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Review

In vitro mechanical loading models for periodontal ligament cells: From two-dimensional to three-dimensional models

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ABSTRACT

Periodontal ligament cells (PDLCs) respond to various mechanical stimuli, including mastication and orthodontic force, and thereby play an important functional role. A number of *in vitro* models have been widely used to study these mechanoresponses. Here, we comprehensively review the various *in vitro* mechanical loading approaches used for assessing PDLCs, including their force generation properties, mechanical characteristics, and simulation of authentic bioprocesses. Furthermore, we highlight the evolution of current cytomechanical studies, from conventional two-dimensional to novel three-dimensional (3-D) cell cultures. Some representative 3-D PDLC culture and mechanical loading systems are also described, with the advantages and limitations of these discussed. From this review, we can conclude that optimal mechanical loading models must be chosen to match the specific research purpose, and that novel 3-D PDLC culture and mechanical loading models are promising for future studies.

Contents

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1. Introduction

The periodontium is a structure that encompasses the tooth and functions to support it during mastication. It consists of gingiva, alveolar bone, cementum, and periodontal ligament (PDL).[1] The PDL is a highly specialised, fibrous connective tissue situated between the cementum and alveolar bone, and although it is physically small, it is also functionally important.[2–4] There has long been interest in PDL as a unique connective tissue capable of responding discriminatorily to various mechanical stimulations, including mastication, lip or tongue pressure, and orthodontic force.[5]

The essential functional components of PDL are heterogeneous cells, including fibroblasts, epithelial cells, endothelial cells, and multi-potential progenitors.[6–9] Among them, PDL fibroblasts are predominant in number and play an essential function role and are often referred to as PDL cells (PDLCs). PDLCs can respond to mechanical loading transferred from the tooth, by repairing the damaged matrix, regulating alveolar bone remodelling, and signalling to the surrounding cells.[10]

During mastication, which is an essential physical bioprocess, PDLCs are subjected to heavy but intermittent mechanical force. When a masticatory force is applied to the tooth, the fluid-filled PDL acts as a shock absorber, stabilising the tooth for an instant, whilst the alveolar bone bends and the tooth is displaced for a short distance along with the bone. This type of intermittent or cyclic mechanical stimulation is necessary for PDL remodelling and maintenance of its homeostasis. It has been reported that lack of mechanical stimulation leads to disuse atrophy of the PDL and the progressive loss of several components in the PDL, such as Sharpey's fibers.[11]

During orthodontic treatment, the sustained force causes tooth micromotion within the PDL space, compressing the ligament at the so-called pressure side and squeezing water out of the PDL.[12] When the water is completely squeezed out, the damping effect greatly deceases, and the PDLCs are directly subjected to static compression. This in turn leads to a series of biochemical reactions that initiate bone resorption and tooth movement, followed by bone formation to stabilise the tooth again.[13] Recently, a review on the role of PDL fibroblasts in osteoclastogenesis has been conducted.[14]

To clarify the responses of PDLCs to various mechanical stimulations, a great number of studies have been carried out using various *in vitro* cellular mechanical loading models. This review provides a comprehensive assessment of these models, interpreting their force-giving approaches concerning simulation of different *in vivo* bioprocesses. Moreover, the evolution from conventional two-dimensional (2-D) to novel three-dimensional (3-D) cell culture techniques using these models is highlighted and discussed.

2. Wide application of the *in vitro* models

To obtain an estimate for the number of studies carried out using various, *in vitro* mechanical loading models on PDLCs, a literature search was performed using PubMed. The following search terms were used in combination to identify appropriate studies: (strain OR stress OR stretch OR mechanical OR force OR compression OR compressive OR shear) AND (cell* OR fibroblast*) AND ''Periodontal ligament'' [Title/Abstract]. The search was limited to studies published from 1960 to 2014.

The search resulted in the identification of 465 articles. Studies were excluded if the full text was inaccessible or if no *in vitro* mechanical loading approach was used or clearly identified. After applying these exclusion criteria, 395 articles were included. We carried out a comprehensive review of the literature, with a focus on mechanical loading techniques and protocols used. In addition, we also read relevant cited papers of these identified articles, to provide a global perspective in this review.

3. Conventional *in vitro* mechanical loading models for 2-D cultured PDLCs

3.1. *Substrate deformation-based approaches*

From the included studies, the substrate deformation-based mechanical loading model was identified as the most widely used technique. Typically, such devices employ a substrate upon which cells are cultured in a 2-D monolayer. In most cases, the substrate is an elastic membrane that is deformed by force, and the cells cultured on it are stretched simultaneously. The substrate can be deformed like a ''dome'' (Fig. 1A), which exerts biaxial stretch-strain on PDLCs.[15–17] Customised devices based on substrate deformation, such as Flexercell, have been widely used in PDLC cytomechanical studies.[18–20] In the most popular Flexercell tension system, a silicone membrane is stretched across a loading post by the application of vacuum pressure and, depending on the shape of the loading post, either a biaxial or uniaxial strain may be applied (Fig. 1B).[21] Characterisation of such devices has been perfected.[22–24] More recently, cyclic compressive and tensile strain have also been imposed on PDLCs using a four-point bending strain unit, another type of substrate, deformation-based, mechanical loading approach.[25,26] This device consists of a cell-culture plate, bent by a digitally controlled actuator on four points with adjustable amplitude and frequency (Fig. 1C). Despite some limitations of these substrate deformation-based systems in precisely defining the strain profile, they still remain widely used to investigate mechanoresponsive genes in PDLCs.