ELECTROSPUN POLYCAPROLACTONE 3D FIBROUS SCAFFOLD FOR HUMAN PERIODONTAL LIGAMENT CELLS MECHANOBIOLOGY

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Introduction

More than 10 % of the world population suffers from severe periodontitis with a high risk for teeth lost due to the periodontal supporting tissue damage. Currently, regenerating the periodontal ligament (PDL), connecting the bone to the tooth, remains a real technical and clinical challenge. In that context, periodontal tissue engineering appears as a relevant strategy. Providing and efficient *in vitro* engineered PDL relies on the suitable definition of the scaffolds and the applied cues. While periodontal cell stimulation *in vitro* is generally addressed through biomolecular signaling, biomechanical cues are known to play a determinant role in the PDL integrity[1]. *In vivo*, periodontal tissues are subjected to a complex masticatory cyclic biomechanical loading. Nevertheless, the few studies investigating the potential of human periodontal cells (PDLCs) mechanical stimulation *in vitro* were designed to better understand orthodontic tooth movement, hence involving a static loading. A study investigated the influence of a cyclic loading on PDLCs within a dynamic bioreactor[2]. But in this study, cells were seeded within a collagenous gel that may not be suitable regarding load transmission. While, many efforts are made in developing fibrous scaffolds, more representative of the fibrous PDL nature[3]. Regarding periodontal biomechanics, polycaprolactone (PCL) is known to have relevant properties[1]. In that context, the objective of the current study was to assess the potential of a 3D PCL fibrous scaffold to promote PDLCs mechanobiological behavior.

Materials and methods

Human PDLCs were seeded (10^3 cells.mL^{-1}) in a 3D PCL scaffold fabricated through electrospinning with a ring geometry to mimic the PDL geometry. (Figure 1). The loaded group (n = 3) was subjected to a static pre-load for 10 days followed by 11 days under a cyclic axial compressive loading, with a 100 µm displacement and 0.5 Hz frequency. A static group (n = 3) and a control group composed of scaffold without cells (n = 3), were let under static condition in the culture media during 21 days. The stiffness relative difference between day 0 and day 21 calculated. Parallelly, the relative difference of ALP activity and IL-6 protein amount were quantified at day 11 (starting day of the cyclic loading) and at day 21 (end of culture). The scaffolds were also observed using confocal microscopy. Three experimental series were performed, for a total of 9 (3x3) samples in each group.

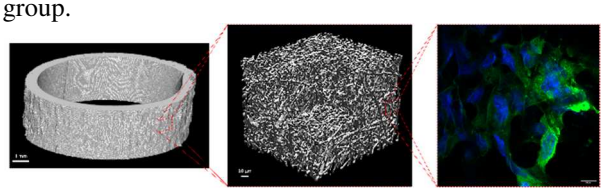

Figure 1: Left and middle: PCL fibrous scaffold observed using microtomography (Scale bars: 1mm, 10 µm, respectively). Right: Confocal image of static scaffold after 21 days of culture (blue: DAPI, green: Phalloidin-Alexa 488; scale bar: 10 µm).

Results and discussion

While the scaffold stiffness of the control group (without cell) only slightly decreased during the 21 days of incubation (-11 %), a significantly larger decrease, compared to the control group, was observed for the loaded and static groups (-37 % and -38 %, respectively, p-value ≤ 0.01, Mann-Whitney). The ALP activity and IL-6 concentrations decreased after the cyclic loading but increased in static conditions between day 10 and day 21. Confocal observations showed a layer of cells on the static scaffold surface (Figure 1, right). Only some isolated cell nuclei were observed on the loaded scaffold surface. Following this result, cells within scaffolds were counted after cells harvesting and detachment using trypsin-EDTA. In average, 15.10^4 and 6.10^4 cells.mL^{-1} were counted on the static and the loaded scaffolds after 21 days of culture, respectively, which confirms the presence of cells on both groups.
Together, these results strongly suggest that the cyclic mechanical loading has an influence on PDLCs behavior. This validates the PCL fibrous scaffold capacity to transmit the mechanical loading to the cells. As a perspective, to evaluate the PDL *in vitro* engineering potential, a more physiological loading will be applied to a cell-seeded fibrous PCL scaffold with an architecture close to the PDL architecture.

References

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