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Physiological Dental Implant Prepared by Stem Cells with β-TCP Coated Titanium and Zirconia Implants

A thesis submitted to the council of the College of Dentistry at the University of Baghdad, in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Prosthodontics

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Abstract

Objective: This study aimed to explore the restoration of missing function in the osseointegrated dental implants due to the absence of periodontal ligament (PDL) and approximation of the artificial dental implant to natural teeth.

Material and methods: A long-pulsed Nd:YAG laser (1064 nm) of single pulse capability was used for coating of Titanium and zirconia substrate. Laser parameters were modulated to achieve the most continuous, homogenous, adhesive, and adherent Beta-tricalcium phosphate (β-TCP) coat layer. Characterization and evaluation of the microstructure, phases, modulus of elasticity, nanohardness of the coating layer and calcium-to-phosphate ratio and composition of the coat was conducted. *In vivo* study involved isolating of bone marrow mesenchyme stem cells (BMMSCs) from rabbit tibia and femur, and collecting periodontal ligament stem cells (PDLSCs) from the freshly extracted lower right incisor. Subsequently, these cells were co-cultured to induce BMMSC differentiation into PDL cells. CD90, CD34, and periostin markers were detected using immunofluorescent assay. A three layered-cell sheets (PDLSC, BMMSC, and co-cultured cell sheets) were constructed using temperature-responsive tissue culture dishes and collagen graft to envelop the implants. The sheets were examined histologically and by Field Emission Scanning Electron Microscope before transplantation. Implants with 3D-engineered tissue were transplanted into the right lower central incisors region of a tooth-extracted rabbit model. Eighty implants (6mm length×2.5mm diameter) were divided into two groups according to the type of implant materials (Titanium and Zirconia). Then, each group (40 implants) was subdivided according to the period of implantation (healing period 45 and 90 days). Afterward each subgroup (20 implants) was subdivided into four groups according to the types of layered-cell sheet stem cells that coat the implant

before transplantation (without cell, PSLSCs sheets, BM-MSCs sheets, and co-culture cells sheets). All groups were subjected to a histological test involving Haematoxylin and eosin staining, immunohistochemistry "periostin", stereoscopic analysis to measure the width of PDL, and Field Emission Scanning Electron Microscope. The natural lower central incisors were used as a control

Results: The laser processes can produce coatings that have acceptable mechanical properties and can bond with high crystallites. EDX results demonstrated no changes in the chemical composition of Titanium, Zirconia, and the coat. The coated Zirconia showed significant increase in surface roughness than coated Titanium. The elastic modulus was low at the surface but gradually increased with depth, it was significantly higher in coated Titanium than coated zirconia at P-value <0.05. Same statistical result was seen when nanohardness for β-TCP coating was compared. The majority of the BM-MSCs and PDLSCs adherent cells were strongly stained with green fluorescence showing positive for CD90, while the majority of the BM-MSCs and PDLSCs adherent cells were negative with no expression for CD34. BMMSCs Co-cultured with PDLSCs successfully induced more PDL cells. The newly induced PDL cells exhibited positive periostin expression. The mean fluorescence green intensity (periostin expression) was significantly higher for the newly induced PDL cells (co-cultured cells) after 1, 2, and 3 weeks when compared with control (BM-MSCs), at 21 days non-significant difference was measured when compared with control (PDLSCs). Haematoxylin and eosin staining and Field Emission Scanning Electron Microscope examination for cross-section of three-layered cell sheets showed stable adhesion. Histological analysis showed that the transplantation of coated (Titanium and Zirconia) dental implants without a cell sheet formed new well-developed bone with osteoblast, with no soft tissue intervention, thereby indicating the presence of an osseointegrated implant at both healing intervals. The periostin gave

negative expression. Mesenchymal tissue layered-cell sheets structured from PDLSCs only, or from Co-cultured (BMMSCs and PDLSCs) were able to form entirely natural PDL-like tissue, including cementum, PDL and alveolar bone on the Titanium and zirconia implants and a biohybrid dental implant was formed after different intervals (45 and 90 days). The periostin green fluorescence was detected clearly around the (Ti and Zirconia) biohybrid implant at 45 and 90 days after transplantation. Field Emission Scanning Electron Microscope showed the PDL-like tissue tightly filled the space between the implant and the implant socket wall, also formation of cementum on the surface of (Titanium and zirconia) bio-hybrid implant and inserted of PDL-like fiber perpendicular to the cementum of biohybrid implant. A non-significant difference was found when the width of PDL round the biohybrid implant, both Titanium and Zirconia, when compared with natural one. There was no significant difference in the PDL width of Titanium biohybrid implant in comparison with that of the zirconia biohybrid implant. Mesenchymal tissue layered-cell sheet isolated from the BM-MSCs failed to form PDL-like fibers around the β-TCP coated Titanium or Zirconia dental implant and the periostin gave a negative expression.

Conclusions: Laser processes can be used in the fabrication of a coat with optimum bonding and desirable mechanical properties for both Titanium and zirconia dental implants. BM-MSCs and PDLSCs can be used in co-culture to increase PDL cells and to produce 3D PDL cell-layered sheets by the novel use of collagen graft as a scaffold, Furthermore, β-TCP coated (Titanium and zirconia) implants were able to generate periodontal tissue and form biohybrid dental implants, when mesenchymal tissue layered-cell sheets were isolated from only PDLSCs or isolated from the co-culture of BMMSCs and PDLSCs.