Gap junctions: the holy grail in stem cell-based suicide gene therapy for head and neck cancer

Jolien Van Den Bosch[1], Karen Libberecht[1,2], Tim Vangansewinkel[1,2], Ivo Lambrichts[1], Esther Wolfs[1]

[1]Faculty of Medicine and Life Sciences, BIOMED, Hasselt University, Diepenbeek, Belgium.
[2]Laboratory of Neurobiology, VIB, Center for Brain and Disease Research, Leuven, Belgium.

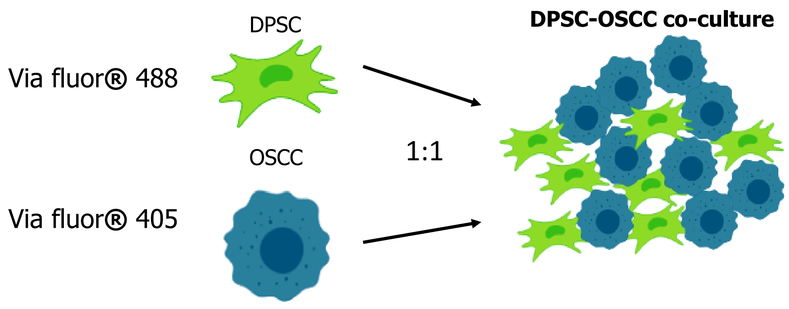
esther.wolfs@uhasselt.be, jolien.vandenbosch@uhasselt.be

Objectives

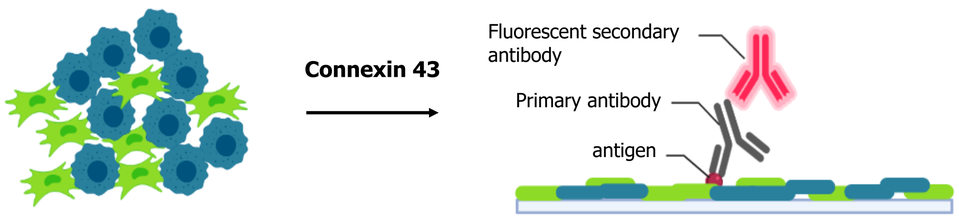
Oral squamous cell carcinoma (OSCC) describes a varied group of malignancies in the oropharynx and the oral cavity. These tumors are often associated with the use of alcohol and tobacco as well as with HPV infection. Because current treatments including radiotherapy, chemotherapy and surgery cause severe discomfort and significant side effects, the development of an alternative and targeted therapy is highly required. This project aims to validate the role of the **gap junctional intercellular communication (GJIC)** between human dental pulp stem cells (DPSC) and OSCC. To confirm this, we visualized Cx43, a gap junctional protein. Furthermore, gap junctional functionality was assessed using micro-injections of the non-permeable dye lucifer yellow.

Methods

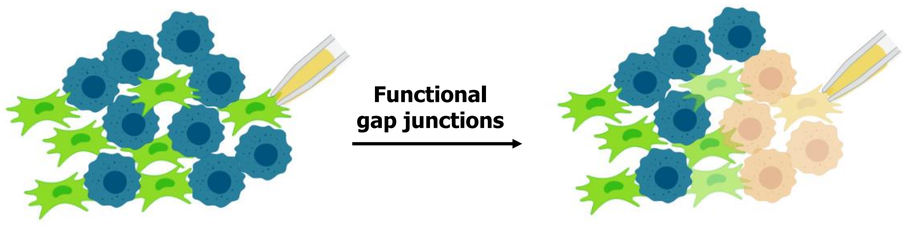
① Staining of DPSC and OSCC in co-culture with Via fluor® dyes



② Immunostaining of Connexin 43 in co-culture



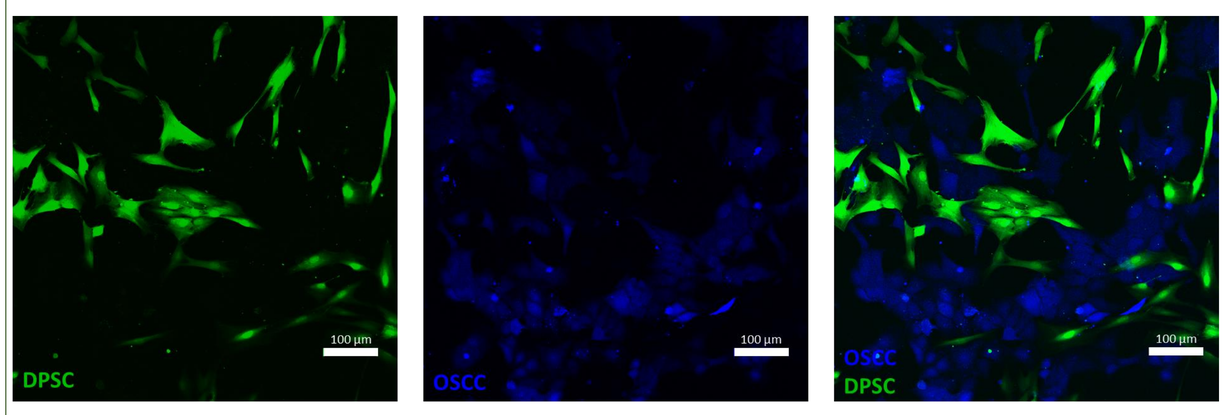
③ Lucifer yellow dye transfer assay



Results

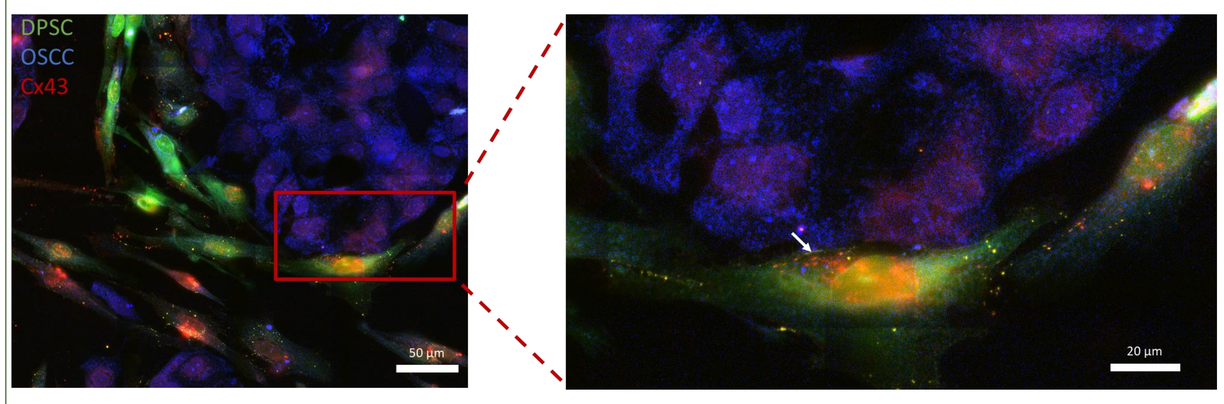
Discrimination between dental pulp stem cells (DPSC) and oral squamous cell carcinoma cells (OSCC) in co-culture.

The visualization of the two different cell types *in vitro* is conducted with the dyes ViaFluor® 405 (DPSC) and ViaFluor® 488 (OSCC).



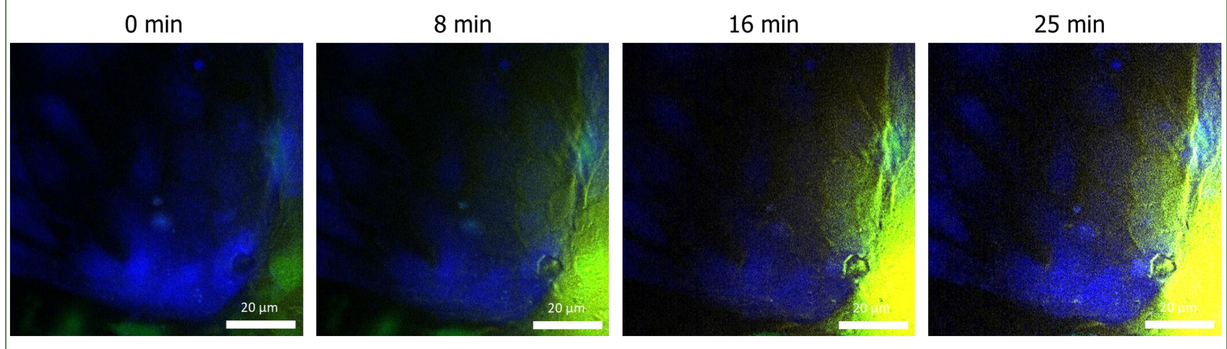
Connexin 43 (Cx43) presence in DPSC and OSCC co-cultures.

Cx43 is one of the most prominent connexins in human gap junctions. For GJIC, gap junction formation between stem cells and tumor cells is essential. However, the functionality of these gap junctions still needs to be researched.



Functionally active gap junctions are observed between DPSC and OSCC.

A single DPSC (green) was micro-injected with Lucifer Yellow (428/536 nm) via micro-injection. Representative images demonstrate transfer of the dye to adjacent OSCC (blue) cells in co-cultures over time.



Discussion

Our results suggest gap junction formation between dental pulp stem cells and oral squamous cell carcinoma cells. Moreover, single cell micro-injection with lucifer yellow dye shows the presence of functionally active gap junctions.
These results provide a good indication for the use of dental pulp stem cells as vehicles for an alternative and targeted therapy.

UHASSELT
KNOWLEDGE IN ACTION