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Soft micropillar interfaces of distinct biomechanics govern behaviour of periodontal cells

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ABSTRACT

A soft micropillar extracellular environment of distinct biomechanics is established by fabricating *polydimethylsiloxane* (PDMS) interfaces with pillar distances of 5, 7, 9 and 11 μm and elasticity moduli of 0.6, 1.0 and 3.5 Mega Pascal. To allow for cell adhesion, the biomimetic concept of pillar head fibronectin (FN) biofunctionalisation is applied. This environmental set-up aims at the analysis of favourable conditions for cell behaviour of three periodontal cell-types, here reflected by the establishment of regular cell morphology and optimal collagen gene expression. Biomechanics of these predefined functionalised model surfaces reveal progressive deterioration of regular cell morphology with increasing pillar distance, independent from pillar elasticity and cell type. Analysis of collagen gene expression demonstrates interdependency to the elasticity and the micropattern of the extracellular environment in all cell types under study. The results suggest that biomechanics of the extracellular environment govern tissue-specific cell behaviour in different periodontal cell types. Moreover, they form the basis for the creation of new biomaterials which address distinct cell functions by specific biomechanical properties.

Introduction

The human periodontium comprises several cell types forming the periodontal tissues including gingiva, periodontal ligament (PDL), root cementum and alveolar bone (AB) (Bartold, 2000; Cho and Garant, 2000). As in other body tissues also in the periodontium cells respond to polypeptide growth factors thereby regulating cell behaviour in tissue homeostasis and regeneration (Giannobile et al., 2003). Under physiological conditions all cell types are embedded in an extracellular environment reflected by the cells' extracellular matrix (ECM). In addition to bind growth factors, one key function of the ECM is to provide a substrate of variable stiffness/elasticity for cell anchorage. This anchorage is crucial for cell spreading and the formation of the regular cell morphology, since only in this state the cells of solid tissues are capable to survive and maintain their ability to proliferate or differentiate by transcribing certain genes. Thus, cell behaviour is not only governed by growth factors, but also by the elasticity and the pattern of cell anchor points, which in sum reflect the biomechanical properties of the cell's environment/ECM. Consequently, understanding of favourable biomechanics is a prerequisite to achieve an optimisation of tissue-specific cell behaviour also under in vitro conditions. Such knowledge will support the elucidation of the molecular basis of tissue homeostasis and regeneration in biomechanical terms. Moreover, it will also be useful in the optimisation of already existing biomaterials or the creation of new materials in regenerative periodontology and medicine.

To reach these goals, quantitative studies of cells adhering to an extracellular environment of defined biomechanics are indispensible. Such kind of studies will be enabled by combining material technologies with life sciences. To create environmental conditions with defined biomechanical parameters, microarrays of elastic pillars or posts are contemporary used (Tan et al., 2003; Lemmon et al., 2005; du Roure et al., 2005; Mohrdieck et al., 2005; Steinberg et al., 2007; Mussig et al., 2008). These pillar microarrays consist of the silicon-based polymer polydimethylsiloxane (PDMS) which has to be biofunctionalised by ECM molecules, e.g. fibronectin (FN) to allow for proper cell adhesion (Steinberg et al., 2007). In addition to this biomimetic concept using FN, other ECM molecules have been already applied as biological implant surface coatings to improve bone formation (Stadlinger et al., 2008).

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By employing a silicon wafer as master, the PDMS-interfaces can be fabricated with pillar arrays of defined distances and defined elasticity as well. Hereby, the effects of a biomechanically predefined extracellular environment on cell behaviour can be evaluated and favourable conditions for tissue-specific cell behaviours, such as evolvement of regular shape or gene expression can be determined.

Under in vivo conditions the biomechanical cell's environment is represented by the ECM. Concerning the periodontal cell types, lamina propria gingival fibroblasts (GF), periodontal ligament fibroblasts (PDLF) and osteoblasts of the alveolar bone (OA) are all synthesising the ECM molecule collagen type-I (COL-I), the matrix constituent which displays the largest biomass in the entire periodontium (Giannobile et al., 2003). While in the lamina propria COL-I is harbouring the GF, this fibrillar collagen constitutes the sharpey fibers in the PDL, thereby interconnecting the alveolar bone and the tooth's root cementum (Cho and Garant, 2000). In case of the alveolar bone, COL-I represents the main proportion of the organic matrix essentially contributing to bone formation (Sodek and McKee, 2000). In the PDL, COL-I is involved in mineralised tissue formation during orthodontic tooth movement, when a specialised PDLF-population originating from the neural crest is assumed to form mineralised tissue, either leading to new cementum or alveolar bone material. This mineralised tissue formation occurs in the context of the tooth movement-associated mechano-sensing as response to the biomechanics of orthodontic treatment forces. This response implies modelling and remodelling processes involving cementum and alveolar bone, with resorption at the force-driven compression site and apposition of these tissues at the juxtaposed tension site (Nanci and Bosshardt, 2006). Among others, non-collagenous constituents abundant in the ECM of periodontal tissues are fibronectin (FN) which facilitates focal adhesion of cells, and alkaline phosphatase (ALP) and osteocalcin (OC) also termed "bone gla" protein, the two last-mentioned also attributed to mineralised tissue formation. Thus, ALP and OC are used as biomarkers for differentiation also in periodontal cells which share the ability to form mineralised tissue, i.e. cementoblasts, alveolar bone cells and the neural crest-descendant PDL fibroblast subpopulation (Nanci and Bosshardt, 2006).

Concerning the biomechanical properties of the extracellular environment, we have previously shown that periodontal keratinocytes from gingival epithelium favour a distinct pattern of micropillar interfaces preferably with small pillar interspaces for early keratinocyte differentiation, one of the essential cell behaviours for normal tissue homeostasis in stratified epithelia (Steinberg et al., 2007). In the present study, we report for the first time about favourable environmental biomechanics, including the two most important biomechanical parameters, microenvironmental patterning and elasticity, for the establishment of regular cell morphology and optimal expression of cell-type-specific genes. Among others, preferentially COL-I expression was quantified in three different cell types, GF, PDLF and OA, the latter two involved in mineralised periodontal tissue formation.

Materials and methods

Micropillar fabrication and biofunctionalisation

Moulds with holes of defined height and diameter were created to achieve a homogeneous thick layer of photoreactive polymers, which defines the structural height onto a solid silicon wafer. The epoxy based SU-8 10 resin (Microresist Technologies, Germany) was spin-coated under clean room conditions onto a clean silicon wafer with a defined speed. Then, the solvent was evaporated by a two step baking process. The wafer was exposed to UV light through a custom-made negative chromium mask. The photo-initiated ring opening reaction was accomplished at 95 °C on a hotplate. Developing is performed in a propylenglycol-mono-ethylether-acetate (PGMEA) containing developer (mr-Dev.600, Microresist Technologies, Germany) for 1 min. For spin-coating, 2 ml of photoresist was spun on a silicon wafer ($\varnothing$ 5.08 cm) in a two step process. To reach a 15 μm thickness, a SU-8 10 resist was spun on with 2000 rpm for 40 s. Next, softbaking of the wafer with 15 μm resist was done on a hotplate at 65 °C for 2 min, followed by baking at 95 °C for 5 min. After cooling for 10 min to room temperature (RT), UV-exposure was performed on a mask aligner (MJB3, Karl Suess, Germany), equipped with a 400 W mercury lamp. Exposure time depends on resist thickness and varies from 2.5 to 4 s. To complete cross-linking in the exposed areas, the wafer chips were heated up to 65 °C for 1 min, followed by heating up to 95 °C for 2 min, and cooled to RT for ~10 min. For developing the structures, it was necessary to re-dissolve the unexposed parts in PGMEA for up to 1 min while shaking. The structure was blown dry with a stream of nitrogen. The silicon-based polymer PDMS (polydimethylsiloxane, Dow Corning, Midland, Michigan, USA) has been mixed vigorously with a short hydrosilane cross-linker in the ratio 10:1. To remove trapped air, the mix was degassed for 30 min under vacuum at 7×10^{-2} mbar. The pillar arrays with different E-*moduli* were generated by pressing the moulds upside down onto a drop of PDMS on a 24 × 24 mm coverslip, followed by curing for 4 h at 65 °C to get an E-*modulus* of 0.6 MPa, 16 h at 65 °C for 1.0 MPa and 16 h at 65 °C plus 2 h at 180 °C for 3.5 MPa. After cooling to RT, the substrates were peeled with a razor blade and overlapping PDMS residues were removed under sterile conditions. Each pillar array presented a dimension of 8 × 8 mm with a cell growth area of 64 mm^2. The pillars had a height of 15 μm, a diameter of 5 μm and were arranged in squares with pillar interspaces of 5, 7, 9 and 11 μm.

For depositing FN only to the pillar tops, drops (100 μl) of a FN solution (10 μ/ml, Sigma-Aldrich GmbH, München, Germany) in PBS were pipetted carefully to the surfaces and incubated for 10 min at RT. Immediately after withdrawing the drop, the surface was rinsed with PBS, and directly used for cultivating cells on the pillar arrays. In this context it is noteworthy, that PDMS is a hydrophobic material and the PDMS pillar microstructure leads to water repellency, also known as the so-called lotus effect (Patankar, 2004). This is important to note because a water drop deposited on the pillar arrays only wets the pillar heads, but not the interspaced region. To control successful biofunctionalisation of the pillar tops only, indirect immunofluorescence (IIF) was performed. Since the green fluorescence was exclusively seen on the pillar heads and the interspaced regions were devoid of fluorescence, it can be assumed that seeded cells are capable to adhere only on the pillar tops (data not shown).

Determination of the E-moduli of PDMS beams

Determination of E-*moduli* from elastic substrates was performed experimentally. An easy experiment described by Pelham and Wang (1997) uses gravity. A rectangular beam of PDMS was prepared, hanged down from a freely suspended "Newton meter" and the rest length l_0 was measured. Then, a force F was applied by drawing with a defined weight of 100 gram, which correspond approximately 0.1 Newton. This value was read off a commercial "Newton meter". The resulting PDMS elongation Δl was then measured by using a caliper. The E-*modulus* E can now be