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Development of a three-dimensional *in vitro* model system to study orthodontic tooth movement

A.F. Heckler [a], Z. Mirzaei [c], I. Pereira [c], C.A. Simmons [b,c,d,*], S.-G. Gong [a,b,**]

[a] Department of Orthodontics, University of Toronto, Canada
[b] Dental Research Institute, Faculty of Dentistry, University of Toronto, Canada
[c] Institute of Biomaterials and Biomedical Engineering, University of Toronto, Canada
[d] Department of Mechanical and Industrial Engineering, University of Toronto, Canada

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ABSTRACT

Few three-dimensional (3-D) models exist to study the cellular aspects and molecular regulation of orthodontic tooth movement (OTM). The aim of this study was to develop a 3-D *in vitro* model to study mechanical loading of human periodontal ligament (PDL) fibroblasts (hPDLF). hPDLF were seeded within collagen gels to form a PDLF analogue. Characterisation of the seeded collagen gels revealed that the gels supported cell proliferation, viability and the emergence of a possible contractile phenotype, replicating the constrained condition of the human PDL *in vivo*. We next developed a 3-D model that incorporated a seeded collagen gel interlocked mechanically at two ends to movable end plates. The movable end plates allowed for static tensile or compressive loading of the hPDLF-seeded collagen gels. Preliminary testings showed that this 3-D model mimicked PDL strains similar to those observed during OTM. Our 3-D model of OTM therefore offers promise for use as a model system in future studies to improve our understanding of the effects of OTM on PDLF.

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1. Introduction

Optimal orthodontic tooth movement (OTM) requires a better understanding of the basic cellular and molecular changes that occur in the tissues surrounding the tooth. It has been said that "life's complexity and organisation.are illustrated in the biological phenomena underlying orthodontic tooth movement".[1] The tooth is surrounded by the periodontal ligament (PDL) that is also attached to the adjacent alveolar bone. During OTM, mechanical stress loaded onto a tooth is transduced to the PDL and results in bone remodelling. The specific nature of the changes that occurs in the PDL and its surrounding structures during OTM is determined by cell-cell and -matrix interactions. Some of the molecules involved during OTM have been identified, *e.g.*, prostaglandins, cAMP, inositol phosphates, integrins and their receptors, RANKL, and its receptor RANK.[2–11] However, the degree of change and the molecular regulation of specific changes following the application of a force to a tooth are still not well characterised. One main hindrance towards a better understanding of the biological and molecular phenomena of OTM is the lack of suitable model systems.

Studies dedicated towards a better understanding of the molecular basis of OTM have so far taken advantage of both *in vivo* animal and *in vitro* models. Animals used in OTM

* *Corresponding author at*: Dental Research Institute, Faculty of Dentistry, University of Toronto, 124 Edward Street, Toronto, ON, Canada M5G 1G6. Tel.: +1 416 946 0548.
** *Corresponding author at*: Dental Research Institute, Faculty of Dentistry, University of Toronto, 124 Edward Street, Toronto, ON, Canada M5G 1G6. Tel.: +1 416 979 4916x4588.
E-mail addresses: c.simmons@utoronto.ca (C.A. Simmons), sg.gong@dentistry.utoronto.ca (S.-G. Gong).
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studies over the last century included dogs, cats, hamsters, rats, rabbits, guinea pigs, and monkeys.[12–17] *In vivo* models present difficulties with regard to the control of the specific characteristics of the mechanical strain and the exact magnitude, location, duration of applied forces and the different anatomical consideration with respect to tooth shape and pattern of eruption. Furthermore, the large number of cellular subtypes *in vivo* makes it difficult in some *in vivo* models to be able to accurately identify specific markers for the cells of interest. *In vitro* models of tooth movement were developed in the '70s and '80s[2,18,19] and some more recently[20–22] and allow the investigator to more closely control the mechanical stimulus. These *in vitro* models generally use specific cell lines of more homogenous populations. The use of specific cell lines leads to easier identification of cellular markers but prevents accurate representation of many of the cascades of events that occur when different cell types interact *in vivo*.[23] Many current cell cultures used in *in vitro* models are two-dimensional (2-D) and do not allow fibroblasts to develop their *in vivo* morphologies. In a three-dimensional (3-D) structure, fibroblasts develop elongated or stellate shapes, developing different matrix adhesions that spread over the entire cell surface.[3] Additionally, the characteristics of the forces applied to 2-D cells cultures may also not be representative of the resultant forces acting on the 3-D PDL.[24] An *in vitro* system that provides a medium for culture of cellular components of the human PDL within a 3-D model could overcome the limitations inherent in pre-existing *in vivo* and *in vitro* models and provide an essential link between these two experimental designs for evaluating OTM. The goal of this current study, therefore, was to develop a model that more closely resembles the 3-D structure of the tooth supporting apparatus than previous *in vitro* models. We had two specific objectives: to characterise the use of human PDL cells in a collagen gel analogue of PDL and to design and test the use of a 3-D model for the application of compressive and tensile forces to the PDL analogue. Our results indicate that our 3-D model has the potential to improve our understanding of the cellular and molecular basis of tooth movement, ultimately translating into improved orthodontic care and delivery.

2. Materials and methods

2.1. Initial characterisation of hPDLF within collagen gels in wells

Human periodontal ligament fibroblasts (hPDLF) (ScienCell Cat#2630; San Diego, CA) were thawed, expanded, and frozen according to manufacturer's instructions. Passage 3 hPDLF cells were mixed with sterile PureCol (type I bovine collagen 3.1 mg/mL; Inamed Biomaterials, Freemont, CA) at a seeding density comparable to previously documented studies, *i.e.* 1×10^5 to 1×10^6 cells/mL of gel.[18,25–31] The gels were polymerised by the addition of 0.01 M NaOH.

For preliminary characterisation of cell viability and growth, 2 mm thick gels in 24-well tissue culture plates was performed at 24 h, 3 days, 7 days, and 14 days (10 gels/time points; total = 40). These times points are consistent with previous studies of 3D cell cultures, and human and animal models of tooth movement that showed early cellular activities such as proliferation and the initiation of recruitment of tissue-remodelling cells.[12,15,32,33] Cells were seeded at a density of 3.33×10^5 cells/mL of gel. Cells and gels were maintained in a sterile environment at 37 °C, in 100% humidity and 5% CO_2, and media was changed every 48 h. At each time period, samples were processed and analysed for subsequent analyses, as described below.

2.2. Development of 3-D model system to apply OTM-relevant forces

The collagen gel was formed in the space constrained on 2 edges only in a mould which comprised the following (Fig. 4; refer to Supplemental Material for exact dimensions):

- a Teflon® base with pin holes determining the position of movable blocks
- 2 movable polycarbonate blocks with both vertical and horizontal pin holes
- 2 stainless steel pins which hold the movable blocks in position
- 2 mm high strip of Velcro at the base of each of the 2 polycarbonate blocks facing the middle of the mould a(to which the collagen gel can attach)

The base and side blocks of the mould were machined from stock Teflon® rods and stock polycarbonate rods, respectively. The pins, of 1 mm diameter stainless steel wire, were 17 mm long and of sufficient length to maintain the position of the side blocks in the mould and yet be able to fit within a closed 6-well plate. 2 mm thick Velcro strips were cut to line the entire width of the side blocks to allow mechanical interlocking of the collagen gel fibrils with the blocks. The loop side of the Velcro strip was used due to the smaller spaces between fibres and improved incorporation of gel within the fibres (Fig. 4; Supplemental Material). The components of the mould were sterilised individually by autoclaving prior to assembly. Within a sterile cell culture hood, the Velcro strips were glued at the inferior margin to the polycarbonate blocks using ethyl-2-cyanoacrylate glue (Krazy Glue) with commercially-available plastic conditioner and allowed to polymerise for 24 h in an incubator. The model was then assembled within a sterile culture hood and autoclaved vacuum grease was used to seal the joints between the blocks and the Teflon® moulds. The collagen gel mixture was then placed in the mould and allowed to gel.

2.3. Finite element analysis of gel strains under tension and compression

The strains generated throughout the collagen gels in the moulds.under both tension and compression were estimated by finite element (FE) analysis. A two-dimensional plane stress model was used to represent the 9.5 mm wide $\times$ 8 mm long gel in the mould (Fig. 5). The FE model contained 525 second-order plane stress elements. The gel was assumed to have a linear elastic modulus, E, of 20 kPa (typical of collagen gels) and a

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