Friday, June 30, 2017

Dear Senior Science Editor

Dr. Nandita Singh

Please consider our manuscript entitled “Parallel decellularization of fetal and adult cardiac tissue explants as 3D *in vitro* microenvironments to dissect cell-ECM crosstalk”, for publication as an Invited Methods Article - JoVE Produced Video in *Journal of Visualized Experiments (JoVE)*.

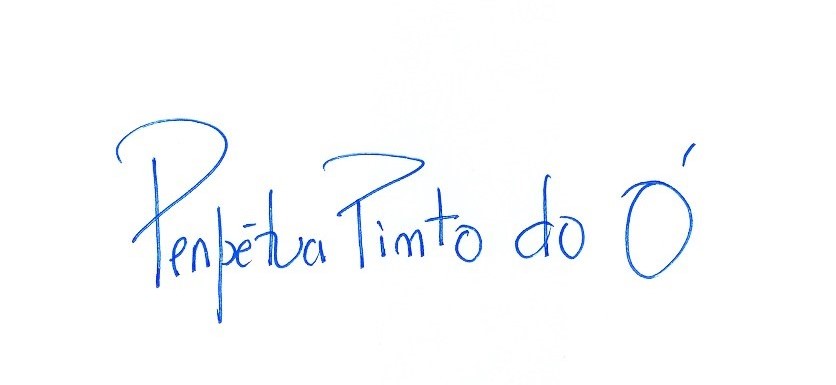
Our laboratory main interest is to **contribute knowledge toward improving the anatomo-functional restoration of poorly regenerative organs**. In this context the heart, an organ with very low cellular turnover, whose function depends upon cells majorly generated during the embryonic development has been a challenge. In brief, for the last five years we have been addressing this problem in a comprehensive strategy directed to the **systematic identification of signals critical for** (i) initiation of the **cardiac molecular program** in the embryo (1Freire AG, 2017), (ii) **emergence and kinetics of the main cell lineages that compose the heart** (2Valente M, 2017, manuscript in revision & *Declaration of Invention*, 2015), and (iii) the **cellular cross-talk with the dynamic extracellular matrix (ECM) that supports the full organ development and growth** (3Silva AC, 2016). The latter publication has been the result of such concerted endeavor and tackles current limited data on microenvironmental cues that sustain survival, proliferation and functional proficiency of cardiac cells. Relative to recent developments, **we have advanced the field by showing unique features of close-to-native three-dimensional (3D) fetal (E18) and adult myocardial ECM and by demonstrating that fetal bioscaffolds are not only more efficiently recellularized with cardiomyocytes and cardiac progenitors as they also direct the cells toward a more regenerative-like profile**.

The herein manuscript is the new protocol, seminal to the work reported on *Biomaterials* (2016) above, developed for obtaining acellular ECM bioscaffolds from fetal- and adult-heart explants under parallel conditions, and thus ensure reliable comparison of the biochemical, biophysical and biological activity features of distinct microenvironments. Noteworthy, this protocol has by now endured testing on different systems and proved, among others, to be easily adaptable also for decellularizing human tissues4 (normal and malignant colorectal samples) and murine lung5 while retaining main ECM elements which include distinctive biochemical and structural meshwork features of the native equivalents, properties which, to the best of our knowledge, have not consistently been shown before. Thus, our experience legitimates the conviction that detailed sharing of this simple three-steps protocol with a broader scientific community will bring further insight into several other tissue-organ models and/or conditions.

A final remark to justify our special request on the possibility of having the herein manuscript published before Oct-Nov 2017. We would be most indebted to your kind understanding that while the first author (and the one to be filmed by the *JoVE* Team while performing the protocol) has been developing her final work for the PhD dissertation in the Laboratory of Dr. McDevitt (PhD co-supervisor) at the Gladstone Institutes in San Francisco (CA), USA for the last year and a half, she might be returning to Portugal before the end of 2017. Attending to that the Iberian Peninsula is not yet in the *JoVE* roadmap for filming, it would be most wise to make the video before Ana Silva departure from McDevitt’s lab.

On the behalf of all authors, I thank you in advance for your original proposal which led to submitting our work to JoVE as well as for the kind and continued attention,

Sincerely,



Perpétua Pinto-do-Ó, PhD

**Stem-cell Microenvironments in Repair/Regeneration Team, Coordinator**

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**Adjunct Professor, Lecturer in Stem Cell Biology, Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, Porto, Portugal**

**President of the Portuguese Society for Stem Cells and Cell Therapy (SPCE-TC**

1Freire AG, Waghray A, Soares-da-Silva F, Lee DF, Pereira CF, Resende TP, Nascimento DS, Lemischka IR\*, Pinto-do-Ó P\* (2017). Transient Hes5 activity instructs mesodermal cells toward a cardiac fate. *Stem Cell Reports* DOI: 10.1016/j.stemcr.2017.05.025 · License: [CC BY-NC-ND 4.0](https://creativecommons.org/licenses/by-nc-nd/4.0/)

2Valente M, Resende TP, Nascimento DS, Cumano A\*, Pinto-do-O P\*. Unique signatures identify sequential stages of cardiomyocyte maturation differentially represented throughout life. (*Dev Cell*., in revision) & Valente M, Resende TP, Nascimento DS, Cumano A and Pinto-do-Ó P (2015). New surface markers discriminate different cardiac populations during development allowing their prospective isolation and the definition of the transcriptional profile at the single cell level. [**Declaration of Invention No I 2015-31 HC**] (Institut Pasteur, Paris, France)

3Silva AC, Rodrigues SC, Caldeira J, Barbosa MAOliveira MJ, Nascimento DS\*, Pinto-do-P.\* (2016). Three dimensional scaffolds of fetal decellularized hearts exhibit enhanced potential to support cardiac cells in comparison to the adult. *Biomaterials* DOI: 10.1016/j.biomaterials.2016.06.062. citations: 2 (RG), 1 (Scopus)

4Pinto ML, Rios E, Silva AC, Neves SC, Caires HR, Pinto AT, Durães C, Carvalho F, Cardoso AP, Santos N, Barrias C, Nascimento D, Pinto-do-Ó P, Barbosa MA, Carneiro F, Oliveira MJ (2017). Decellularized human colorectal cancer matrices polarize macrophages towards an anti-inflammatory phenotype promoting cancer cell invasion via CCL18. *Biomaterials* DOI.org/10.1016/j.Biomaterials.

5Garlíková Z, Silva AC, Rabata A, Potěšil D, Ihnatová I, Dumková J, Koledová Z, Zdráhal Z, Vinarský V, Hampl A, Pinto-do-Ó P**\***, NascimentoDS**\***. Generation of a close-to-native *in vitro* system to study lung cells-ECM crosstalk. (*Tissue Eng Part C*., in revision).