In Vitro Fibroblasts and Osteoblasts Under Orthodontic Compression: Insights into Matrix Metallopeptidases

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Conclusions

This study evidenced a cell-specific response to mechanical forces involving alterations in both morphology and matrix metallopeptidases activity. These results support the hypothesis that the periodontal ligament and alveolar bone contribute differently to tissue remodelling during orthodontic tooth movement.

Introduction

Orthodontic tooth movement results from the application of mechanical forces to teeth, which induce periodontium remodelling, thereby allowing for tooth repositioning. This process creates two distinct zones: a compression side, associated with resorption, and a tension side, where tissue apposition occurs. Fibroblasts within the periodontal ligament (PDL) and osteoblasts at the PDL/alveolar bone interface are key mechanosensitive cells involved in this biological response. Both cell types produce matrix metallopeptidases (MMPs), which are enzymes responsible for the degradation of the extracellular matrix and play a critical role in PDL remodelling during orthodontic tooth movement. Despite their relevance, the types and precise behaviour of MMPs under compression mechanical stimuli still require further clarification. **The objective of this in vitro study is to simulate orthodontic forces and clarify the presence and enzymatic activity of matrix metallopeptidases in PDL fibroblast and osteoblast cultures exposed to compression forces.**

Materials and Methods

PDL fibroblasts (HPLF) and osteoblasts (MG-63) were cultivated in a three-dimensional collagen gel and subjected to a static compressive force for 24 hours using a weight-based technique. Cell viability **(1)** was assessed using staining techniques, while morphology **(2)** was analysed under an inverted optical microscope. The enzymatic activity of MMPs **(3)** was evaluated through collagen zymography, in comparison with control cells.

Fig. 1| Methodology comparison of applying compressive forces for 24h through the action of a weight to PDL fibroblasts and osteoblasts cultured in a collagen matrix, and the standard situation of cells not subjected to weight.

Results

Preliminary findings indicated that cells exposed to compressive force:

1. Retained their viability, as demonstrated both by fluorochrome staining (PI+DAPI) and by the metabolic activity assessed in the MTT assay;
2. Changed their morphology, appearing to be less elongated and with fewer citoplasmatic extensions, as illustrated by arrows;
3. Modulated collagenolytic activity, as observed in zymography.

1. Cell Viability Tests

Fluorochrome staining:

PI ⇨ Stains non-viable cells red

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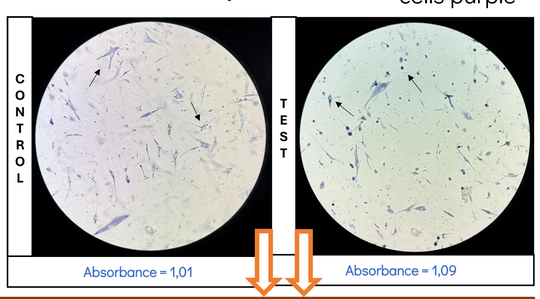
DAPI ⇨ Stains all cells blue

Only blue fluorescence was observed **in the cells subjected to compression and control**
- Viable cells

Fig. 2| PDL fibroblasts and osteoblasts from the control and test groups were fixed with the fluorochromes propidium iodide (PI) and 4',6-diamidino-2-phenylindole (DAPI).

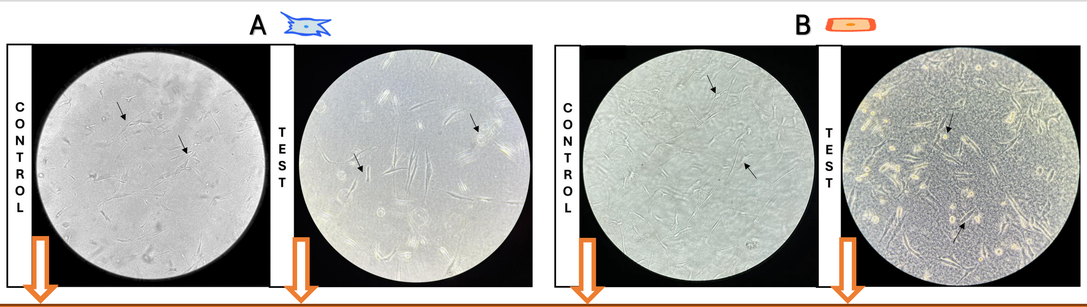
MTT assay: MTT ⇨ Stains viable cells purple



Viability of PDL fibroblasts **identical in cells subjected to compression and control**
- Viable cells

Fig. 3| PDL fibroblasts from the control and test groups observed under an inverted optical microscope after being subjected to the 3-4,5-dimethyl-thiazol-2-yl-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. Images were acquired at 100x magnification. The change of cells to a purple coloration and the absorbance values are indicators of cell viability. Compressed cells show a more intense purple coloration due to the thinner gel resulting from the compression of the collagen gel.

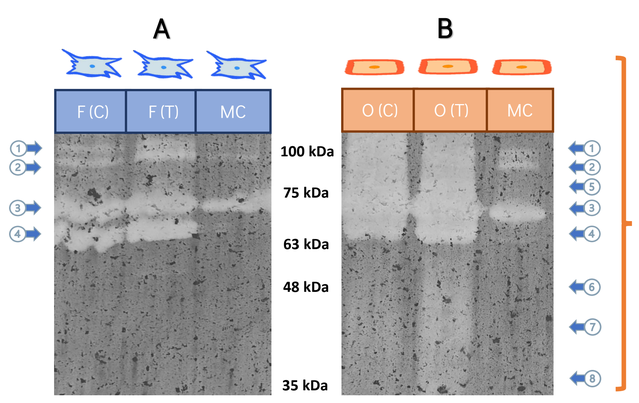
2. Cell Morphology:



Control cells presented a characteristic fibroblast morphology. However, **changes in their morphology** were detected **after compression**, suggesting that there has been an **alteration in their interaction with the matrix**. Compressed cells also show greater clarity, resulting from the concomitant compression of the collagen gel.

Fig. 4| PDL fibroblasts (A) and osteoblasts (B) from the control and test groups observed under an inverted optical microscope. Images were acquired at 200x magnification in phase contrast.

3. MMP Enzymatic Activity by Collagen Zymography:



PDL Fibroblasts:
4 bands of proteolytic activity are visible, with apparently **greater intensity in the supernatant of cells subjected to compression.**

Osteoblasts:
At least 8 bands are visible, with apparently **greater intensity in the supernatant of cells subjected to compression**, in particular for molecular mass bands with values between 35 and 45 kDa.

PDL fibroblasts and osteoblasts show different proteolytic activity profiles

More intense bands are visible in cells subjected to compression

Band 1: proteins ≈ **101-104 kDa**
Band 2: proteins ≈ **94-98 kDa**
Band 3: proteins ≈ **73-75 kDa**
Band 4: proteins ≈ **64-66 kDa**
Bands smallers than 63 kDa:

HYPOTHESES[9]

- Pro-MMP-9 **(92 kDa)**
- Pro-MMP-2 **(72/75 kDa)**, Pro-MMP-8 **(75 kDa)**
- MMP-2 **(64 kDa)**, Pro-MMP-13 **(65 kDa)**, Pro-MMP-14 **(66 kDa)**
- Pro-MMP-1 **(57 kDa)**, MMP-1 **(52 kDa)**, Pro-MMP-3 **(57 kDa)**, MMP-3 **(45 kDa)**, MMP-8 **(57 kDa)**, Pro-MMP-10 **(57 kDa)**, MMP-10 **(44 kDa)**, MMP-13 **(48 kDa)**, MMP-14 **(54 kDa)**

Fig. 5| Collagen zymography of supernatants of PDL fibroblast samples (A) from the control group - **F (C)** - and the test group - **F (T)** and osteoblasts (B) from the control group - **O (C)** - and the test group - **O (T)**. Culture medium **(CM)** was used as a negative control. Samples with 15 µg of protein were applied. The bands of proteolytic activity are identified by being light areas on the dark background and are marked numerically. The molecular mass was calculated in Image J by comparison with the calibration curve.

Conflict of Interest

The authors declare no conflicts of interest related to this study.

Funding

This work was carried out with institutional support from Egas Moniz School of Health and Science.

References

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