PERIPHERAL NERVOUS SYSTEM AND BONE: ADVANCED TOOLS TO STUDY AN ESSENTIAL INTERACTION

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Introduction

Bone innervation has proven to be a critical step in bone homeostasis/regeneration where soluble factors produced by nerve fibers have been associated to changes in bone cells activity [1, 2]. Thus in this study we have established two different non-contiguous coculture systems to mimic the *in vivo* scenario where nerve fibers can be found in bone microenvironment.

Methods

Cocultures between osteoblasts and sensory neurons were performed using porous inserts enabling the study of paracrine effects.
Exploring an alternative approach that would allow the segregation of cell soma but at the same time the contact between the nerve fibers and bone cells, microfluidic chambers emerged as a potential tool to perform these studies. Embryonic or adult primary dorsal root ganglia (DRGs) and MC3T3-E1 or primary osteoprogenitor cells were used and cell density tests were performed.

Aiming to mimic the properties of tissue extracellular matrices, osteoblasts were seeded in the axonal side upon laminin, collagen or within 3D functionalized RGD alginate matrices.

To quantify the axonal outgrowth in a non-subjective and user bias independent manner it has become necessary to develop a custom-made solution for quantifying axonal outgrowth in the specific framework of the microfluidic cocultures platform. Therefore it was our aim to develop a simple and automated approach which would allow the quantitative analysis of neurite outgrowth in microfluidic devices along the longitudinal axis.

Results and Discussion

Metabolic activity of osteoblasts was not affected and sensory neurons differentiation was achieved using porous inserts cocultures. Serum concentration showed to be a key limitation of this system, which implied coculture periods no longer than 24h.
Regarding the microfluidic chambers, the time of adhesion and readout of axonal outgrowth was improved by the alignment of DRGs, with the axis of microgrooves, which showed to be a crucial step for the designed experiments. For the first time, cocultures of entire DRGs from adult origin with osteoblasts were performed showing DRGs extended projections towards the axonal compartment, reaching bone cells, with no evidences of axonal degeneration. Moreover, axonal outgrowth was still observed when culturing the osteoblasts upon different substrates.

Coculture images were analyzed with the developed algorithm and it was observed that the collagen and laminin substrates displayed a higher amount of axons reaching the axonal side. Nonetheless, axons were able to reach longer distances in the presence of 3D functionalized RGD alginate matrices.
Overall, both explored methods revealed to be a suitable tool to study the interaction between peripheral nervous system and bone cells in different contexts mimicking the *in vivo* scenario.

References

[1]Franquinho, F. et al. 2010.
[2]Sisask, G. et al. 1996.