2D and 3D modeling of Parkinson's disease: lysosomal and pH dysregulation in 3K-SNCA neuron-like cells

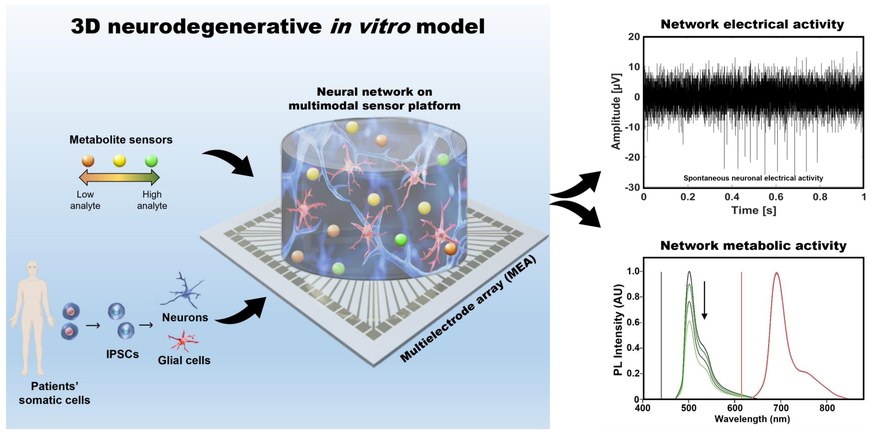
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RATIONALE

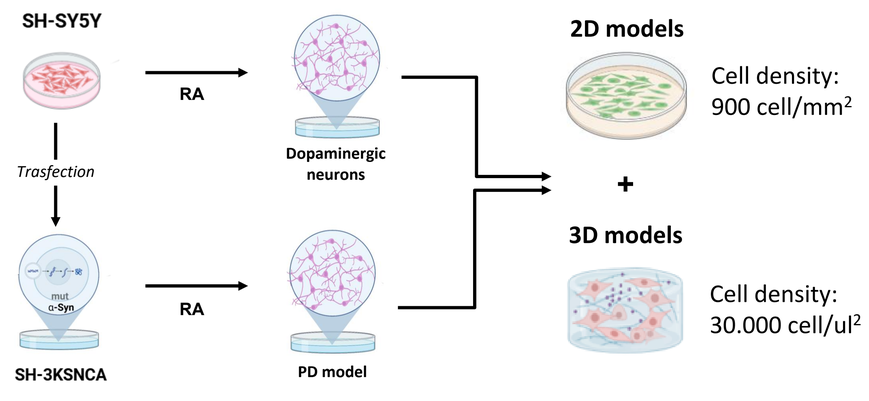
The endolysosomal system is crucial for neuronal function, mediating trafficking and signaling. Its dysfunction is central to diseases like Parkinson's (PD), where α-synuclein aggregates due to impaired lysosomal degradation at abnormal pH. These aggregates disrupt cellular trafficking and lysosomal clearance, leading to neuronal self-destruction.[1,2] Advanced sensors for measuring intralysosomal pH at the single-cell level provide critical insights into these dysfunctions.[3] Correlating pH changes with neuronal electrical activity can enhances our understanding of how lysosomal alterations impact neuronal signaling. Real-time monitoring of lysosomal pH helps assess degradative efficiency and detect early dysfunctions. Integrating these data through sensing platforms could identify early biomarkers and support targeted therapeutic strategies for neurodegenerative diseases.

AIM

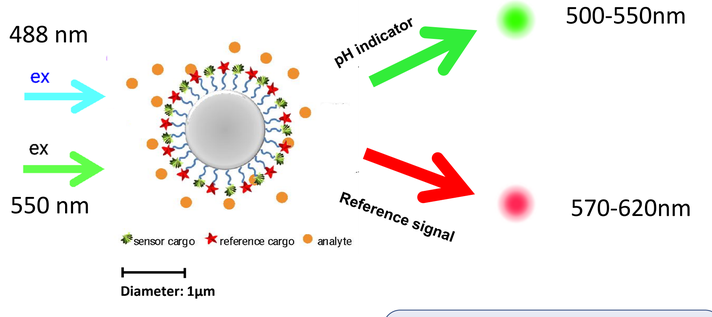


METHODS

Human neuroblastoma cell lines as *in vitro* model of Parkinson's Desease:



Ratiometric Optical pH-Sensor microparticles:



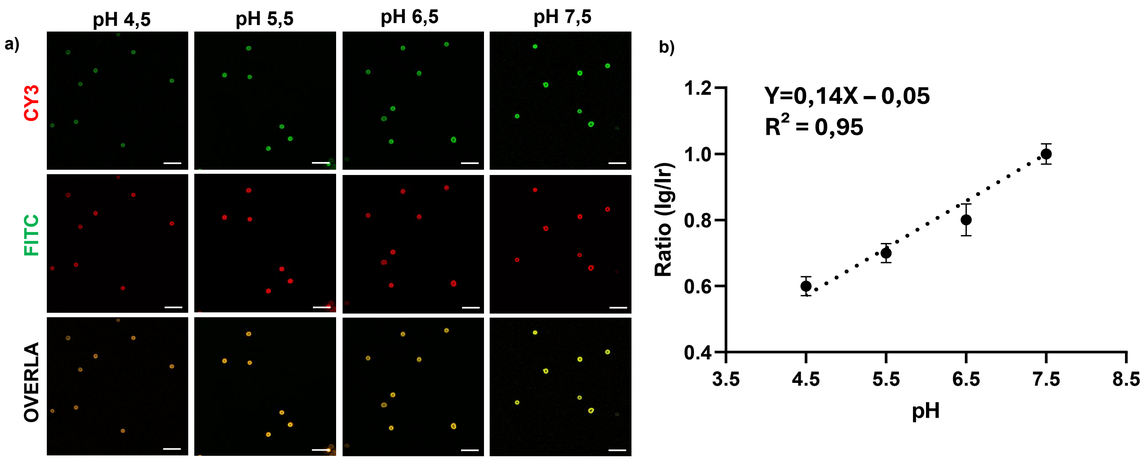
$$\text{RATIO} = \frac{\text{FITC}}{\text{Cy3}}$$

Confocal microscopy acquisitions

- Raw data collection using ImageJ software
- ROIs selection and cropping
- Post processing using Origin software
- Generation of FITC/Cy3 Graphs over time
- pH derivation from calibration curve

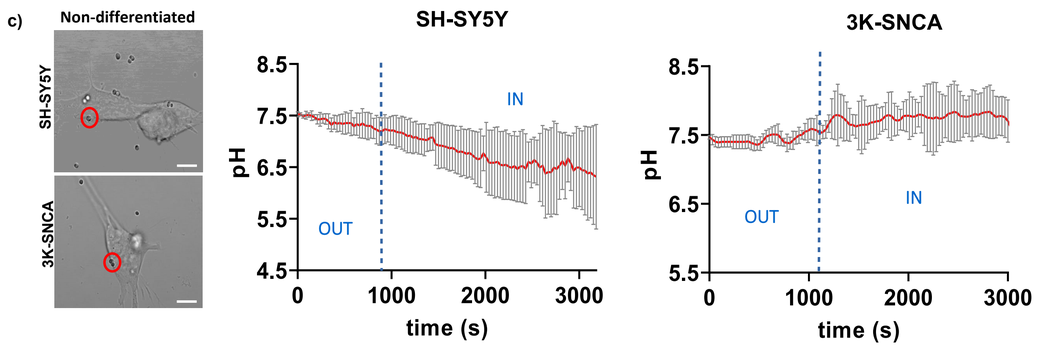
RESULTS

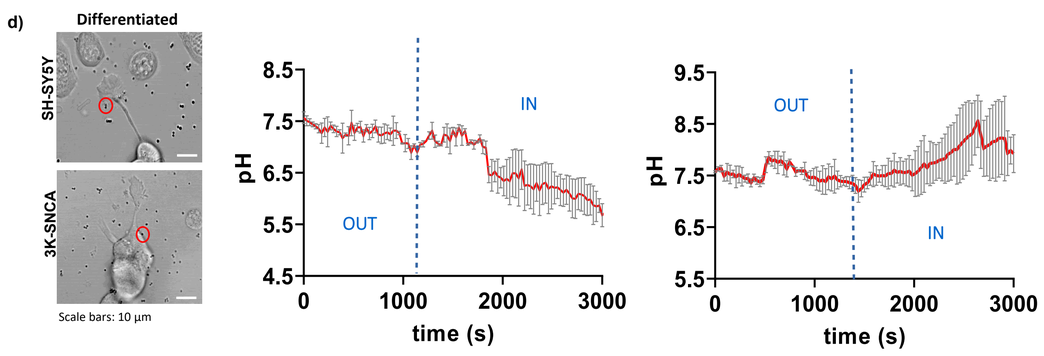
pH tracking in 2D non-differentiated and differentiated cell cultures



Scale bars: 5 μm

Calibration of pH sensors. **(a)** CLSM micrographs showing the pH-dependence of senors fluorescence in pH-adjusted cell medium (4.5, 5.5, 6.5, and 7.5). **(b)** Ratiometric calibration curve of sensors on fluorescence intensity ratio of green and red channels (lg/lr) derived from fluorescence micrographs.

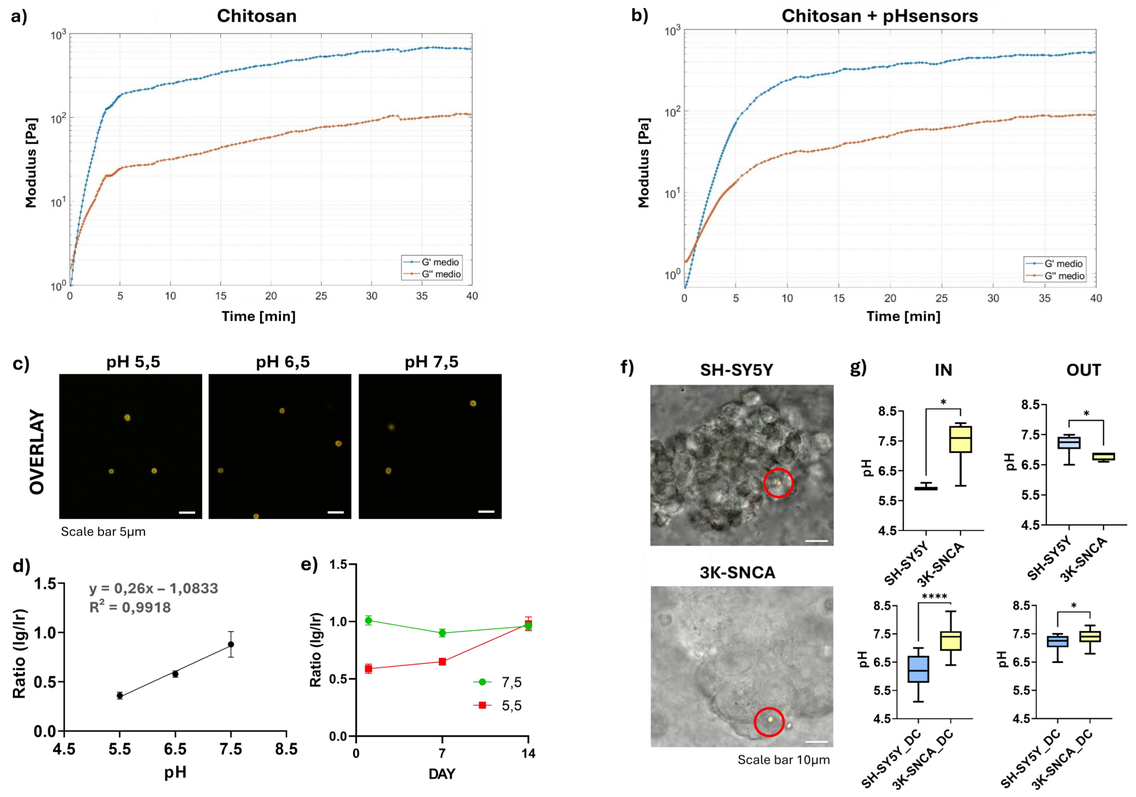




Scale bars: 10 μm

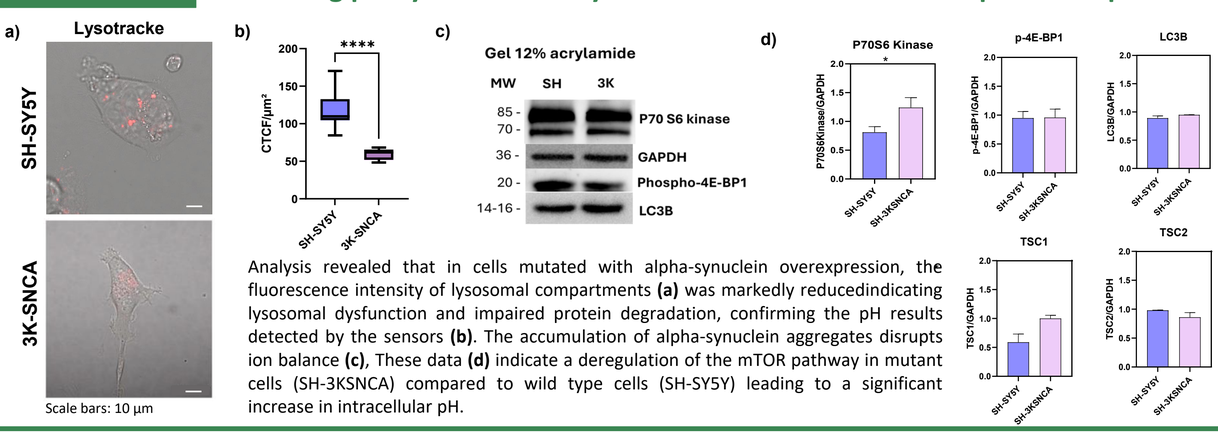
In SH-SY5Y cells, sensor internalization is accompanied by a typical decrease in pH, while in 3K-SNCA cells, the intracellular pH increases, shifting towards more basic values. This contrasting trend is consistent in both non-differentiated **(c)** and differentiated **(d)** cell models, indicating that α-synuclein overexpression disrupts normal pH regulation.

Characterization and pH tracking 3D *in vitro* model



(a) Time-dependent trends of the storage modulus (G') and the loss modulus (G") of Chitosan and **(b)** chitosan +pH microsensors **(c)** CLSM images showing pH-dependent sensor fluorescence in Chitosan (pH 5.5–7.5). **(d)** 3D Ratiometric calibration curve of sensors (lg/lr) from fluorescence micrographs. **(e)** stability plot of ratio values assessed at DIV1, DIV7, DIV14; **(f)** 3D model of SH-SY5Y and 3K-SNCA. **(g)** pH intracellular values both in SH-SY5Y and 3K-SNCA non-differentiated and differentiated 3D cultures.

Correlating pH dynamics with lysosomal characterization and protein expression



Scale bars: 10 μm

Analysis revealed that in cells mutated with alpha-synuclein overexpression, the fluorescence intensity of lysosomal compartments **(a)** was markedly reducedindicating lysosomal dysfunction and impaired protein degradation, confirming the pH results detected by the sensors **(b)**. The accumulation of alpha-synuclein aggregates disrupts ion balance **(c)**, These data **(d)** indicate a deregulation of the mTOR pathway in mutant cells (SH-3KSNCA) compared to wild type cells (SH-SY5Y) leading to a significant increase in intracellular pH.

CONCLUSIONS

Data suggest a potential link between dysfunction in maintaining intracellular pH of SH-3K-SNCA cell line, likely due to the accumulation of α-synuclein, a pathological protein characteristic of PD.

REFERENCES

[1] M. E. Gegg and A. H. Schapira, 'Lysosomal-endolysosomal dysfunction and neurodegenerative diseases', Neurobiology of Disease, 2022.
[2] A. D. Klein and J. R. Mazzulli, 'Is Parkinson's disease a lysosomal disorder?', 2018.
[3] Chandra A., Prasad S., Alemanno F., De Luca M., Rizzo R., Romano R., Gigli G., Bucci C., Barra A., del Mercato L.L. Fully Automated Computational Approach for Precisely Measuring Organelle Acidification with Optical pH Sensors (2022), ACS Applied Materials & Interfaces, 2022, 14, 16, 18133–18149