EORS Exchange Travel Grant

Visit goals	1 week	2 week	3 week	4 week
• Visit the laboratories at i3s Institute in Porto and explore the research field of orthoregeneration concerning intervertebral disc injuries. • Plan the activities for the next weeks.	X			
Improve my knowledge and acquire new skills concerning cell-free regenerative approaches by providing support to expert researchers		X	X	
Summarize and discuss the achieved goals with host mentor and supervisors				X

1st-2nd weeks: Working Plan and Training

During the first week I had the possibility to explore the laboratories at i3s Instituto de Investigação e Inovação em Saúde, Universidade do Porto and I was involved in mandatory training sessions (e.g. cell culture access, safety in the lab., fire extinguisher use ect.). Simultaneously, under the supervision of Prof. Raquel Gonçalves with the collaboration of Dr. Joana Caldeira from Biofabrication research group and Susana Santos from Stem Cells in Regenerative Biology and Repair Laboratory planned the work and activities for the next weeks. Our work focused on the employment of natural molecules to treat degenerated nucleus pulposus cells. Specifically, we used Naringenin and Hesperidin which are flavonoids found in citrus fruits, with a wide range of biological and pharmacological activities, including antioxidant, anti-inflammatory, cytoprotective and antitumor actions. The goal of this approach was to study the potential senolytic and anti-inflammatory effect of natural molecules-loaded extracellular vesicles (EVs) in intervertebral disc degeneration (IDD).

2nd - 3rd weeks: Improvement of my knowledge and acquisition of new skills

I started working for cell culture expansion and EVs isolation and characterization. Indeed, I learnt to isolate EVs by differential (ultra)-centrifugation from immortalized bone marrow mesenchymal stem/stromal cells (iMSC) and characterized them by electron microscopy (TEM), WB and nanoparticle tracking analysis.
For hesperidin or naringin iMSCs-EVs loading, three different methods were tested: incubation, electroporation and sonication.

4th week: Discussion of achieved goals and future plans

The three different methods used to load Naringenin and Hesperidin on EVs were suitable as TEM images confirmed that loading methods do not affect EVs morphology However, drug loading capacity (LC) and encapsulation efficiency (EE) for each method were evaluated by Fluorescence/ Spectrophotometry with some limitation related to the concentration of the drug. So, the next step will be to perform functional assay on adult bovine nucleus pulposus cells treated with flavonoids loaded-EVs to establish the best concentration and test their potential senolytic effect.
Obviously, this collaboration provides further analysis and investigations with the last goal of a joint publication.

Note: During my experience as visiting researcher at i3s, I had the opportunity to attend many seminars and events. Among these, I was invited by J.Caldeira (CEO and co-founder of Fetalix) and M.Fiordalisi (CTO and co-founder of Fetalix) as speaker at Spine Innovation Forum with leading experts, innovators and other stakeholders in the field of spinal health as Raquel Gonçalves, as well.