GINGIVAL FIBROBLAST DEDIFFERENTIATION: AN OPTION IN REGENERATIVE DENTISTRY?

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Mafalda Laranjo, PhD | Anabela Paula, DMD, MSc | Ana Cristina Gonçalves, PhD
Ana Bela Sarmento-Ribeiro, MD, PhD | João Casalta-Lopes, MD | Ana Margarida Abrantes, PhD
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State of the art

Tooth decay
Trauma
Periodontal disease

↓

Partially or complete tooth loss

- × Functional problems
- × Psychological problems
- × Economical cost for patients and nations

↓

Artificial substitutes:
- Dental composites
- Dental implants
- ...

→ **Problem:**

Regenerative Dentistry

Dentin
Pulp complex
Bone

State of the art

Regenerative Dentistry

Bioengineering

- Most explored option
- Matrices with proper characteristics + selection and differentiation of dental stem cells into the desired target tissues

Problems:

- × To obtain dental stem cells (extraction of the tooth)
- × Number of cells
- × Specific cultivation process allowing their identification and separation is required
- × Some sub-types exist only in developing teeth

Reproduction of dental embryogenesis

State of the art

Regenerative Dentistry

Bioengineering

Reproduction of dental embryogenesis

- Some researchers develop pulp regeneration techniques based on more accessible stem cells, such as, bone marrow or adipose tissue stem cells

Problems:

- × Harvesting (surgical procedure)
- × Number of cells
- × Ethical issues
- × Rejection problems (allografts)

State of the art

Regenerative Dentistry

Bioengineering

Problems:
- × To obtain dental stem cells (extraction of the tooth)
- × Number of cells
- × Specific cultivation process allowing their identification and separation is required
- × Some sub-types exist only in developing teeth

Reproduction of dental embryogenesis

GOAL:

TO OBTAIN STEM CELLS FROM METHODOLOGIES THAT AVOID ALL THESE CONSTRAINS

Problems:
- × Harvesting (surgical procedure)
- × Number of cells
- × Ethical issues
- × Rejection problems (allografts)

Aim

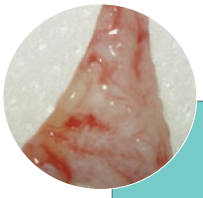
To obtain odontoblast-like cells from dedifferentiated gingival fibroblasts

Why odontoblasts?
- Post-mitotic cells
- Produce dentin (the larger dental tissue)
- Fundamental in tooth regeneration

Why gingival fibroblasts?
- Easy harvesting at dental office (simple surgical procedure)
- Rapid tissue regeneration
- No ethic or compatibility problems
- Similar micro-environment (oral cavity)

Why dedifferentiation?
- Technique potential
- Methodology applied to other tissues

Materials and Methods



Gingival Fibroblast Cultures

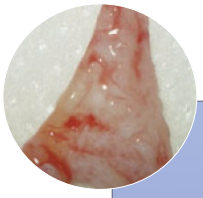
- Explant methodology
- Balb/C, male, 8 weeks
- Gingival fragments
- Mechanical dissociation (1mm^3 fragments)
- Enzymatic dissociation (type I collagenase and trypsin)
- 80% confluence

Dedifferentiation

- 100.000 cel/ml
- 1) Negative control, untreated cells
- 2) Vehicle control, 5µL DMSO
- 3) 5µM agent X without medium change
- 4) 5µM agent X applied daily without medium change
- 5) 5µM agent X applied daily with medium renewal

CIMAGO

Materials and Methods



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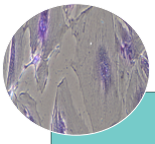
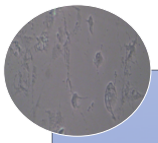


Cell morphology

- Live cells confocal microscopy
- Crystal violet stained cells
- May-Grünwald-Giemsa staining

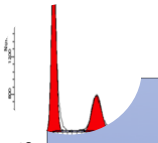


Cell proliferation

- Viable cells counted in Haemocytometer

Clonogenic efficiency

- 500, 2500 and 5000 cel/well
- 12 days
- Colonies with more than 50 cells



Cell cycle analysis

- Flow cytometry with propidium iodide labelling

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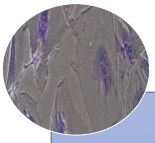

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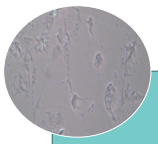

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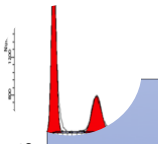

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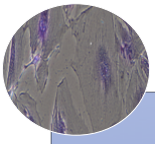

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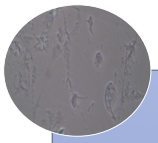

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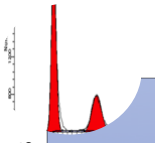

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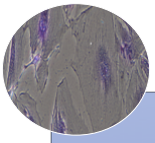

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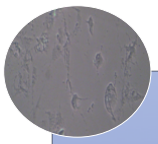

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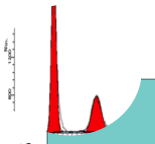

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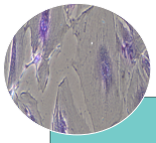
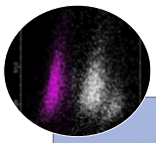


DNA content

- Crystal violet assay
- CV bind DNA
- Produced colour is proportional to cell number
- Absorbance quantified at 590nm

Metabolic activity

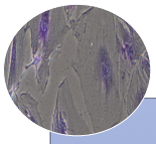
- MTT assay
- Evaluation of metabolic viable cells
- Absorbance quantified at 570nm



Cell death

- Flow cytometry
- Annexin V and propidium iodide labelling
- Live, apoptotic, late apoptotic and necrotic cells

Materials and Methods



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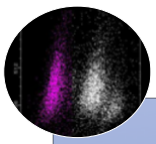

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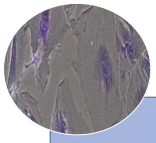

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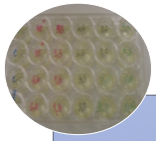

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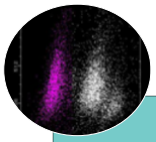

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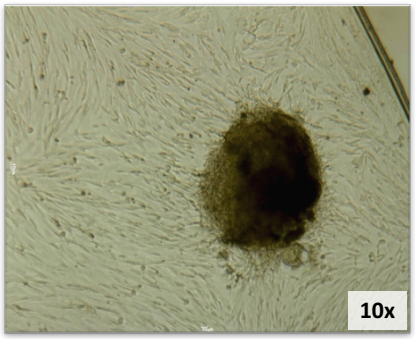
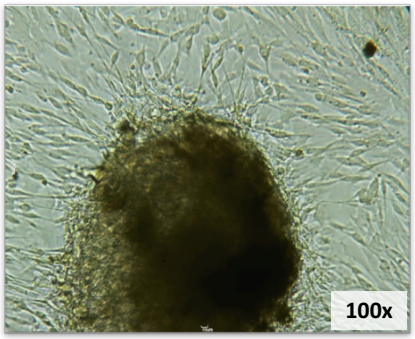
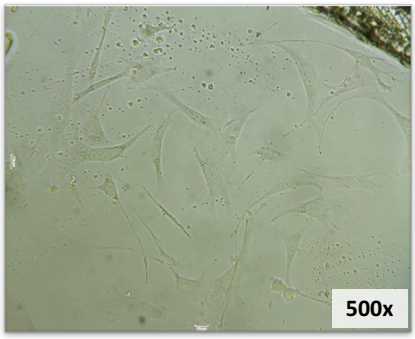
Results and Discussion

Gingival fibroblast cultures







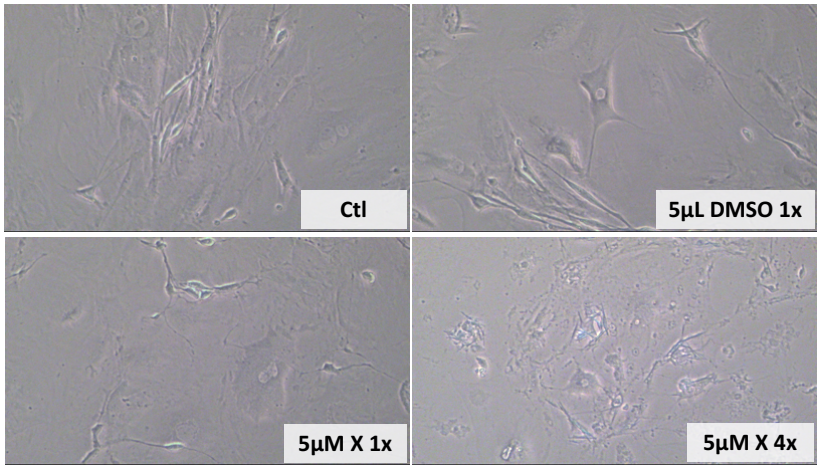
Explant methodology

✓ Great efficiency in cell cultures

✓ Great efficiency in cell number

Results and Discussion

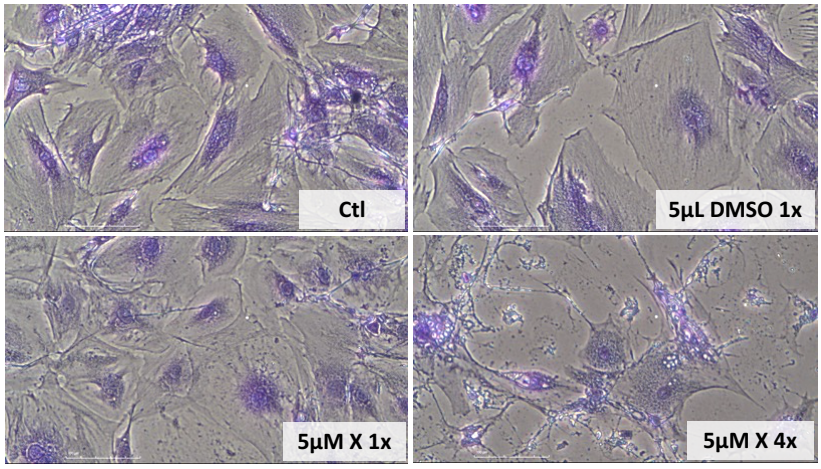
Cell Morphology after deddiferentiation – **Light microscopy**



- ✓ Some cell death
- ✓ Agent X 5µM with and without medium renewal promote an increase in cell size and spherical appearance in survival cells, compatible with stem-like phenotype

Results and Discussion

Cell Morphology after deddiferentiation – **Crystal Violet**



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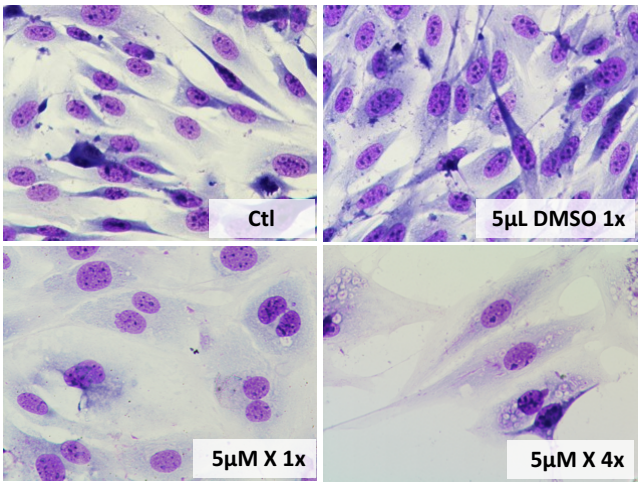
Results and Discussion

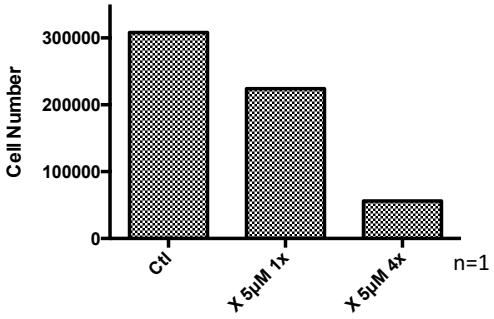
Cell Morphology after dedifferentiation – **May-Grünwald-Giemsa staining**



- ✓ Some cell death
- ✓ Appearance of a population with increased DNA, probably due to tetraploid formation

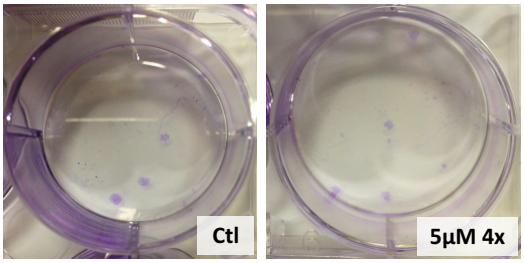
Results and Discussion

Cell Proliferation



- ✓ Agent X application promoted a dose-dependent decrease in cell proliferation

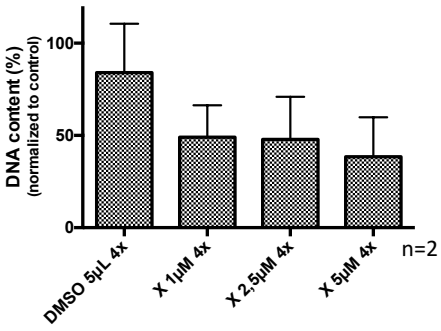
Clonogenic efficiency



- ✓ Agent X application promoted a decrease in clonogenic efficiency

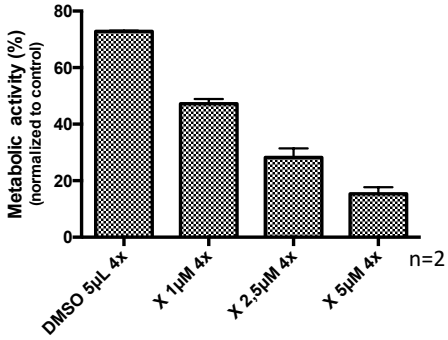
Results and Discussion

DNA content



✓ Agent X application promoted a dose-dependent decrease in cell proliferation

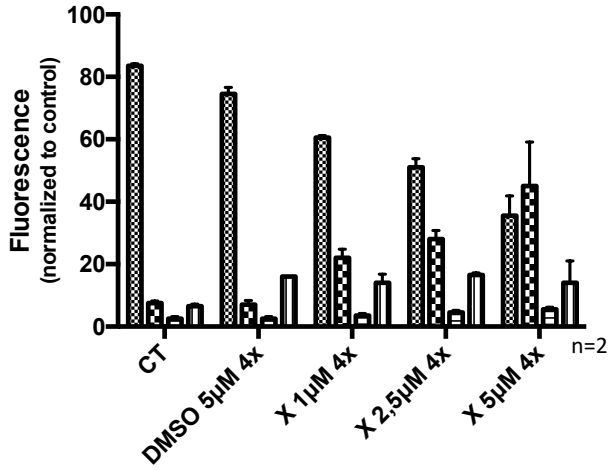
Metabolic Activity



✓ Agent X application promoted a dose-dependent decrease in metabolic activity

Results and Discussion

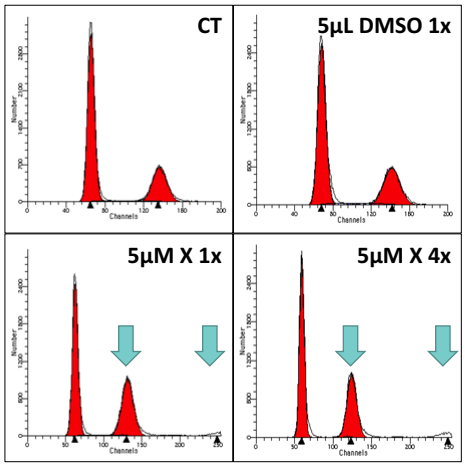
Cell Death

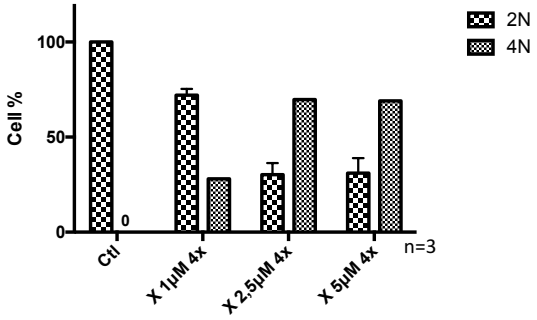


✓ Agent X application promoted a dose-dependent cell death mainly by apoptosis

Results and Discussion

DNA analysis – **Flow cytometry**





- ✓ Agent X lead to a G2/M increase
- ✓ Appearance of a population with double DNA amount, probably due to tetraploid formation

Conclusions

- ✓ Agent X succeeds in promoting gingival fibroblast dedifferentiation, with surviving cells presenting morphological and genetic changes compatible with stem-like phenotype:
  - ✓ Increase in cell size
  - ✓ Round shape
  - ✓ Increase in DNA content
- ✓ Some cell death, which increases with agent concentration
- ✓ This methodology could potential provide stem-like cells for regenerative procedures

Future perspetives

- ✓ International Society for Cellular Therapy criteria characterization will be completed
- ✓ Dedifferentiated cells used for regenerative dentistry research (odontoblastic differentiation)
- ✓ Proof-of-concept in human tissues
- ✓ Differentiation in other tissues (bone, nervous...)

Acknowledges

Funding

- ✓ SPEMD - Portuguese Society of Stomatology and Dentistry | Sociedade Portuguesa de Estomatologia e Medicina Dentária – SPEMD Scholarship for Scientific Dissemination
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Experimental Pathology Institute | Biophysics Unit |Dentistry Area | Laboratory of Oncobiology and Hematology

Centre of Investigation on Genetics and Oncobiology (CIMAGO)| Institute for Biomedical Imaging and Life Sciences (IBILI)

Faculty of Medicine, University of Coimbra, PORTUGAL

CIMAGO

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