Biocompatibility of epiretinal prostheses extended by an integrated circuit (IC) based optical capturing via photodiodes (OPTO-EPIRET)

Kim Schaffrath1, Tibor Lohmann1, Claudia Werner2, Pascal Raffelberg2, Florian Waschkowski3, Reinhard Viga4, Rainer Kokozinski5,6, Wilfried Mokwa7, Peter Walter8, Sandra Johnen1

1Department of Ophthalmology, University Hospital RWTH Aachen, Aachen, Germany, 2University Duisburg-Essen, Electronic Components and Circuits, Duisburg, Germany, 3Institute of Materials in Electrical Engineering 3, RWTH Aachen University, Aachen, Germany, 4Fraunhofer Institute of Microelectronic Circuits and Systems, Duisburg, Germany

Purpose

Epiretinal prostheses can regain certain functionality in blind patients suffering from retinal degenerative diseases, e.g., retinitis pigmentosa. Microelectrodes, embedded in prostheses, electrically stimulate the remaining functional retinal ganglion cells. Within the OPTO-EPIRET proposal, the common approach is extended by an integrated circuit (IC) based optical capturing of the visual field. A photodiode array at the front side of the IC records the images that normally fall directly onto the retina. The optical information is converted by the IC into appropriate stimulation pulses that are forwarded to the microelectrodes on the backside. The biocompatibility profiles of single basic structures (basic wafer) and the IC including photodiode structures (sensor chip) were investigated after direct and indirect cell contact in terms of cell proliferation, gene expression and viability.

Methods

Manufacturing of the structures was carried out by our partners. For indirect contact, L-929 and retinal precursor (R28) cells were cultivated in medium pre-incubated with the different structures separately and growth rates were analysed using a luminescent cell viability assay. A fluorescein-diacetate/ propidium iodide-based life-death assay was performed to evaluate survival according to the direct cell contact. Quantitative real-time PCR was used to analyse the gene expression of R28 cells.

Results

- Both cell lines showed no cytotoxicity
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Survival rates of L-929 and R28 cells were analysed in cultures incubated with increasing dilutions (1:1 - 1:4) of extractive media obtained from certified positive (RM A, RM B) and negative (RM C) reference materials as well as different wafer structures. The dotted lines designate mean values of the glass approaches (negative control). Bars represent mean ± SD (one-way ANOVA with Dunnett’s post hoc test, basic wafer: for L-929 cells n=4, for R28 cells n=6; sensor chip: for L-929 and R28 cells n=8, n.s. not significant, * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001).

Gene Expression

- Significant downregulation for S100B (retinal marker gene) and p53 (tumor suppressor gene)
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Real-time PCR was performed with cDNA templates of R28 cells to quantify the expression of different genes involved in the cell cycle and representing neuronal/glial and retinal markers. Using the comparative CT (2-ΔΔCT) method, the relative gene expression ratio of cells cultured on glass was set to 1. Regarding cultivation on the different wafer slices, values >1 denote upregulation and values <1 denote downregulation of gene expression. Each column represents the median, maximum, minimum, and the 95th percentile of the data for 4 distinct LightCycler runs (one sample two-tailed t-test; * p<0.05, ** p<0.01, white bars: retinal marker; light grey bars: neuronal marker; dark grey bars: cell cycle/ oncogenes).

Regarding indirect contact, extractive media of the structures had no significant influence on cell growth rates, as compared to positive reference materials that showed defined levels of cytotoxicity. Regarding direct contact, both cell types exhibited good proliferation properties on both structures and showed less than 1.1 % and 0.5 % dead cells for L-929 and R28, respectively. Regarding gene expression, a slight decrease in S100B and p53 gene expression was observed for the basic wafers and in S100B expression for the sensor chips. All other genes do not show any statistical difference.

Conclusions

The single parts of the photodiode extended epiretinal prosthesis showed good biocompatibility profiles without any aspects of cytotoxicity, neither after direct nor indirect cell contact. These results are the first step towards the subsequent biocompatibility testing of the final OPTO-EPIRET structures in vivo in a rabbit model.