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

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Purpose

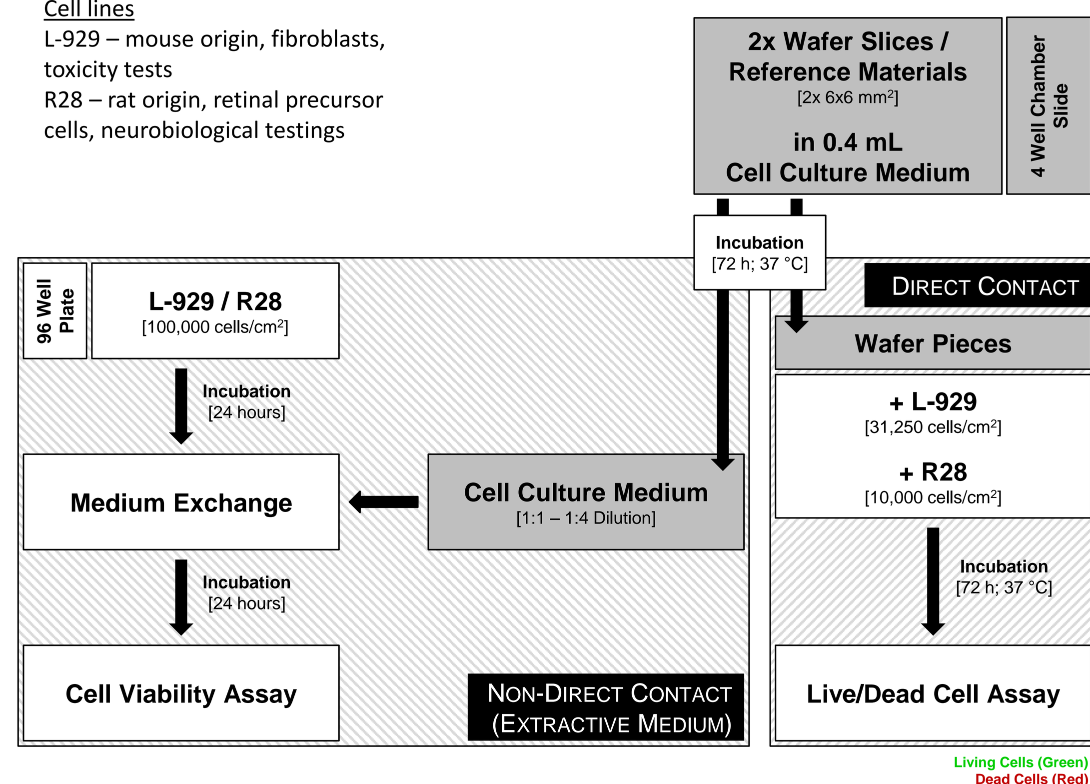
Epiretinal prostheses can regain certain functionality in blind patients suffering from retinal degenerative diseases, e.g., retinitis pigmentosa. Microelectrodes, embedded in prostheses, electrically stimulate the remaining functional retinal ganglion cells. Within the OPTO-EPIRET proposal, the common 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-dead 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.

accredited reference materials (RM)
RM A – **z**inc **d**i**e**thyldithio**c**arbamat (ZDEC), moderate level of cytotoxicity
RM B – **z**inc **d**i**b**utylthio**c**arbamate (ZDBC), weak level of cytotoxicity
RM C – **p**oly**e**thylene (PE), no cytotoxicity

Cell lines
L-929 – mouse origin, fibroblasts, toxicity tests
R28 – rat origin, retinal precursor cells, neurobiological testings

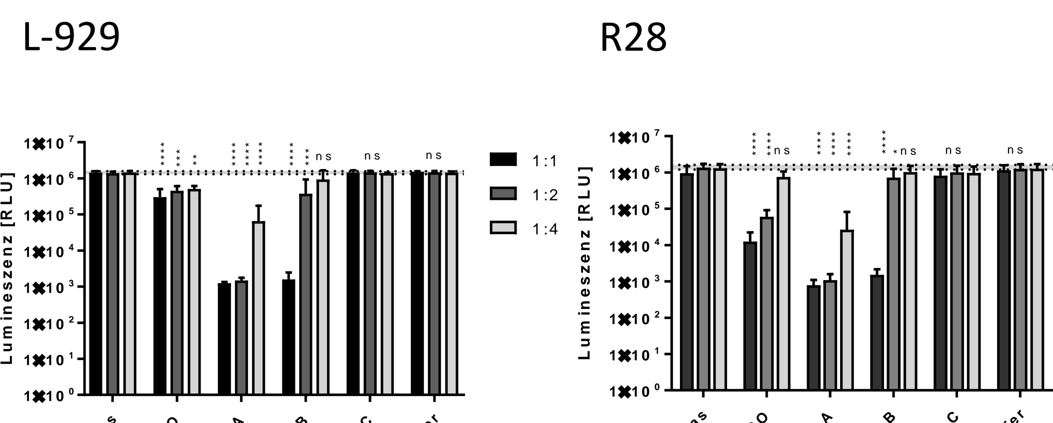


The flow scheme illustrates the protocol to evaluate the survival rate of L-929 and R28 cells after contact to different wafer structures. **Direct contact** was examined via a fluorescein-diacetate/ propidium iodide-based life-dead assay. For **non-direct contact,** a luminescent cell viability assay was performed with extractive media pre-incubated with the structures compared to different reference materials (RM) with defined levels of cytotoxicity. RM A, RM B and dimethyl sulfoxide (DMSO) were used as positive controls of cytotoxicity, RM C and glass were used as negative controls. All tests were conducted referring to the ISO 10993 standard "biological evaluation of medical devices", tests for in vitro cytotoxicity (part 5) and sample preparation and reference materials (part 12).

Results

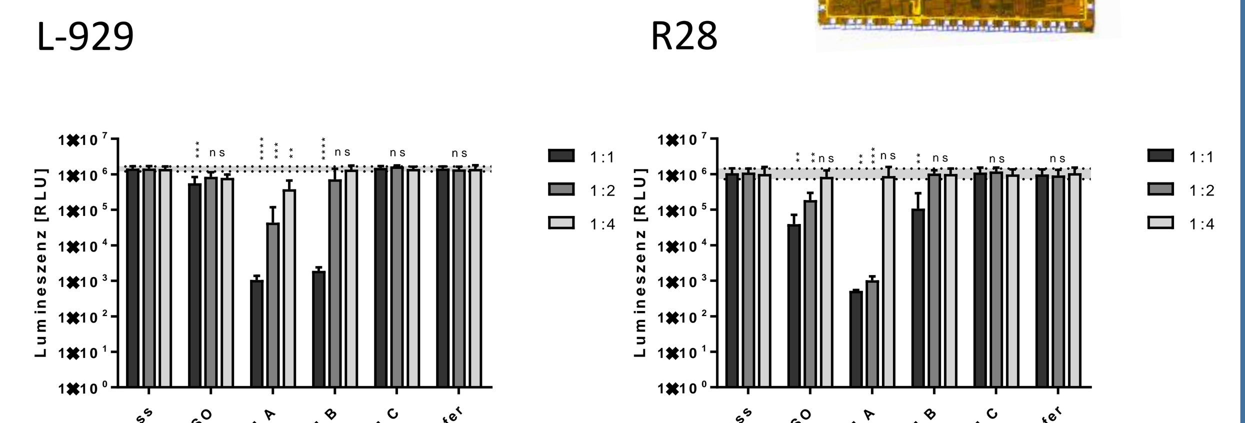
Basic wafer

Indirect Contact



⇨ Both cell lines showed no cytotoxicity

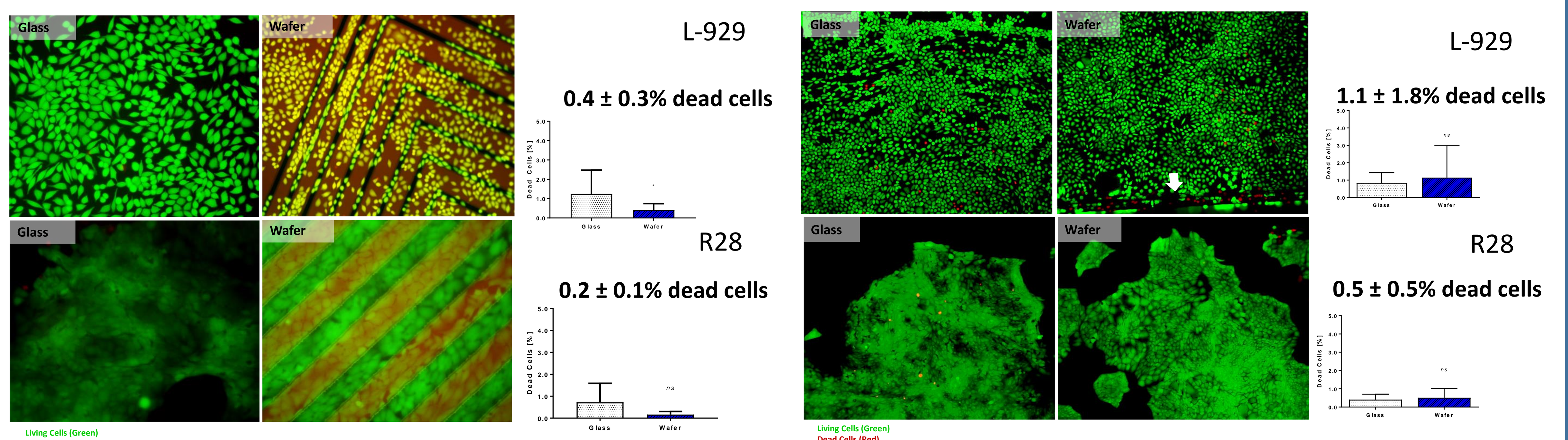
Sensor chip



⇨ Both cell lines showed no cytotoxicity

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; ns: not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$).

Direct Contact



Viability of L-929 and R28 cells directly cultivated on glass and wafer structures 72 hours after seeding (basic wafer: 100x magnification for L-929 cells on wafer and 200x for L-929 on glass and R28 on glass and wafer; sensor chip: 100x magnification). Immediately after cell staining, fluorescence microscopy was performed to differentiate between vital (green) and dead (red) cells. The quantity of dead cells is presented as percentage of the total cell amount. For each substrate, 3 to 6 randomly selected image sections (original magnification, x100) were analysed. Bars represent mean ± SD (unpaired t-test; basic wafer: for L-929 cells n=4, for R28 cells n=5; sensor chip: n=8; ns: not significant, * $p<0.05$).

Gene Expression

significant downregulation for *S100B* (retinal marker gene) and *p53* (tumor suppressor gene)

significant downregulation for *S100B* (retinal marker gene)

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 cultivated 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 50th 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.

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