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Establishment of an in vitro Fluid Flow Shear Stress Model to Investigate the Impact of Mechanical Stress on Human Periodontal Ligament Cells

Dissertation

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List of abbreviations

CFD	Computational fluid dynamics
dyne/cm^2	1 Pascal = 10 dyne/cm^2
FSS	Fluid shear stress
hMSC	Human mesenchymal stem cells
hOst	Human osteoblasts
hPDLCs	Human periodontal ligament cells
mOcyt	Mouse osteocytes
mOst	Mouse osteoblasts
OTM	Orthodontic tooth movement
PDMS	Polydimethylsiloxane
RT-qPCR	Reverse transcription quantitative polymerase chain reaction
SaOS-2	Human osteosarcoma cell line SaOS-2

List of publications

Nile M, Folwaczny M, Wichelhaus A, Baumert U, Janjic Rankovic M (2023). Fluid flow shear stress and tissue remodeling-an orthodontic perspective: evidence synthesis and differential gene expression network analysis. Front. Bioeng. Biotechnol. 11: 1256825.
PMID: https://www.ncbi.nlm.nih.gov/pubmed/37795174
PMC: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10545883/
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Nile M, Folwaczny M, Kessler A, Wichelhaus A, Janjic Rankovic M, Baumert U (2024). Development of a Custom Fluid Flow Chamber for Investigating the Effects of Shear Stress on Periodontal Ligament Cells. Cells 13(21):1751.
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DOI: https://doi.org/10.3390/cells13211751

Your contribution to the publications

1.1 Contribution to paper I

Engaged in conceptualizing and designing the research, including formulating the P.I.C.O. research question and crafting the search formula. Utilized the predefined search formula to query the PubMed database. Reviewed 6,974 studies by screening titles and abstracts. A full-text assessment was performed for the remaining 218 studies, followed by exclusion based on predefined criteria. Applied the risk of bias criteria and conducted methodological and reporting quality assessments. Carried out data extraction for 120 included studies, encompassing qualitative and quantitative details such as cell types, detailed information regarding cell characteristics, types of fluid flow, apparatuses utilized in each experiment, and specified fluid shear stress (FSS) parameters. Furthermore, extracted expressed genes and analytes, including the methods employed for measurement. Analysed various *in vitro* FSS models, assessing parameters like magnitude, duration, and frequency of stress. Compiled and analysed gene/metabolite regulation data across all included studies, identifying prevalent loci and categorizing them into tissue formation, tissue degradation, and inflammation groups. Drafted the original manuscript and incorporated revisions based on co-authors' feedback.

1.2 Contribution to paper II

Under the guidance of my supervisor, I designed the fluid flow shear stress (FSS) *in vitro* experiments after reviewing and collecting data regarding the magnitude, duration, and fluid flow profile based on paper I and additional relevant literature. Designed and constructed a FSS setup consisting of a parallel flow chamber, a peristaltic pump, a bubble trap, and a pulse damper. The parallel flow chamber model was subjected to rigorous testing using a flow meter and computational fluid simulation (CFD) to confirm the stress distribution and exclude fluid turbulence areas. I implemented polydimethylsiloxane (PDMS), subtractive manufacturing, and 3D printing to build the parallel flow chamber and custom-made cell seeding gasket wells. These seeding gaskets made it easy to seed the cells in an FSS-uniform area on a microscopic slide. Calibrated the temperature of the circulated cell culturing media inside the chamber with the temperature of the water heating bath to achieve ≈37 °C of circulating media. The chamber was tested *in vitro*, by applying a FSS to a human osteosarcoma cell line (SaOS-2) and human periodontal ligament cells (hPDLCs) over one hour, then evaluated cell attachment and viability.

Performed primary cell cultures of hPDLCs and applied FSS, conducted subsequent sample preparation, reverse transcription, quantitative real-time polymerase chain reaction (RT-qPCR), and western blot. Additionally, conducted primer testing to determine the annealing temperature of newly selected primers including efficiency testing of each primer.

Calibrated western blot settings (voltage, current, and time) for electrophoresis and wet transfer. Contributed to the analysis of RT-qPCR and western blot data. Drafted the initial versions of the manuscript and iteratively refined them based on the recommendation of co-authors and reviewers.

2. Introductory summary

2.1 Introduction

The doctoral research project titled “Establishment of an *in vitro* Fluid Flow Shear Stress Model to Investigate the Impact of Mechanical Stress on Human Periodontal Ligament Cells” was done at the Department of Orthodontics and Dentofacial Orthopedics, University Hospital, Ludwig-Maximilians-Universität München, under the supervision of PD Dr. rer. nat. Uwe Baumert, Prof. Dr. Dr. Matthias Folwaczny, and Prof. Dr. med. dent. Andrea Wichelhaus. Within this project, two studies were conducted and subsequently published to meet the criteria for PhD completion.

Orthodontic treatment has long been utilized to address dentofacial misalignment. The principles of orthodontic tooth movement are straightforward. However, the intricate cellular mechanotransduction pathways governing such movement remain not well understood. Different mechanical stresses are exerted on the teeth throughout orthodontic treatment independent of the orthodontic appliances employed. Factors such as force level and duration significantly influence orthodontic interventions' speed, stability, and outcome (Krishnan and Davidovitch 2006).

Mechanical cues encountered during orthodontic tooth movement (OTM) stimulate various cells within the periodontium, including those in the bone, periodontal ligament, cementum, blood capillaries, and nerve tissues (Nile et al. 2023). These stimuli generate biological signals essential for tissue remodelling and, ultimately, for facilitating tooth movement. Compression and tension emerge as the primary stimulating mechanical cues affecting the periodontium during orthodontic treatment (Janjic et al. 2018; Sun et al. 2021). These stresses may directly impact the cells or secondary contribute to the generation of FSS, a stress induced by the shifting (movement) of interstitial fluid of the periodontal ligament or bone (Krishnan and Davidovitch 2006).

Interstitial fluid constitutes a significant portion of body mass, typically present within the extracellular matrix, and is exchanged between blood capillaries and lymphatics by the osmotic and hydrostatic pressure differences. The generation of interstitial fluid movement requires a force influencing the geometry of a porous tissue, causing an alternation in pressure within it (Ashrafi et al. 2020). Therefore, alterations of flow, including shear stress, are perceptible to the surrounding cells. The mechanosensing capability of cells through mechanoreceptors, cytoskeleton, extracellular matrix, and signal transduction pathways can detect fluid-flow movement/shear stress, triggering genes and protein/metabolites signals to facilitate tooth movement (Krishnan and Davidovitch 2009).

Fluid flow movement can vary from tissue to tissue (e.g., bone vs vascular system), health and disease, and the nature of force (e.g., cyclic movement vs sudden movement) driving it. For instance, fluid flow within the bone can be steady laminar by sudden movements (e.g. changing position from sitting to standing) (Piekarski and Munro 1977), oscillatory laminar by cyclic movements (Lu et al. 2012; Wang et al. 2024) (e.g., mastication or walking), or pulsatile laminar by heart pumping effect (e.g., arteries) (Hwang et al. 2003).

Different experimental models have been introduced to mimic the fluid microenvironment based on the fluid profile and estimated shear stress in these tissues (e.g. custom-made parallel flow chambers, orbital shakers, rocking plate, and cone and plate). There has been growing evidence investigating the effect of FSS on bone remodelling, but little is known about the FSS microenvironment in periodontal ligaments during OTM. Due to the limited experimental research in this field, (1) we aimed in this project to include and analyse all 2D *in vitro* studies that determined the

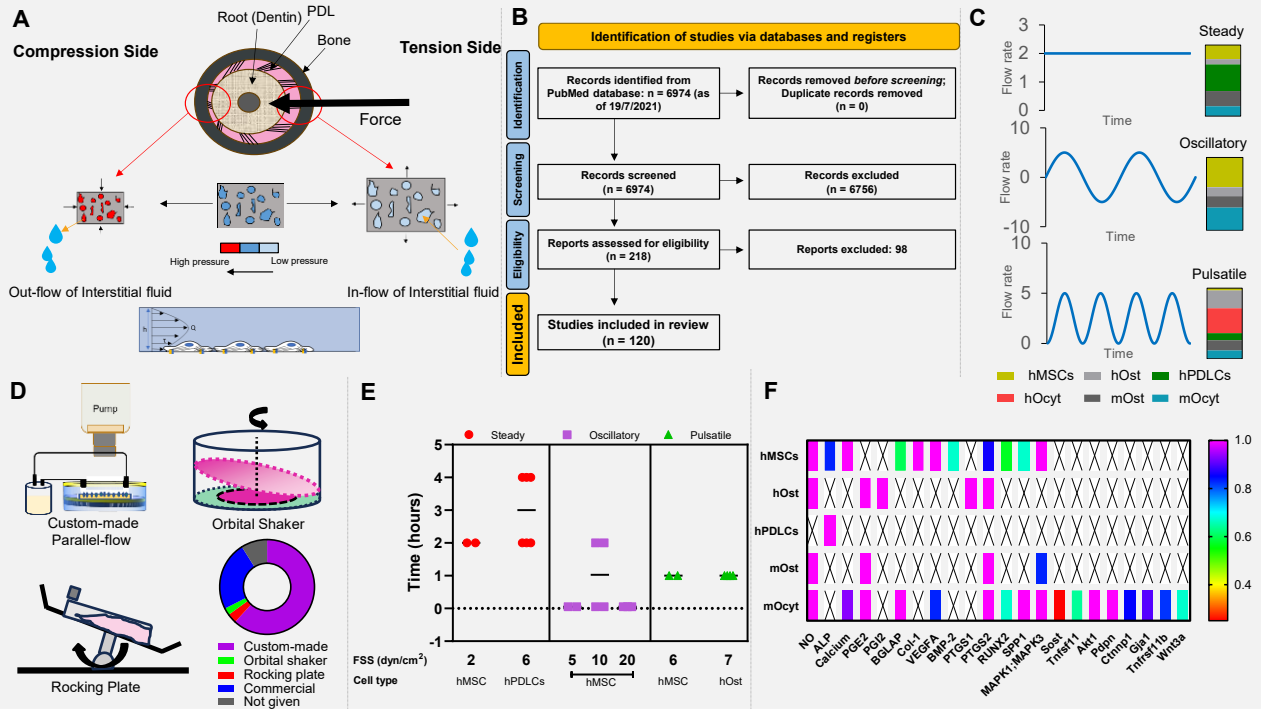
influence of FSS on mesenchymal stem, bone, and periodontal ligament cells of human and mouse origin using a predefined search strategy. We extracted all information about the experimental setups, experimental methods for evaluating FSS-related cell response, and gene/ metabolite regulation. An extensive quantitative/qualitative summary and analysis of the commonly used fluid flow profiles, apparatuses, durations and magnitudes of applied FSS, and the commonly upregulated genes and metabolites was performed. The relationship between the nature of investigated tissue, pore size, and movement (displacement) in relation to fluid flow profile and the encountered FSS magnitude were analysed and discussed. The extracted information was also used for performing protein-protein (PPI) network construction and enrichment analysis, to get an overview of the protein-to-protein interaction and the important biological process and signalling pathways involved in FSS-related periodontal ligament (PDL) or bone interaction.

(2) In the project's second phase, we established an *in vitro* FSS setup after analysing/reviewing all the designs extracted from the first part of the project. We considered the simplicity of the design, biocompatibility of the material, and multiple and ease of usage. Computer-aided design, additive, and subtractive manufacturing were used in the chamber fabrication process using PDMS. The setup was extensively evaluated *in silico* and the chamber's performance was tested to confirm flow rate, flowing medium temperature, and cell attachment and viability. Fluid shear stress was applied to hPDLCs, focusing on gene expression related to mechanosensing, tissue formation, and inflammation.

2.2 Short summary of the publications

2.2.1 Paper I. Fluid flow shear stress and tissue remodeling—an orthodontic perspective: evidence synthesis and differential gene expression network analysis

Graphical abstract paper 1 (G.A. 1)



As introduced in the previous section, FSS is caused by the shifting (movement) of interstitial fluid inside the PDL during OTM owing to a shift in pore pressure (G.A. 1A.). Due to the limited number of studies (=7) that investigated the influence of FSS on hPDLCs during OTM, our objective was to include all published studies examining the impact of FSS on periodontal ligament (hPDLCs) and bone cells of human or mouse origin *in vitro*.

In this study, we summarized the force specifications and regulations of the genes/metabolites and identified the biological processes and pathways potentially influenced by FSS. A total of 6,974 publications were identified using a predefined search formula (Nile et al. 2023). After screening the titles, abstracts, and full texts of relevant literature, 120 studies were considered for risk of bias evaluation and data extraction, **G.A. 1B**. The data extraction covered the mechanical characteristics of the different FSS setups, and the genes/proteins/metabolites reported (Nile et al. 2023). Using the extracted data of the investigated genes, a network analysis was performed. Overrepresentation gene analysis and set enrichment analysis were performed using preselected High-throughput gene expression studies.

2.2.1.1 Type of fluid flow characteristics and apparatuses for shear stress application

Fluid flow can be delivered on cells in different forms including steady, pulsatile, and oscillatory laminar. In our systematic review, we found that different cell groups had different investigated fluid flow profiles, based on their research objective. As shown in **G.A. 1C**, oscillatory laminar was

most frequently applied in hMSC (10/21; 47.6%) and mOcyt (21/58; 32.6%) cell groups; steady laminar in hPDLC (5/7; 71.4%) and mOst (9/22; 40.9%) cell groups; pulsatile laminar in hOcyt (100%), hOst (10/14; 71.4%) and mOst (9/22; 40.9%) cell groups (Nile et al. 2023).

Different fluid flow chambers were implemented in each experiment (Nile et al. 2023), **G.A. 1D**. Custom-made fluid flow chambers were most frequently utilized (76/123; 61.7%). The most used commercial fluid flow chambers were from Flexcell Inc. (12/123; 9.7%) and ibidi (6/123; 4.8%) and can deliver different fluid flow profiles. The Focht Chamber System 2, the Bioflux system, and the PeCon parallel plate system are commercial fluid flow chambers used for live imaging, each was identified once. Rocking culture systems (4/123; 3.2%) and rotational orbital shakers (3/123; 2.4%) were utilized to generate a culturing environment of oscillatory fluid flow.

2.2.1.2 Magnitudes, durations, and frequencies of FSS

FSS magnitude and durations showed great variation between cell groups independent of their origin (human or mouse) and fluid flow profile (Nile et al. 2023). These were summarised for human cells in **G.A. 1E.**

Frequency: the most frequently utilized fluid flow frequency was 1 Hz in the hMSCs cell group and 5 Hz in the hOst, hOcyt, and hPDLCs cell groups (Nile et al. 2023). In the mOst cell group, a frequency of 5 Hz was used twice as often as 1 Hz, while in the mOcyt cell group, both frequencies were used equally (Nile et al. 2023).
Selection of force parameters: this study considered analysing different cell groups representing the cells found within the periodontium. While not all studies had the same objective and due to the limitation of the number of studies investigating the effect of FSS during OTM, we found that these studies were directed to the general overview of FSS created by forces during speech, tooth movement, or mastication rather than justifying the FSS magnitude, duration and flow profile used during the experiment (Maeda et al. 2007; Tang et al. 2014; van der Pauw et al. 2000; Zheng et al. 2016; Zheng et al. 2019). For instance, the fluid flow profile created by cyclic movement differs from that of instant movement, see below.

In our study, we gathered and summarized all the relevant experimental and biological details. We then correlated this information with finite element analysis studies, such as those determining the FSS in ligaments and tendons (Chen et al. 1998) and those explaining the direction of flow during OTM (Ashrafi et al. 2020). While fluid flow in bone cells/tissue was extensively investigated compared to PDL, we found that cyclic movements such as walking and mastication/chewing consist of cyclic loading and unloading that may result in a different fluid flow profile (physiological: oscillatory fluid flow) compared to postural change (e.g. changing posture from sitting to standing) or therapeutic force application (e.g. OTM) that causes a unidirectional fluid flow (steady fluid flow) (Ashrafi et al. 2020; Jacobs et al. 1998; Lu et al. 2012; Ortún-Terrazas et al. 2020; Ponik et al. 2007). Therefore, the fluid flow profile during speech or mastication/chewing may not resemble the fluid flow profile during OTM. Also, the cellular response may differ when cells are exposed to different flow profile (Jacobs et al. 1998).

2.2.1.3 Genes, proteins, and metabolites investigated

Based on the collected data from each study, we found that despite the cell type FSS activated genes and metabolites responsible for tissue formation and inflammation and downregulated genes accountable for tissue degradation (Nile et al. 2023). Regulatory biological changes triggered by FSS application were identified and measured, followed by a summary of gene/pro-

tein/metabolites expression patterns. At least three studies that reported common genes/proteins/metabolites independent of the analysed cell types were included in the analysis. Twenty-two genes, proteins, and metabolites were part of the analysis. The colour represents the ratio of gene/proteins/metabolite upregulation in each cell group, summarized in **G.A. 1F** (Nile et al. 2023).

2.2.1.4 Network analysis

Differentially expressed genes induced by FSS in different species were curated from identified studies, excluding high-throughput expression data. RT-qPCR-derived gene expression data were included, focusing on clear FSS-dependent expression patterns. Protein expression data were omitted due to methodological heterogeneity. Adjusting the STRING-DB cut-off score facilitated the integration of additional genes into the networks, primarily supported by text-mining results. Lowering the cut-off score affected network statistics, potentially influencing cluster and hub gene identification. Clustering algorithms revealed variations in network structure based on the chosen cut-off score, impacting downstream enrichment analysis.

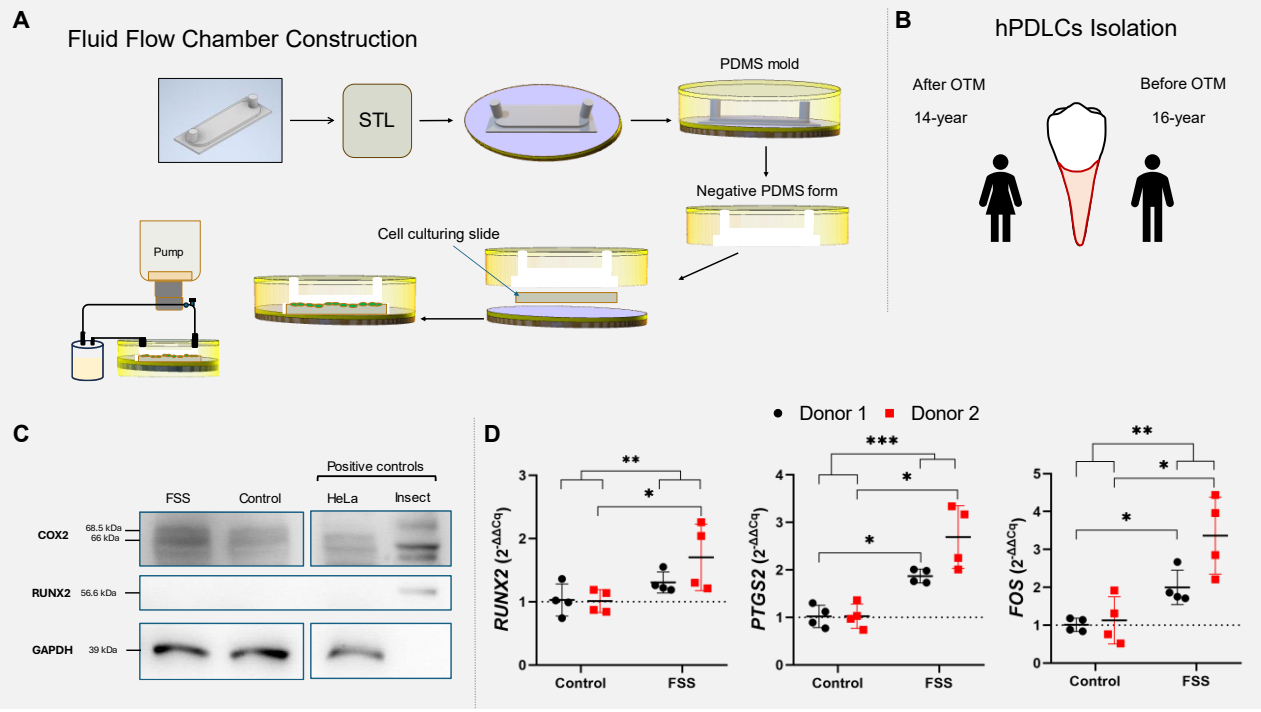
Overrepresentation analysis was utilized to uncover biological processes and signalling pathways influenced by FSS in both human and mouse DEG lists. In humans, enriched terms cantered on cytoskeleton/extracellular matrix reorganization and ossification, alongside pathways like ERK1/ERK2 and Toll-like receptor signalling. Community detection identified clusters focusing on ossification, smooth muscle cell proliferation, and inflammation. The 11 genes identified as hubs were primarily associated with clusters #1 (IGF1, RUNX2) and #2 (IL1B, IL6, JUN, MMP1, MMP2, TGFB1, TIMP1, VEGFA) (Nile et al. 2023). FGF2 was the sole hub gene in cluster #3, while cluster #4 had no hub genes.

Mouse DEG analysis revealed enrichment in organ/tissue development, ossification, and WNT-related signalling, with clusters emphasizing WNT and BMP signalling, cell proliferation/migration, and bone remodelling. Identified hub genes were predominantly associated with these clusters, providing insights into the regulatory mechanisms underlying FSS response. The 12 genes identified as hubs were largely associated with clusters #1 (DKK1, LRP5, SFRP1, WNT1, WNT3A, WNT4, WNT5A) and #2 (ACTB, IL6, JUN, TNF), with a single hub gene located in cluster #3 (CTNNB1) (Nile et al. 2023).

Gene set enrichment analysis: five high-throughput gene expression studies in humans and mice were found, focusing on FSS impact on hMSCs and MLO-Y4 cells. Enriched pathways in hMSCs included inflammation, TNF-, MAPK-, and Toll-like receptor signalling, alongside metabolic pathways and cytosolic DNA sensing. In MLO-Y4 cells, enriched pathways encompassed ribosomal RNA processing, inflammatory response, chemotaxis, and signalling pathways involving NF-KappaB, TNF, IL17, and cytokine receptor interaction, identified across multiple studies.

2.2.2 Paper II. Development of a Custom Fluid Flow Chamber for Investigating the Effects of Shear Stress on Periodontal Ligament Cells

Graphical abstract paper 2 (G.A. 2)



During OTM, applied forces create compression and tension regions in the PDL, leading to interstitial fluid displacement. FSS is a natural consequence of these forces, though it has been less studied than static or cyclic compression/tension alone. In the first part of this thesis (Nile et al. 2023), we highlighted the limited research on FSS-related topics in dentistry, with only 7 identified studies exploring this topic.

Correlating the magnitude of FSS *in vitro* to *in vivo* conditions during OTM is challenging because of the complex tissue architecture. Due to the need for more research to fill the gap we found in the literature and to clarify the biological relevancy of FSS to OTM, we constructed an *in vitro* setup to uncover the effect of FSS on hPDLCs. The experimental setup described below focused on the biological response of hPDLCs to FSS in strictly controlled simplified *in vitro* conditions. Unidirectional laminar flow was chosen to mimic the unilateral forces proposed in OTM. The use of a flow chamber and the applied FSS levels (1-6 dyn/cm^2) were selected based on previous research and summarized in the first part of this project. In addition, studies have shown that FSS levels of 1-6 dyn/cm^2 promote PDL cell proliferation, while higher levels (9 dyn/cm^2) inhibit it (Shi et al. 2022), aligning with FEM analysis estimates of 12.1 dyn/cm^2 in the collagen fibrils' narrowest zones in ligaments (Chen et al. 1998).

2.2.2.1 Construction and assessment of the apparatus

Different FSS apparatuses were used in literature, each with inherited advantages and limitations as discussed by the first publication. In the second part, the goal was to design and build a parallel plate system that could deliver uniform FSS to cells. We focused on ease of use, cost-effective construction, and the ability to handle a large cell density for downstream analysis. A 3D-designed

apparatus was developed taking into consideration the possibility of assembly and disassembly, **G.A. 2A**. The parallel flow chamber consists of different components: a chamber made of PDMS, threaded nozzles, closing lid, closing frames, and clamps. The fluid flow system consists of additional components including but not limited to a water heating bath, media reservoir, peristaltic pump, pulse damper, and bubble tap. All components are connected through silicon tubing.

The chamber was validated *in silico* to confirm the distribution of FSS, determine areas of turbulence, and locate a stress-constant cell seeding area. The fluid flow rate and temperature of the running media inside the chamber were calibrated during the pre-experimental testing. In addition, the possibility of leakage, assembly and disassembly, and sterilization/disinfection of the parts were evaluated in the pre-experiments. Custom-made cell seeding gaskets were constructed to seed cells in a shear-constant area of the microscopic slide.

For the *in vitro* experiment, the cells were seeded on the microscopic slides after coating them with collagen using custom-made cell culturing gasket wells. After incubating the cells overnight, the slides were inserted inside the chamber, which was then closed and secured by clamps. The cells were then subjected to FSS for 1h 6 dyn/cm^2, **G.A. 2A**. The chamber was tested *in vitro* using SaOS-2 and hPDLCs to confirm the cell attachment and viability under the selected stress level. Overall, the cells showed minimal de-attachment, and the viability remained unaffected with only a limited number of dying cells.

2.2.2.2 Study design

To explore the impact of FSS on gene and protein expression in hPDLCs, several genes and metabolites were selected for analysis including those involved in tissue formation, mechanosensing, and inflammation. These genes and metabolites were commonly investigated and are involved in bone and periodontal ligament remodelling (Nile et al. 2023).

hPDLCs cells from two donors (cells were harvested before or after clinical OTM, male and female, respectively) were selected for the experiments, **G.A. 2B**. The cells were exposed to a laminar fluid flow of 6 dyn/m^2 FSS for 1 hour (Nile et al. 2024). Cell lysates were collected to determine gene (using RNA lysis buffer) and intracellular protein expression (using ice-cold RIPA buffer). Following the MIQE guidelines (Bustin et al. 2009; Bustin et al. 2010), which recommends assessing the stability of reference genes for each specific study, and using more than one reference gene, multiple reference genes were selected. Consequently, RNA cell lysates were used to evaluate the stability of several reference genes, using the RefFinder program (Xie et al. 2012). Based on this evaluation, *RPL0* and *RPL22* were identified as the two most stable reference genes and selected for further target gene analysis (Nile et al. 2024). RT-qPCR was used to examine the expression of target genes, concentrating on tissue formation (*VEGFA, RUNX2, SP7, TNFRSF11B/OPG*), mechanosensing (*FOS*), and inflammation (CXCL8/*IL8, IL6, and PTGS2/COX2*) (Nile et al. 2024). Cell lysates extracted using RPA buffer were analysed by Western blot using primary antibodies targeting COX2, RUNX2, and GAPDH. HeLa and baculovirus-insect cell lysates were used as a positive control for GAPDH and RUNX2/COX2 respectively (Nile et al. 2024).

2.2.2.3 Results

The effect of FSS on mechanosensing of hPDLCs: after one hour of application of 6 dyn/cm^2 FSS on cells, the expression of *FOS*, a gene related to mechanosensation, significantly increased compared to the control in both donors (Nile et al. 2024), **G.A. 2D**. However, donor 2 (cells obtained prior OTM) showed a two-fold change compared to donor 1 (cells obtained after OTM),

with only a one-fold change (Nile et al. 2024). Previous studies highlighted the importance of *FOS* in osteoblasts' cell cycle control and differentiation as well as regulating the bone's adaptive response to mechanical stimuli through *PTGS2*/*COX2* (Grigoriadis et al. 1993; Wagner and Eferl 2005). Therefore, our data repropose FSS as a potential mechano-stimulatory stress acting on cells during OTM in addition to compression and stretching.

The effect of FSS on osteogenesis of hPDLCs: for genes involved in bone formation, *RUNX2* showed significant upregulation in donor 2 compared to its corresponding control after the application 1h 6 dyn/cm^2 FSS (Nile et al. 2024), **G.A. 2D**. Although donor 1 showed upregulation of *RUNX2*, this was not statically insignificant. In contrast to our RT-qPCR data for *RUNX2* in both donors, our Western blot result showed no expression of RUNX2 proteins, **G.A. 2C** (Nile et al. 2024). Similarly, *VEGFA* showed significant upregulation in donor 2 in relation to its control compared to donor 1 after 1h 6 dyn/cm^2 FSS (Nile et al. 2024). No regulation of *SP7* and *TNFRSF11B/OPG* in response to FSS was observed in either donor (Nile et al. 2024). Although we did not observe any regulation of *SP7* and *TNFRSF11B/OPG* immediately after 1 hour of FSS, previous research has reported significant upregulation of *SP7* after at least 6 hours of post-FSS incubation (Tang et al. 2014) and *TNFRSF11B* after 72 hours of FSS (Aisha et al. 2015; van der Meijden et al. 2016).

Effect of FSS on inflammation of hPDLCs: Applying 6 dyn/cm^2 on cells for one hour led to the upregulation of pro-inflammatory genes (*PTGS2/COX2, CXCL8/IL8, IL6*) in both cells (Nile et al. 2024), **G.A. 2D**. Overall, the expression of these genes was greater in donor 2 compared to donor 1. FSS resulted in significant upregulation of *PTGS2/COX2 and IL6* in donor 2, while it was significant for *CXCL8/IL8* and *PTGS2/COX2* in donor 1 (Nile et al. 2024). Similarly, the western blot data showed overexpression of PTGS2/COX2 proteins in FSS-treated cells compared to its controls with a greater difference observed between FSS-treated cells of donor 2 compared to those from donor 1, **G.A. 2C** (Nile et al. 2024).

2.2.2.4 Strengths and limitations:

The fluid flow chamber offers several advantages, including biocompatibility, durability, adaptability to diverse research questions, decomposability for sample retrieval, sufficient sample size for subsequent analysis, and cost-effectiveness. However, limitations include the usage of high media volume, the need for disinfection for reuse, and performing experiments outside the incubator. Also, the chamber is incompatible with live microscopy, thus limiting real-time cellular analysis and turbulence assessment. Lastly, additional genes should be included to capture the broader biological responses to FSS fully, and more cells from different donors are needed to address biological variability.

2.2.3 Conclusions

In conclusion, the need for constructing a custom-made apparatus was determined by the first part of the PhD project including the selection of the most appropriate fluid flow character, shear stress magnitude, and duration. Nevertheless, we gained an understanding of how FSS influences the expression of different cells, including human and mouse periodontal ligament and bone cells. In the second phase of the PhD project, a fluid flow apparatus was designed and constructed taking into consideration the ease of handling, reusability, and sample collection for further analysis. The chamber was constructed from PDMS using negative moulding technique. The chamber was then extensively pre-tested to evaluate the fluid flow rate, temperature of media inside the chamber, and the possibility of leakage. Then, it was evaluated with cells to confirm

cell attachment and viability. The most appropriate reference gene was determined for the experiment by testing and assessing several reference genes. Moreover, the influence of 6 dyn/cm^2 FSS for 1 h on genes related to mechanosensing, tissue formation, and inflammation was determined in hPDLCs of two donors. The findings of the *in vitro* experiment support the conclusion that we determined by the first part of the PhD project. This project highlights the important role of FSS as a cellular modulatory factor together with compression and tension during OTM.

3. Paper I

Nile M, Folwaczny M, Wichelhaus A, Baumert U, Janjic Rankovic M (2023). Fluid flow shear stress and tissue remodeling-an orthodontic perspective: evidence synthesis and differential gene expression network analysis. Front. Bioeng. Biotechnol. 11: 1256825.
PMID: https://www.ncbi.nlm.nih.gov/pubmed/37795174
PMC: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10545883/
DOI: https://doi.org/10.3389/fbioe.2023.1256825

4. Paper II

Nile M, Folwaczny M, Kessler A, Wichelhaus A, Janjic Rankovic M, Baumert U (2024). Development of a Custom Fluid Flow Chamber for Investigating the Effects of Shear Stress on Periodontal Ligament Cells. Cells 13(21):1751.
PMID: https://www.ncbi.nlm.nih.gov/pubmed/39513858
PMC: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC11545369/
DOI: https://doi.org/10.3390/cells13211751

Summary

In the first part of this project, we represented an extensive extraction and analysis of mechanical stress and biology data. It summarized the most frequently used apparatus, magnitudes, durations, and the character of fluid flow, and then compared and discussed these findings in the context of functional and biological situations. The study investigated the regulation of genes, proteins, and metabolites, followed by network analysis and gene set enrichment analysis, providing deeper insights into the biological mechanisms underlying FSS. Several research gaps were identified, including the lack of experimental data regarding the level of FSS in periodontal ligament during OTM, the misconception between fluid flow profile during daily activities (e.g., mastication/chewing) and therapeutic means (e.g., OTM), and the limited research that concentrated on FSS in relation to OTM.

In the second part of this PhD project, a fluid flow apparatus was designed and constructed taking into consideration the ease of handling, reusability, and sample collection for further analysis. The chamber was constructed from PDMS through a negative moulding process. The chamber was then extensively pre-tested to evaluate the fluid flow rate, media temperature inside the chamber, and the possibility of leakage. Then, it was assessed with cells to confirm cell attachment and viability. The most appropriate reference gene was determined for the experiment by testing and assessing a panel of reference genes. Exposure to 6 dyn/cm^2 FSS for 1 h increased the expression of genes responsible for mechanosensing, tissue formation, and inflammation in the hPDLCs of two donors. The findings of the *in vitro* experiment support the conclusion that we determined by the first part of the PhD project. These findings as well as the differences among donors provide valuable insights for further experiments. It also highlights the role of FSS as a cellular modulatory factor together with compression and tension in OTM.

References

Aisha MD, Nor-Ashikin MN, Sharaniza AB, Nawawi H, Froemming GR (2015). Orbital fluid shear stress promotes osteoblast metabolism, proliferation and alkaline phosphates activity in vitro. Exp Cell Res; 337(1):87-93.

Ashrafi M, Ghalichi F, Mirzakouchaki B, Zoljanahi Oskui I (2020). Numerical simulation of hydro-mechanical coupling of periodontal ligament. Proc Inst Mech Eng H; 234(2):171-178.

Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT (2009). The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem; 55(4):611-22.

Bustin SA, Beaulieu JF, Huggett J, Jaggi R, Kibenge FS, Olsvik PA, Penning LC, Toegel S (2010). MIQE precis: Practical implementation of minimum standard guidelines for fluorescence-based quantitative real-time PCR experiments. BMC Mol Biol; 11:74.

Chen CT, Malkus DS, Vanderby R, Jr. (1998). A fiber matrix model for interstitial fluid flow and permeability in ligaments and tendons. Biorheology; 35(2):103-18.

Grigoriadis AE, Schellander K, Wang ZQ, Wagner EF (1993). Osteoblasts are target cells for transformation in c-fos transgenic mice. J Cell Biol; 122(3):685-701.

Hwang J, Ing MH, Salazar A, Lassegue B, Griendling K, Navab M, Sevanian A, Hsiai TK (2003). Pulsatile versus oscillatory shear stress regulates NADPH oxidase subunit expression: implication for native LDL oxidation. Circ Res; 93(12):1225-32.

Jacobs CR, Yellowley CE, Davis BR, Zhou Z, Cimbala JM, Donahue HJ (1998). Differential effect of steady versus oscillating flow on bone cells. J Biomech; 31(11):969-76.

Janjic M, Docheva D, Trickovic Janjic O, Wichelhaus A, Baumert U (2018). *In vitro* weight-loaded cell models for understanding mechanodependent molecular pathways involved in orthodontic tooth movement: a systematic review. Stem Cells International; 2018:3208285.

Krishnan V, Davidovitch Z (2006). Cellular, molecular, and tissue-level reactions to orthodontic force. Am J Orthod Dentofacial Orthop; 129(4):469 e1-32.

Krishnan V, Davidovitch Z (2009). On a path to unfolding the biological mechanisms of orthodontic tooth movement. J Dent Res; 88(7):597-608.

Lu XL, Huo B, Park M, Guo XE (2012). Calcium response in osteocytic networks under steady and oscillatory fluid flow. Bone; 51(3):466-73.

Maeda A, Soejima K, Bandow K, Kuroe K, Kakimoto K, Miyawaki S, Okamoto A, Matsuguchi T (2007). Force-induced IL-8 from periodontal ligament cells requires IL-1beta. J Dent Res; 86(7):629-34.

Nile M, Folwaczny M, Wichelhaus A, Baumert U, Janjic Rankovic M (2023). Fluid flow shear stress and tissue remodeling-an orthodontic perspective: evidence synthesis and differential gene expression network analysis. Front Bioeng Biotechnol; 11:1256825.

Nile M, Folwaczny M, Kessler A, Wichelhaus A, Janjic Rankovic M, Baumert U (2024). Development of a Custom Fluid Flow Chamber for Investigating the Effects of Shear Stress on Periodontal Ligament Cells. Cells; 13(21):1751.

Ortún-Terrazas J, Cegoñino J, Pérez Del Palomar A (2020). In silico study of cuspid' periodontal ligament damage under parafunctional and traumatic conditions of whole-mouth occlusions. A patient-specific evaluation. Comput Methods Programs Biomed; 184:105107.

Piekarski K, Munro M (1977). Transport mechanism operating between blood supply and osteocytes in long bones. Nature; 269(5623):80-2.

Ponik SM, Triplett JW, Pavalko FM (2007). Osteoblasts and osteocytes respond differently to oscillatory and unidirectional fluid flow profiles. J Cell Biochem; 100(3):794-807.

Shi Q, Zheng L, Na J, Li X, Yang Z, Chen X, Song Y, Li C, Zhou L, Fan Y (2022). Fluid shear stress promotes periodontal ligament cells proliferation via p38-AMOT-YAP. Cell Mol Life Sci; 79(11):551.

Sun C, Janjic Rankovic M, Folwaczny M, Otto S, Wichelhaus A, Baumert U (2021). Effect of Tension on Human Periodontal Ligament Cells: Systematic Review and Network Analysis. Front Bioeng Biotechnol; 9:695053.

Tang M, Peng Z, Mai Z, Chen L, Mao Q, Chen Z, Chen Q, Liu L, Wang Y, Ai H (2014). Fluid shear stress stimulates osteogenic differentiation of human periodontal ligament cells via the extracellular signal-regulated kinase 1/2 and p38 mitogen-activated protein kinase signaling pathways. J Periodontol; 85(12):1806-13.

van der Meijden K, Bakker AD, van Essen HW, Heijboer AC, Schulten EA, Lips P, Bravenboer N (2016). Mechanical loading and the synthesis of 1,25(OH)2D in primary human osteoblasts. J Steroid Biochem Mol Biol; 156:32-9.

van der Pauw MT, Klein-Nulend J, van den Bos T, Burger EH, Everts V, Beertsen W (2000). Response of periodontal ligament fibroblasts and gingival fibroblasts to pulsating fluid flow: nitric oxide and prostaglandin E_2 release and expression of tissue non-specific alkaline phosphatase activity. J Periodontal Res; 35(6):335-43.

Wagner EF, Eferl R (2005). Fos/AP-1 proteins in bone and the immune system. Immunol Rev; 208:126-40.

Wang Z, Du S, Zhu H, Yi K, Tang Z, Li Q (2024). A finite element analysis of periodontal ligament fluid mechanics response to occlusal loading based on hydro-mechanical coupling model. Arch Oral Biol; 164:106008.

Xie F, Xiao P, Chen D, Xu L, Zhang B (2012). miRDeepFinder: a miRNA analysis tool for deep sequencing of plant small RNAs. Plant Mol Biol; 80:75-84.

Zheng L, Chen L, Chen Y, Gui J, Li Q, Huang Y, Liu M, Jia X, Song W, Ji J, Gong X, Shi R, Fan Y (2016). The effects of fluid shear stress on proliferation and osteogenesis of human periodontal ligament cells. J Biomech; 49(4):572-9.

Zheng L, Shi Q, Na J, Liu N, Guo Y, Fan Y (2019). Platelet-Derived Growth Factor Receptor-α and β are Involved in Fluid Shear Stress Regulated Cell Migration in Human Periodontal Ligament Cells. Cell Mol Bioeng; 12(1):85-97.

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