg). After orotracheal intubation, anesthesia was maintained by ventilation with N_2O/O_2 (70%/30%) and halothane (0.3%-0.8%) or isofluorane (0.5-1.5%). Continuos monitoring of rectal temperature (38°C), end-expiratory CO_2 (3.8%-4.2%), electrocardiogram, and reflexes were used to control the level of anesthesia. Anesthesia was maintained by ketamine hydrochloride (Ketanest, 1-5/kg i.m.) and/or propofol (propofol, 0.02-0.05 mg/kg/h).

Visualization was achieved with the aid of an operating microscope (Zeiss, Germany). The pupils were dilated with topical cyclopentolate hydrochloride 0.5 % and tropicamide 1 % eye drops. Retraction of the nictitating membrane was achieved by topical administration of phenylephrine hydrochloride 2 %. Tissue plasminogen activator (25 mg, Actilyse, Biberach a. d. Riß, Germany) was injected (n = 2, 33.3 %) through the pars plana to induce a posterior vitreous detachment enzymatically by autologous generated plasmin (Hesse et al., 2000c; Hesse et al. 2001). Surgery for implantation of the retina electrode followed 1 week or more after enzyme injection in those cases. The removal of the posterior vitreous cortex should allow a better contact between the retina and the epiretinal array.

Surgery began with a 1 cm opening cut of the nictitating membrane at the temporal side to gain exposure. A 180 degrees superior peritomy was performed, followed by a superior limbal incision of 2.5 mm. Capsulorhexis on the anterior lens capsule between 3 and 6 mm size through a temporal corneal incision was done after injection of 1 ml Healon chamber (Pharmacia, Freiburg, Germany) into the anterior.

The implantation of this new generation epiretinal implant requires total lens removal. In all cases the lens was removed by phacoemulsification (Geuder, Heidelberg, Germany) and subsequent irrigation/aspiration (Figure 19). The irrigation solution was composed of 500 ml Ringer's lactate, 40 mg refobacine and 5000 IU heparin. Two additional corneal incisions were made 3 clock hours temporal and nasal to the superior paracentesis. A temporal limbus paracentesis was used for fluids infusion. The infusion bottle with the delivering solution was positioned at about 35 mmHg. The vitreous cutter was used to create a hole of about 3 mm diameter in the posterior capsule. The large anterior chamber of the cats was filled with a single air bubble, allowing easy visualization of the complete retina.

Additional lens systems such as macula lens was utilized if a large air bubble in the anterior chamber was not achieved (Figure 20).

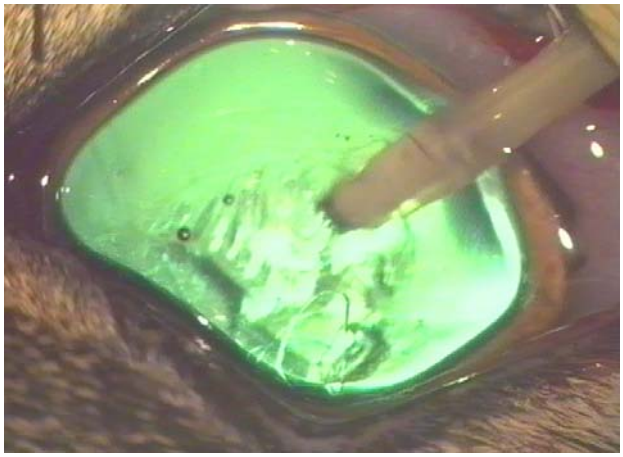

Figure 19: Capsulorrhexis varied between 3 and 6 mm. The lens was removed by phacoemulsification.

After anterior chamber intervention, vitrectomy was then performed. Then, 1 ml indocyanine green dye (0.05%) (Pulsion, Munich, Germany) was injected (n= 2) in the posterior vitreous to obtain good visualization and complete removal of the vitreous tissue remnants. After 1 minute, the indocyanine green was removed with the vitreous cutter (Figure 21). Aspiration pressure of the vitreous cutter was increased up to 150 mmHg, the same technique used to remove posterior cortex of idiopathic macular holes in humans. The engaged vitreous was raised up slowly, and once it was far from the retina, cutting and aspiration were again initiated. To verify the complete removal of the vitreous cortex a silicone soft-tip extrusion needle with active suction was swept across the retinal surface in all cases. No "fish-strike" sign was seen in any cats, indicating a successful complete stripping of the posterior hyaloid. The peripheral vitreous was carefully removed by diaphanoscopic indentation (Schmidt et al., 2000).

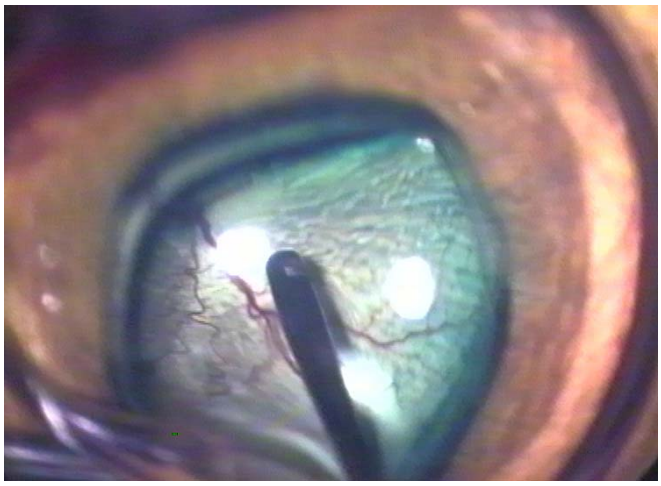

Figure 20: A air bubble in the anterior chamber allowed good visualization of the posterior pole during vitrectomy.

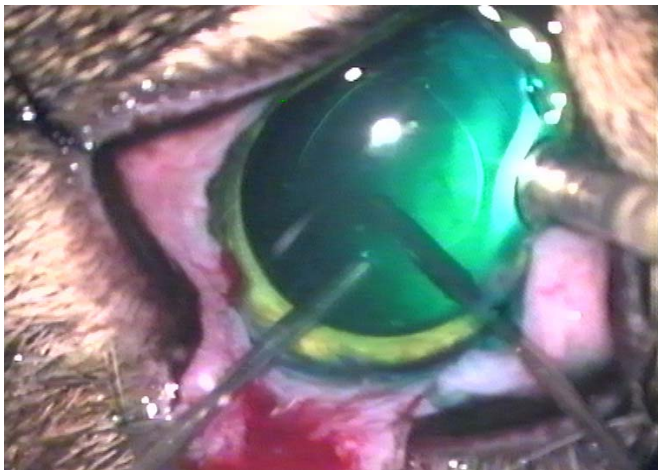

Figure 21: Indocyanine green dye was injected in four eyes in a small volume (1 ml) and concentration (0.05 %). After 1 minute indocyanine green was removed from the vitreous cavity.

In three cases, perfluorocarbon liquid (2 ml, F-Octane, Neu-Ulm, Germany) was injected in the vitreous cavity to support the entry of the implant and protect the retinal tissue before epiretinal implant insertion. The epiretinal implant was introduced through a 9 mm corneoscleral incision into the anterior chamber, and implanted as follows: The tip of the electrode film was subcutaneously directed into the subconjunctival space. The epiretinal implant was implanted through the limbus with care not to touch the endothelium (Figure 22). By using intraocular forceps the microelectrode array was inserted through the posterior

capsulotomy and the vitreous cavity. The implant was guided to its final position in the vicinity of the posterior chamber, where it was released, thus allowing the foldable part to unfold onto the perfluorocarbon liquid bubble. After inserting the epiretinal implant in the sulcus, perfluorocarbon liquid was carefully removed in (n = 3) so that the flexible microfolie and the microelectrode array would slowly move towards the posterior vitreous and touch the retinal tissue. While the perfluorocarbon liquid bubble was removed, the posterior part of the epiretinal implant slipped slowly towards the posterior vitreous, and achieved a parafoveolar position at the posterior pole. Table 4 illustrates the variations in the surgical techniques applied in this study.

In all cases the epiretinal implant was implanted after manually folding the flexible microfolie onto the electronic intraocular lens shortly before implantation, and carefully implanted towards the capsule sulcus. In three cases (50%), the electronic intraocular lens piece was sutured to the microelectrode array with a prolene 10-0. Suturing was done at the anterior part of the electronic intraocular lens, so that the whole implant final location was near the equator. The epiretinal implant was inserted folded, and after centering it in the middle of the eye, the suture was cut intravitreally by a microvitreoretinal blade, so that the flexible microcable and microelectrode array would slowly move towards the retina.

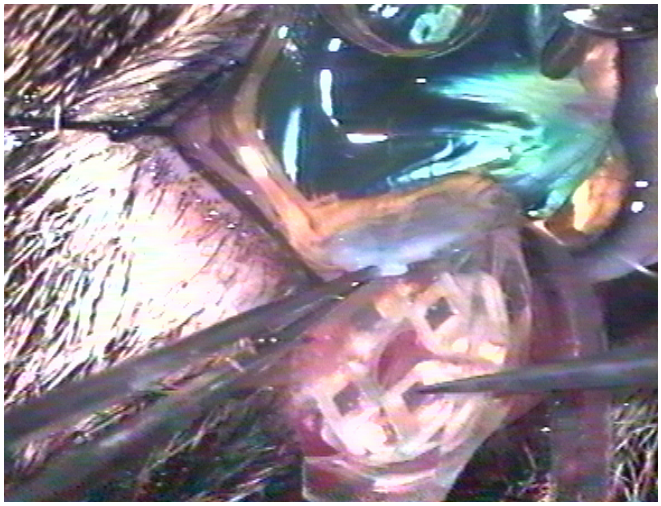

Figure 22: Moment of implant insertion. The cornea had to be raised for complete implantation. The flexible microfolie is folded towards the electronic intraocular lens.

Fixation of the epiretinal implant may be divided in two groups: it was performed either by reinjecting a great perfluorocarbon bubble (3 ml) in the vitreous cavity (n= 2) plus the effect of a in-the-bag position of the electronic intraocular lens; or by suturing the anterior part of the implant temporal and nasal to the scleral tissue (n= 4). In the first group,

care was taken to implant the electronic intraocular lens in the capsular bag so that an additional mechanism of capsular fibrosis may auxiliate in the fixation process. The technique for scleral fixation utilized in this investigation is described briefly. A two-needle prolene 10-0 was penetrated through nasal scleral tissue. A forceps inserted through the limbus took the needle into the posterior chamber. This same needle was inserted and sutured to the temporal part of the electronic intraocular lens. The same maneuver was repeated in the nasal side. After electronic intraocular lens insertion in the posterior chamber, the second two needles remaining outside the eye were passed through the sclera and firmly sutured to the scleral wall.

Corneal incisions were closed with 10.0 nylon (Alcon, Freiburg, Germany). The conjunctiva was closed with interrupted 6-0 vicryl sutures (Ethicon, Somerville, NJ). A sub-tenon's injection of gentamicine and dexamethasone phosphate was administered at the end of surgery. For up to 2 weeks after surgery, a topical antibiotic and steroid ointment were instilled.

3.5 Clinical follow-up

The cats were examined under narcosis and received atropine sulfate (0.03 mg/kg) prior to any surgical procedure in order to reduce salivation. Anesthesia was started by intramuscular injection of a mixture of ketamine hydrochloride (Ketanest, 10-15 mg/kg) and xylazine hydrochloride (Rompun, 1 mg/kg).

Animals were examined 1 day before and after the operation, one week post-operatively, and then every month after the surgery. Final follow-up varied from 6 months to 18 months. A follow-up period of a minimum of 6 months was chosen, i.e., a length of time after which the outcome is unlikely to change as far as vitreoretinal procedures are concerned. Standart ophthalmic instruments were used for examination, including slit-lamp for anterior segment biomicroscopy. Intraocular pressure measurement was carried out with a Shiotz tonometer and by bidigital touch measurements. An indirect ophthalmoscope was the instrument utilized for vitreous and retinal visualization. The position of the implant on the retinal surface was identified by an indirect ophthalmoscope and a high-power magnification surgical microscope. Optical coherence tomography, and A- and B-scan ultrasound examination were performed in one eye postoperatively, as good media transparency allowed visibility of the retinal surface (Meyer et al., 2003a; Meyer et al., 2003b).

Color fundus photography was performed using 100 ASA color film Nikon Dimage 7 Camera (Tokyo, Japan). The degree of corneal edema was clinically evaluated by using the following criteria: 0, no opacity; 1, a scattered or diffuse area is present, but details of iris clearly visible; 2, an easily discernible translucent area, but details of iris slightly obscured; 3, an opalescent area with no visible details of iris or pupil; and 4, opaque cornea and invisible iris (Ohguro et al., 1991).

If epiretinal implant dislocation occurred, it was quantified by “no” to “+++” as follow: no dislocation (no); light dislocation, but the microelectrode array sits on the retina and microelectrode array stimulation is possible (+); moderated dislocation with the microelectrode array touching the retina but microelectrode array stimulation was not achieved (++); severe dislocation without any contact between the microelectrode array and the retina (+++).

3.6 Electrical stimulation and data analysis

For a successful epiretinal implant functioning, electrical stimulation requires generation of short voltage or current impulses transmitted by electrodes into neural tissue. The visible light impulses for implant's activation in in-vivo experiments should be avoided. The diode has a viewing angle of 16 degrees and provides a light irradiation at 875 nm with 900 mW/sr for current impulses of 1 *A* amplitude and 100 μs duration. A corresponding area of 6 mm diameter or 28.3 mm^2 may be irradiated with 55 mW infrared light under optimal conditions. This is a power density of near 2 mW/mm^2. Therefore the epiretinal arrays should create a short circuit current of about 280 μA. Considering the 80% attenuation of the infrared beam after passing through the ocular media up to the retina, the photodiodes should produce a current of 56 μA. This value should be enough for an epiretinal stimulation, since recent data show that the threshold for successful epiretinal stimulation should be under 10 μA (Schanze et al., 2002).

The retina implant prototype was activated with infrared light irradiated from the optical driving unit positioned in front of the eye. The driving unit was controlled by a programmable wave form generator. Impulses of 100 and 200 μs duration at different light intensity and distances between the electrodes and the eye. For recording of the currents generated by the implanted retina stimulator and evoked neuronal responses a contact lens electrode was placed onto the eye and positioned a stainless steel electrode under the skin onto the bone above primary visual cortex at the occipital pole of the head. As reference an electrode located at the rectal body temperature sensor or a tongue electrode was utilized. The electrodes were connected to differential amplifiers. Stimulation waveforms and the amplified band passed electrode signals (1 Hz – 10 kHz), and were displayed on a digital oscilloscope and recorded at 50 kHz sampling rate with a computer-based data acquisition system (Multichannel Systems, Reutlingen, Germany) for subsequent data analysis.

Computer-controlled axial positioning of concentric bundles of three or seven occipital cortex fibre electrodes was achieved with a modified Eckhorn manipulator drive, specially adapted for retinal stimulation. We verified the movement of the fibre electrodes and their close retinal contact optically. This approach was completed by the recording of spontaneous or visually evoked retinal activity. A schematic overview of the experimental set-up used for stimulation experiments with fibre electrodes is presented in the figure 23.

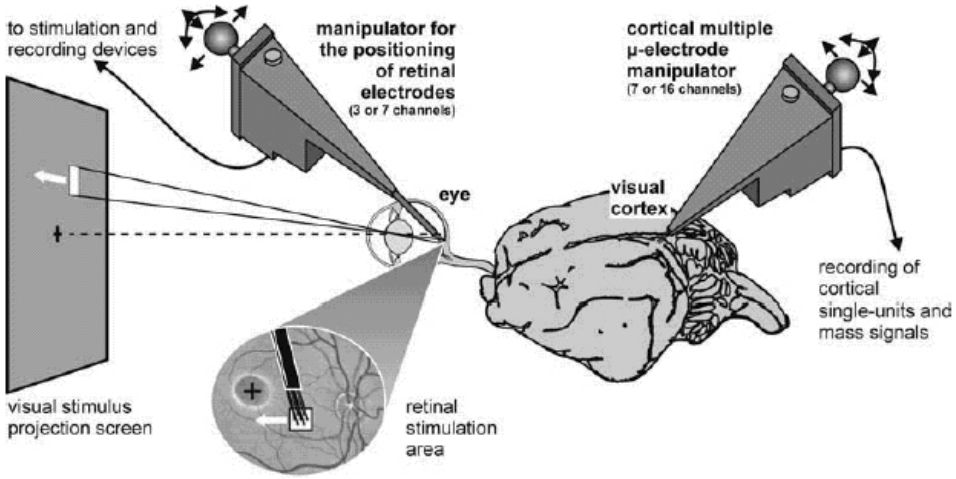

Figure 23: Esquematic representation of the experimental set-up for testing electrical retina stimulation by cortical responses in the anaesthetized cat. Reprinted from Shanze et al., 2002.

4. Results

4.1 In-vitro testing of the epiretinal implant functioning under different intraocular liquids

Of the five intraocular fluids tested for electric conduction with two electrodes, ringer lactate, glucose 5 %, and viscoelastic showed to be good electric conductors. Silicone oil and perfluorocarbon liquid did not allow electric current of the two electrodes, and were concluded to be bad electric conductors.

Electrical conduction of the active microelectrode array was tested with a constant of 100 mV/1KHz, and the following results were obtained:

Under air, the zero measurement was 62 mV. Inside the liquids, the currents below were obtained:

Ringer lactate: 57 mV

Silicone oil: 64 mV

Silicone oil plus ringer lactate: 64 mV

Perfluorocarbon: 64 mV

Perfluorocarbon plus ringer lactate: 57 mV

Silicone oil adhered strongly to the epiretinal implant material. Perfluorocarbon could easily be totally removed from the epiretinal implant.

In the eye, the epiretinal implant generated the following electric currents:

Zero measurement: 56.2 mV

Perfluorocarbon: 56.2 mV

Perfluorocarbon plus ringer lactate: 54 mV, after 3 hours: 57.7 mV

After thoroughly vitreous removal,

New zero measurement was: 56.5 mV

Silicone oil: 58.3 mV

The silicone oil adherent to the chip was mechanically removed, then:

New zero measurement: 63.4 mV

Silicone oil, better epiretinal implant positioning: 57.7 mV

Viscoelastic: 57.7 mV

Ringer lactate: 57.7 mV

When perfluorocarbon was injected followed by ringer lactate, the epiretinal implant remained positioned on the retina, not dislocating. The chip must touch the retina to allowing a good conductance (Table 3).

Table 3

Electric conduction capacity and advantages of different intraocular liquids

Liquid	Two eletrodes conduction	Electric current under liquids (mV) *	Electric current in eye (mV) †	Advantages as intraocular fluid for EI
Glucose 5 %	good	ND	ND	low costing good electric conductor
Ringer Lactate	good	57	57.7 §	does not adhere to the EI good electric conductor
Perfluorocarbon	bad	64	56.2	does not adhere to the EI presses the EI against the retina
Silicone oil	bad	64	58.3 §	good interaction with RL viscous structure avoid trauma
Viscoelastic	good	ND	57.7 §	viscous structure avoid trauma good electric conductor
Perfluorocarbon + RL	ND	ND	54	advantages of both perfluorocarbon RL

*: Zero measurement: 62 mV

†: Zero measurement:: 56.2 mV

§: Measurements obtained after thoroughly vitreous removal

ND: No data

EI: Epiretinal implant

RL: Ringer lactate

4.2 Implantation surgery

A less traumatic surgical technique was developed for the implantation of a new generation epiretinal implant. The differences in approach were the result of adaptations for a better implantation. Timing for any surgery is important to make the procedure less traumatic as possible, as it is known that time of vitrectomy is related to post-operative complications. Surgery in this work varied between 1 hour and 45 minutes to 2 hours and 30 minutes (Table 4).

Table 4
Surgery technique in each animal model

Animal	Duration the surgery (hours)	Diameter of capsulorrhexis (mm)	Mechanical PVC removal	Use of ICG	PFC intra-operative
1	2	4	no	no	yes
2	1.75	6	no	no	yes
3	2.5	5.5	yes	yes	yes
4	2.5	4.5	no	yes	no
5	2.3	3	yes	yes	no
6	2.3	4	no	yes	no

PVC: posterior vitreous cortex; ICG: indocyanine green; PFC: perfluorocarbon liquid

Our surgical technique consisted of phacoemulsification for assurance that no lens cortex would remain in the eye, which has similarities to some of the previous works in the literature (Table 4, 5 and 6). Phacoemulsification with low energy pulse was utilized. No residual cortex remained inside the eye in any case after the phacoemulsification.

The anterior capsulorrhexis was performed without rupturing in all cases. The size of the capsulorrhexis varied between 3 and 6 mm. The posterior opening in the capsule was successfully accomplished with the vitreous cutter in all eyes. In one case an intraoperative rupture of the posterior capsule was seen as epiretinal implant insertion was performed. The anterior chamber maintained fully filled during the whole procedure. No iris contraction was observed during the procedure. One eye was operated in a two-step procedure. The two step-procedure aimed to decrease post-operative inflammation and this goal was achieved. Between the two operations, no intraocular symptoms of inflammation were observed; the cornea remained transparent and no haze or intraocular cells were visible.

Limbus vitrectomy plus lens removal with the phacoemulsification technique could be safely performed in cats using a limbus approach. In no cases cortex residues were found in the vitreous cavity post-operatively. The air bubble in the large anterior chamber of cats allowed good vitreous and retinal visualization in all cases. When more precise visualization was warranted, a macular lens was placed on the cornea. The posterior hyaloid could easily and successfully be removed in all eyes, with the help of syneresis by the tissue plasminogen activator in two cases. No residual posterior cortex was observed postoperatively. Indocyanine green proved to be a helpful adjunct to find the residual vitreous, allowing recognition of several blocks of vitreous remnants (Figure 24).

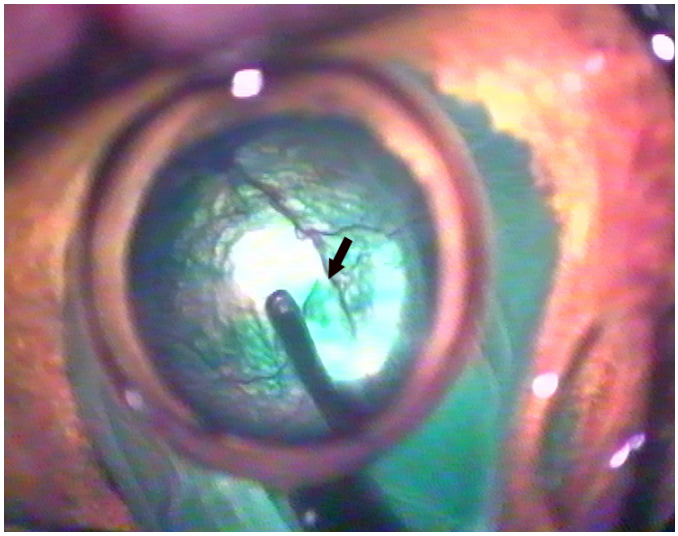

Figure 24: Indocyanine green made observation of the remaining vitreous cortex possible (black arrow).

The limbus incision to insert the epiretinal implant was safe and allowed an easy implant introduction in the eye. While implantation could be possible with a smaller incision, a less traumatic epiretinal implant insertion was achieved when a 9 mm limbus opening was made. The epiretinal implant was successfully implanted in all cases. Insertion and positioning of the epiretinal implant could be performed with a low rate of complications. The maneuver of inserting epiretinal implant with perfluorocarbon liquid and then having to remove the liquid was technically difficult due to the large size of the epiretinal implant and its design. At the time of implant insertion, several observations were made. When scleral fixation was performed, the time of surgery increased from 2 hours compared to a minimum of 2 hours and 20 minutes (Table 4). The cornea was temporarily folded at the implant insertion through the large 9 mm opening, and this maneuver was considered reasonable traumatic for the current standards of ophthalmic surgery. This observation is important because it reminds the demand for smaller epiretinal implant. Although the epiretinal implant implantation was performed as quickly as possible, care had to be taken to avoid touching the corneal endothelium. In two cases (33.3%), intraoperative vitreous hemorrhage occurred intraoperatively a few minutes after epiretinal implant implantation. The source of vitreous hemorrhage was likely to be the sclera in one case, and the superficial retinal vessels in the other case. Concerning the use of perfluorocarbon to protect the retina against traumatic damage, perfluorocarbon proved to be useful for epiretinal implant insertion. Severe retinal trauma was not observed intraoperatively during the epiretinal implant intraocular introduction procedure. In one case the microelectrode array touched the retina slightly

during the implantation. Although the implant was easily introduced into the posterior segment, several movements had to be accomplished to achieve a paramacular posterior position and this may have led to damage on the intraocular tissues. By the end of the surgery the epiretinal implant was good positioned in all 6 eyes (Figure 25).

Implant fixation was obtained by two different techniques in this study. One first group (n= 2) the perfluorocarbon liquid bubble in the posterior segment would fixate the epiretinal implant. In two eyes, 3 ml of perfluorocarbon liquid were reinjected after the end of the implantation to serve as a fixation tool. Additionally, the electronic intraocular lens was carefully implanted in the capsular bag so that the it would be involved by a fibrosis as it occurs in the cataract surgery. In a second group (n= 4) the electronic intraocular lens was fixated to the sclera to maintain a stable position. This technique was very successful once only one case a mild dislocation has ensued. In three other cases the epiretinal implant was centrated for several months post-operatively (Table 5). All wounds were closed successfully with no occurrence of suturing rupture. Table 6 compares our surgical technique to those already tested in the literature.

Table 5

Surgical techniques to implant and fixate the epiretinal implant in each animal model

Animal	EI insertion	Scleral suturing	PFC	Sulcus/ in the bag	Tack	Dislocation
1	folded	No	Yes	in the bag	No	++
2	folded	No	Yes	in the bag	No	+++
3	folded	Yes	No	sulcus	No	no
4	sutured	Yes	No	in the bag	No	no
5	sutured	Yes	No	sulcus	No	+
6	sutured	Yes	No	sulcus	No	no

PFC: Perfluorocarbon liquid; EI: epiretinal implant

Dislocation grading: no dislocation (no); light dislocation, but the microelectrode array sits on the retina and retinal stimulation is possible (+); moderated dislocation with the microelectrode array touching the retina but retinal stimulation was not achieved (++); severe dislocation without any contact between the microelectrode array and the retina (+++).

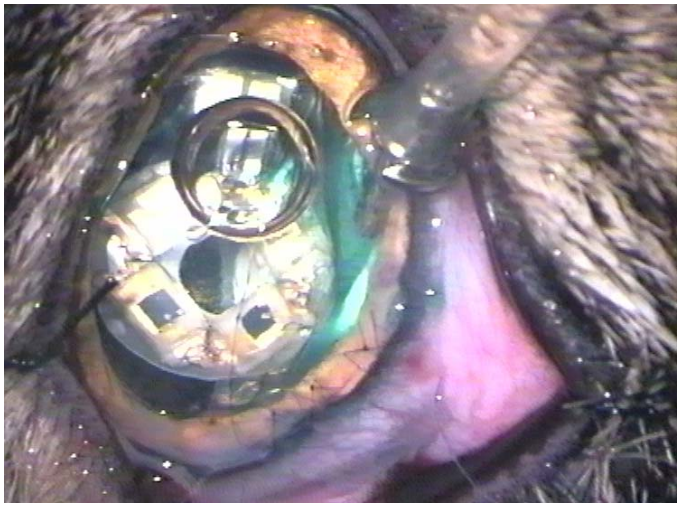

Figure 25: Positioning of the epiretinal implant before the infusion was removed. The epiretinal implant was centrated in all cases by the end of the surgery.

Table 6

Comparison of results of surgical techniques tested for epiretinal implants in animals in the recent literature and present study

	Animal	Approach	Sclerotomy Sites	One or two steps procedure	Fixation	Fixation area	EI Insertion through
Walter et al 1999	rabbits	lens sparing PPV	1 mm behind limbus	2	1 tack * to retina	inferior	pars plana; enlarged sclerotomy
Majii et al 1999	dogs	lens sparing PPV	3.5 mm behind limbus	1	2 tacks	ND	sclerotomy superotemporal
Hesse et al 2000	cats	lens removal (phaco), vitrectomy	limbus	1	cyanoacrylate	para-foveolar	limbus
Vobig et al 2000	rabbits	lens removal (phaco), vitrectomy	ND	1	capsular bag structure + tack	ND	ND
Walter et al 2000	rabbits	lens sparing PPV	yes	1	PFC †	onto the visual streak	one of the sclerotomies
Gerding et al 2001	primates	lens sparing PPV	pars plana	1	hybrid tack	ND	pars plana
Kerdraon et al 2002	ovines	lens removal (phaco), vitrectomy	pars plana	1	suturing	on the retina	limbus
Schanze et al 2002	cats	lens sparing vitrectomy	limbus	1	PFC	para-foveolar	limbus
This work	cats	lens removal (phaco) vitrectomy	limbus	2	suturing PFC	para-foveolar	limbus

* previous laser coagulation for preparation of the tacking
† array was connected to the external stimulator with a wire
ON: optic nerve; PPV: pars plana vitrectomy; EI: epiretinal implant
ND: no data; PFC: perfluorocarbon

4.3 Positioning of the epiretinal implant

The retcam digital fundus camera has proved to image all implants with good quality. Successful intraoperative epiretinal implant positioning was achieved in all eyes. Throughout the minimum 6 months follow-up time, the implant was located at its original fixation in three eyes (50%) (Figure 26, 27), as the microelectrode array situated well behind the iris in the posterior chamber. While in four eyes the epiretinal implant was fixated by scleral suturing, in one of those eyes, there was a mild dislocation to the anterior chamber after 1 month post-operatively, though the epiretinal array kept in contact to the retina (Table 5). Of the two eyes which only perfluorocarbon was utilized as epiretinal implant fixation, some dislocation of the microelectrode array from the retinal surface was observed in two of them. Observation of the dislocated epiretinal implant was visualized since the second examination at 1 week postoperatively. One epiretinal implant dislocated posterior and nasally, and the second one to the anterior chamber (Figure 28).

Figure 26: One month post-operative picture. The epiretinal implant is well positioned in the anterior chamber. The cornea and the anterior chamber are intact and transparent.

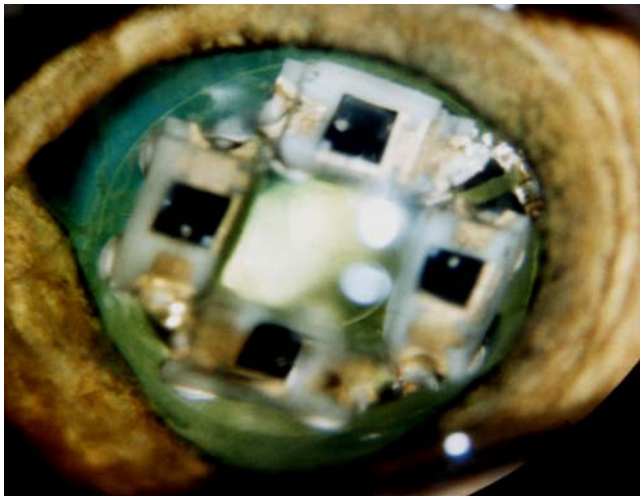

Figure 27: Epiretinal implant is centrated five months post-operatively. The cornea is transparent, and anterior chamber deep.

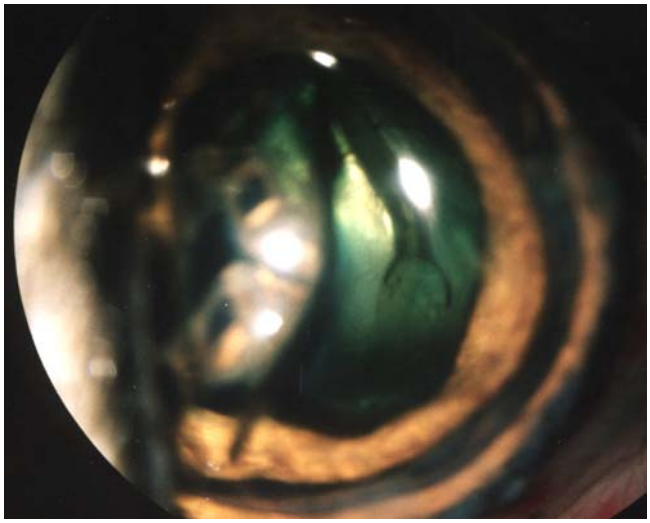

Figure 28: Three months postoperative picture. The epiretinal implant is dislocated. However, the microeletrode array sits on the retina and touches it.

Close examination with a 78 dioptries lens, a high-power magnification surgical microscope, and an indirect ophthalmoscope revealed no mechanical degradation of the epiretinal array in five eyes (83.3%). In one of the two cats which had perfluorocarbon postoperatively, there was a white fibrinous substance around the microelectrode array and

flexible microfolie. The structural integrity of the implanted arrays was also observed macroscopic by routine postoperative examinations. Although it was not possible to quantify the amount of mechanical pressure of the epiretinal array and the microflexible cable to the retina, the microelectrode array caused mechanical damage to the retina in one of the 6 eyes and in five eyes no retinal damage was visualized.

4.4 Influence of the implant on the eye tissues and clinical follow-up – biocompatibility issue

The follow-up period of the implanted eyes was no shorter than at least 6 months. The mean follow-up time was 7 months and varied from 6 to 14 months. Moderate conjunctival hyperemia was observed in the first week in all eyes but improved afterwards. No corneal suture rupturing was visualized. While in 5 eyes the lens capsule was damaged and could no longer be visualized in later examinations, in one eye the capsulotomy was large and remained partially intact.

Corneal alterations were observed in the first month post-operatively in four eyes. In two cases, one resolved totally and in the other eye a slight limbus neovascularization remained in the long-term follow-up time. In two other cases the corneal alterations were found over 6 months post-operatively. Corneal damage for more than 6 months after the surgery developed in the eyes (n= 2) where perfluorocarbon was left intraocularly. These developed corneal alterations were diffuse corneal stromal and epithelial edema early in the first week, and they persisted in two eyes. After the six months follow-up period, one of those eyes showed corneal edema grade 2, and another grade 4 (Figure 29,30,31). In the former, no concomitant severe anterior chamber inflammation occurred. In the one month examination, the corneal edema developed to a slightly opacified corneal lesion. After the six months follow-up period, the cornea was opaque, but the anterior chamber was clear, as well as vitreous cavity. In the latter case, severe anterior segment inflammation was observed concomitantly with the corneal damage. Endothelial opacities and thickening appeared in the early postoperative period and persisted. After the 1-month examination, corneal opacity, corneal stroma edema secondary to endothelial dysfunction, anterior chamber inflammation (cells and flare), and new vessels were documented. With prolonged edema, vascularization of the peripheral corneal stroma occurred. However, these abnormal inflammatory reactions were limited to the anterior eye segment. In this eye, vitreous was clear, and the retina could be visualized. The posterior segment was calm, and no abnormal finding such as vitreous hazing, inflammatory membranes, proliferative vitreo-retinopathy and retinal necrosis were observed.

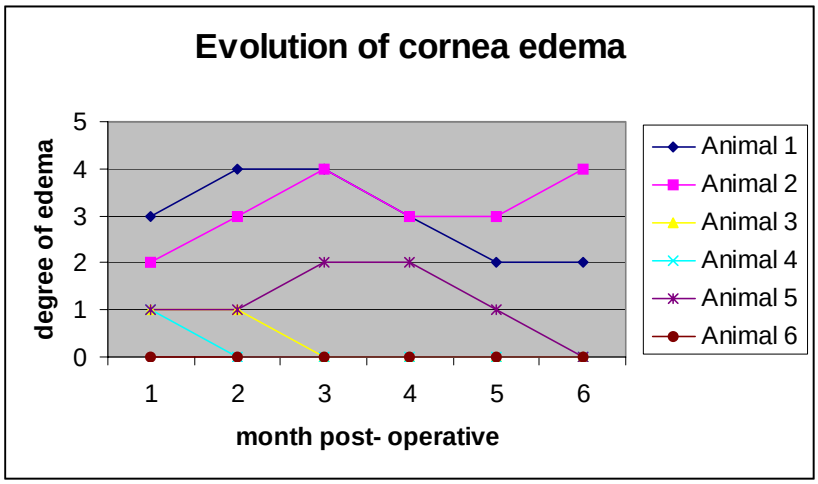

Figure 29: Evaluation of corneal edema in six eyes. At six months follow-up important corneal alterations remained in two eyes (66.67%).

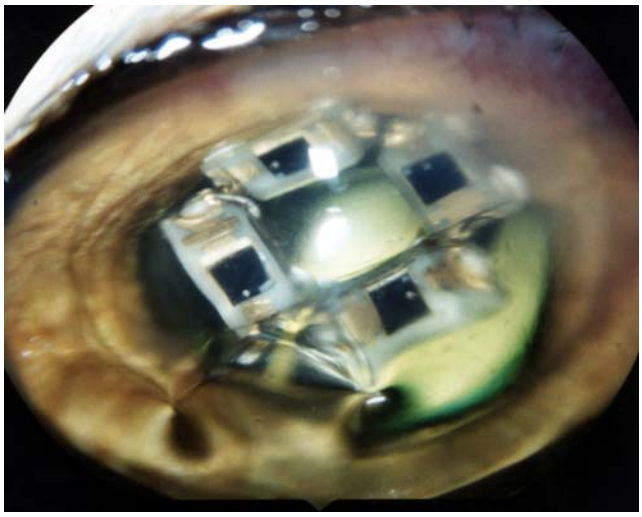

Figure 30: Anterior chamber 2 months post-operatively. Corneal fibrosis and new vessels developed in the superior cornea. The corneal edema was quantified as degree 2. The epiretinal implant is slightly superiorly dislocated. There are no other signs of endophthalmitis as hypopion, flare, or fibrin.

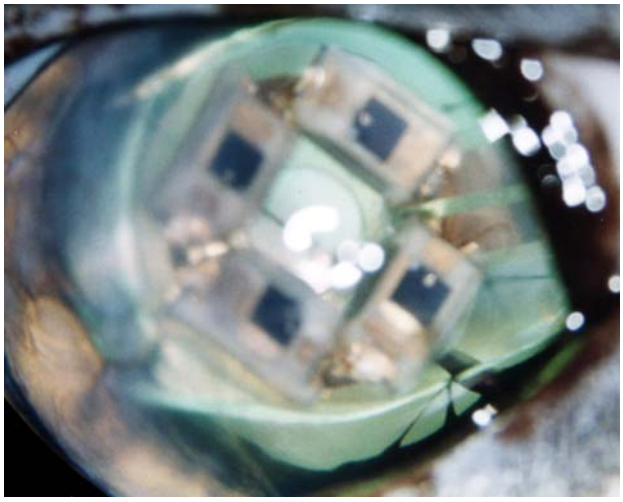

Figure 31: Five months post-operative picture. The epiretinal implant is well centrated. This eye demonstrated corneal edema degree 4 with moderate corneal opacity (left).

The vitreous and the retina was less damaged in this work than the anterior segment structures. Around the epiretinal array, no retinal hyper or hypopigmentation occurred in no eyes. Neither retinal detachment nor retinal fold developed postoperatively. No vitreous hazing followed implantation. Slight damage to the tapetum associated with epiretinal membrane formation was observed in one case. Retinal thinning was observed by fundus examination and accompanied these two previous clinical findings. No gross anatomic globe distortion was seen ophthalmoscopically. There was no retina compression by the microelectrode array in any eyes. No mechanical or chemical degradation of the silicone or the polyimide epiretinal implant material was revealed. Retinal ganglion cells layer seemed not to be affected macroscopically (n= 5) by the epiretinal array pressure. Although intraoperative vitreous hemorrhage occurred in two eyes (33.3%) soon after the epiretinal implant positioning, it resorpted fast so that after the 1 month examination in one case and 3 months examination in another case no hemorrhage was seen anymore. The cause of the vitreous hemorrhage could be either superficial retinal vessels damage, or from the sclera during the epiretinal implant fixation procedure. We postulated that in one case the source of the hemorrhage was the sclera, and in a second the superficial retinal vessels. No choroidal detachment, hemorrhage or hypotony was seen post-operatively (Figure 32,33).

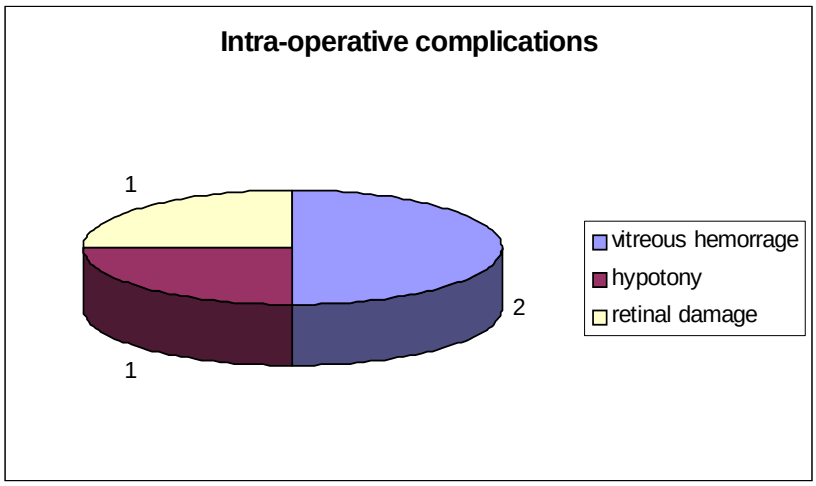

Figure 32: Intraoperative complications. Vitreous hemorrhage ensued in two cases; hypotony in one eye, and retinal damage in one eye.

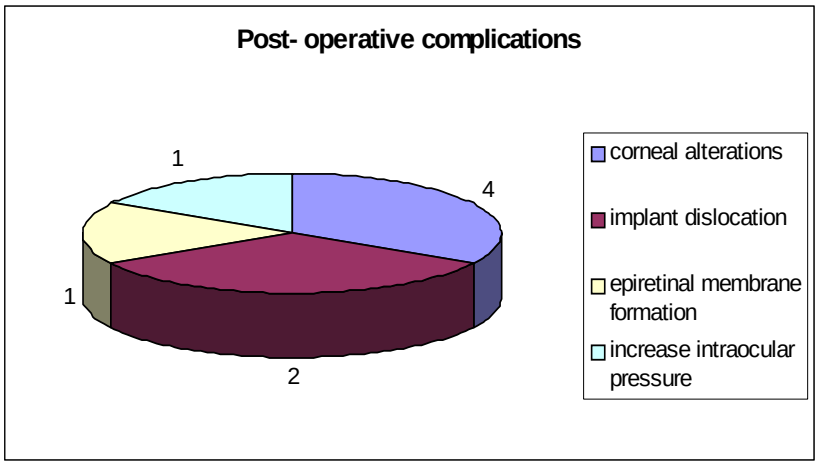

Figure 33: Post-operative complications.

Elevated intraocular pressure of 25 mmHg was observed in one animal in the first month postoperatively, but normalized after the second month. In the other cases the intraocular pressure was at normal levels under 21 mmHg through the follow-up period. The epiretinal implant dislocation did not cause angle closure glaucoma by exerting pressure to the angle in any of the cases. Anterior chamber angle remained opened in all cases. Figure 34 matches the use of perfluorocarbon liquids and the incidence of complications.

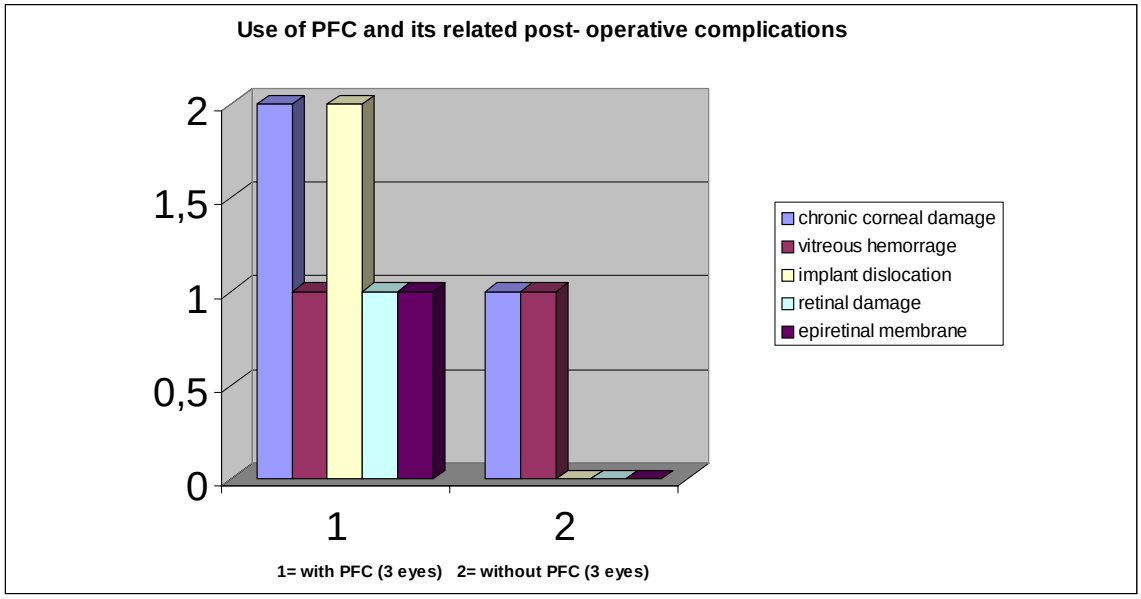

Figure 34: Use of perfluorocarbon liquids (PFC) and incidence of complications.

Table 6

Clinical observation of implanted epiretinal implants in this work and in the literature

	Examination techniques	Follow-up time	Complications	Enucleation	Histology
Walter et al 1999	Indirect Ophthalmoscopy ERG, VEP	6 months	10% total RD, cataract retinal folds	yes	Intact ganglion cells retina compressed
Majii et al 1999	Funds camera, FAG ERG, VEP, enucleation	3 months	vitreous hemorrhage vitreous haze retinal folds *	yes	Loss of tapetum under the tack Every other retinal layer remained not damaged
Hesse et al 2000	78 D lens Ophthalmoscopy Cortical recordings	ND	electrode displacement without fixation	no	no
Vobig et al 2000	Ophthalmoscopy ERG, VEP	6 months	Limited proliferative changes	yes	Confirmation of the proliferative changes
Walter et al 2000	Cortical evoked potentials, EEG	ND	no	no	no
Schanze et al 2002	Cortical recordings	removed immediately	no	no	no
Kerdraon et al 2002	CT scan	removed after CT scan	transient hypotony	yes	no
Rodrigues 2003 (this work)	Ophthalmoscopy ERG, VEP, OCT Funds camera Cortical recordings	6-12 months	corneal edema implant dislocation	no	no

RD: retinal detachment; ERG: Electroretinogram; VEP: Visual evoked potential

FAG: Fluorescein angiogram; OCT: Optic coherence tomography; CT: Computer tomography

* all three findings were self limited

ND: no data

4.5 Epiretinal implant function - electrical stimulation and evoked cortical responses

After the visual observation for spurious artifacts the electrode signals were averaged with respect to stimulation onsets. The electromagnetic induced artifacts were subtracted in order to isolate the stimulation artifacts generated by the implanted retina stimulator. True neuronal responses were identified by comparing the response waveforms or spontaneous activity, including pseudostimulation with a light barrier with those obtained for implant activation in a time window of 5 to 100 ms post-stimulation onset.

In the fabrication of the epiretinal implant, the fast high temperature silicone encapsulation procedure turned out to be inappropriate since all implants encapsulated with this process showed severe malfunctions in preliminary investigations. It may be assumed that this may be occurred due to the large thermal stress leading to broken contacts of the electronic part of the implant. The implants fabricated with slow low temperature process resulted in fully functional retina implants.

In all eyes the epiretinal implant has functioned postoperatively. The functioning status of epiretinal implants was observed for a duration of 6 up to 16 months. Electroretinogram and visual evoked potential examinations were performed in all cases at least twice. Cortical recordings were performed in all eyes, but recording parameters were reliable in only two of the six eyes, and during a subacute period of seven days (Figure 35). As expected, the contact retina-chip observed in the electrophysiological tests were highly correlated to the incidence of dislocation shown in table 5. In three cases a good contact between the microelectrode array and the retina was achieved chronically (Table 7).

Concluding, good mechanical contact between microelectrode array and the retina was possible in three eyes postoperatively in a long-term period. In all cases the epiretinal implant was functioning in the whole long-term period. However, cortical stimulation of active epiretinal implants was obtained by the epiretinal device in only two cases and in a short-term period for seven days. The reasons for a bad long-term cortical recording, although the epiretinal implant was functioning in all eyes and thought to be on the retina, could be a formation of an epiretinal membrane between the array and the retina, a small space separating the array and the retina, or even a deep array pressure on the retina surface. No long-term stimulation of an active epiretinal implant with corresponding cortical recordings was achieved.

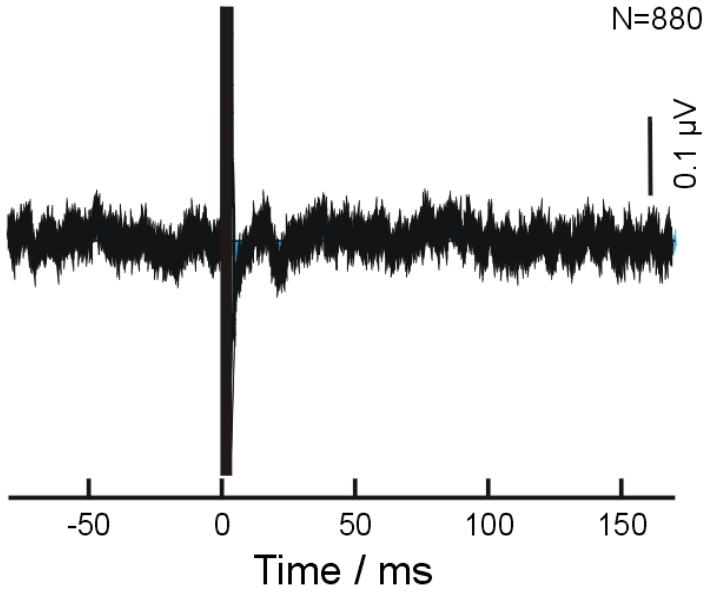

Figure 35: Averaged cortical response to stimulation with the epiretinal implant (7 days post implantation). For stimulation a train of 10 short infrared light impulses (200 µs interimpulse duration). The signal was recorded with an electrode placed onto the skull above primary visual cortex. The deflections within 0 up to about 5 ms are the stimulation artifacts. The cortical response occurs about 20 ms post stimulation onset.

Table 7

Electrophysiological testing of the EI after intraocular implantation

Animal	EI functioning duration (months)	Number of ERG/ VEP examinations	Number of Cortical Recordings	Contact retina-chip *
1	14	9	3	poor
2	16	4	1	poor
3	9	3	2	good
4	11	4	1	good
5	6	2	1	fair
6	8	3	1	good

EI: Epiretinal implant; ERG: Electroretinogram; VEP: Visual evoked potential

* Notice the correlation of contact retina-chip in this table to the dislocation represented in table 5

5. Discussion

5.1 General remarks

To our knowledge, this is the first study that evaluates the long-term effects of a functioning intraocular electronic prosthesis. The development of a retinal prosthesis represents a great challenge. Considering an epiretinal approach, e.g., positioning of an microelectrode array on the inner retinal surface for electrical stimulation of the inner retinal cells, and a stable long-term fixation of the array are two very important aspects.

The cost of housing and care of the animals is very high, which limited the number of animals available in this study. Although other animal models such as the dog and ovine allow a better comparison and adaptation to the human eye, reliable cortical recording the epiretinal prosthesis is not described in the literature. In those animals the visual pathway is not well understood, and cortical recording after retinal stimulation has not been reported. Conversely, the cat is along with the monkey the best-understood visual pathway to study the functioning of the implants.

The operative technique used in this work is based on current vitreoretinal surgical techniques, but it is specifically adapted to the task of implanting the microelectrode array in the cat's eye. The surgeries in the cats to implant the microelectrode arrays were performed by adapting and applying the techniques and instruments normally used for humans.

5.2 In-vitro testing of electrical stimulation under intraocular liquids

These set-up of experiments aimed different goals. First, simple electrical conduction in contact with several commonly used intraocular fluids was tested. Both silicone oil and perfluorocarbon showed to be weak electric conductors. Perfluorocarbon, ringer lactate, viscoelastic, and glucose 5 % conducted electricity well.

In the second setup experiment, how much electric current in the epiretinal implant can be conducted under ringer lactate, perfluorocarbon, silicone oil, viscoelastic, and glucose 5 % was tested. Ringer lactate allowed good electric conduction because of the 59 mV in comparison to the 62 mV of the zero measurements. Silicone oil did not allow electric current of the chip, a current of 64 mV was achieved. Even after mixing with ringer lactate, silicone oil, remained as a bad conductor, 64 mV.

Perfluorocarbon represented the same bad conduction of 64 mV. When perfluorocarbon was mixed with ringer lactate, a good conduction, 57 mV, was achieved. A layer of ringer lactate is supposed to remain in contact with the epiretinal implant. One of the conclusions is that silicone oil is very adhesive to the epiretinal implant, and once silicone oil gets in contact to the silicone material of the epiretinal implant tested, no other fluid interacts with the epiretinal array.

Finally, the influence of the several intraocular fluids on the epiretinal implant in contact to the retina was experimented. The first zero measurement was 56.2 mV, when the epiretinal implant touched the retina. Additional perfluorocarbon injection did not disturb electric conduction; the same 56.2 mV value was obtained. Later removal of vitreous remnants changed the zero measurements to 56.5 mV. Silicone oil resulted in the disturbance of the electric conduction, because a value of 58.3 mV was then achieved. Based on the previous set-up, the silicone oil adherent to the epiretinal implant was removed. A new zero measurement of 63.4 mV arose. The epiretinal implant was again carefully repositioned on the retina and a good conduction of 57.7 mV could be observed. Viscoelastic and ringer lactate additionally did not change the conduction value of 57.7 mV. One of the most important observations was that the chip must intimately contact the retina surface to allow a good electric current, and a thoroughly vitreous removal must be achieved before any epiretinal implant stimulation.

Every intraocular fluid may therefore have its advantages or disadvantages. Two properties are important for an intraocular fluid to be useful as adjuvant to a retinal prosthesis. It must be a good electric conductor, and it should auxiliate to hold the epiretinal implant stable and press it to a better retinal contact.

Silicone oil is lighter than water and the smaller layer of ringer lactate that remains below the silicone oil may allow a good electric transmission. The second advantage of silicone oil is its viscous structure, which may avoid abrupt movements of the microelectrode array and flexible microfolie. However, silicone oil has the disadvantages that silicone oil itself is not a conductor, it adheres strongly in the microelectrode array surface, and its high specific gravity does not press the microelectrode array against the retina so much.

Perfluorocarbon liquid has two great advantages: it does not adhere to the microelectrode array material, and it presses fairly the epiretinal implant to the retina. The first disadvantage of perfluorocarbon liquid is that it does not conduct electric current very well. Second, perfluorocarbon liquid has potential toxic effects when chronically used. Third, perfluorocarbon liquid does not mix with other intraocular fluids. These fluids may enter the space between the epiretinal implant and the retina, changing its electric transmission.

Compared to the four intraocular fluids tested, ringer lactate has few advantages as an intraocular fluid in the presence of an epiretinal implant, such as very good conduction properties and no adherence to the epiretinal implant. However, as disadvantages we may cite that it should not prevent movements of the microelectrode array and flexible microcable.

Viscoelastics have the advantage of being a good conductors, they may mix with ringer lactate, and due to their viscous properties may keep the microelectrode array and flexible microcable in the posterior pole. Though, viscoelastics may increase intraocular pressure since it is not drained by the trabecular meshwork.

5.3 Surgical technique and epiretinal implant fixation

5.3.1 Vitrectomy, phacoemulsification and implantation

Vitreoretinal and lens surgery techniques have evolved and several ocular diseases can be safely managed nowadays. The application of those surgical techniques to implant an electronic device inside a cat's eye requires many adaptations. Pars plana vitrectomy is more difficult in cats than in humans because of the large anterior segment. The optimal place for a pars plana in cats would be 5 mm behind the limbus (Johnson et al., 1992), but if sclerotomies are incised in this place the instruments must be inserted at the equator of the eye, and the mobility of the instruments is hampered by the neighboring orbital bone (Hesse et al., 2000b). Besides, cats have a highly vascularized sclera, and a large area should be cauterized if the 5 mm behind the limbus approach is chosen, especially for retina implant surgery. Further, the large cornea of cats impairs the image quality of wide-field lens systems for vitreoretinal surgery. Removal of the lens and subsequent injection of air into the large anterior chamber provides an excellent view of the posterior segment and allows more accurate anterior insertion of the surgical instruments and specially the epiretinal array through the corneal incisions. Another reason for the good retina visibility without a lens system is the high reflectibility of the retina tapetum in cats. The same principle of removing the lens and performing pars plana vitrectomy has been reported by others in rabbits with a low rate of complications (Vobig et al., 1999).

The limbus for epiretinal implant insertion was the approach chosen in this study. The concept of limbus epiretinal implant insertion comes from the cataract surgery. Intraocular lens of about 5 or 6 mm in diameter for the substitution of natural lens are routinely inserted through the limbus. Epiretinal implant insertion through the limbus in cats seems more physiological than the other possibility, through the sclera. Implant insertion through the sclera would be technically difficult because our device is composed of three parts; one piece should be directed anteriorly and the other posteriorly. It would be difficult from a posterior approach to position and fixate the epiretinal implant to the capsule bag or sulcus from a posterior capsulotomy, which is surgically more difficult. Also, the risk of bleeding upon the insertion of the epiretinal implant through the sclera in cats' eyes is very high. Besides, a large scleral opening brings to the eye a high chance of retinal damage when inserting instruments into the vitreous. Walter et al. reported insertion of a small epiretinal implant through one sclerotomy in rabbits (Walter et al., 1999). Majii et al. also implanted a one-piece epiretinal implants through the sclera in dogs (Majii et al., 1999). However, those

epiretinal implants were inactive, a one-piece type, and much smaller than those used in this study. In this study new generation active epiretinal implants were tested. These complex epiretinal implants were implanted not only to test the biocompatibility but also the functionality of the whole prototype, i.e. to receive the stimulus of a camera and to transmit to the retina to active stimulate the retina, what is the goal of the retinal prosthesis itself. There are, indeed, reports of others who successfully implanted an inactive prototype of epiretinal implant similar to the ones used in this work through the limbus in ovines (Kerdraon et al., 2002). In the future, smaller new generation of active epiretinal implants will substitute these used in the present. It is then possible that smaller epiretinal implants could be implanted through the sclera. Table 6 shows characteristic and fixation techniques of several different epiretinal implants described in the literature.

To permit insertion of the epiretinal implant through the limbus, phacoemulsification, capsulorrhexis and a posterior capsulotomy were performed routinely in this work. Phacoemulsification is reported with low rate of postoperative inflammation in cats (Gwon et al., 1993), and it must always be performed when implanting the currently used 3 pieces epiretinal implant to allow space of its anterior part. Capsulorhexis is a major surgical advance that contributes to long-term intraocular lens centration in the cataract surgery, and reduces the likelihood of needing intraocular lens removal or exchange. Despite capsulorhexis, intraocular lens decentration may occur by capsule contraction syndrome. A large capsulorhexis prevents this complication. An eccentric capsulorrhexis may allow the capsulorhexis edge to influence in the mechanism of decentration. In all six cases in this study a centrated, good-sized capsulorrhexis was performed. Posterior capsulotomy was performed routinely in this study. Since the epiretinal implant was inserted through the limbus, the goal of the posterior capsulotomy was to create a space in the capsular bag for inserting the flexible microcable and microelectrode array towards the posterior vitreous. The anterior part of the epiretinal implant, called the electronic intraocular lens, should be located in the capsule bag or in the sulcus. The posterior capsulotomy may also serve as a fixation support for the electronic intraocular lens. However, in only one case we observed an intact the structure of the bag observed postoperatively. In the other five eyes, the capsule bag was damaged post-operatively. Based on these findings, one can assume that the posterior capsule is not capable to auxiliate supporting the current large electronic intraocular lens in cats.

A lower rate of inflammatory reactions and retinal detachment is reported to occur in the two-step procedure. Walter et al. (1999) implanted microelectrode array in a two-step procedure in rabbits. In this work, a two-step procedure was performed in one eye, and no

inflammation was observed post-operatively. The two-step procedure has the disadvantage that the limbal incision must be made twice, causing corneal deformations more frequently. Besides, the chance of endophthalmitis may be proportional to the number of procedures to be performed.

5.3.2 Vitreoretinal interface cleaning and microelectrode array contact

Close contact of stimulating electrode to the retina is essential for a low electrical stimulation threshold. Although considered a different structure of the retina, the vitreous is intimately related to the inner retinal surface. The vitreous is a viscoelastic extracellular matrix composed of 98% water, and it normally exists in a gel state as a result of the intricate organization of its macromolecular components (Figure 36). The most posterior anatomical structure of the vitreous is called the vitreo-retinal interface, a connective tissue situated between the retina and the vitreous. Several biochemical elements form the vitreoretinal interface interposing the space between the posterior vitreous cortex and the inner retinal surface (Hesse, 2001). Cleaning the vitreoretinal interface would facilitate the electronics-retina communication of an epiretinal device. However, the total removal of the vitreoretinal interface and creation of an intimate contact tissue-electronic are still one of the main hurdles for a successful retinal prosthesis. After a standard pars plana vitrectomy, rest of vitreous tissue or the adherent vitreo-retinal interface could prevent intimate contact of the electrode array with the retinal tissue (Hesse et al., 2000b).

Posterior vitreous cortex may be removed basically by two means: mechanically or biochemically. The mechanical removal of the posterior vitreous cortex may be performed either with the vitreous cutter, with or without indocyanine green guidance, or with a soft-tip extrusion needle. The vitreous cutter may remove the posterior vitreous cortex by moving the cutter around the optic nerve, and with a high aspiration catching the posterior cortex. This maneuver was successfully performed in all six eyes in this work. A complete vitrectomy is necessary with complete posterior hyaloid separation to avoid vitreoretinal traction, as well as for device displacement by vitreous fibers. Guidance of a vital dye may facilitate posterior vitreous recognition. Indocyanine green is a tricarbocyanine dye that has been used as a contrast in diagnostic ophthalmology for a long time. It has recently been reported to stain the internal limiting membrane for surgical treatment of macular diseases (Rodrigues et al., 2003a). The indocyanine green seems to stain the posterior vitreous cortex, allowing a better

vitreous visualization and removal. Vitreous staining with indocyanine green was performed in four cases (66.67%). It was proven to be very useful for vitreous recognition and removal of all vitreous remnants. In future experiments, the removal of the internal limiting membrane should try to increase the contact between the epiretinal implant to the inner retinal layers, assuring that all vitreous remnants are removed. The group may try the use of indocyanine green or trypan blue for internal limiting membrane staining, the latter a new dye for retina staining (Rodrigues et al., 2003c). Then, with the internal limiting membrane removal, the inner retinal cells should be totally exposed. To verify the complete mechanical removal of the vitreous cortex, one may also sweep the retinal surface with a silicone soft-tip extrusion needle with active suction. This technique auxiliated total vitreous removal in this work. A two-step procedure for ocular surgery is also claimed to induce posterior vitreous detachment and facilitate the removal of the vitreous cortex in the second vitrectomy (Walter et al., 1999).

Cleavage of the vitreoretinal interface by biochemical means (enzymatic vitrectomy, or pharmacological vitreolysis) should be a desirable approach as an adjuvant to epiretinal implant implantation. Enzyme-induced posterior vitreous detachment may help to remove vitreous residues before or during pars plana vitrectomy, allowing close contact between the epiretinal implant and the inner retinal cells. Enzymes (collagenase or hyaluronidase) are either being tested to liquefy the vitreous body, named vitreolysis, or to cleave or dehiscence (dispase, plasmin, tissue-plasminogen activator, chondroitinase, urokinase) the posterior vitreous cortex and the retina, named syneresis (Hesse, 2001; Sebag, 2002). Plasmin, a serine protease mediating the fibrinolytic process, hydrolyses several glycoproteins at the vitreoretinal interface, including laminin and fibronectin. These both glycoproteins support the attachment of the vitreous cortex to the internal limiting membrane. The result is that intravitreal injections of plasmin may induce the formation of posterior vitreous detachment. Intravitreal injections of tissue plasminogen activator may chemically induce a posterior vitreous cleavage enzymatically by autologous generated plasmin, clearing the inner retinal surface (Hesse et al., 2000c). Injection of tissue plasminogen activator to auxiliate removal of all vitreous tissue was performed in this study (n= 2) to achieve good contact between the epiretinal array and the retina. Our work group has previously reported successful implantation of epiretinal electrodes 1 week or more following enzyme injection (Hesse et al., 2000b). The technique of enzymatic vitrectomy was reported to introduce epiretinal implants by others (Vobig et al., 2000). However, the efficacy of biochemical removal of the vitreoretinal interface is yet to be determined.

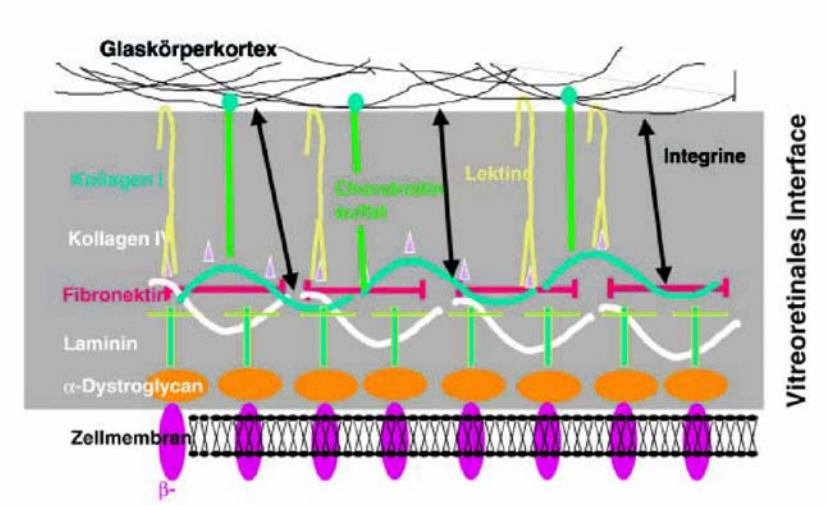

Figure 36: Schematic presentation of the vitreoretinal interface as connection between the retina and vitreous. Main elements are laminin, fibronectin and collagen IV, which sit on the cell membrane and the Müller cells. Chondroitine, elactin, integrin or specific matrix proteins are molecules of connection. Reprinted from Hesse, 2001.

5.3.3 Implant fixation and final position of the epiretinal implant

A stable array position of the epiretinal implant on the retina is critical to maintaining steady image resolution and stimulation current levels. The implant must be fixed in such a stable manner that its interference on other intraocular tissues is minimized, and electronic stimulation can be transmitted in a regular way. However, the long-term contact between the retina and the microelectrode array is a difficult task. Stable and long-term fixation of the electronic intraocular lens inside the capsule bag requires resistance against several compressive forces.

Electrode arrays may be susceptible to ocular rotational movements that can reach a high speed. The electronic intraocular lens-flexible microcable- microelectrode array structure must retain its support and fixation structure. The chip and its intraocular lens structure must behave stable not only on healthy eyes, but also in vitrectomized eyes. There

is no consensus as to what fixation technique should be the most appropriate available in the literature. The several fixation possibilities of the three-piece new generation epiretinal implant fixation are illustrated in figure 37.

Evidence that the array is in a stable position was verified through fundus photography in this study. In the first group (n= 2) the electronic device was not sutured to any structure inside the eye. Perfluorocarbon liquid filled the vitreous cavity in a way that it could hold the epiretinal implant in place as care was taken to implant the electronic intraocular lens in the bag. These two implants did not keep the same position as they were implanted inside the eye. The first one dislocated posteriorly and nasally, and the second one to the anterior chamber. One of the reasons why they dislocated could be an asymmetric positioning of the chip inside the eye. The folie of the implant runs only to one side, and it is not possible to assure that a good equilibrium was achieved. Then, it can have simply slipped, either posteriorly or anteriorly. Of the four eyes in which its suturing the electronic intraocular lens to the scleral tissue was done, three retained stable positioning, though one was little dislocated. Currently available prototypes of an inactive compound retinal prosthesis consisting of an intraocular lens connected to a microelectrode array were implanted by several groups. Although some described no adverse reactions related to the epiretinal implant suturing, proliferative reactions in the posterior segment were reported as a complication of this fixation procedure (Vobig et al., 2000). Figure 37 illustrates the several fixation possibilities for the epiretinal approach. Following the different fixation possibilities are discussed.

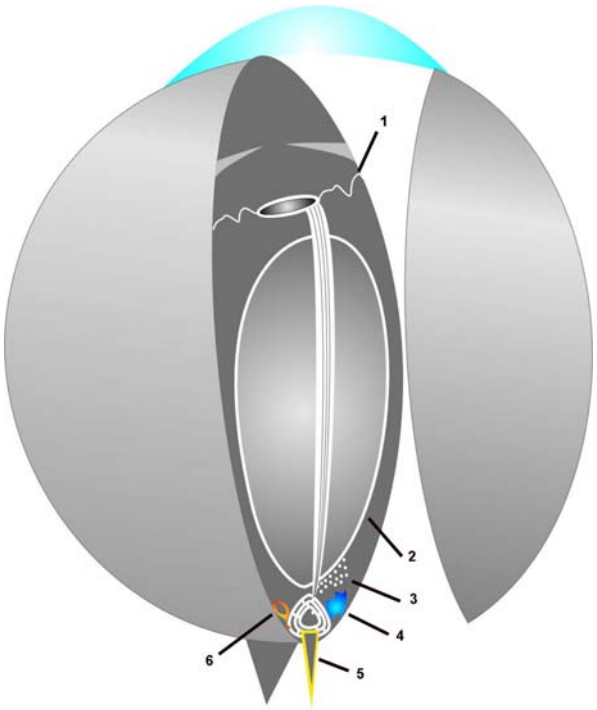

Figure 37: Esquematic drawing showing 6 alternatives to fixate an epiretinal implant. The implant has three parts. The first sits in the lens sulcus (electronic intraocular lens). The second part is a flexible microcable, that transmits the electric impulses to the microelectrode array. Fixation- 1: Suturing the electronic intraocular lens. 2: Intraocular heavy tamponades as perfluorocarbon liquid or S6F8 gas. 3: Biochemical fixation. 4: Bioadhesives. 5: Scleral tack. 6: Suture of the microelectrode array.

5.3.3.1 Heavy liquids

Of all heavy liquids used in vitreoretinal procedures, perfluorocarbon liquids are the most common. Perfluorocarbon liquids, as a heavier-than-water temporary vitreous substitutes, have become an important adjunct in the treatment of complex vitreoretinal disorders. They have been useful for the stabilization of the retina during dissection of retinal membranes, management of complicated retinal detachments, and in cases of luxated intraocular lenses. However, the safe use of perfluorocarbon liquids is restricted to intraoperative application. Indeed, they should be removed at the end of the surgery to prevent mechanical or toxic damage to the retina and the corneal endothelium. Although intraocular perfluorocarbon liquids for 5 days have been reasonable well tolerated (Bottoni et al., 1993), the chronic perfluorocarbon liquid use as intraocular tamponade in humans has

been associated with corneal and retinal toxicity (Eckardt et al., 1991). Perfluorocarbon liquids may be toxic to the corneal endothelium and may damage the retina through their sheer weight if left chronically in the eye (Miyamoto et al., 1984; Eckardt and Nicolai, 1993). After chronic exposure of the cornea to perfluorocarbon liquid, examination showed descemet's folds, as well as stromal and epithelial edema. Inflammation and fibrin have also been reported 2 weeks after perfluorocarbon liquid use by others. After a week to several weeks of chronic perfluorocarbon liquid use, profound retinal changes occurred. Histological examination of excised retinal tissue demonstrated extensive destruction of the normal architecture with decreased number of cells in the nuclear layers (LoCascio et al., 1985).

In general, short-term exposure of the anterior chamber and vitreous cavity to purified perfluorocarbon liquids is well tolerated (Bottoni et al., 1993). Nevertheless, in the anterior chamber perfluorocarbon liquid may lead to corneal endothelium loss with subsequent corneal opacity (Eckardt and Nicolai, 1993).

The first reason of perfluorocarbon liquid use in this study was to mechanically protect the retina during the epiretinal implant insertion. The most remarkable property of the perfluorocarbon liquids is the specific gravity, which is higher than that of water. Typically, the specific gravity ranges from 1.7 to 2.03 g/ml in comparison to 0.97 g/ml of silicone oil. Perfluorocarbon liquid is easy to be surgically handled, and the low viscosity (1.4-2.8 mPas) of perfluorocarbon allows easy introduction and aspiration from the vitreous cavity, while their high specific gravity exerts a flattening force on the retina which is over three times greater than that exerted by the same volume of other intraocular fluids such as silicone oil (Kobuch et al., 2001). While perfluorocarbon liquid prevented direct retinal trauma by the epiretinal implant in this work, right after epiretinal implant positioning the aspiration of the perfluorocarbon liquid led to microelectrode array movements at the retinal surface which could have damaged the inner retinal layers.

The second purpose of perfluorocarbon liquids was to keep the epiretinal implant (n= 2) chronically attached to the retina based on its high specific gravity properties. If the whole vitreous cavity were filled with a heavy intraocular liquid, the epiretinal implant would sit stable inside the eye. This property made the researchers to inject perfluorocarbon liquid for chronic intraocular tamponade in two eyes. Perfluorocarbon liquids could facilitate contact of the microelectrode array to the ganglion cells and improve stimulation quality. In both cases where perfluorocarbon liquid was utilized, the epiretinal implant dislocated. For short-term epiretinal implant insertion and stimulation, perfluorocarbon liquid has been found to be a useful tool for acute experiments (Walter and Heimann, 2000; Hesse et al., 2000b). However, this is the first study to test the chronic fixation of epiretinal implant by

the perfluorocarbon liquid. In conclusion, perfluorocarbon liquids did not maintain a long-term stable epiretinal implant positioning.

In this work, iritis, uveitis, and anterior inflammatory reaction have been noted postoperatively, and may be associated to the chronic exposure of intraocular tissues to perfluorocarbon. It was not possible to determine whether a definite relation exists between inflammation and internal tamponade with perfluorocarbon liquid. Retinal toxicity could have occurred in one eye in this study, and the most likely cause could be direct perfluorocarbon liquid damage.

There is a new type of long-term artificial vitreous substitute: the semifluorinated alkanes and their oligomers. Semifluorinated alkanes have a low specific gravity of 1.35 g/ml, which means that these liquids could exert successful mechanical pressure to keep the epiretinal implant close to the retina. Although their long-term use has been allowed in Europe, clinically severe adverse effects to the retina have been described. Severe fibrin reaction in the anterior chamber was visualized. Epiretinal membranes and necrotic appearance of the retina were described (Roider et al., 2002).

Another alternative for perfluorocarbon liquid as internal tamponade for long-term fixation would be the use of silicone oil. However, silicone oil specific gravity is low (0.97 g/ml), and it would probably not be able to keep the epiretinal implant stable positioned in the vitreous (Kobuch et al., 2001). Further, silicone oil is less dense than water and tends to remain in the superior surface of the vitreous.

5.3.3.2 Biochemical fixation

A less traumatic technique for epiretinal implant fixation would be the intraocular injection of biological growth factors. The use of biochemical growth factors seems feasible to attach the microelectrode array to the retinal tissue. In order to promoting stable implant fixation in animal models, others reported injection of growth factors into the vitreous cavity leading to ingrowth of retinal Müller cells through and around the microelectrode array (Laube and Bornfeld, 1999). Conversely, this approach seems to be very biocompatible since the risk of auto-immune reactions is low.

The formulation of the biochemical fixation demands two aspects. First, the silicone surface of the epiretinal implant must be modified in a biochemical way by coating the contact area with proteins, peptide, or growth factors to improve the biocompatibility of the

silicone and to induce the proliferation of Müller cells. With the use of matrix proteins (Laminin, Fibronectin) in combination with growth factors (A1-a5 and C1-c5), the time of Müller cells growth doubled in comparison to the control culture in previous investigations of the Epi-Ret group (not published data). The second aspect refers to the biochemical fixation of the implant by proliferating Müller cells. A goal is to induce a limited proliferation of Müller cells into the pores of the microelectrode array after performing irradiation with the Excimer Laser. In animal experiments after trauma to the retina, Müller cells reentered a cell cycle and started regeneration in a multilayer pattern, and this could enter the microelectrode array pores and fixate the epiretinal implant. In response to damage, others described Müller glial cells in the retina as a potential source of retinal regeneration (Fischer and Reh, 2001). Injections of insulin and fibroblastic growth factor into the eye led Müller cells to start differentiation (Fisher et al., 2002).

One test of biochemical fixation was made by others with a microfilm made of polydimethylsiloxane graft polymerized with polyacrylic acid used to biochemically establish contact between the epiretinal array and the retina. The perforated epiretinal implants became ingrown by microvilli-like retinal cell processes of ganglion cells. Biological attachment was, in this case, based on a locally limited and controlled proliferation of retinal glial cells, which grew into the pores of the microelectrode array to establish firm connections to the retina (Laube and Bornfeld, 1999).

Biochemical fixation may be improved by the application of proteins regulating the differentiation or activation of myofibroblasts. Fibroblasts and myofibroblasts are involved in the foreign body reaction to biomaterials. In porcine cells, cellular differentiation into myofibroblasts was inhibited by basic fibroblastic growth factor and antibodies to transforming growth factor-beta (Khouw et al., 1999). Acidic fibroblast growth factor, transforming growth factor, and basic fibroblastic growth factor were found in the epiretinal membranes and are a potential fibroblastic growth stimulator. Others reported transforming growth factor beta 1 and beta-receptor II as the main inducer of myofibroblastic differentiation in epiretinal membrane in proliferative diabetic vitreoretinopathy and proliferative vitreoretinopathy (Bochaton-Piallat et al., 2000).

The use of growth factors is not free of potential side effects. Biological growth factors may cause exaggerated proliferative reactions and increase the resistance between the stimulator and the target cells, as well as a change in the stimulus current necessary for successful electric stimulation. Examples of such exaggerated epiretinal membranes constitute the end-stage of evolution of proliferative vitreoretinopathy and proliferative diabetic vitreoretinopathy (Bodanowitz and Kroll, 1996). Any tissue between the stimulator

and the target cells, including epiretinal membranes will enhance the minimal current for an electric stimulus. Yet, the dose, administration route, and which growth factors can create a retina-implant adhesion are not known. Growth factors may not affect specifically one particular type of cell and thus, they are likely to have unwanted and possibly serious adverse effects by acting on cell types other than the ones targeted. For example, because of the mitogenic activity, growth factor may also cause vasoproliferation and problems with the blood-retinal barrier.

5.3.3.3 Bioadhesives

The use of bioadhesives to glue the microelectrode array to the retinal tissue may be a further possibility to fixate epiretinal implant intraocularly. This concept has already been applied in the anterior segment surgery to fixate several tissues using bioadhesives. Bioadhesives such as cyanoacrylate and fibrinogen were proved an effective alternative to sutures in sclera tunnel cataract surgery (Alio et al., 1998). The use of fibrin was also beneficial in assisting corneoscleral wounds closure (Shigemitsu and Majima, 1996).

In one study, nine commercially available bioadhesive compounds were examined for their suitability as intraocular adhesives in rabbits. One type of adhesive, a hydrogel (SS-PEG hydrogel, Shearwater Polymers Inc.) proved to be strongly adherent and non-toxic to the retina (Margalit et al., 2000).

Cyanoacrylate adhesive was reported to successfully attach epiretinal electrodes temporally to the retina. Cyanoacrylate undergoes immediate polymerization and adhesion on contact with fluid, which is an advantage because of the fluidic content of the vitreous cavity (Hesse et al., 2000b). Another different adhesive, a polymer in the form of polyethylene glycoldiacrylate, attached polyamide arrays successfully to the retina (Loewenstein et al., 1999).

Some authors have reported disappointing results with bioadhesives as fixation adjuvants for epiretinal implant fixation (Walter et al., 1999). Among the several reasons, first, it may disturb the retina-electrode interface. Many adhesives clinically tested so far elicited severe inflammation in the eye and have proven to be highly toxic to the retinal tissue. Others did not have sufficient adhesive strength. Most of the polymeric adhesives form very rapidly via a highly exothermic reaction. The heat and the chemical impurities discharged during the bioadhesive chemical reaction may cause toxic damage and

inflammatory response (McCuen, 1989). Although rapid formation is a desired characteristic, it should be balanced with the need for a biocompatible nontoxic adhesive. Early forms of cyanoacrylate adhesives may degrade into cyanoacetate and formaldehyde, which are reported as cause of histotoxicity and inflammation. Indeed, in rabbits cyanoacrylate was associated with severe retinal tissue toxicity (Margalit et al., 2000).

5.3.3.4 Tacks

The purpose of utilization of tacks of previous studies in ophthalmology has been to provide intraoperative fixation of the retina to the eye wall so as to facilitate adhesion. Different tacks have been tested so far to attach the retina tissue in vitreous surgery, including titanium, hydrogel, and hybrid tacks. While titanium and alloy retinal tacks have been successfully implanted thereby causing minimal injury to the retina and ocular structures (Ohira et al., 1991; De Juan et al., 1985), prior cryopexy was required because of the high risk of bleeding. Although hydrogel tacks may be a good type of tack for epiretinal implant fixation, one of its disadvantages is the difficulty of their manipulation.

Several authors have published their experience with retinal tacks for epiretinal implant fixation (Walter et al., 1999; Majii et al., 1999; Heimann, 1999; Vobig et al., 2000; Gerding et al., 2001). Berk and co-workers have demonstrated that the retinal visual function the retinal architecture underneath the implant in rabbits seemed to be unaffected by the implantation of a retinal stimulator and fixation with tacking to the inner surface (Berk et al., 1999). Tack fixation of microelectrode arrays on the inner retinal surface was a good approach in long-term implantation of an epiretinal implant, since chronic clinical and electrophysiologic data did not indicate any adverse effect caused by the implant or the tack itself. Taneri and co-workers have reported a hybrid tack well suited for the epiretinal fixation in a highly biocompatible way (Taneri et al., 1999). A two-step vitrectomy, enzyme assistance, focal laser coagulation, phacoemulsification, and tack fixation of the array were the techniques to implant a epiretinal prosthesis by Vobig and co-workers (Vobig et al., 2000). After unsuccessful experiences with glues and fluid-gas exchange for fixation of microelectrode arrays, retinal tacks have been used with low rate of complication by others, but the pretreatment with laser coagulation around the tack area to prevent choroidal bleeding is recommended (Walter et al., 1999). Retinal tacks and the electrode array remained

firmly affixed to the retina for up to 1 year of follow-up with no significant clinical or histological side effects (Majii et al., 1999).

To avoid inducing proliferations in the macular region, the place of tack insertion for an epiretinal implant should be out of the macula and have two parts. A combination of tack fixation with its extension to the macula region is optimal. Between this extension and the retinal surface a biochemical fixation could be used. The result should be covalent bonds between the active area of the array and the internal retinal surface (Walter et al., 1999).

Although tacks have been reported for epiretinal implant fixation, they were not used in this study for several reasons. Retinal tacks to fixate an implant epiretinally may not be appropriate in cats because of the very thin posterior sclera. Tacks are also technically difficult, and pulling out may result in epiretinal implant displacement. Retinal tacks may also subject the eye to a risk of severe choroidal bleeding, proliferative vitreoretinopathy, retinal folds, and retinal damage. Besides, hiperpigmentary reactions around the tack site have been reported. There is no scientific evidence that tacking induces a stable functional contact between the electronic array and the retina. The mechanical force to insert the tack may could also cause retinal folds close to the perforation sites. Majii et al. described the occurrence of retinal folds adjacent to the placement of an epiretinal implant (Majii et al., 1999). Although few publications mention the use of tacking as a useful adjunctive technique in the repair of complicated retinal detachments (de Juan et al., 1985), there is currently no indication of a retinal tack for the treatment of vitreoretinal diseases in humans. Tacks were found to cause fibrovascular reaction when used for rhematogenous retinal detachment (Ohira et al., 1991). Severe proliferative reactions and retinal detachment were induced after intraocular tacks application (Algvere and Jahnberg, 1990). In conclusion, there is so far little evidence that the utilization of tacks is safe and efficacious.

5.3.3.5 Suturing

Suturing the electronic intraocular lens to the scleral tissue presents as a feasible alternative for epiretinal implant fixation. Mechanical fixation with scleral suturing was performed in four eyes (66.67%) in this study. The principle of scleral suturing comes also from the cataract surgery. When support for the intraocular lens is lost by capsule rupture, intraocular lens is sutured to the sclera. This technique has long been applied for secondary intraocular lens fixation with low risk of complications (Schmidt et al., 2002). Epiretinal

implant is heavier than the intraocular lens for the cataract surgery, and surgical adaptations should be done to support the usually large epiretinal implant. Besides, the fixation of epiretinal implant with suturing poses technical problems. First, the sclera in cats is thin and the risk of bleeding when passing a suture through is high. In 33.3% of the cases in this study intravitreous hemorrhage occurred and it was most likely caused in one case by the scleral fixation technique. Second, the electronic intraocular lens is a large component, and the chance of tilting exists. In this work, partial dislocation due to tilting may have occurred in one eye. To avoid tilting, three-points fixation may be tried in the future.

The posterior part of the epiretinal implant, the microelectrode array, could eventually be sutured through the retina and the choroid and sclera. Indeed, successful suturing through the retina, choroid, and scleral tissue was described when trauma to those tissues occurred (Schmidt, Vitreous Society, Video, 2003). However, this technique may subject the eye to high risk of proliferative reactions or retinal hemorrhage. The abundant vascular supply of the retina and the underlying choroid complicates surgical manipulation because of the risk of excessive bleeding.

5.3.3.6 Others

There may be some other possibilities to fixate the epiretinal implant in the eye. Magnets could be used to keep epiretinal implants on the retina. With this technique the retina implant should be easy to replace. Although magnets have the advantage that the implant should be easy to replace (Majii et al., 1999), their use could contra-indicate the use of magnetic resonance examination.

Preshaped electrodes that follow the posterior pole curvature, named cantilever design, are an alternative to keep the implant attached to the retina. A preshaped electrode was proposed initially to avoid piercing of the retina, retinal pigment epithelium and choroid with a retinal tack. Those designs may be very physiologic to the eye. Since there are variations in the eye structure, it could be difficult to find the perfect curvature for each eye structure, and the risk of epiretinal implant dislocation is high (Wyatt and Rizzo, 1996). Besides, if the flexible microcable or microelectrode array touches the retina tissue, the chance of proliferative vitreoretinopathy increases.

If the microelectrode array could be injected in high speed towards the retina, it would attach firmly to the retinochoroidal tissue. Intraocular foreign bodies found to enter the eye in high speed adhere to the retinochoroidal tissue. Indeed, intraocular foreign bodies

are very difficult to remove. This fixation seems feasible, but it would probably damage the retina, in a way that electrical transmission would be hampered.

5.3.3.7 The future and solutions to fixating an epiretinal implant

The epiretinal concept places the vitreo-retinal surgeons to the major challenge to finding out if it is possible to fixate the epiretinal implant. While several approaches are being tested by several groups, there is no consensus and no safe technique yet. As the epiretinal concept has two major pieces, the electronic intraocular lens and the epiretinal array, it must be decided if one or both of those pieces must be fixated intraoperatively.

With the current epiretinal implants available, the anterior part, the electronic intraocular lens, should be fixated due to its large size. Once the anterior part is stable inside the eye, the risk of dislocation is reduced extremely. However, the most delicate aspect of the epiretinal implant's fixation is related to the posterior microelectrode array, because it sits in intimate contact to the retina tissue. This interaction, microelectrode array and retina, may cause destructive and severe reactions against the eye, as exampled by the proliferative vitreo-retinopathy and epiretinal membranes. Nevertheless, the solution for fixation may come from the understanding and control of those epiretinal glial reactions. The next goal of our epiretinal implant study group is to modify the material of the microelectrode array, in order to induce a limited and benign glial reaction around the microelectrode array itself. Once it is achieved, the problem of dislocation may be overwhelmed.

5.4 The epiretinal implant and the reaction of the ocular tissues – The biocompatibility issue

Two basic biological factors determine the success of replacement of degenerated photoreceptors by a technical implant. First, the neuronal tissue in the residual retina of patients selected for implantation must still be capable of processing technically generated signals. Second, the implant itself must be biocompatible with ocular tissue, i.e. it may not itself induce further degeneration, tissue damage, or immunological reactions. In this section, the biocompatibility issue is addressed.

5.4.1 Intraocular positioning of the epiretinal implant

Stable and long-term fixation of the electronic intraocular lens structure inside the capsule bag requires resistance to compression forces. The electronic intraocular lens structure must retain its support and fixation structure in an intact or ruptured capsule. Good positioning of the epiretinal implant was found postoperatively in three eyes in this study showing that an active epiretinal implant can be implanted to the inner retinal surface, and it may be mechanically stable.

Dislocation of the epiretinal implant was observed spontaneously in 3 eyes (50%) within the first 2 weeks. Pathogenesis of an epiretinal implant dislocation may be related to several factors:

A) Absence of electronic intraocular lens fixation: in two eyes in which epiretinal implant dislocation occurred, no electronic intraocular lens scleral fixation was accomplished. The electronic intraocular lens part of epiretinal implant is reasonable large, and every intraocular device implanted, including the intraocular lens, should have a mechanical support to avoid dislocation.

B) Intraoperative dislocation: if the epiretinal implant was implanted asymmetrically intraocularly, it may have slipped and dislocated towards the vitreous. Intraocular epiretinal implant good positioning was achieved in all cases. Then, this factor as a cause of epiretinal implant dislocation is unlikely.

C) Internal forces: internal forces include either a size disparity between the electronic intraocular lens and the site of fixation, capsular scarring, and capsular contraction. The epiretinal implant is a very large structure in comparison to the ocular anteroposterior

diameter. Then, a disparity between its size and the intraocular cat's eye may have occurred. The hypothesis of epiretinal implant dislocation secondary to capsular contraction comes from the cataract surgery. In the cataract surgery, capsular contraction syndrome consists of a fibrous reaction created by the remaining lens capsule leading to intraocular lens dislocation (Taylor et al., 1983). A large capsulorrhexis was performed in the animal's eyes. Scarring and peripheral anterior synechiae were not seen in any cases. Since capsule rupture occurred in all cases but one, capsule contraction most likely did not contribute to stable epiretinal implant positioning.

D) External forces: external forces include abrupt movements or eye trauma. The asymmetric shape of the epiretinal implant is subject to rotational eye movements, and those movements in conjunction with lack of fixation were the possible cause of dislocation in this study. External forces such as rubbing or eye trauma may have happened in the experimented animals.

The design of the electronic intraocular lens is similar to an unfoldable rigid intraocular lens composed of 1 piece (Figure 17). Although complications from intraocular lens were common the early days of cataract surgery, improvements in the surgical technique and intraocular lens design and quality have reduced the intraocular lens-related complications in the cataract surgery. The same principle may be applied for electronic intraocular lens. As there was a learning curve to implanting any intraocular lens, it is possible that improvements the surgical technique and increasing experience may decrease the rate of complications when implanting new generations of epiretinal implant in the future. Intraocular lens have also evolved to the sixth generation, which includes modern foldable small-incision designs as well as improved rigid polymethylmethacrylate design. The same design development may occur in the future for electronic epiretinal implants as for the cataract surgery has occurred. Newer epiretinal implants designs, improved surgical techniques, and a greater understanding of epiretinal implants fixation technique should reduce the incidence of dislocation.

5.4.2 Epiretinal implant related intraocular damage

There are so far no studies reporting the behavior of electrically active intraocular epiretinal implants. Only biocompatibility testing of electrically inactive and one-piece implants have been studied until now. The retinal tissue tears easily and may be susceptible to

mechanical compression by the epiretinal array. Anatomically, the retinal underneath may have been damaged (n= 1, 16.67%) by the epiretinal implants in this study, as described by others earlier (Walter et al., 1999). There was tapetum damage and retinal thinning after the one-month examination. Mechanical biocompatibility of inactive was tested in dogs by others, and no cases of retinal detachment or lesions were described in 4 eyes of dogs. Retinal pigment epithelium changes were observed near the tacks used for epiretinal implants fixation of the epiretinal implant (Majii et al., 1999). In another study, 9 out of 10 rabbits were implanted with an epiretinal device. The implant was stable at its original fixation area and no change in retinal architecture underneath the implant was found by light microscopy. Mild cataract formation was seen in 3 cases, and, in one case, a total retinal detachment was found after 6 months of follow up (Walter et al., 1999). The presence of an intraocular device may cause several other complications such as gross anatomic globe distortion, prolonged elevated intraocular pressure, and hypotony. In one case temporary elevated intraocular pressure has occurred, but it resumed after one month follow-up. Intraoperative hypotony was observed in one eye, but it did not keep post-operatively.

Several causes of epiretinal implant-related intraocular tissue damage may be listed: mechanical trauma, intraocular inflammation by the own implant or fixation substances, infection, epiretinal implant material non-biocompatibility, or tissue damage by electronic components or heat of the implant.

Table 2 shows in detail the composition, size, and fixation techniques used for different retina implants testing. Table 6 shows the examination and surgical techniques, as well as complications secondary to epiretinal implant observed by others.

5.4.2.1 Mechanical trauma

Intraocular mechanical trauma caused by an intraocular prosthesis may occur at the time of original surgery or it may be the result of ongoing intraocular injury. Surgical trauma to the corneal endothelium leads to endothelial cell loss, which may cause corneal decompensation. If the electronic intraocular lens is positioned in the anterior chamber, it may also produce mechanical trauma to the iris, ciliar body, or the anterior chamber angle, potentially compromising aqueous outflow and leading to glaucoma. Mechanical injury to the uveal tissue may produce eye atrophy and chronic uveitis.

In one case in this study anterior epiretinal implant dislocation and corneal touch were observed. Endothelial cell loss related to the electronic intraocular lens may be caused by direct surgical trauma, postoperative dislocation, eye rubbing, or breakdown in the blood-aqueous barrier secondary to chronic eye inflammation. Besides pulling the microelectrode array far from the retina, anterior dislocation may damage the corneal endothelium, leading to corneal damage. Corneal damage is one of the most serious complications after intraocular surgery. The disturbance of barrier function of the endothelium and damage to the pump that actively removes fluid which leaks into the stroma are causes of endothelium damage. Understanding the histology and physiology of the cornea may facilitate comprehension of the traumatic lesions. The cornea is a transparent converging lens that is comprised of 5 layers. The last corneal layer, the endothelium, consists of a monolayer of nonregenerative cells responsible for maintaining corneal clarity by its pump function. The endothelium serves to maintain stromal deturgescence and transparency. The cells that make up this layer are among the most delicate type in the human body. The endothelial pump, named Na/K-ATPase, consists of enzymes that are integrated in the plasma membrane and that transport ions from the corneal stroma into the anterior chamber. With disturbances in endothelial function, water diffuses into the stroma and disrupts the parallel arrangement of collagen fibrils, resulting in corneal opacity. In future studies, we intend to study the endothelial alterations after retinal implantation with specular microscopy examination (Bonanno, 2003).

5.4.2.2 Intraocular inflammation

A potential damage secondary to the presence of an intraocular epiretinal implant is incitation of inflammation or uveitis. Evidence of uveitis on clinical examination includes anterior chamber cells and flare, keratitic precipitates, and pigment debris in the inferior trabecular meshwork. In fact, traumatic uveitis may have occurred in one eye because inflammatory signs such as anterior chamber cells and flare, corneal new vessels were observed during the follow-up period.

Post-operative uveitis secondary to epiretinal implant insertion may have several causes. Improper electronic intraocular lens positioning and sizing during surgery may cause lens-induced inflammation. The release of toxic substances from the implant can provoke the inflammatory response, specially secondary to biodegradation, may lead to a syndrome called toxic lens syndrome. This syndrome is associated with polishing and sterilization of the

implant. Although the central nervous system and the eye are immunologically privileged sites, the natural course of inflammation is similar to elsewhere in the body once an incitement of inflammation has initiated. Emigration of lymphocytes from the bloodstream is mediated by circulating antigens and chemotactic factors. Macrophages are engaged in phagocytosis of injured cells, and plasma cells, which are involved in immunoglobulin mediated reactions (Rocha et al., 1992).

Another mechanism of inflammation may be mechanical uveal damage post-operatively. The electronic intraocular lens may touch the uvea and breakdown of the blood-aqueous barrier. Besides, the electronic intraocular lens may erode the uveal tissue liberating inflammatory mediators, such as prostaglandin, which may damage the corneal endothelium. These inflammatory products may contribute to progressive corneal endothelial cell loss. Touching the iris may also lead to the development of a fibrovascular membrane (Smith et al., 1988).

Increased intraocular pressure may be caused induced by chronic uveitis secondary to uveal trauma. Increased intraocular pressure was observed transiently in one eye in this work, and it may have been caused by intraocular lens by several mechanisms. Inflammatory cells or debris may occlude the trabecular meshwork and elevated intraocular pressure. Uveitis associated to the adherence of the iris to the intraocular lens optic may cause pupillary block. Outflow may be reduced by direct mechanical injury to the trabecular meshwork or peripheral anterior synechiae. The angled flexible microcable could eventually cause angle closure by blocking the trabecular meshwork. The incidence of glaucoma after intraocular lens implantation is around 2% (Worthen et al., 1980). However, no clinical correlation between the inflammation and increase of intraocular pressure was found in this study.

5.4.2.3 Infection

A more serious form of inflammation related to the epiretinal implant may be an injury resulting from infectious endophthalmitis. Clinical signs include conjunctival inflammation, episcleral inflammation, corneal stroma edema secondary to endothelial dysfunction, anterior chamber inflammation, including hypopyon, cells, flare, keratic precipitates, secondary glaucoma, uveal inflammation, inflammation of the posterior segment including vitreous hazing, inflammatory membranes, and retinal necrosis. It is unlikely that endophthalmitis occurred in the eye where severe inflammation occurred for several reasons,

including the transparent and healthy appearance of the vitreous cavity, whereas anterior chamber inflammation was present. The surgery in this study was done under sterile conditions, which makes the hypothesis of endophthalmitis also unlikely. While infectious endophthalmitis is an uncommon complication after modern cataract surgery with intraocular lens insertion, endophthalmitis is usually not directly attributed to the intraocular lens. Bacterial infections are often delayed and appear to be due in part to the host's inability to respond properly to infections. Their origin is frequently distant infected sites in the body or skin flora, while infected implants and surgical or nursing staff are less often origin. Attempts to avoid bacterial colonization on implants may be accomplished by coating polymers of the epiretinal implant with proteins or antibiotics. An electronic intraocular lens is essentially a foreign body and like any prosthetic device, it requires additional surgical manipulation for implantation (Jackson et al., 2003).

5.4.2.4 Epiretinal implant materials biocompatibility

Besides microelectronic aspects and the demand for developing minimal invasive implantation techniques, the biocompatibility of the implant materials is an important issue for the development of long-term technical implants. Since the sensitivity to different materials may vary from one cell type to another, it is of great importance to test those cells that actually come in contact with the implanted materials. There are also a number of potential complications of an electronic device in the eye that must be investigated: infection, inflammation, carcinogenic effect, toxicity, heat and corrosive damage (Margalit et al., 2002). Conversely, the ocular tissue can also damage the implant by degradation and corrosion of polymers and metals, mechanical dislocations of the implant by fibrous and glial tissue growth, and impairing electronic components of the implants by the ionic reactions. The electrical components of the prosthesis are in contact with all intraocular fluids, which could corrode the thin metal of the electrodes and impair the ability of the epiretinal implant to transmit electrical impulses to the inner retina. Products of electrical impulse transmission through metallic electrodes are generally toxic to living cells, and must be diminished in order to ensure minimal chemical devastation of the retina. The electrodes themselves must also be anchored to the retina with sufficient strength to accommodate physical agitation due to daily activity. As in all retinal surgical procedures, the implantation of a retinal implants subjects the eye to risk of retinal detachment and infection of the associated membranes, both of

which would exacerbate, rather than restore vision loss. These concerns have not yet been addressed in this stage of the research, because no long-term clinical trials of the prosthesis have been undertaken.

The quality of the electrodes charge will influence the retinal prosthesis. Most of the precious metals (platinum, gold) may corrode under certain conditions of electrical stimulation. The two most widely used stimulating electrodes are platinum and its alloy with iridium, due to its resistance to corrosion and good charge carrying capacity. Iridium oxide is a new category of electrodes that is very resistant to corrosion. Its electrodes have been proved to withstand more than 2 billion 10 mA current pulses without degradation. Titanium nitride has higher charge injection limit than platinum or iridium, but it may cause damage to retina cells (Ziaie et al., 1997). Some researches are experimenting electrode arrays of titanium nitride in animals (Zrenner et al., 1999; Margalit et al., 2002).

An implantable epiretinal array must have its components encapsulated for isolation from the eye fluids. This encapsulation has been investigated so far by various types of materials: silicone, polymethyl methacrylate, silicone plus polyamide, acryl polymethyl methacrylate, polymethyl methacrylate plus polypro, silicium oxide, silicium nitride, and iridium (Hetke et al., 1994). Most of the tests with silicium oxide, silicium nitride, and iridium had good biocompatibility, except for the reduced cell survival for the titanium nitride (Weiland and Anderson, 2000; Margalit et al., 2002). Silicone has been shown to be well tolerated for intraocular use as a component of silicone foldable lens. Silicone and platinum metal are well tolerated in the cochlea as part of the cochlear implant electrode array (Yamanaka, 1991; Brabyn, 1982). Platinum is used in iris clip lenses with a low rate of adverse reactions (Brummer and Turner, 1977). Platinum has the advantage of being resistant at high charge limits, taking a long period before interfering with the vitreoretinal interface.

In this work, our epiretinal implant made of polyimide was coated with parylene and finally encapsulated with silicone. In previous experiments this epiretinal implant demonstrated to be highly biocompatible (Schanze et al., 2003). Others also tested isolation with the silicone-polyamide interface and silicone-platinum substances for epiretinal implant materials. Silicone-teflon for encapsulation was not effective, allowing leakage current. Although polyamide can be used as material to make an epiretinal array, its disadvantages are the risk of saline leaking with consequent low durability and its weakness as material for interfaces. Moreover, polyamide-based microelectrodes have been reported to induce regeneration of optic nerve axons in rats (Heiduschka et al., 2001).

Different materials of a multi-photodiode array were tested with mammalian retinal cells using retinal cell cultures. In the experiments, retinal cells were seeded onto different

chip materials and cultivated over a period of several weeks. Survival rates of retinal nerve cells on different chip materials were measured. Measurements carried out over a period of 4 weeks determined the percent of each cell type which survives on different chip materials in comparison to a control group. While most materials exhibited good compatibility, the survival rate on titanium nitride was only 50%. This material therefore should require coating its surfaces with glycoproteins to improve their biocompatibility (Guenther et al., 1999).

The retina of cats and pigs are reported to remain intact for more than 2 years after implantation of a subretinal device (Kohler et al., 2001). In rats, the implant remains remain stable in the subretinal position and kept long-term functioning. Implanted subretinal devices were somehow damaged over time due to accumulation of silicon oxide on their surfaces and disintegration of the gold electrodes (Zrenner et al., 2001). Chow and Chow proposed that small micro-electrodes located on each micro-photodiode serve to transfer current from the chip into the adjacent tissue (Chow and Chow, 1997). Micro-photodiode arrays were manufactured on a silicon wafer. After completion of the electrically active structures, a passivation layer consisting of silicon oxide is applied which consists of a thin layer of high temperature oxide and about 0.5 μm of tetra-ethyl-ortho-silicate. Firstly, a photoresistant layer is applied and micro-patterned. Subsequently, contact holes are etched into the passivation layer in order to provide for an electrical connection of the micro-electrodes to the micro-photodiodes. A layer of titanium nitride is applied and finally micro-patterned by lift-off to create micro-electrodes. Microchip diameter is typically 1 to 3 mm, thickness 30 to 70 μm. Table 6 describes follow-up and complications in several groups after retina implants testing.

5.4.2.5 Electrical and heat intraocular injury

Different components of the visual prostheses can produce excessive heat and cause damage to any neuronal tissue if not kept below a certain limit. Placing the electronics in contact with the retina, either epiretinally or subretinally, has a high risk of causing heat injury. Therefore, any electronic component that produces relatively large amount of heat must be placed as far away as possible from the retina.

Some authors studied the retina's ability to dissipate and tolerate heat generated by an intraocular electronic heater in 16 dogs. No more than 50 mW of power over a 1.4 mm^2 area can be applied onto the retina for more than one second. A power of 500 mW in the center

of the vitreous for 2 hours did not damage the retina. Thermal damage occurred at powers of 50 mW or greater if the heat was induced on the retina. Piyathaisere and co-workers also investigated the differences of heat effect in two separate groups (Piyathaisere et al. , 2003). The first animal group was subjected to heat dissipation on the retina. In the second study group, the heat was mechanically held in the vitreous cavity, dissipating 500 mW for two hours. While the retina in group one was frequently damaged, the animals in which heat was dissipated in the vitreous demonstrated no retinal damage, but only slight temperature increase in the retina was reported. Temperature increase was under 2 degrees Celsius even at high heat generation (Piyathaisere et al., 2001). Therefore, a reasonable amount of power energy can be generated if the implant is inserted in the vitreous cavity.

It is very unlike that heat damage have caused corneal damage in this work since only a short circuit current of 56 μA may be produced by our epiretinal implants. One must consider phacoemulsification in the differential diagnosis to heat injury, because it has been associated with cats' corneal opacification. The reasons may be the heat energy released by the low frequency ultrasound, corneal mechanical touch, lost of anterior chamber pressure, and excessive amplitude of vibration (Kelman, 1967).

5.5 Future perspectives in the development of a retinal prosthesis

Whereas this work was a little contribution to the development of an efficient retinal prosthesis for the blind, several work groups in the world continue their efforts to achieve this major goal. Microelectronics should not be a barrier for the development of a visual prosthesis (Rizzo et al., 2001). The most difficult step should be the decodification of the retina-implant communications. In the last thirty years eye surgeries have been used even more successfully, and many diseases which were considered not curable in the past are not anymore. The same story may occur in the aspect of a visual prosthesis, as advances in engineer and ophthalmology should allow the creation of an efficient visual prosthesis.

The researches for the creation of a retinal prosthesis have come to a stage of small pilot tests in humans. Humayun and co-workers have described implantation of an epiretinal prosthesis in humans (Humayun et al., 2003). Chow and co-workers implanted subretinal implants in several blind patients (Chow et al., 2003). Short tests in humans with retinal implants are planned in a near future in Germany. At the same time, tests in animals must continue to clarify several still opened questions.

Conclusions

1. A surgical technique to implant the epiretinal implants in cats was defined. Both phacoemulsification and vitrectomy are necessary to introduce surgically the epiretinal implants. Epiretinal implant insertion in cats is preferentially performed through the limbus to avoid scleral trauma. Mechanical posterior cortex removal may be accomplished successfully. The use of dyes for vitreous visualization is recommended. Perfluorocarbon liquids is not long-term tolerable and should not be desirable technique for long-term fixation. The most difficult step is the implantation into the eye of the large second generation epiretinal implant.

2. In four eyes (66.67%) the epiretinal implant was biocompatible for at least 6 months and no major complications ensued. In one of those eyes mild corneal damage occurred post-operatively related to corneal touch by the implant. Severe intraocular inflammation occurred in one eye, caused either by perfluorocarbon liquids or to epiretinal implant-related damage. Slight retinal damage was observed in only one case. Currently available prototypes of an active compound epiretinal prosthesis consisting of a intraocular lens connected to a microelectrode array could be implanted with a moderate rate of complications.

3. In four cases (66.67%) the implant was long-term stable. Small dislocation occurred in one eye. In one eye the dislocation was severe and may have contributed to damaging the ocular tissues. Inserting the electronic intraocular lens in the capsular bag and leaving the perfluorocarbon as intraocular tamponade was not enough for long-term fixation. Scleral suturing was a successful approach to fixate the epiretinal implant.

4. In all cases our active epiretinal implant was functioning in the long-term period. A good contact between microelectrode array and the retina was observed macroscopically in three eyes postoperatively. Short-term functioning and cortical stimulation was obtained by the epiretinal device in two cases for seven days. However, no long-term stimulation of an active epiretinal implant with corresponding cortical recordings was achieved. The reasons could be a formation of an epiretinal membrane between the array and the retina, a small space separating the array and the retina, or even a too deep array pressure on the retina.

5. Major advances have been achieved in the development of visual prosthesis. Several groups in Europe, Asia, and North America are working intensely to develop a functioning visual prosthesis. And several pilot studies in humans are planned for the next few years.

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8. Résumé - Summary

Electronic implants which can bypass the damaged neurons of the visual system to restore vision in blind patients have been researched in the last few years. Most of concepts for a visual prosthesis are based on neuronal electrical stimulation at different locations along the visual pathways within the nervous system. Retina and optic nerve implants may restore vision in patients with progressive retinal degenerations (retinitis pigmentosa and age-related macular degeneration) by means of electrical stimulation of neurons. Cortex prosthesis may restore vision in a larger amount of blind patients due to the more posterior location in the visual pathway.

A number of key questions remain to make visual prosthesis feasible. The goal of this thesis is to address some of those questions. The feasibility of an intraocular active epiretinal prosthesis is investigated. A new generation epiretinal implant is tested in cats and long-term functioning is followed. The long-term biocompatibility and biostability of the implant for at least 6 month period is evaluated. A surgical technique for implantation and fixation of the electrically active epiretinal implant into the retina of cats and the secondary tissue reaction to the implant are investigated. Besides, an overview of the current and future concepts of cortex and optic nerve as well as the retinal prostheses are provided, and this is done in a general and historic perspective.

Data from experiments in cats with a epiretinal prosthesis prototype in this work suggest the feasibility of this concept. Vitrectomy, phacoemulsification, and epiretinal implant insertion were successfully performed in cats. While perfluorocarbon was not effective as a fixation tool, scleral fixation has raised as efficient alternative for epiretinal implant fixation. Biocompatibility of epiretinal implants for at least 6 months was achieved in most operated eyes. Implantation of an epiretinal implant was surgically feasible with little chance of damage to the underlying retina.

In conclusion, we here determined the surgical technique to implant a new generation of electronic epiretinal implants and proved the biocompatibility and functionality of the epiretinal approach. While major advances have been made in the field of visual prosthesis for the blind in the last decade, further research is warranted.

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10. Curriculum Vitae

Education

1993-98 School of Medicine– Federal University of Santa Catarina. Brazil

1997 Clinical elective. Bascom Palmer Eye Institute– University of Miami

Post-Graduate Training

Residency in ophthalmology

1998-2001 Hospital Regional de São Jose - Brazil

2001 Brazilian Council of Ophthalmology. National examination for Ophthalmologist degree. Pass

Clinical and Research Fellowships

2001 Surgical Retina Fellowship

Federal University of Goias, CBCO, Brazil, Tutor: Prof. Marcos Ávila

2002-03 Retina and Vitreous Fellowship.

Philipps-Universität Marburg, Germany, Tutor: Prof. Peter Kroll

Grant and Research Support

2002-03 DAAD German Exchange Academic Service

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Topic: Surgical intraocular implantation and cortical recording of active epiretinal implants in cats

Publications

6 papers published in peer-review international journals.

12 papers *in press* in peer-review international journals.

14 papers under review in peer-review international journals.

Lectures and Presentations

2003 "Intravitreous use of indocyanine green." German retina society. Tübingen. Germany. Lecture

2003 "Visual prosthesis for the blind". Retinitis pigmentosa meeting. Barcelona. Spain. Invited Lecture

2003 "Rosai-Dorfman disease presenting as bilateral serous retinal detachment." Congress of the Brazilian Retina and Vitreous Society. Goiânia. Brazil. Lecture

2003 "Potential effects of suctioning in LASIK procedures on vitreoretinal structures."

Annual Meeting of German Ophthalmic Surgeons. Nürnberg. Germany. Poster

2002 “Optic pit maculopathy secondary to blunt trauma”. German Congress of Ophthalmology. Berlin. Germany. Lecture

2001 “Hypertensive retinopathy in pregnancy”. VI South Brazilian Congress. Florianópolis. Brazil. Lecture

2000 “Indications for vitrectomy”. National Congress of the Brazilian Retina Society. Belo Horizonte. Brazil. Lecture

2000 “Ectodermic Displasy presenting with uveitis”. National Congress of the Brazilian Retina and Vitreous Society. Belo Horizonte. Brazil. Lecture

1999 “Strabismus in childhood- Prevalence in 587 children” National Brazilian Congress of Blindness Prevention. Lecture

1998 “ Corneal foreign body: correlation with professional activity” South-Brazilian Congress. Curitiba. Brazil. Lecture

1998 “ Leiner Syndrome ” Regional Congress of Dermatology. Florianópolis. Brazil. Poster

1997 “Prevalence of Neurocisticercosis by patients with epilepsy” Internal Medicine Congress. Florianópolis. Brazil. Poster

Congress

1997-2003 Participation in 78 Congresses of Ophthalmology

Spoken Languages

English, German, Portuguese, Spanish

Volunteer Activities

Participation in national eye camping in Brazil as volunteer:

1996 Glaucoma Prevention – Ação Global-Sesi

1999 Olho no Olho

2000 Campanha Nacional de Prevenção a Cegueira

2001 Diabetes 2003.