

# STEM CELLS AS IN VITRO MODEL FOR TESTING NANODRUG DELIVERY PROPERTIES OF SELENIUM NANOPARTICLES

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# Selenium

- selenium and its compounds possess a strong antioxidant activity
- Se supplementation modulates the expression of selenoproteins and selenoenzymes
- decrease in incidence of certain types of cancer
- biological effects of selenium are highly concentration dependent



Figure 1. Selenium in foods  
(<https://www.drnicollemd.com/>)



Figure 2. selenium mineral  
(<https://optimisingnutrition.com/>)

# Nanoselenium

- enhanced biological activity and reduced toxicity
- development of SeNPs-based chemotherapeutics with the potential to cross the blood-brain-barrier (BBB)
- extensive toxicity testing prior to their application in medicine



Figure 3. Depiction of SeNPs synthesis

# Induced pluripotent stem cells (iPSC)

- powerful tool to test the toxicity and activity of novel chemotherapeutics
- can be differentiated to specific cell types and further be utilized as a drug screening platform

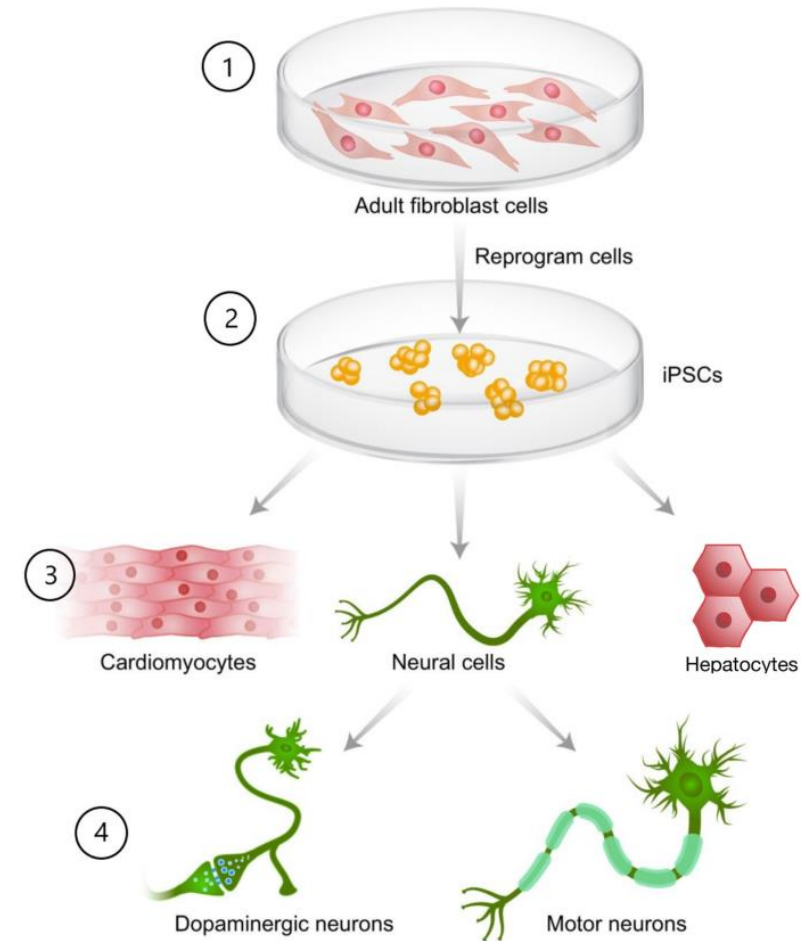


Figure 4. Reprogramming the iPSC (Scarfone et al. 2020)

# Aim

- culture the cells obtained from Parkinson's disease patient that were characterized by abnormal expression of alpha synuclein
- differentiate the cells to midbrain dopaminergic neurons
- evaluate the effects of 2 types of SeNPs – Polyvinylpyrrolidone (PVP) stabilized and Polysorbate (PS) stabilized, on viability and alpha synuclein expression

# Cell maintenance

- cells previously transduced with lentiviral vector carrying the LMX-1A transcription factor, which facilitates ventralization of dopaminergic neurons, under the control of the neural NESTIN enhancer
- cell culture dishes coated with matrigel and media conditioned by irradiated murine embryonic fibroblasts. The media was then collected, filtered through 0.22  $\mu\text{m}$  filter and supplemented with fibroblast growth factor (FGF)
- after reaching 80% confluence the cells were used to generate embryoid bodies

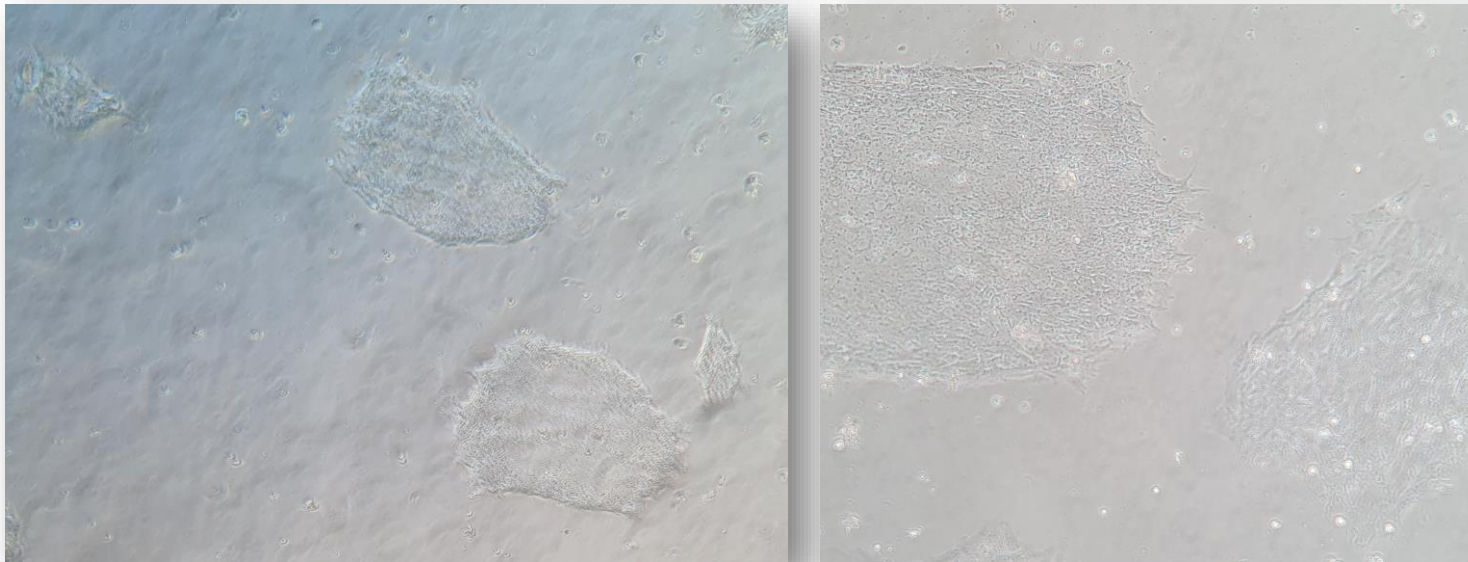


Figure 5. Cell line obtained from a patient with Parkinson's disease observed under the optical microscope

# Generation of embryoid bodies

- cells were detached from the surface of the dish using EDTA and collected in a tube.
- cell suspension was added to each well of a 96-well plate with conical bottom.
- plate was centrifuged for 10 min at 800 x g, and put in an incubator for 24h.
- EBs were then detached from the plate and put in a 6 cm “low attachment” dish.
- media was changed every day for the next 2 days

# Embryoid bodies

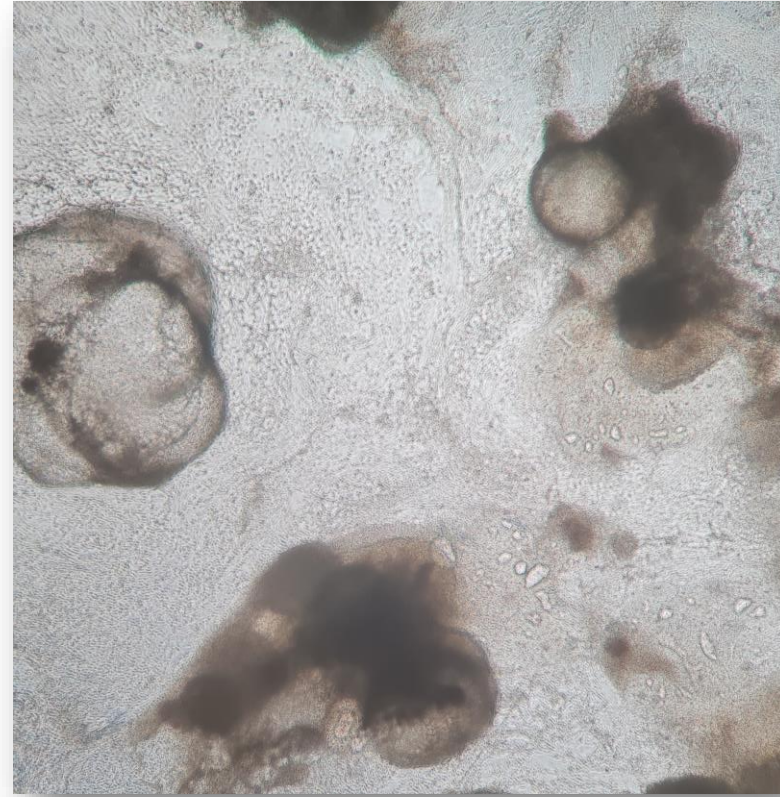
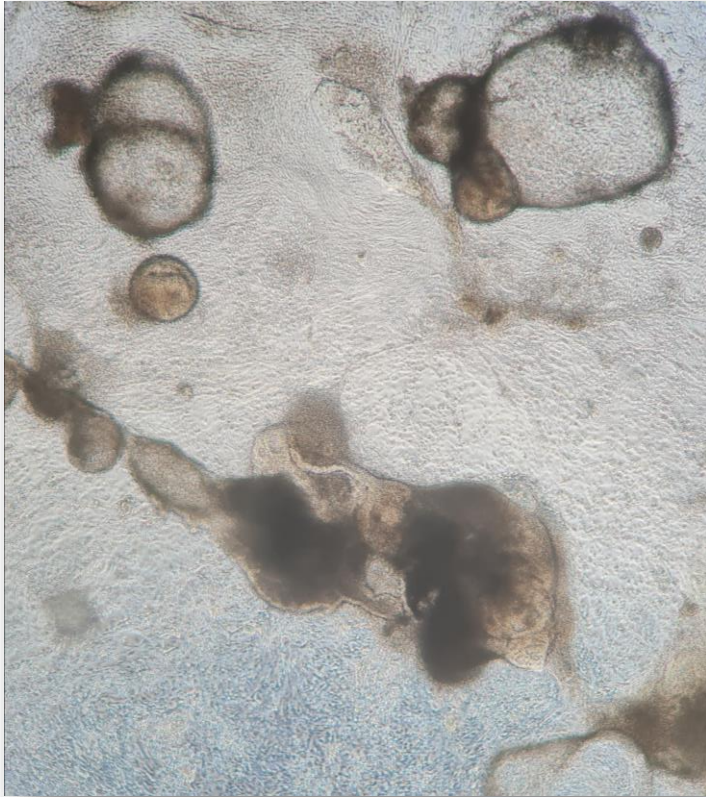


Figure 6. The EBs observed under the optical microscope

# Differentiation of dopaminergic neurons

- EBs were transferred to N2B27 media.
- media was changed every 2 days
- EBs were transferred on top of PA6 cell to allow further differentiation
- EBs were allowed to differentiate in co-culture with PA6 for another 4 weeks and the media was changed every 2 days
- dopaminergic neurons were successfully generated, as confirmed by staining the cells with antibodies specific for tyrosine hydroxylase (TH) and neuron-specific class III beta-tubulin (TUJ1)

# Depictions of AP6 cells and dopaminergic neurons

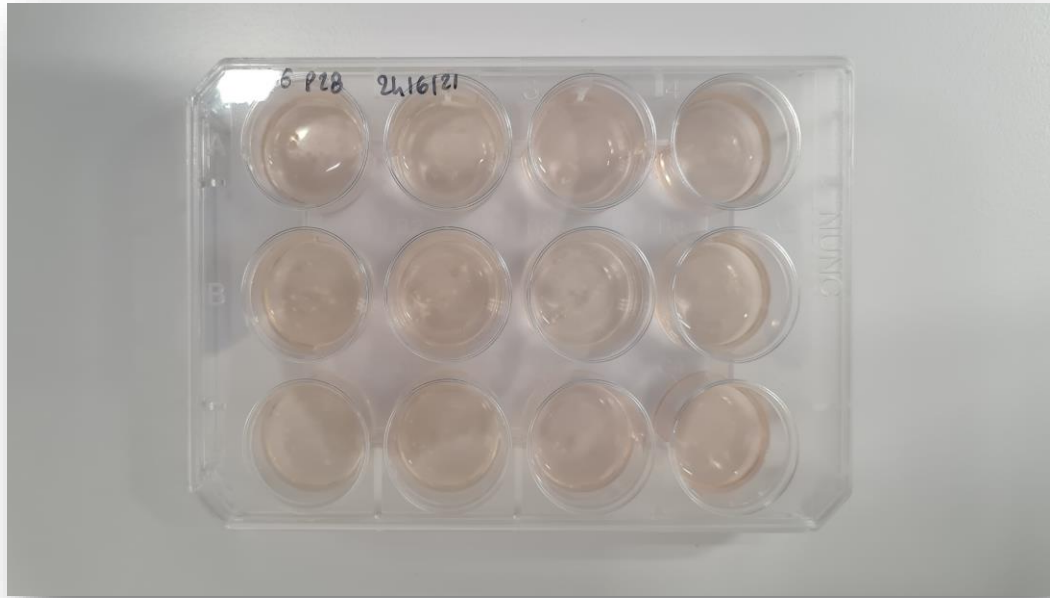


Figure 7. AP6 cells cultured in 12 well culture plate

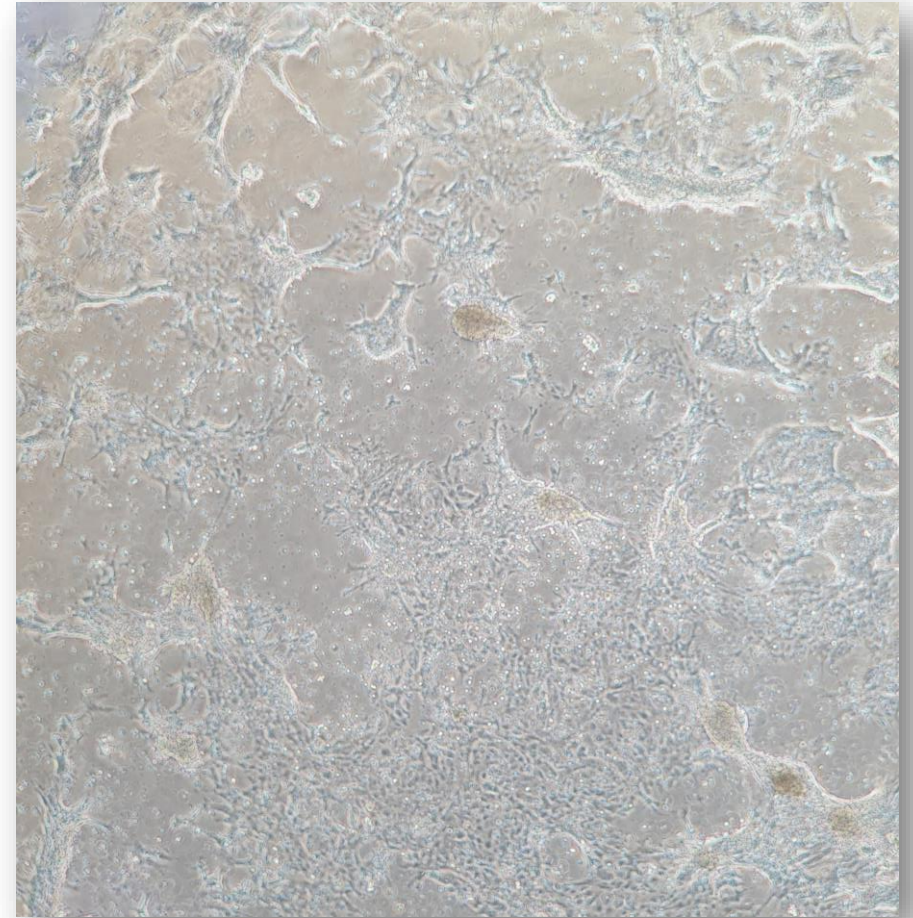


Figure 8. Differentiation of dopaminergic neurons

# Treatment with SeNPs

- To seed the cells for the experiment with selenium nanoparticles, the EBs were disaggregated using trypsin or acutase and seeded in 12-well plates.
- The other testing protocol did not include the disaggregation step.
- The neurons were treated with two types of SeNPs in concentrations of 0,1 ppm to 10 ppm, to evaluate its effect viability and Alpha-synuclein accumulation

# Conclusions

- iPSC-derived neuronal cells were sensitive to treatment with SeNPs
- The majority of cells were apoptotic
- The protocol for seeding resulted in a reduced viability of neuronal cells → modification of the protocol for cell seeding
- SeNPs had no effect on Alpha-Syn accumulation
- Carefull consideration if using SeNPs to target the CNS



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