



Topical Application of Plasmid-based Rinse for Oral Cancer Screening

John C Ahn BS¹, Yolanda E Hartman BS², Ben Kasten PhD³, Angela D Haskins MD², Joshua J. Kain MD⁴, Luke N

Bailey, MD², Jason M Warram PhD²

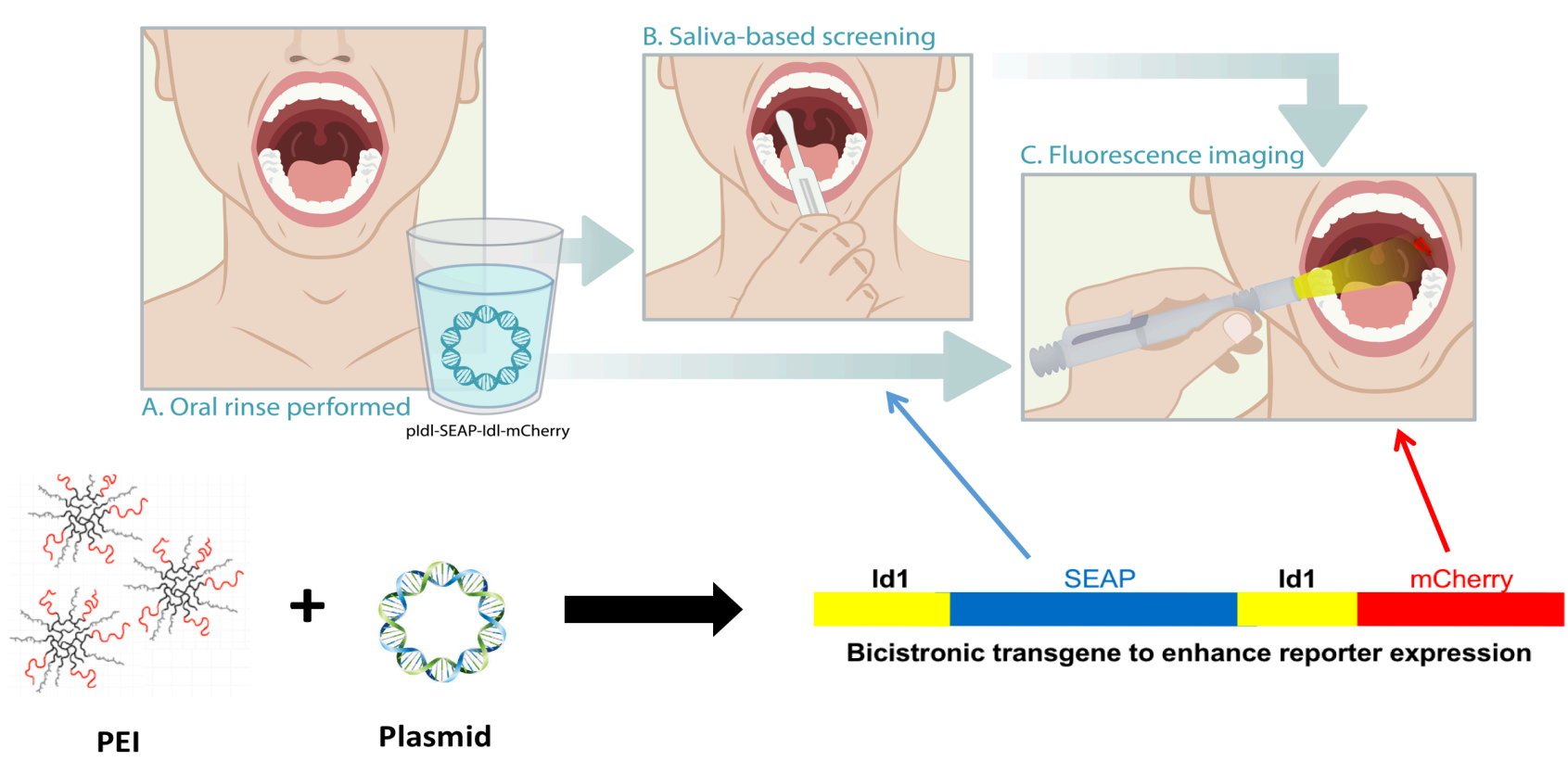
¹School of Medicine, ²Department of Otolaryngology, ³Department of Neurosurgery, University of Alabama at Birmingham, ⁴Department of Otolaryngology- Head & Neck Surgery, University of California-Davis

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INTRODUCTION

- Current strategies for oral cancer screening rely on physical examination alone using visual inspection and manual palpation, which is non-specific and makes accurate diagnosis of possible malignancies very difficult.
- Further assessment methods are costly, invasive, and involve many adverse complications.
- Timely detection and accurate diagnosis of oral cancer has remained a formidable challenge.
- A highly sensitive, cost-effective, and non-invasive method for oral cancer screening is needed so that necessary treatment may be initiated more quickly.
- We hypothesize that a topically applied, cancer-specific plasmid with a combined saliva-based and imaging screening system can be used to rapidly and noninvasively screen for oral cancer.



Inhibition of Differentiation 1 (Id1)

- Ectopic over-expression of Id1 has been found in over 20 cancers, including oral squamous cell carcinoma (OSCC)
- Normal adult tissue does not express the Id1 protein
- Level of Id1 expression is proportional to the aggressive phenotype of cancer

Secreted embryonic alkaline phosphatase (SEAP)

- Phosphatase inhibitor resistant and heat tolerant
- Simple blood based assay detects picogram levels of SEAP
- Enzyme of human origin = no immunogenicity

- Linear polyethylenimine (PEI)**
- A superior transfection agent that provides robust transfection efficiency
 - PEI compacts DNA into positively charged complexes
 - Provides protection against degradation of the DNA complexes by acting as a "proton sponge" and buffering the pH within the endosome
- mCherry**
- Fluorescent protein that is visible to the human eye
 - Allows for simple localization of cancers using a penlight

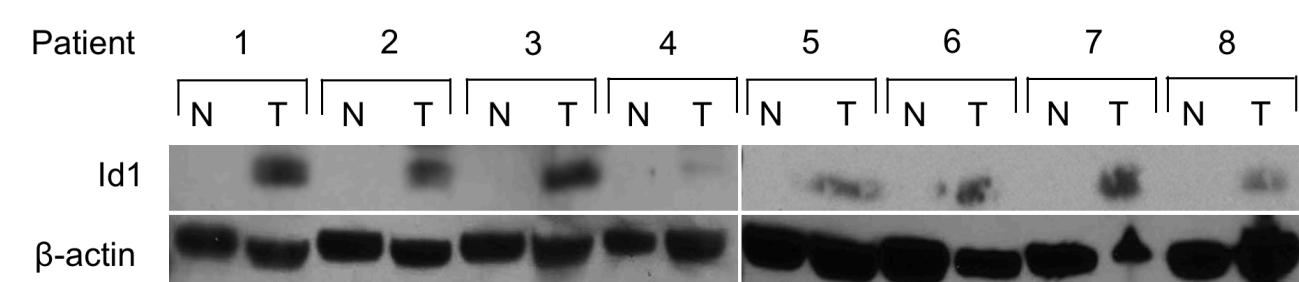


Figure 2. Id1 expression was consistently expressed in patient tumor tissue while not expressed in patient-matched normal tissue. Tissue lysates of tumor and normal tissues obtained from eight patients demonstrated cancer-specific expression of Id1.

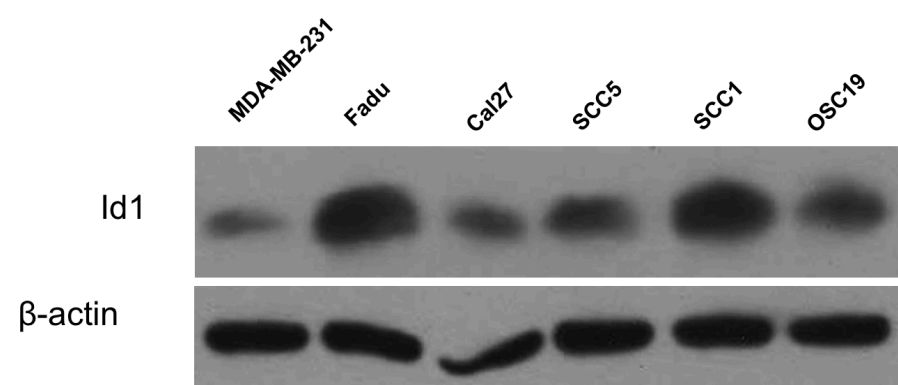


Figure 3. Robust Id1 expression is shown in the head and neck cancer cell panel. Lysates from five head and neck cancer cell lines (Fadu, Cal27, SCC5, SCC1, OSC19) were probed for relative Id1 expression using conventional western blot analysis.

OBJECTIVES

- Determine the optimal PEI:plasmid ratio to achieve the highest disease-specific SEAP and mCherry expression.
- Characterize the Id1 expression in the head and neck cancer cell panel.
- Correlate Id1 expression with SEAP and mCherry expression.

METHODS

For the experiments, 5×10^4 - 1×10^5 cells were plated in triplicate in 24-well plates 24hrs prior to addition of PEI. Following manufacturer protocol, PEI/plasmid ratios of 2, 3, 4, 5, and 10 were added to respective wells and incubated for 24 and 48 hrs. A positive control with no addition of PEI was included in the 24-well plate. In vitro SEAP assays were performed to measure enzyme secretion and the plates were imaged for mCherry expression.

RESULTS

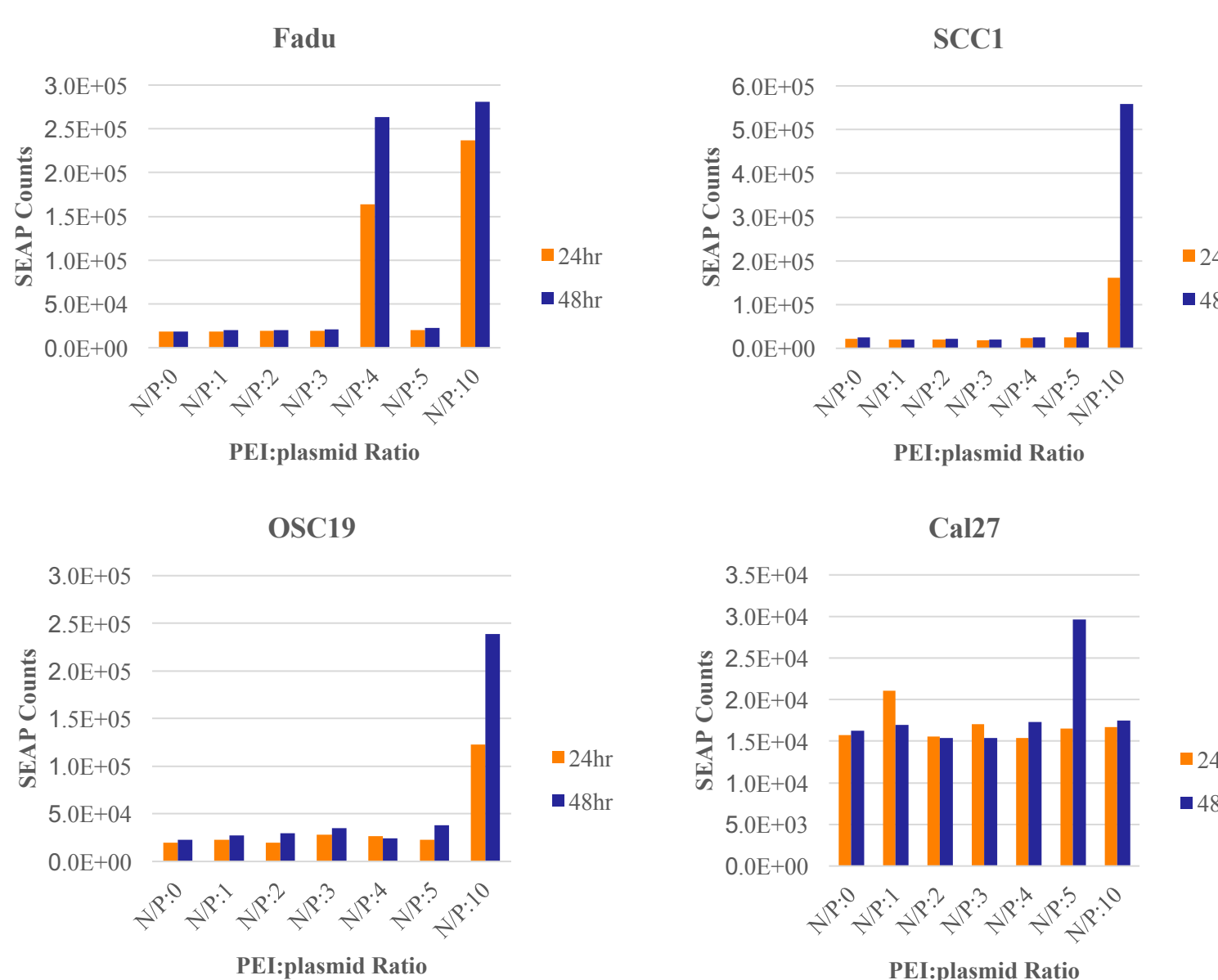


Figure 4. pId1-SEAP expression in cancer cell lines with varying degrees of Id1 expression. At 24 and 48hrs post-administration of transfection agent, SEAP expression was measured through supernatant collection and compared between cell lines.

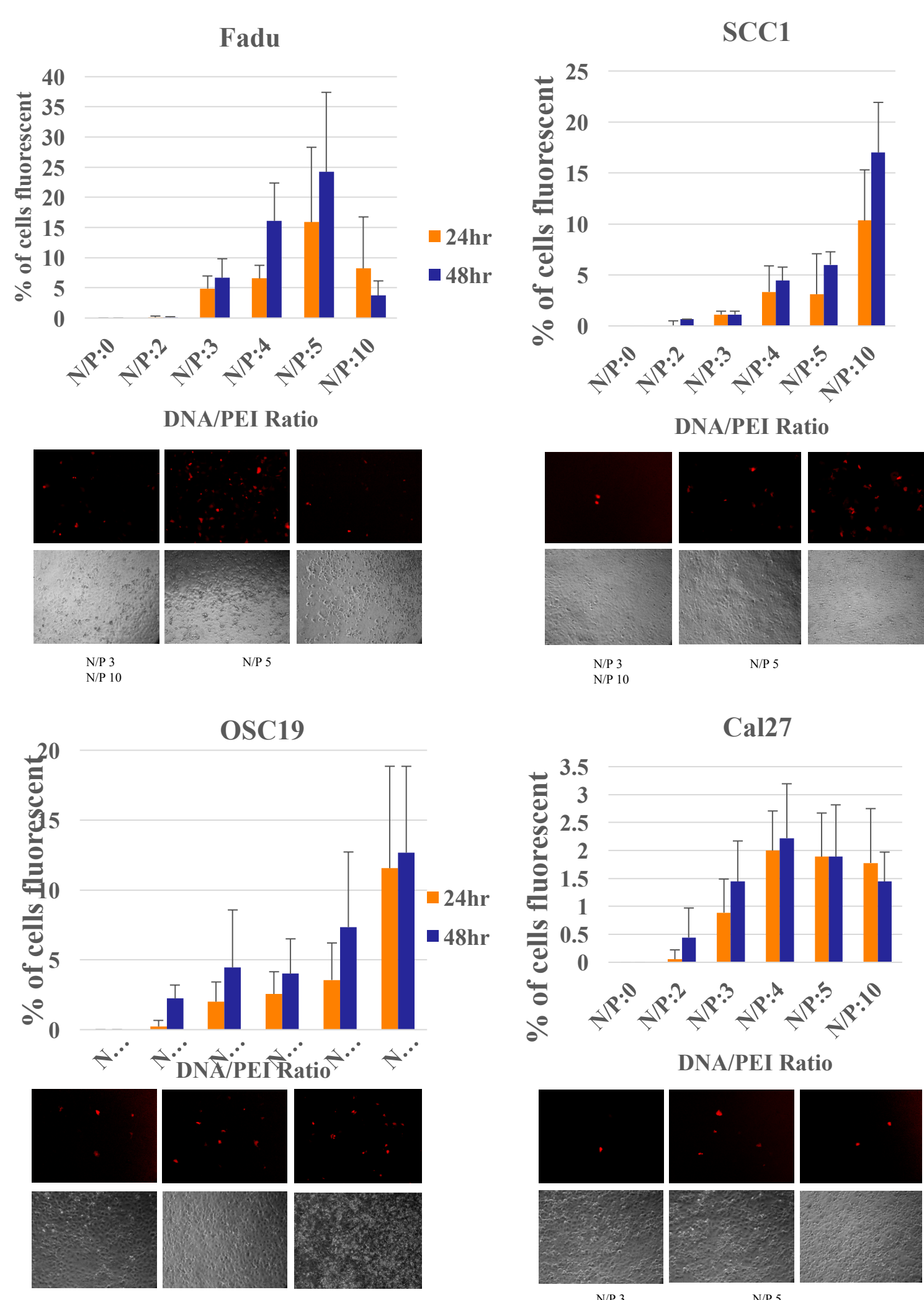


Figure 5. pId1-mCherry expression and fluorescence imaging of cancer cell panel. Using the Nuance multi-spectral camera, fluorescence imaging and respective brightfield imaging of the 24-well plate was performed in triplicate at 24 and 48hrs post-transfection. Images were analyzed and blindly graded for fluorescence quantity, intensity, and cytotoxicity.

RESULTS

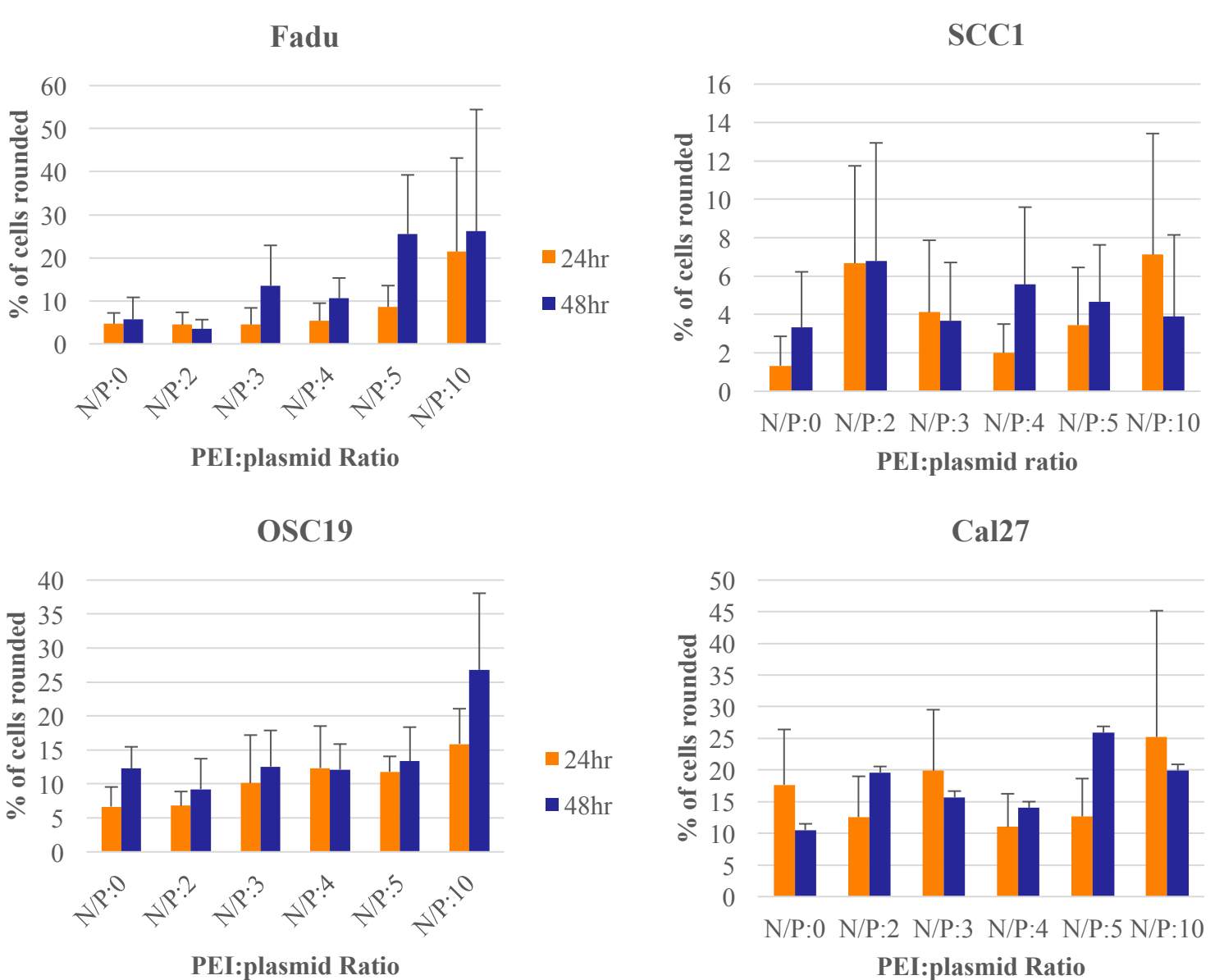


Figure 6. Measurement of PEI dose-dependent cytotoxicity. Brightfield images were analyzed and blindly graded for cytotoxicity. Increasing the N/P ratio appeared to have cause increased cell death in Fadu and OSC19, while SCC1 and Cal27 did not demonstrate dose-dependent cytotoxicity.

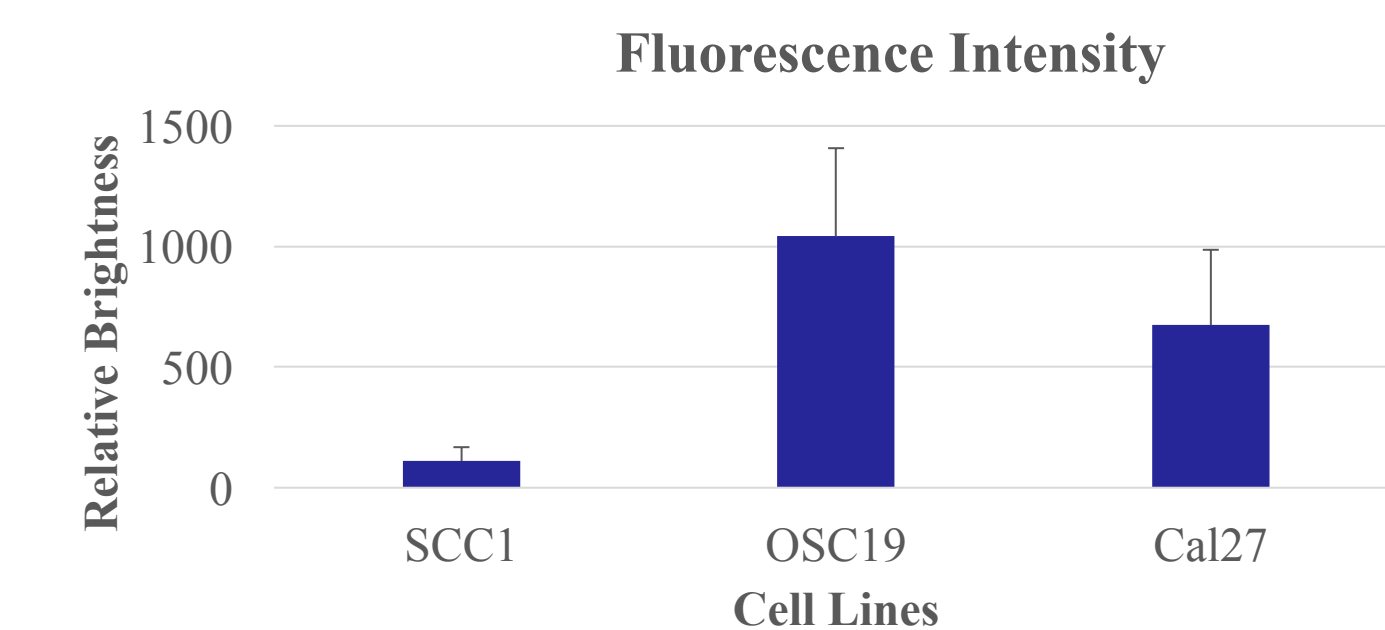


Figure 7. Autoexposure of fluorescence images. Using the Nuance multi-spectral camera, each image was autoexposed based on fluorescence intensity of mCherry expression. The relative brightness intensity in SCC1, OSC19 and Cal27 cells treated with N/P:5 at 48hrs was compared. Autoexposure of Fadu cells was not performed because this component of imaging was not identified during the first part of the project.

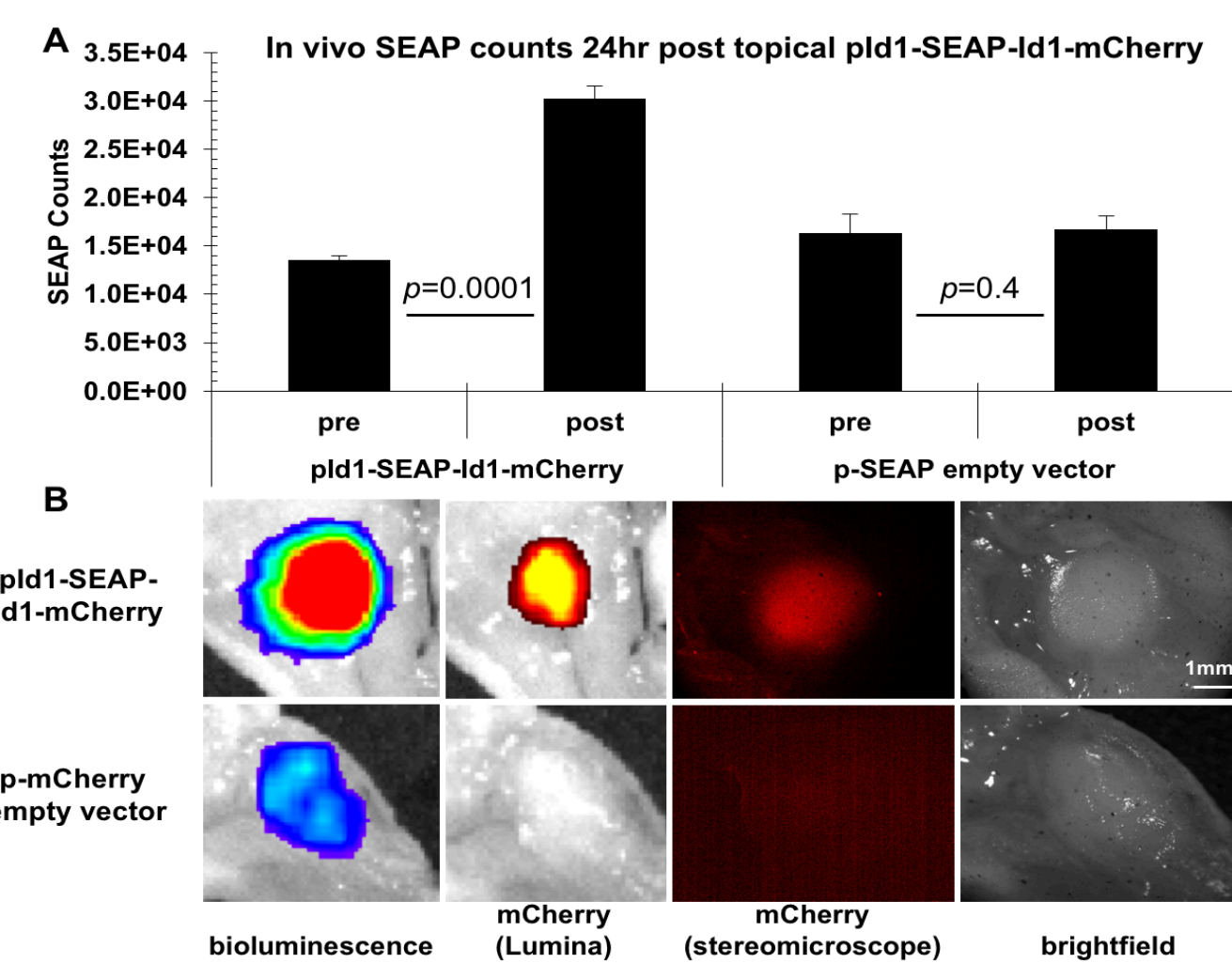


Figure 8. Topical application of the diagnostic plasmid in an in vivo oral flap model demonstrates feasibility of the approach for enzyme and imaging-based detection.

CONCLUSION

- Higher ratio of PEI leads to higher transfection efficiency and greater reporter expression.
- SEAP expression correlates with Id1 expression.
- N/P = 10 produced greatest levels of SEAP expression in Fadu, SCC1, and OSC19, while levels varied in Cal27.
- Optimal N/P ratio for mCherry expression varied within the cancer cell line panel.
- Higher N/P ratio appears to have higher cytotoxicity.