



Dottorato di ricerca in Scienze Veterinarie XXXVII CICLO - Anno di corso: 2°
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Curriculum: Scienze di Base
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Development and validation of complex systems of primary cell cultures, including embryonic, adult and induced pluripotent stem cells, for toxicity studies and modeling of rare and neurodegenerative diseases

Objective: Development and validation of a protocol for the decellularization of Spinal Cord (SC) **(i)** and set up a protocol for 2D **(ii)** and 3D **(iii)** *in vitro* models from decellularized SC for the Extracellular Matrix (ECM) study.

Materials and Methods: Neural Stem Cells (NSCs) isolated from embryonic (E13.5) rat forebrain growth on 2D-coating and 3D-ECM-gel [1] obtained from decellularized rat SC.



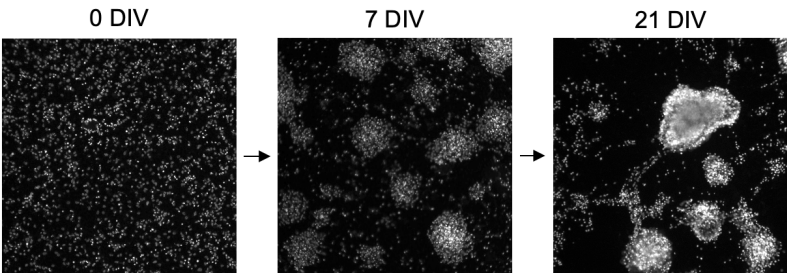
Results:

(i) Production of Decellularized SC



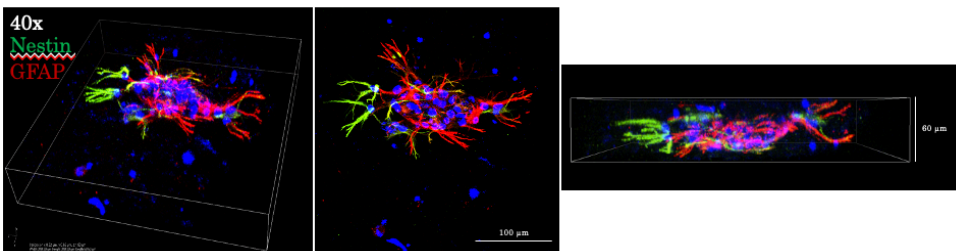
Macroscopic structure maintenance; Very low residual DNA quantity; Absence of intact cells in the decellularized tissue; Quick and easy execution.

(ii) Clusters formation on 2D - coating



The increasing amount of ECM proteins affects the connections between NSCs; Formation of large 3D structures; Increased connections between clusters.

(iii) Spheroids formation on 3D - ECM-gel



Clusters formation and cells/spheroids survival after 3 Days *In Vitro* on 8 mg/mL ECM-gel concentration.

Conclusions: Those experiments have defined an efficient method to decellularize the spinal cord and tested its effect in 2D and 3D systems to evaluate the contribution of ECM on NSCs in physiological conditions.

Future Proposal: Evaluate the ECM effect from Spinal Cord Injury on NSCs in 2D and 3D *in vitro* systems.

References: 1. Xu Y, Zhou J, Liu C, Zhang S, Gao F, Guo W, Sun X, Zhang C, Li H, Rao Z, Qiu S, Zhu Q, Liu X, Guo X, Shao Z, Bai Y, Zhang X, Quan D. Understanding the role of tissue-specific decellularized spinal cord matrix hydrogel for neural stem/progenitor cell microenvironment reconstruction and spinal cord injury. *Biomaterials*. 2021 Jan;268:120596. doi: 10.1016/j.biomaterials.2020.120596. Epub 2020 Dec 10. PMID: 33341040.