ADVANCED PLATFORM FOR IN VITRO STUDYING CELL BIOLOGICAL RESPONSE TO CONTROLLED STRETCH STIMULATION – PERIODONTAL LIGAMENT STEM CELLS APPLICATION

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Introduction

In vivo, mechanical cues are fundamental in promoting cell and tissue development and in maintaining homeostasis [1]. Thus, advanced investigation platforms able to replicate and combine native-like physical stimuli represent essential tools for investigating *in vitro* the biological response of cells under defined mechanical stimuli. In this study, we designed, characterized, and tested an investigation platform, based on a flexible substrate and a stretch bioreactor [2], for exposing adherent cells to controlled uniaxial stretch culture protocols in view of mechanotransduction studies, and we performed explanatory biological tests on human periodontal ligament stem cells (hPDLSCs).

Methods

The proposed investigation platform is composed of a flexible substrate combined with a previously developed stretch bioreactor [2]. The substrate presents two parallel rectangular wells for enabling test parallelization and optimization of the culture medium volume. To guarantee a planar and uniform uniaxial strain, the substrate design was supported by finite element (FE) analyses (Abaqus, Dassault Systèmes). A uniaxial displacement of 3 mm (15% strain) was imposed at one side of the substrate while the opposite one was fixed, mimicking the bioreactor stretching. Once identified, the optimal substrate design was fabricated in polydimethylsiloxane (PDMS, Sylgard 184). Digital image correlation (DIC) method was adopted (VIC-2D system, isi-sys GmbH) to measure the substrate surface strain under uniaxial stretch (n=3). Explanatory biological tests were carried out on hPDLSCs from healthy donors. After coating with collagen I, the substrates were seeded with hPDLSCs, clamped in the bioreactor (Fig. 1A), and subjected to intermittent cyclic uniaxial stretch (8% constant pre-strain + 7% cyclic strain for 90 s (n=3) or 5 min (n=3) at 1 Hz every 6 h) for 3 days [3]. Cell-seeded substrates were cultured statically as control. Cell alignment was visually inspected by light microscopy and the expression of the osteogenic markers alkaline phosphatase (ALP), collagen type I (COLL 1), osteocalcin (OCN), tenogenic markers tenomodulin (TNM), periostin, and runt-related transcription factor 2 (RUNX2) was quantified by real-time PCR.

Results

FE and DIC analyses showed similar results (Fig. 1B), with a planar and mostly uniform strain distribution at the substrate well bottom with a mean strain value along the stretching direction (ε_{xx}) of 13.4% and 14.4±0.25%, respectively. Biological tests revealed cell alignment and significant over-expressions of OCN and RUNX2 genes with respect to the control when cells underwent the stimulation protocol with cyclic stretch lasting for 90 s. For cells exposed to longer stimulation (5 min), a slight decrease of osteogenic gene expression was observed compared to shorter stimulation (90 s) (Fig. 1C), while 2-fold and 3-fold increase in periostin and TNM respectively was obtained.

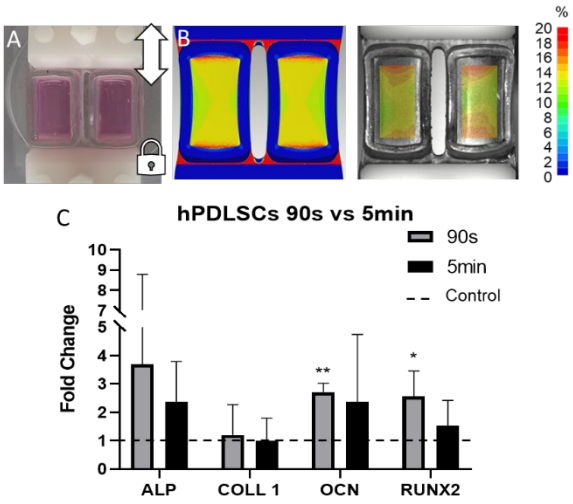

Figure 1: (A) Substrate clamped in the bioreactor; (B) FE (left) and DIC (right) analyses; (C) Genes expression after 3 days of culture under 90 s or 5 min cyclic stretch conditions.

Discussion

Providing controlled physical stimuli *in vitro* is fundamental for in-depth understanding the cause-effect relationship between the applied mechanical cues and the cellular response. Here, we developed and tested an investigation platform able to provide by stretching controlled planar and uniform strain to adherent cells. Also, the presented platform, allowing for various strain protocols, gives the possibility to separately unravel the effect of each stimulation parameter. Preliminary biological results showed that timing in stimulation can play a crucial role in promoting hPDLSCs alignment and differentiation. Further biological tests are ongoing to confirm the obtained results.

References

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2. Putame et al, Med Eng Phys, 84:1-9, 2020
3. De Jong et al, J Biomater Tissue Eng, 7:1303-1312, 2017