Investigating the Phenotypical Effects of An Adaptable Multi-Modality pH-Sensitive Nanoprobe for Real-Time Monitoring of Engineered Cells and Tissues.

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RESEARCH MOTIVATION

The use of stem cells and tissues is inevitable in regenerative application, disease modelling and drug screening. According to BioFAb USA's and NCMC's roadmap, a versatile tool for monitoring cells and tissues in the biomanufacturing industry is lacking.

Through this work, we investigated the functionality and phenotypical effect of a ratiometric pH probe previously developed by a team from ASU to measure cell viability in real-time. We studied the probe in different industrially applicable culture systems

METHODOLOGY

- Cells were cultured following protocols for the different systems: 2D, microcarriers (MC) and matrigel
- Cells were labeled with pH probe on day 1 post culture for NPCs and day 7 for neurons on 2D and matrigel. Neurons on MCs were differentiated from NPCs for 30 days and labeled on day 30.
- Labeling efficiency was performed with flow cytometry and microscopy
- Cells were used for characterization assays including immunofluorescence, qPCR, Calcium imaging and proliferation.

RESULTS

Fig 1

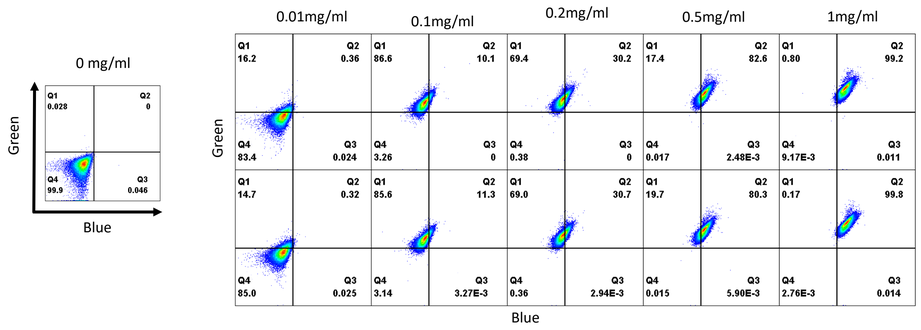
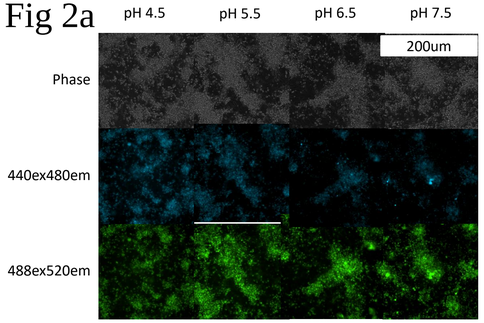
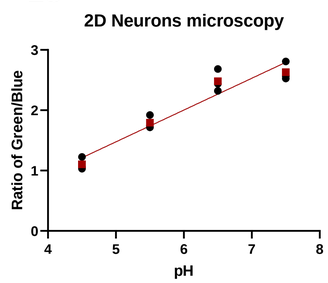


Fig 2a



2b



2c

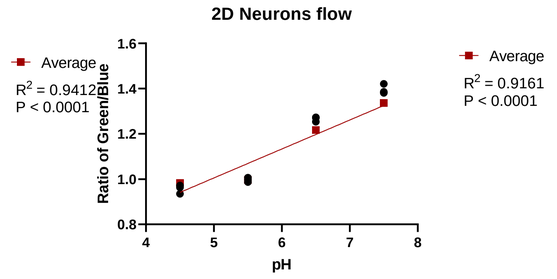


Fig 3

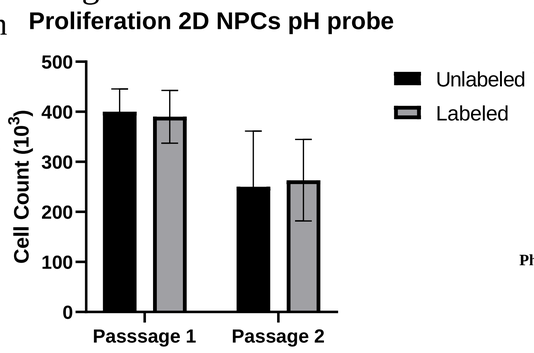
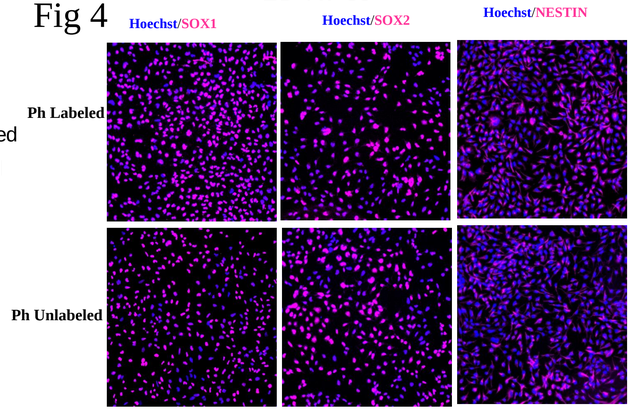


Fig 4



CONCLUSION

Efficiency of the probes in labeling cells were validated in 2D, microcarrier and matrigel culture systems using both NPCs and neurons.
Additionally, cells were characterized post labeling and comparing with unlabeled cells demonstrated that labeling do not affect cell phenotype or proliferation.
Through this experimental work, the pH sensitive probe demonstrated reproducible pH dependency while maintaining its functionality without any obvious toxicity across different culture systems.

REFERENCES

Scan for reference

FIGURES DESCRIPTION

Fig 1. Flow cytometry showing 2D NPC cells labeled with different probe concentration with percentage of cells fluorescing green, blue, both or none. **Fig 2a;** Fluorescence images showing pH dependency with decreasing blue fluorescence and increasing green fluorescence with pH. **Fig 2b, 2c;** pH dependency plot using microscopy and flow respectively for 2D neurons. **Fig 3;** Proliferation of NPCs post labeling with probe. **Fig 4;** Immunofluorescence data showing NPC specific markers Sox1, Sox2 and Nestin for both labeled and unlabeled cells with Hoechst DNA stain

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