17TH INTERNATIONAL SYMPOSIUM OF THE PORTUGUESE SOCIETY FOR METABOLIC DISORDERS

THE 2020S TOOTH FAIRY: FROM LOOSE TOOTH TO NEURONAL CELL CULTURES, a method to establish neurologic Lysosomal Storage Diseases *in vitro* – an update

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INTRODUCTION

PO 04

Lysosomal storage disorders (LSD) are a group of rare diseases caused by mutations in genes that encode lysosomal enzymes, membrane proteins or transporters. This leads to an accumulation of undegraded substrates, which ultimately causes a broad range of highly debilitating clinical symptoms affecting multiple organs/systems, including the central nervous system. Yet, most therapies for LSD are limited to treating non-neurological signs. Thus, there is an urgent need for the development of new ones that can tackle the neuronal pathogenesis. Fortunately, the portfolio of innovative therapeutic approaches under development has been growing tremendously and the need for proper models grows alongside.

To address this concern, we propose the **development** and **characterization** of innovative patient-derived cell models for early onset neurodegenerative LSD using **dental pulp stem cells** (DPSC) from deciduous "baby" teeth. DPSCs hold potential to give rise to a variety of cells including functionally active neurons. Nevertheless, to the best of our knowledge, this sort of technology hasn't yet been applied to samples obtained from LSD patients. This will be a total innovation in the field and we believe it holds potential to set a new trend for investigating LSD as it relies on a non-invasive, cost effective approach that can be set as a routine in any lab with standard cell culture conditions.

Here we present an update on this project, summarizing its rationale and current results, while giving an overview of the whole protocol and discussing its potential applications.

METHODS' OVERVIEW

PO 04

1.1. Preparation of "tooth kits" to be sent to LSD patients' families

👩 ***Clinicians following LSD-affected children & Patient Association representatives:*** identification of potential subjects to be enrolled in this study

👩‍🔬 ***Lab members:*** preparation of a "tooth kit" to be sent to his/her family

- 🧪 Falcon tube + transport media
- 📄 Informed consent forms + Project summary
- 📄 Return instructions + Biohazard bag + pre-filled and pre-paid Easy Mail Envelope.

1.2. Establishment of LSD DPSC cultures

DPSC reside deep in the pulp of the tooth. Thus, teeth need to be be broken or bored to access the pulp tissues. Then, the pulp tissues are digested with appropriate enzymes (dispase II and collagenase)

DPSC → expanded → passaged → frozen

1.3. Differentiation of LSD DPSC into mixed neuronal cultures

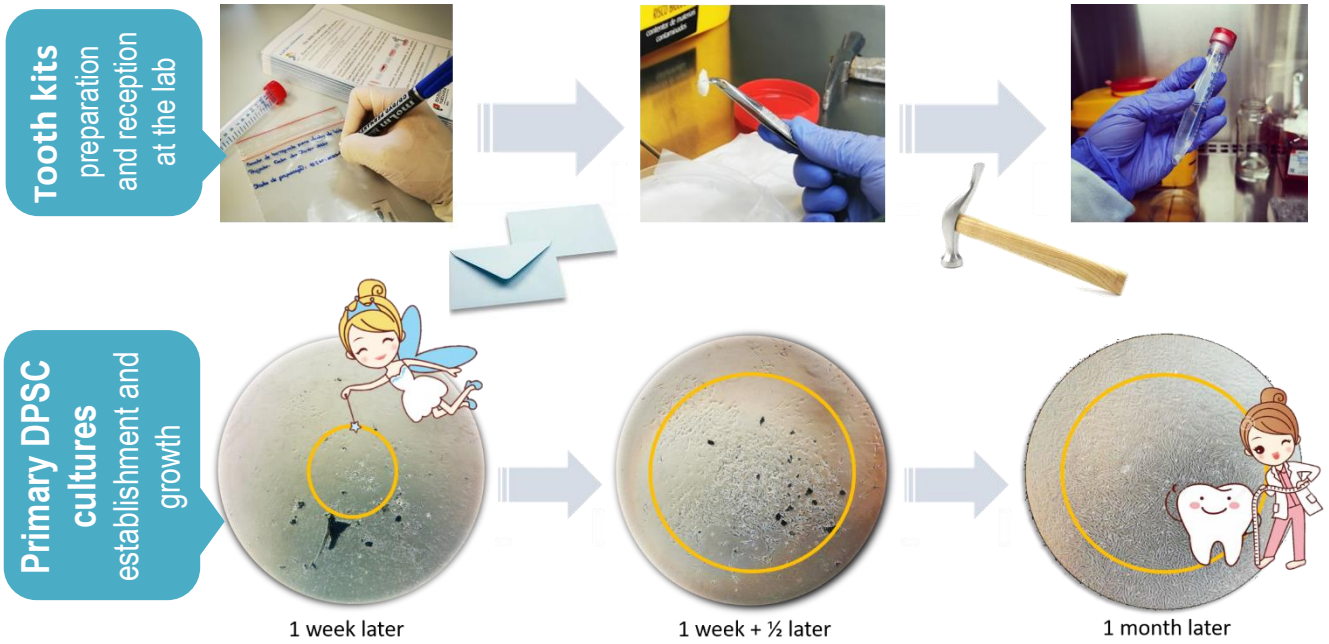
DPSC will be incubated in epigenetic reprogramming media (48 h) → change to neural differentiation media (3 days) → change to maturation media (until they reach maturity) → check for the presence of neuronal markers (MAP2 and GFAP)

RESULTS' OVERVIEW - I

PO 04

Briefly, over the last months, we have **successfully implemented** the protocol for the establishment of DPSC cultures in our lab and are currently working on the differentiation protocol, which will allow the formation of mixed neuronal and glial cultures.

Thanks to a well-succeeded **call for volunteers** (see **PO 05**), we were able to collect baby teeth from over 50 unrelated controls and established more than 30 different DPSC lines.



RESULTS' OVERVIEW - II

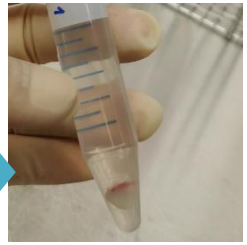
PO 04

Recently, we have also established our very first **LSD-derived DPSC line**.

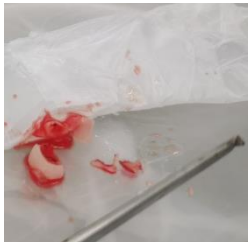
↓

Mucopolysaccharidosis type VI

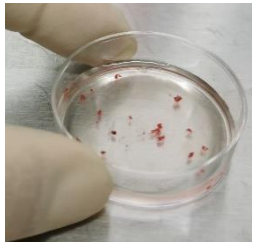
Primary DPSC culture establishment



Tooth reception



Dental pulp (DP) extraction

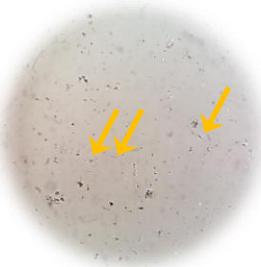


DP minced into small fragments

Day 1 culture
(with typical debris → dark matter)

Primary DPSC culture growth

Day 3 culture
(before medium change)



Day 3 culture
(after medium change;
1st visible DPSCs highlighted)

1 week later

2+ ½ weeks later
(DPSC culture at approximately 80% confluence, prior to passage)

CONCLUSION - I

PO 04

Traditionally, one of the best ways to get insights over the molecular mechanisms that drive pathogenesis is the analysis of **patient-derived cells**. Nevertheless, not every patient-derived cell holds potential to recapitulate relevant features of the disease. For neurodegenerative diseases such as the LSDs we are adressing, it is challenging to grow neuronal cultures that accurately represent them because of the obvious inability to access live neurons from a sufficient number of individuals.

Still, this whole scenario changed significantly, since Yamanaka *et al*. published their protocol for induction of pluripotent stem cells (SC) from adult human fibroblasts. From then on, a series of differentiation protocols to generate neurons from those **induced pluripotent stem cells** (iPSC) was developed and extensively used for disease modeling, especially for early proof-of-concept studies. Currently, human iPSC and iPSC-derived cell models have been generated for a number of LSD. Nevertheless, iPSC generation is a laborious and expensive protocol that still holds significant limitations not only in terms of production but also of its subsequent uses, particularly for therapeutic purposes. Here we present a tempting alternative to establish LSD patient-derived neuronal cell lines, in a much more expedite way.

LSD patient-derived DPSC have never been used for differentiation into specific cell types even though they represent a natural source of SC, which may be used to investigate human disease. This will be a total innovation in the field and we do believe it holds potential to set a **new trend** for investigating the cellular and gene expression changes that occur in these **monogenic diseases** as it relies on a **non-invasive**, **cost effective** approach that can be set as a routine in virtually any lab with standard cell culture conditions.

CONCLUSION - II

PO 04

Currently, we are actively working with patients' associations and a team of expert pediatricians from the major reference centers for treatment of LSD to identify potential volunteers for baby teeth collection, having already approached several families, who are now actively involved in the project and willing to send us deciduous baby teeth, as soon as they fall.
Therefore, the number of LSD patient-derived DPSC lines and the catalogue of LSDs included in this project is expected to significantly increase in the near future.

Nevertheless, if you find this study interesting, and want to get involved, **there's still time for you to join the 2020s Tooth Fairy Team**!

We have just been informed by the Portuguese national funding agency for science, research and technology (FCT) that we will be getting funds to proceed this study in 2022 (EXPL/BTM-SAL/0659/2021). So, if you want to get involved simply e-mail us and we'll get back to you with all further details:

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APL
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LITTLE DROPS OF WATER