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Integrin mediated attachment of periodontal ligament to titanium surfaces

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ABSTRACT

Objective. Reducing the force between the implant and the bone by recapitulating a similar matrix has the potential to reduce implant failure. To begin to pursue the goal of creating a periodontal ligament interface between a dental implant and bone, the mechanism of cellular attachment to dental implant surfaces must be characterized.

Methods. In this study we examined the role of integrin receptors in the attachment of periodontal ligament fibroblasts to titanium surfaces utilized on dental implants; those surfaces included smooth polished titanium, acid pickled titanium, ground titanium, sandblasted and acid etched titanium, non-oxidized titanium that has been sandblasted and acid etched, hydroxyapatite coated titanium, titanium plasma sprayed or uncoated titanium. For these studies integrin mediated fibroblast attachment was blocked by the integrin blocking peptide GRGDSP or anti-integrin β1 antibody or a combination of the two. Quantitation of periodontal ligament fibroblast attachment was completed by counting cells on the various implant surfaces after culturing in vitro for 24 h with and without the integrin receptor blockers.

Results. Antibody and peptide treatment significantly reduced the number of fibroblasts cells attached to the various implant surfaces but this effect varied significantly depending on the surface. Moreover, increased levels of peptide further decreased fibroblasts attachment in a dose dependent manner.

Significance. Blocking studies suggest first, that integrin receptors function in periodontal ligament attachment to titanium surfaces and second, that different integrin subunits are important in attachment to a particular surface.

1. Introduction

Recent studies show a long term (i.e., >5 years) failure rate for dental implants ranging from a little above 2% to approximately 14% [1–4]. Complications from dental implants have occurred with a frequency ranging from 10 to 77% in various 3-year studies [5–9]. One factor contributing to complications is loading of the implant. Impact forces are greater with implant restorations than with natural teeth because implants lack the stress release or cushioning associated with a periodontal ligament thus, impact loading to restorative materials and the bone is potentially more damaging [10,11]. Complications from overloading implants are more common in posterior restorations and are more common in the maxilla than the

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mandible [7,8]. Bone loss around endosseous implants is not fully understood but likely occurs for several reasons, some of which are related to occlusal loading [12,13]. Experiments in humans and animals support the idea that repetitive overloading beyond a physiologic threshold can cause osteoclastic activity resulting in bone loss [14–17]. One potential solution to reduce impact forces is to restore a cushioning interface between the bone and implant. In vivo, periodontal ligament functions to cushion impact forces on teeth but understanding the mechanism of how these cells attach to surfaces is necessary to effectively recapitulate a periodontal ligament interface between a implant and the bone [18].

A large number of studies have focused on improving bone attachment to implant materials but our studies will focus on non-osteogenic (i.e., excluding osteoblast and osteoclast) cell types. Studies of the interface between various non-osteogenic cell types and implant surfaces showed that fibroblasts are adjacent and in contact with the titanium surface of implants [19–21]. A few studies have focused on improving non-osteogenic cellular attachment to different surfaces or coatings on titanium that include collagen [22], polymers [23], acid etched surfaces [24], fibronectin [25,26], and hydroxyapatite [27,28]. Titanium surface coatings or textures lead to differences in implant fibroblast cell attachment but few studies have focused on the molecules required for this attachment process. Recent studies have shown the extracellular matrix molecule, laminin, functions in fibroblast attachment to an implant surface [29]. Periodontal ligament has been shown to express multiple integrin protein subunits including α4, α5, α 6 and β1 [30–33]. Integrin subunit β1 has been found in the periodontium of rats and humans and has been determined to function in the attachment of cells in these tissues [30–35]. Integrin proteins bind to many different ligands including extracellular matrix proteins, plasma proteins, and integral membrane proteins [36] but the role of integrin molecules in periodontal ligament attachment to titanium implant materials has not been studied.

Integrins serve as specialized adhesive molecules functioning in cell to cell and cell to substrate adhesion [37]. Integrin receptors are composed of varied combinations of proteins of two general groups termed α and β that combine to αβ heterodimers. Because the α and β group each has multiple proteins, combinations of these different proteins to form the αβ heterodimer can lead a large number of integrin receptors with varied structure and function. In addition, the different subunits of the α and β heterodimers produce receptors with different ligand specificities. For example, the β_1 subunit was found to interact with collagen, fibronectin and vitronectin in adhesion of gingival fibroblasts to amalgams [38] and gingival and periodontal ligament fibroblasts attachment to cementum proteins [32]. The integrin super family has many different genes but most will recognize and bind primarily proteins containing the amino acid sequence Arg–Gly–Asp (RGD) [36,39].

In this study we characterized the role of integrin receptors during attachment of fibroblasts to eight different titanium materials. Understanding the molecules involved in the attachment mechanism will allow for future design of implant coatings that optimize cellular attachment.

2. Materials and methods

2.1. Periodontal ligament fibroblast isolation

Primary, human periodontal fibroblast cells were isolated following an Institutional Review Board approved protocol from teeth that were extracted for clinical necessity. Extracted teeth were rinsed in phosphate buffered saline (PBS) containing penicillin and streptomycin three times and then once in minimal essential media. The extracted tooth was held in place by a clamp on the crown of the tooth, the apical side facing up. Using a low-speed handpiece and a diamond disk, irrigated with 1× PBS, a section at a depth of ~0.5 mm was removed from the extracted tooth (Fig. 1A and B). The section was placed in minimal essential media supplemented with 10% fetal calf serum and antibiotics and placed in a 5% CO_2 atmosphere [40]. Proliferative cells with periodontal ligament morphology cultured from the explant (Fig. 1C) plated on glass slides and processed for immunohistochemistry after 24 or more hours in culture.

2.2. Immunohistochemistry

Cells cultured on slides were fixed (4% formaldehyde in 1× PBS) for 30 min blocked (10% normal goat serum in 1× PBS) and incubated in a 1:500 dilution of the monoclonal antibody specific for collagen III (Chemicon, Temecula, CA). After an overnight incubation at 4 °C the slides were rinsed three times in PBS, incubated with FITC conjugated goat anti-mouse 1:500 (Vector Labs, Burlingame, CA), rinsed three times in PBS and mounted.

2.3. Cell culture on titanium implants

Five different types of flat, circular titanium discs were used in these experiments having various surfaces consisting of a smooth polished surface, acid pickled surface, ground surface, coarse grit blasted and acid etched (SLA) surface or a non-oxidized coarse grit blasted and acid etched (SLA active) surface (a generous gift of Institut Straumann AG, Waldenburg Switzerland). SLA active disks were never exposed to air and were supplied in an aqueous solution to prevent oxidation (Institut Straumann AG). Three different types of titanium cylinders were also used in these experiments with a hydroxyapatite (HA) coated titanium surface, titanium plasma sprayed (TPS) surface or an uncoated titanium surface (Biocon Inc., Boston, MA). The surface area for each of the titanium disks or titanium cylinders was approximately 180 mm^2.

Experiments were completed by gently adding 200 μl of α-MEM with 5% FBS and 40,000 periodontal ligament fibroblasts to each of the different titanium disks or cylinders placed in individual wells of a 24-well plate. Disks or cylinders were maintained at 37 °C in a humidified 5% CO_2 atmosphere to allow for cellular attachment [41]. Fifteen minutes before adding media with cells to the titanium surfaces either peptide [5,50 or 200 μM of peptide GRGDSP (Bachem, King of Prussia, PA)] and/or antibody [20 μg/ml of anti-integrin β1 antibody (PharMingen, San Diego, CA)] or nothing was added (control) to the media. After a 3-h incubation in this media, with or