

A versatile in vitro bioassay for the screening of neurotrophic factor gene therapy systems to combat blindness

Glaucoma is the world's second leading cause of blindness after cataracts, affecting about 80 million people worldwide annually. The disease is characterized by neurodegeneration involving a progressive visual field loss from the periphery towards the center, and eventually leads to complete blindness. The deterioration of vision is mainly attributed to elevated intraocular pressure-induced (IOP; >22 mm Hg) damage to retinal ganglion cells (RGCs), the population of neurons in the retina that forms the optic nerve which relays visual signal from the eye to the brain. Presently, patients suffering from glaucoma are primarily treated with IOP reducing regimens through drug therapy or surgical interventions. While combinations of these treatments could effectively decrease IOP, they do not provide support to protect or restore damage to RGCs. Consequently, many patients continue to suffer from visual field deterioration as a result of continuous RGC degeneration.

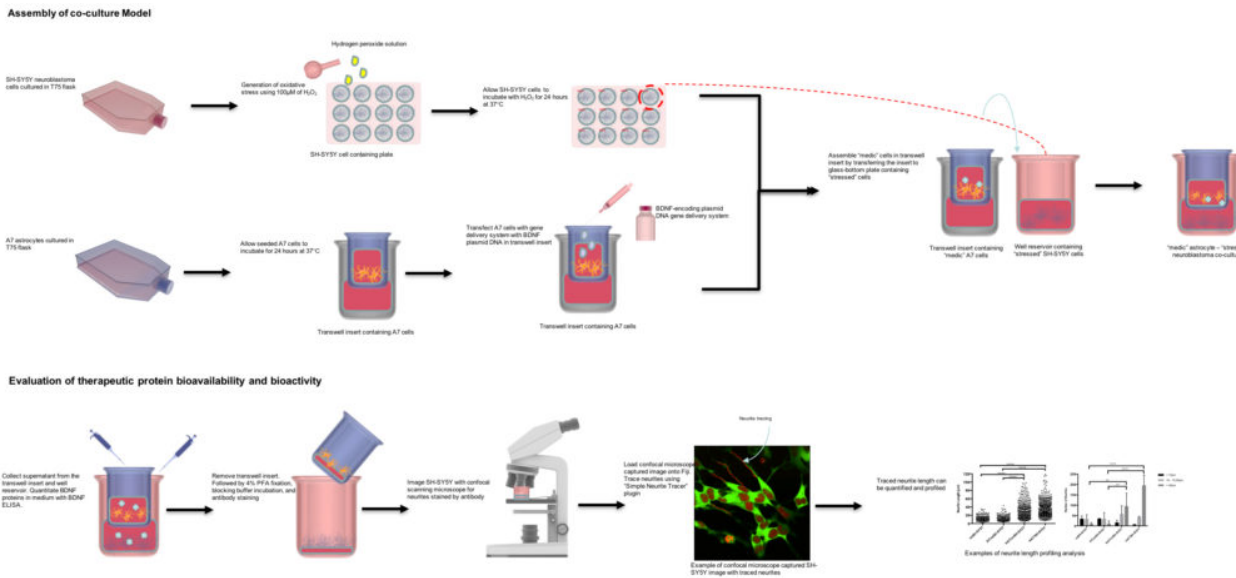


Fig. 1. Summary of the astrocyte-neuronal co-culture model and the bioavailability and bioactivity evaluation workflow.

Non-viral gene therapy is a novel class of gene-based therapeutics, utilizing nanotechnology and nucleic acids to deliver unconventional therapeutic possibilities that could address the unmet needs in glaucoma management. More specifically, treatments such as brain-derived neurotrophic factor (BDNF) plasmid DNA gene therapy can genetically modify and transform patient's retinal cells into

“medic retinal cells”, through the delivery of BDNF-encoded plasmid DNA. Medic cells could then mediate localized production and release of BDNF proteins within the patient’s eye. As BDNF have been shown to provide promising therapeutic possibilities toward neurons, BDNF released from medic retinal cells could provide a means of support to RGCs that are already damaged, or to prevent damage.

To identify the most effective gene delivery systems, there is a need for an *in vitro* assay that helps screen transfection efficiency and bioavailability of large number of formulations that can be advanced into further investigations in live animals. Therefore, we have developed a 3-dimensional astrocyte-neuronal co-culture model to simulate the “medic” and “stressed” cells microenvironment *in vitro*. In addition, through the incorporation of enzyme-linked immunosorbent assay (ELISA) and immunofluorescent imaging and tracing techniques into the process, we could assess the therapeutic potential of candidate gene delivery systems.

To provide a proof-of-principle for the bioassay in evaluating the therapeutic potential of novel gene therapy systems, we have utilized a commercially available non-viral gene delivery system, K2®, as a model system. Astrocyte cells, cultured on a transwell insert, were genetically modified by K2® nanoparticle system, and transformed into BDNF-expressing astrocyte cells (medic astrocyte cells), while neuroblastoma cells cultured on the bottom of the co-culture setup were exposed to hydrogen peroxide to simulate neurons undergoing oxidative stress (stressed neuroblastoma cells). The quantity of BDNF released from medic astrocyte cells was assessed using ELISA, generating concentrations reaching up to $10,367.1 \pm 390.8$ pg/mL over 72 hours. The BDNF released resulted in neurite outgrowth stimulation in the recipient stressed neuroblastoma cells. Specifically, the fluorescent microscopic analysis showed that stressed neuroblastoma cells co-cultured with medic astrocyte cells had up to 10.8 times more neurites with extended lengths ($>20\mu\text{m}$) compared to stressed neuroblastoma cells that were cultured in the absence of medic astrocyte cells over 72 hours.

In summary, we have shown through this study that the described bioassay is a feasible and versatile system that can be used as a tool in identifying promising non-viral gene delivery systems. Furthermore, the development of innovative gene therapy regimens using BDNF as one of the promising neurotrophic factors, can provide neuroprotection in the retina and be part of the extended treatment that could help prevent blindness in glaucoma.

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