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Effect of 3D microgroove surface topography on plasma and cellular fibronectin of human gingival fibroblasts

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ABSTRACT

Objectives: Fibronectin (FN), an extracellular matrix (ECM) glycoprotein, is a key factor in the compatibility of dental implant materials. Our objective was to determine the optimal dimensions of microgrooves in the transmucosal part of a dental implant, for optimal absorption of plasma FN and expression of cellular FN by human gingival fibroblasts (HGFs).

Methods: Microgroove titanium surfaces were fabricated by photolithography with parallel grooves: 15 μm, 30 μm, or 60 μm in width and 5 μm or 10 μm in depth. Smooth titanium surfaces were used as controls. Surface hydrophilicity, plasma FN adsorption and cellular FN expression by HGFs were measured for both microgroove and control samples.

Results: We found that narrower and deeper microgrooves amplified surface hydrophobicity. A 15-μm wide microgroove was the most hydrophobic surface and a 60-μm wide microgroove was the most hydrophilic. The latter had more expression of cellular FN than any other surface, but less absorption of plasma FN than 15-μm wide microgrooves. Variation in microgroove depth did not appear to effect FN absorption or expression unless the groove was narrow (~15 or 30 μm). In those instances, the shallower depths resulted in greater expression of cellular FN.

Conclusions: Our microgrooves improved expression of cellular FN, which functionally compensated for plasma FN. A microgroove width of 60 μm and depth of 5 or 10 μm appears to be optimal for the transmucosal part of the dental implant.

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1. Introduction

Soft tissue integration is a prerequisite for implant success and requires an effective seal between the soft tissue and implant to protect the underlying bone from microorganism invasion.[1] Peri-implantitis begins at the soft tissue of implant is a risk factor in implant failure.[2] The soft tissue interface consists of two zones: a slim epithelium and a thicker connective tissue. Connective tissues have poor mechanical resistance at the implant interface compared to natural teeth.[3] Furthermore, as Jansen et al. state: "the quality of the connective tissue in the transitional area is apparently more important for the long-term prognosis of oral implants than the epithelial attachment".[4] Epithelial down-growth and attachment to the implant can be inhibited by a firm connection between the underlying connective tissue and the implant.[3] Our study focused on reinforcing the attachment between connective tissue and titanium implants via well-defined surface topographies.

New advances in micro-electromechanical systems (MEMS) allow the fabrication of biological MEMS (BioMEMS) and have been used in the investigation of cell response to microgrooved surfaces.[5] Microgrooves integrated into the structure of an implant have advantages compared with the traditionally smooth implant surface. For instance, epithelial down-growth could be inhibited by microgroove-induced contact guidance of connective tissue during growth.[6] The microgroove surface has also affects cell interaction and behaviour, including modulation of cell adhesion, proliferation and gene expression.[7,8] However, how the specific microgroove dimensions affect the extracellular matrix (ECM) has not yet been studied.

The organization and composition of the ECM mediate cell adhesion by controlling the degree to which cells can attach. Fibronectin (FN) is a high-molecular weight (~440 kDa) glycoprotein of the ECM that is a typical marker of the biocompatibility of new materials for connective tissue in dental implant studies.[9] FN occurs in two forms: soluble plasma FN in blood, and insoluble cellular FN in ECMs or on cell surfaces.[10] Plasma FN is a major protein component of blood plasma and is produced in the liver by hepatocytes.[10] Cellular FN is secreted by various cells, primarily fibroblasts, as a soluble protein dimer and is then assembled into an insoluble matrix in a complex cell-mediated process.[11] In both forms, FN is involved in tissue repair. The plasma form of FN is incorporated into fibrin clots, affecting platelet function and mediating the early stages of wound healing. Plasma FN also can be bound to the cell surface and assembled into extracellular fibrils.[12] Cellular FN is synthesized and assembled by cells for attachment to the ECM as they migrate into a clot to repair damaged tissue.[10] Both forms of FN are vital for establishing and maintaining tissue architecture and for regulating cellular processes and behaviours, such as adhesion, spreading, proliferation, migration and differentiation.[10–12]

Plasma proteins are spontaneously adsorbed onto an implant surface within seconds and therefore help determine the bioactivity of implants.[13,14] Plasma FN plays an important role in the interactions of implants with their surrounding ECMs by enhancing the attachment of cells to implant materials.[15,16] Furthermore, the surface structure and wettability of implant materials determine the extent of protein adsorption.[17,18] However, observations regarding the effects of surface wettability on protein have not been consistent. Though many previous studies have shown that FN absorption is enhanced on hydrophobic surfaces,[19–21] it has also been reported that FN adsorption is greater on hydrophilic surfaces.[22,23] Anisotropic wetting is attributed to a liquid contact line encountering a physical discontinuity (e.g., a solid edge along a microgroove present on solid surfaces).[24] The microgroove surface leads to anisotropic wetting and these micro-scale topographical surface structures may encourage macroscopic changes in droplet wetting behaviour. What is more, to the best of our knowledge there has been only a few studies[23] on anisotropic wetting and the dimensions of microgrooves affecting plasma FN absorption.

Cells continuously synthesize their own FN matrix, which they deposit and organize as a three-dimensional network. Material-mediated cellular FN reorganization is an important factor in determining the biocompatibility of a material,[25] cell attachment is required for cellular synthesis of FN.[26] Cells react to micromaterial corrugation, possibly through membrane deformation and stretching.[27] Thus, forces can be generated from just the process of cells recognizing topographical or other cues.[28,29] This is relevant because mechanical forces are necessary for efficient (integrin-mediated) cellular FN assembly to fibrillar matrices.[30–32] Furthermore, the forces placed upon cells, stimulated by material topography, can influence cellular FN assembly.[27–32] Since topographical features alter surface wettability, adsorption of preferred proteins might result. We hypothesized that the dimensions of some microgroove surfaces might influence the synthesis and assembly of cellular FN more effectively than others.

The tissue repair function of FN is ubiquitous and useful for dental implant biocompatibility studies. The aim of this work was to investigate the effect of 3D microgroove surfaces on plasma and cellular FN. Through this effort, we hoped to identify an optimal set of dimensions for microgroove surfaces suitable for the transmucosal part of a dental implant. Plasma FN adsorption on different surface topographies was measured by immunofluorescence and ELISA. Human gingival fibroblasts (HGFs) were chosen for this study as they are most abundantly found in gingival connective tissues and have been used in numerous in vitro studies of implant integration.[33,34] Cells were cultured on the topographically modified surfaces; real-time PCR and Western blotting were used to confirm enhanced cellular FN activity. The structure and properties of the microgrooves, including topography, groove dimensions, surface wettability in FN adsorption and synthesis are discussed in detail.

2. Materials and methods

2.1. *Fabrication of micro-structured substrates*

Microgroove surfaces were fabricated by photolithography with a micro-structured silicon substrate and an overlying 200-nm thick layer of titanium sputtering. Groove widths of