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Supplementary Materials: Fasudil Loaded PLGA Microspheres as Potential Intravitreal Depot Formulation for Glaucoma Therapy

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MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole)-assay was used to determine the viability of fasudil-treated immortalized trabecular meshwork cells (HTM-N), trabecular meshwork (TM) cells, Schlemm's canal (SC) cells and fibroblasts. MTT is a dye (yellow), which is reduced by cellular enzymes to formazan (blue dye). This transformation is only possible in viable cells; therefore, the amount of formazan is proportional to the number of viable cells [1]. Cells were seeded in a 96-well plate and cultivated for 24 h. Then, cells were washed once with DPBS and the culture medium was replaced by 100 µL of fasudil dilutions ranging from 5 to 1000 µm in culture medium supplemented with 10 or 0.35% FBS and incubated again for 24 h. Two hundred microliters of MTT (625 µg/mL in culture medium without serum) was added and further incubated for six hours at 37 °C. Then, the supernatant was carefully removed, and the MTT crystals were dissolved in 60 µL PBS containing 10% (*w*/*v*) sodium dodecyl sulfate (SDS). The cells were incubated for additional 48 hours in the dark and room temperature. The absorbance of formazan was measured at 570–690 nm using a microplate reader (FLUOstar Omega; BMG Labtech GmbH, Ortenberg, Germany). As positive and negative control served cells treated with DPBS and 0.1% (*w*/*v*) SDS, respectively. The results were calculated as the mean percentage of viability in relation to the positive control. Viabilities below 70% were considered as cytotoxic. (n = 6).

Figure S1 shows the cell viability data of fasudil-treated HTM-N cells, TM cells, SC cells and fibroblasts cells. Data indicate that all analyzed cell types tolerated well a fasudil concentration of 25 µm.

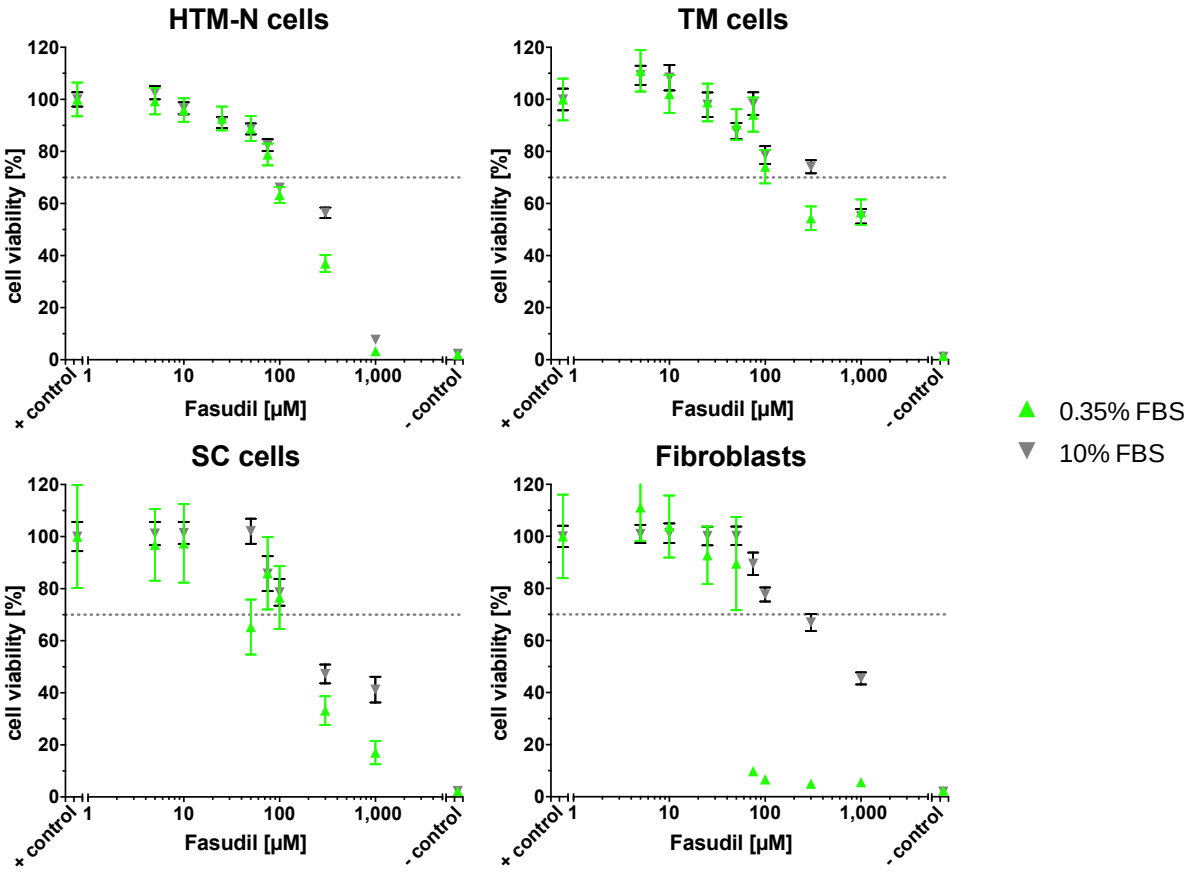

Figure S1. Fasudil at a concentration of 25 µm is well-tolerated by HTM-N cells, trabecular meshwork (TM) cells, Schlemm's canal (SC) cells and fibroblasts. MTT assay data are shown. Cells were incubated for 24 hours with different fasudil concentrations ranging from 5 to 1000 µm, in culture medium supplemented with 0.35 or 10% FBS. All analyzed cell types tolerated well a fasudil concentration of 25 µm. Concentrations below 70% (dashed line) were considered as cytotoxic. Data expressed as mean percentage of viability in relation to the positive control ± standard deviation of the mean (n = 6); + control—positive control (DPBS); - control—negative control (0.1% (w/v) SDS).

References

1. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55–63, doi:10.1016/0022-1759(83)90303-4.