Senescence Assay for AD drug screening in OrganRX™ Plate

Objective

Biopico's OrganRX™ plate is used to assess disease modeling and drug testing on cellular aging, or senescence, in multi-organ cultures. The objectives are

- To develop a 3-D brain model to study senescence
- To screen AD drugs with senescence mechanism
- To study the contribution of multi-organs in the senescence of brain cells

Abstract

The OrganRX™ plate is a microfluidic organ plate that enables the culturing and screening of a range of 3D multi-organ and tissue disease models. Here, we investigate the senescence signaling pathway in the OrganRX plate for multi-organs. In the present study, we showed that amyloid-β oligomers (Aβ), one of the core pathological players of Alzheimer's Disease (AD), significantly upregulated the expression of senescence markers, and senescence-associated β-galactosidase (SA-β-gal) in SK-N-SH neuroblastoma cells. This study revealed that Aβ exposure markedly increased senescence, and the AD drug rescued Aβ-induced cellular senescence. In conclusion, our findings clearly demonstrate that exposure of Aβ causes the senescence of multiple human cells in 3-D organs and pre-treatment with AD drugs reduces the senescence activity.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder that becomes more prevalent with age and progresses irreversibly[1-4]. It is associated with cellular senescence, characterized by distinct phenotypic traits such as senescence-associated β-galactosidase (SA-β-gal) activity, cell cycle arrest, persistent DNA damage response (DDR), and the release of senescence-associated secretory phenotype (SASP) components, including inflammatory cytokines, growth factors, matrix metalloproteinases, and other proteinases. These factors contribute significantly to the onset and worsening of AD.

Senescent cells exhibit three primary features: a decline in their ability to proliferate or regenerate, alterations in metabolic functions, and resistance to apoptosis. They also

secrete a variety of biologically active molecules, collectively known as the senescence-associated secretory phenotype (SASP). A widely recognized marker for senescence is the increased activity of senescence-associated beta-galactosidase (SA-β-Gal). This assay is typically colorimetric and can be observed using a light microscope.

Methods

Organ Culture

OrganRX™ Plate was seeded with SK-N-SH neuroblastoma cell in fibrinogen-thrombin gel to model the 3D brain organ culture. Cells were seeded in the brain compartment of the OrganRX™ Plate with 6 units (Fig. 1) and maintained with EMEM media for 7 days until sufficient growth. Senescence was induced by adding Aß oligomers to cultures with or without drug pretreatment.

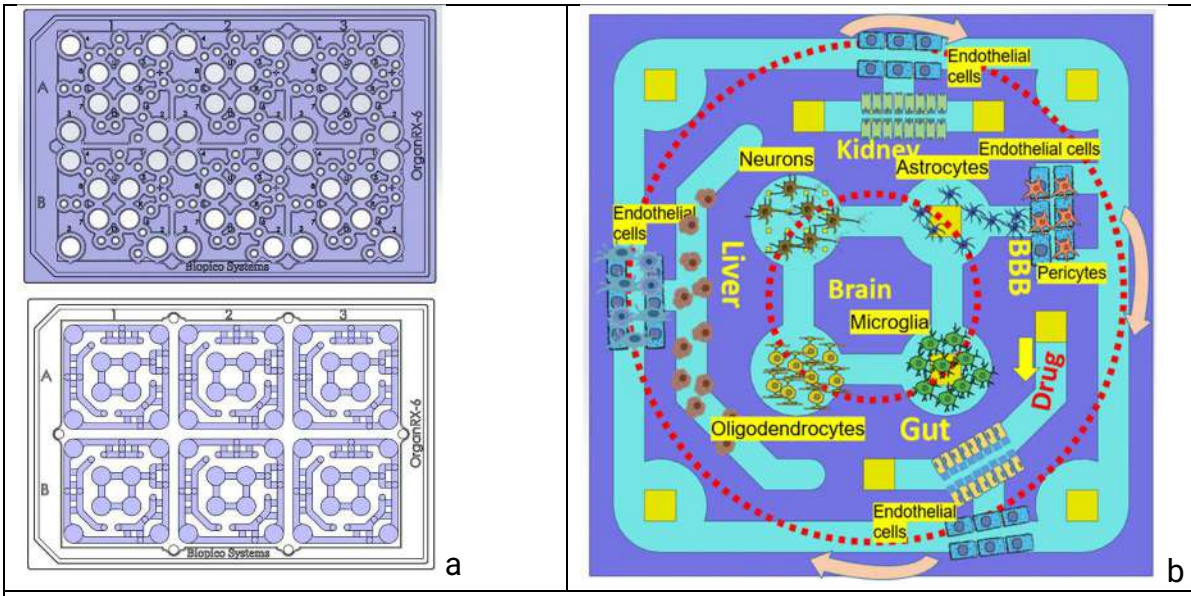

Fig. 1 OrganRX™ Plate. (a) Top: Wells. Bottom: Channels connecting the wells (b) multi-organs culture populated with cells for brain-liver-kidney-gut organs

Senescence Model

We have optimized the OrganRX™ recirculation system (Fig. 2a) to give a recirculation shear flow rate of 0.5 dynes/cm^2 to the brain organ in culture with endothelial cells (Fig. 2b). The organs cultured are monitored using a NyOne imaging station (Fig. 3a) with 20 Z-stacks across 1 mm depth on 34 frames per unit. To induce senescence, 5uM of Aß oligomers were added to organ cultured for 7 days under recirculation and incubated for 3 days before performing senescence assay.

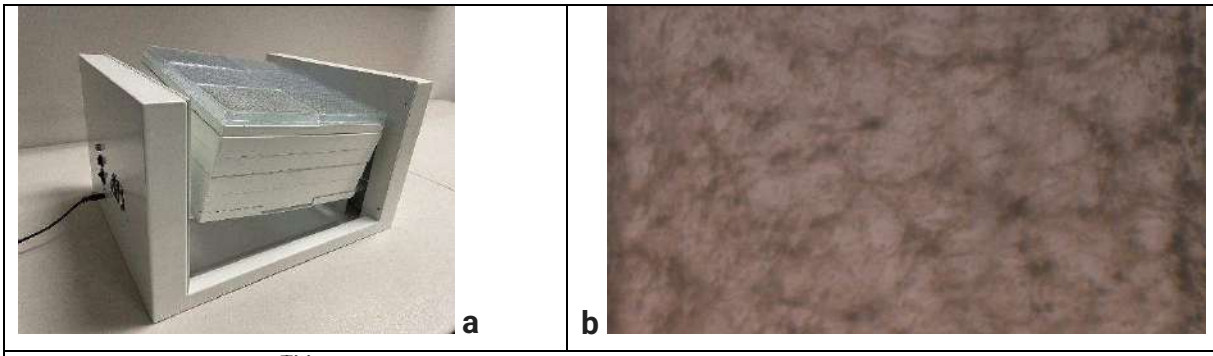

Fig. 2. (a) OrganRX™ recirculation system (b) Shear flow validated for vascular network

Drug Testing

We have studied multiple AD drugs in the organ plate and Senescence Assay was performed to study drug effects of the organ challenged with Aß oligomers. The drug solution was added to organ cultures on day 8 of culture. Organ cultures were treated with 0, 0.5, 1, 2, 4, or 8 µM of tacrine. We have also tested[5] the organ cultures with a cocktail of both Quercetin (0, 2.5, 5, 10, 20, 30 uM) and Dasatinib (0, 0.25, 0.5, 1, 2, 3 uM).

Assays

On day 11, SA-ß-gal staining was used to assess senescence, and SA-ß-gal activity was measured using absorbance at 406 nm using a Synergy2 plate reader (Fig. 3b) for

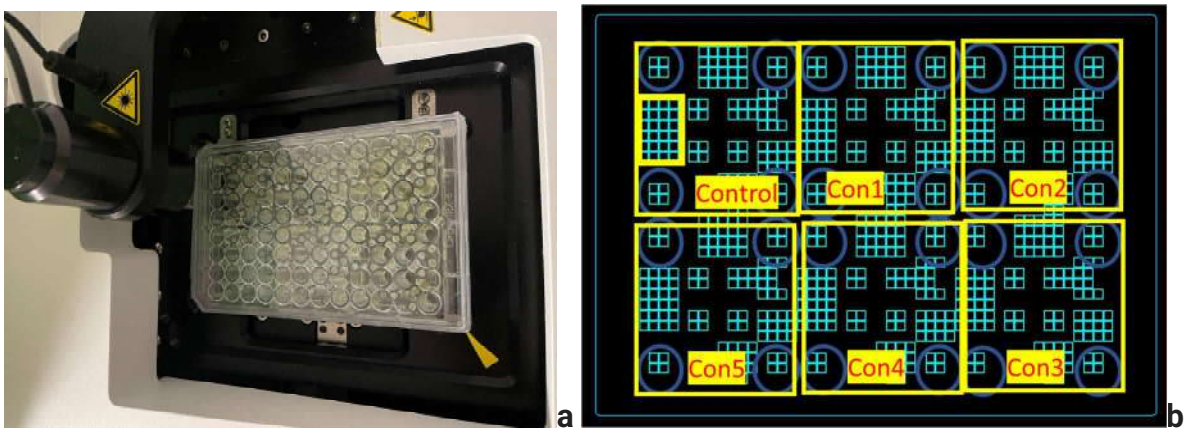

Fig. 3. (a) Organ growth monitoring using NyOne imaging Station. (b) Selected wells in the 1536 well plate for measuring senescence assay using plate reader

quantitative measurements. Organ cultures were also imaged using a microscope to qualitatively view the senescent cells.

Results

Neuronal cell senescence was examined by the detection of SA-β-gal activity to study AD drugs. The SA-ß-gal assay produces blue-green staining in the cytoplasm of positive cells which is quantified using absorbance at 406 nm. The cells were pretreated with a drug of interest and followed by 5uM Aβ treatment, and the tacrine drug inhibited the Aβ-induced cell senescence. We used a wide range of drug concentrations specifically for brain cells (SK-N-SH). By staining of SA-β-gal, we observed that Aβ oligomer (5 μM, 72 h) significantly increased the number of SA-β-gal-positive cells. This was attenuated by pretreatment with tacrine for 24 h in a dose-dependent manner (0.25 to 3.25 μM) as shown in Fig. 4. The absorbance of the senescence organs at 406 nm wavelength using the microplate reader showed increased senescence compared to the organs not treated with drugs. The increase of drug concentration decreased the senescence activity as

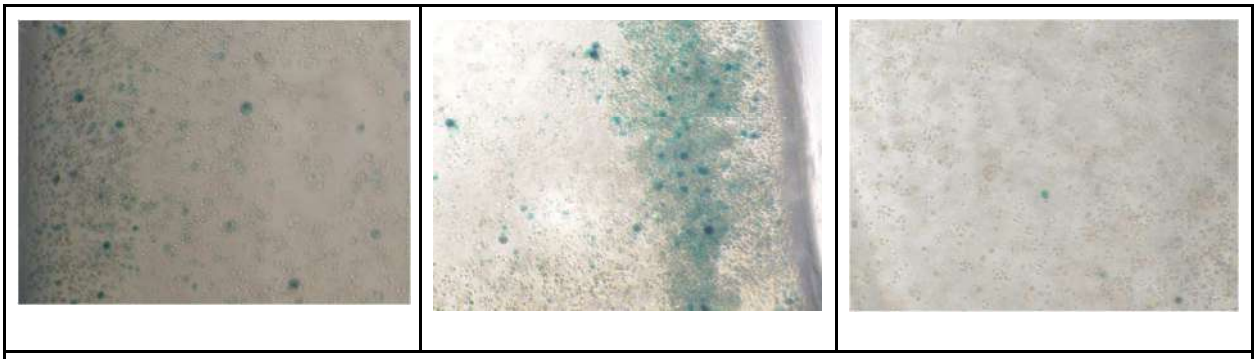

Fig. 4. SA-β-gal staining (a) on SK-N-SH cells. (b) on Aß-treated SK-N-SH cells. (c) on Aß-treated SK-N-SH cells and pretreated with 8uM Tacrine drug

shown in Fig 5. It is important to study the effect of multiple organs interacting with the brain organ to activate senescence. In this context, we have also carried out preliminary studies with multiple organs such as liver, kidney and gut organs using the senescence assay protocol as presented in Fig. 6. These organs are developed by HepG2 cells for liver, HEK 293 cells for kidney and T81 cells for gut. The multi-organ experiments will be systematically repeated, incorporating each organ one at a time.

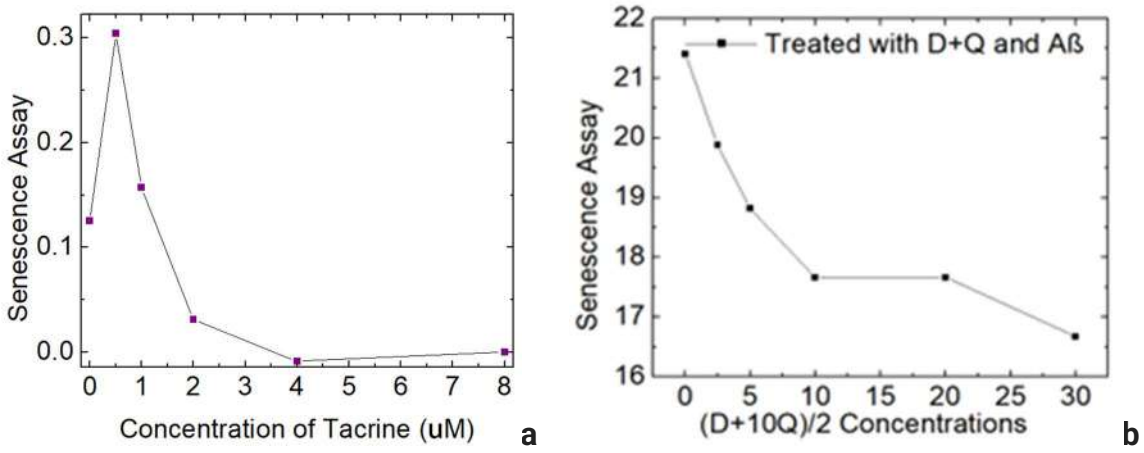

Fig. 5. Senescence Assay results with the drugs (a) Tacrin (b) D+Q

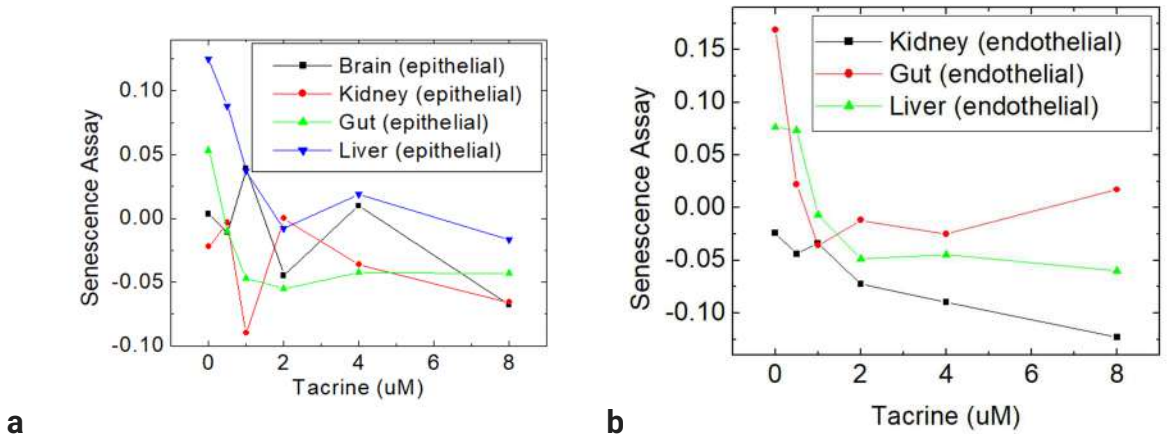

Fig. 6. Senescence Assay results for tacrine with multi-organ (a) epithelial cells (b) HuVEC cells adjacent to epithelial cells

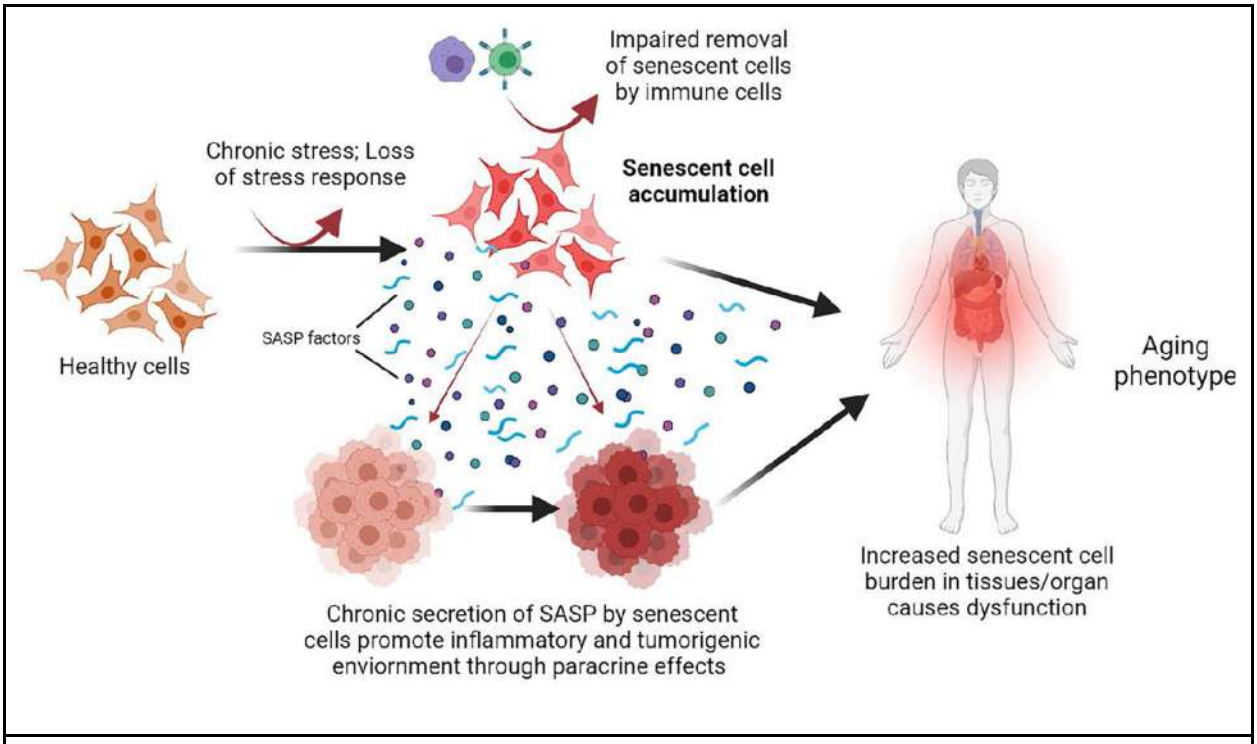

Fig. 7. Overview[6] of the development and accumulation of senescent cells during aging.

Conclusions

We observed that the number of SA-β-gal-positive cells within Aβ-treated neuronal cells-based organs were remarkably declined by the pre-treatment with AD drugs tacrine and Quercetin/ Dasatinib. Multi-organs are developed and studied the effect of senescence. Our ongoing research is exploring the impact of senescent cells, particularly their senescence-associated secretory phenotype (SASP), on neighboring healthy cells in multiple organs. This research aims to understand how SASP can influence nearby cells through paracrine mechanisms, potentially contributing to the aging phenotype[6,7] as presented in Fig. 7.

References

1. Li, Y., Lu, J., Hou, Y., Huang, S., & Pei, G. (2022). Alzheimer's Amyloid-β Accelerates Human Neuronal Cell Senescence Which Could Be Rescued by Sirtuin-1 and Aspirin. Frontiers in Cellular Neuroscience, 16, 906270. https://doi.org/10.3389/fncel.2022.906270

2. Li, Y., Lu, J., Cao, X., Zhao, H., Gao, L., Xia, P., & Pei, G. (2020). A Newly Synthesized Rhamnoside Derivative Alleviates Alzheimer's Amyloid- β -Induced Oxidative Stress, Mitochondrial Dysfunction, and Cell Senescence through Upregulating SIRT3. Oxidative Medicine and Cellular Longevity, 2020, 1–16. https://doi.org/10.1155/2020/7698560

3. Li, Y., Lu, J., Cao, X., Zhao, H., Gao, L., Xia, P., & Pei, G. (2020). A Newly Synthesized Rhamnoside Derivative Alleviates Alzheimer's Amyloid- β -Induced Oxidative Stress, Mitochondrial Dysfunction, and Cell Senescence through Upregulating SIRT3. Oxidative Medicine and Cellular Longevity, 2020, 1–16. https://doi.org/10.1155/2020/7698560

4. Wang, P., Wang, P., Luan, H., Wu, Y., & Chen, Y. (2023). Midazolam alleviates cellular senescence in SH-SY5Y neuronal cells in Alzheimer's disease. Brain and Behavior, 13(1). https://doi.org/10.1002/brb3.2822

5. H.Wong, G. Katara, C. Collins, J. A. J. Jose, J. Kohana, A. Collins, S. Samantha, J. Collins, Advancing Senescence-based In Vitro Drug Testing for AD: Targeting Aβ and p-Tau Models in a Gut-Liver-Brain Multiorgans, SFN Annual Meeting, Washington DC (2023).

6. R. Sharma, Emerging Interrelationship Between the Gut Microbiome and Cellular Senescence in the Context of Aging and Disease: Perspectives and Therapeutic Opportunities, Probiotics and Antimicrobial Proteins (2022) 14:648–663 https://doi.org/10.1007/s12602-021-09903-3

7. Tecalco-Cruz AC, Zepeda-Cervantes J, López-Canovas L, Ramírez-Jarquín JO, Pedraza-Chaverrí J, Briones-Herrera A. Cellular Senescence and ApoE4: Their Repercussions in Alzheimer's Disease. CNS Neurol Disord Drug Targets. 2021;20(9):778-785. https://doi.org/10.2174/1871527320666210628102721