

# THE 2020s TOOTH FAIRY: FROM LOOSE TOOTH TO NEURONAL CELL CULTURES, a method to establish patient-derived MPS III neuronal cell lines

Maria Francisca Coutinho<sup>1,2</sup>, Juliana Inês Santos<sup>1</sup>, Liliana Matos<sup>1,2</sup> and Sandra Alves<sup>1,2</sup>

<sup>1</sup> Research and Development Unit, Department of Human Genetics, National Health Institute Dr. Ricardo Jorge, Porto, Portugal

<sup>2</sup> Center for the Study of Animal Science, CECA-ICETA, University of Porto, Porto, Portugal



SNS SERVIÇO NACIONAL DE SAÚDE



## INTRODUCTION

Poster

**Mucopolysaccharidosis type III** is a subgroup of rare, neuronopathic **Lysosomal Storage diseases (LSD)**. Over the last decade, we have molecularly characterized the Portuguese MPS III population. More recently, we have also designed RNA-based methods to either correct specific mutations or actively promote substrate reduction by inhibiting its biosynthesis.

Whatever the case, the **therapeutic potential** of the RNA drugs under development has been addressed in patients' fibroblasts with promising results. Ideally however, **we should test each drug on its target cells/tissues**. Thus, taking into account that one of our major goals is to achieve **brain delivery of therapeutic RNA drugs**, we recognize that it would be crucial to test those same molecules also in **patient neuronal cells**. Still, the inability to access live neurons from affected individuals remains a challenge.

## RATIONALE

Poster

Here we present an alternative approach to generate **patient-derived MPS III neuronal cell lines** through a **non-invasive, cost effective** approach using **dental pulp stem cells (DPSC)**.

The plasticity of DPSCs has been demonstrated by several independent teams, who have shown that they self-maintain through several passages, holding 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**.

Our goal is to establish one such protocol in house, to **generate MPS III neurons** from normally shed deciduous **"baby" teeth**. Once established, those neuronal cell lines will constitute a valuable platform to address further address RNA drugs' efficiency *in vitro*.

## METHODS I

Poster

### 1.1. Preparation of "tooth kits" to be sent to MPS II patients' families

**Clinicians following MPS III children:**

Identification of **potential subjects** to be enrolled in the study

Once a subject is identified for our study, a "tooth kit" will be prepared at the lab and sent to his/her family,

- ✎ Falcon tube + transport media
- ✎ Return instructions
- ✎ Biohazard bag +
  - informed consent forms
  - (and any other required paperwork)
  - + pre-filled FedEx form.

## METHODS II

Poster

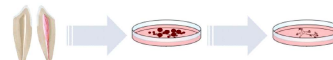
### 1.2. Establishment of MPS III DPSC cultures

(DPSC reside deep in the pulp of the tooth)

Teeth will first be broken or bored to access the pulp tissues. <sup>\*collagenase</sup>  
 digested with appropriate enzymes <sup>\*dispase II</sup>  
 DPSC → expanded → passaged → frozen

### 1.3. Differentiation of MPS III 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  
\*\*GFAP

## Let our TOOTH FAIRY explain...

Poster

*What's the advantage of using baby teeth as a sample?*

It's a **non-invasive, absolutely 'pain-free'** sample!  
 No need for painful biopsies! Those teeth will fall anyway; all we ask is for the family to keep them in proper conditions and mail them to our lab.



*How easy is it to go from loose tooth to neuronal cells?*

Everything takes its time at the lab, that's for sure. Under ideal conditions, the entire protocol from the arrival of the exfoliated tooth to the establishment of a mature neuronal cell line will take at least 6-8 weeks.

*Are there any special requirements for tooth collection or storage?*

- For the whole process to succeed it is **extremely important** that:
- ✓ the tooth is placed in the transport media within **20 min** from exfoliation!
  - ✓ it is sent to the lab within the **first 48h!**

## TAKE-HOME MESSAGE

Poster

Our current goal is to drive our peers' attention to this project and promote it among the Portuguese medical community on inherited metabolic disorders.

In summary, this is a **call for collaborators**: **if you're a clinician following up MPS III children who are currently losing their baby teeth, please consider contacting us.**

*How can you become enrolled?*

Are you a clinician following up MPS III children? Simply e-mail us ([francisca.coutinho@insa.min-saude.pt](mailto:francisca.coutinho@insa.min-saude.pt)) and we'll get back to you with all further details.



*Who is supporting this study?*

We have just received an **incubator grant** from the Sanfilippo Children's Foundation, to support this pivotal work.



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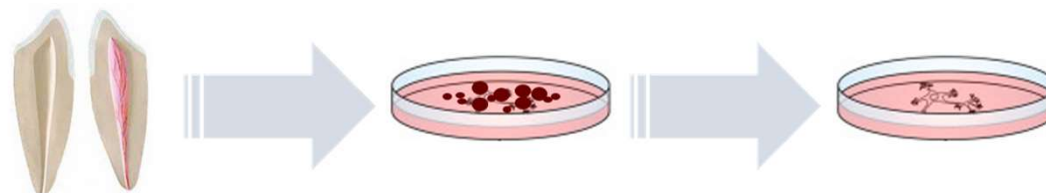
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