Clinical and Radiographic Assessment of Dental Implants Activated by Cold Atmospheric Plasma (CAP) and Plasma Rich in Growth Factors (PRGF)

(A randomized clinical trial)

A Thesis

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Dedication

Acknowledgement

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APP	Atmospheric pressure plasma
A-PRF	Advanced platelet-rich Fibrin
BAFO	Bone area fraction occupancy
BFGF	Basic fibroblast growth factor
BIC	Bone implant contact
BMP	Bone morphogenic protein
CaP	Calcium phosphate
CAP	Cold atmospheric plasma
CBCT	Conebeam Computed Tomography
EGF	Endothelial growth factor
HA	Hydroxyl apatite
IGF	Insulin like growth factor
i-PRF	Injectable platelet-rich Fibrin
L-PRF	Leukocyte platelet-rich Fibrin
L-PRP	Leukocyte platelet-rich plasma
MMP	Matrix metalloproteinase
NTP	Non-thermal plasma
PDGF	Platelet derived growth factor
P-PRF	Pure platelet-rich Fibrin
P-PRP	Pure platelet-rich plasma
PRF	Platelet rich fibrin
PRGF	Plasma rich in growth factor
PRP	Platelet rich plasma
SLA	Sandblasted large grit acid etched
TGF	Transforming growth factor
TGF-β	Transforming growth factor- β
TPS	Titanium plasma sprayed
VEGF	Vascular endothelial growth factor

REVIEW OF LITERATURE

Osseo-integration was defined as highly differentiated tissues making a direct functional and structural connection between ordered living bone and a load-carrying implant ***(Branemark et al. 1977)***. Also it was defined as a biological term that describes the contact between living tissues as bone and implant surface placed inside the bone ***(Mavrogenis et al. 2009)***. It is very critical to implant stability and considered as a precondition for implant loading and long term success, its essence is to achieve permenant stability for the implant which in turn ensures the proper route of the healing process and the acceptance of the implant by the living tissues. This process is very imporntant to initate osteogenesis and determines the stability and sustainability of the implant ***(Wrobel et al. 2010).***

The process of osseointegration includes many mechanisms that closely resembles those included in the healing of the fractured bone and includes a cascade of cellular and extracellular events. This tissue response to the foreign body like implant will eventually leads to formation of new bone on the implant surface reaching biological stability ***(Fini et al. 2004).*** This cascade of biological events is controlled by many growth and differentiation factors that is released by activated blood cells at the bone implant interface ***(Davies 1998).***

Many in vitro and in vivo researches were conducted to determine the cell response to the implant surface to select the specific characteristics and properties of the implant material including surface bio modification, chemical composition and sterilization procedures which can directly influence the long term stability of the implant and the course of osseointegration ***(Wrobel et al. 2010).***

Bone response to implant or trauma during implantation had been thoroughly studied both histologically and mechanically with increasing interest in the molecular biology of this process. The bone response to implant is influenced by the presence of some factors related to implant characteristics which include surface roughness, coating and surface topography, also surgery related factors like implant stability and intraoperative heating injury might influence the bone response ***(Mavrogenis et al. 2009)***.

Major stages of bone response to implantation-related injury and key histological events after insertion and mechanical fixation of implants include hematoma formation and mesenchymal tissue development, woven bone formation through the intramembranous pathway, and lamellar bone formation on the spicules of woven bone ***(Mavrogenis et al. 2009)***.

Surgical placement of the implant causes bone injury to which the body responds with pathological mechanisms similar to bone fracture. Blood originating from the injured vessels is the first biological component to come in contact with the surface of endoosseus implants. Blood cells include red cells, platelets, and inflammatory cells such as polymorphonuclear granulocytes and monocytes emigrate from post-capillary venues, and migrate into the tissue surrounding the implant. Platelets entrapped at the implant interface are activated and release cytokines and other soluble growth and differentiation factors ***(Davies et al.1998).***

Initial interactions with the implant surface leads to clot formation, several seconds after implant placement plasma proteins such as fibrin become adsorbed on the implant surface, then platelets become associated with the surface and undergo biochemical and morphological changes like adhesion, spreading and aggregation and release of phosphotyrosine,

intracellular calcium increase, and hydrolysis of phospholipids, as a result coagulation mechanisms take place and degranulation of its cytoplasmic granules releasing growth factors as transforming growth factor–β(TGF-β), platelet derived growth factors(PDGF) and other vasoactive factors as serotonin and histamine. These cytokines are chemoattractants that can stimulate the migration and proliferation of various types of cells guiding the periimplant tissue healing ***(Dereka et al.2006),*** PDGF is considered a very potent myogenic growth factor affecting several types of cells as osteoblasts, leukocytes and fibroblasts ***(Helden and Westermark 1999)***.

Osteoconduction is influenced by the formation of fibrin matrix that act as a scaffold for osteogenic cells migration and osteoinduction takes place by their eventual differentiation in the healing compartment. The three dimensional structure of the fibrin matrix and the migrating effects expressed by the growth factors released by the first arriving cells is very important in creating an osteoprogenitor reservoir at the implant interface ***(Park and Davies 2000).*** Osteogenic cells form osteoid tissue and new trabecular bone that eventually remodels into lamellar bone in direct contact with most of the implant surface which will lead to osseointegration ***(Berglundh et al. 2003).***

Osteoblasts and mesenchymal cells seem to migrate and attach to the implant surface from day one after implant insertion, depositing bone-related proteins and creating a non-collagenous matrix layer on the implant surface that regulates cell adhesion and minerals binding. This matrix is an early-formed calcified afibrillar layer on the implant surface, involving poorly mineralized osteoid similar to the bone cement lines and laminae limitans that forms a continuous, 0.5 mm thick layer that is rich in calcium, phosphorus, osteopontin and bone sialoprotein ***(Meyer et al. 2004).*** This was termed as de novo bone formation through contact osteogenesis ***(Davies 2003).***

The newly formed bone was laid down on the reabsorbed surface of the old bone after osteoclastic activity. This suggested that, the implant surface is positively recognizable from the osteogenic cells as a biomimetic scaffold which may favor early peri-implant osteogenesis, A few days after implantation, the osteoblasts in direct contact with the implant surface began to deposit collagen matrix directly on the early formed cement line/lamina limitans layer on the implant surface A thin layer 20-25 nm consists of flat osteoblast like cells, calcified collagen fibrils, and mineralized area at the titanium implant – bone interface was reported by ***(Murai et al. 1996).***

The early deposition of new calcified matrix on the implant surface is followed by the arrangement of the woven bone and bone trabeculae. This is appropriate for the periimplant bone healing process as it shows a very active wide surface area, adjoining with marrow spaces rich in vascular and mesenchymal cells ***(Franchi et al. 2005)***. Initially rapid Woven and trabecular bone fill the initial gap at the implant-bone interface, arranged in a three-dimensional regular network, it offers a high resistance to early implant loading ***(Probst & Speigel 1997)***.

The early peri-implant trabecular bone formation ensures tissue anchorage that leads to biological fixation of the implant, this begins at 10 to 14 days after surgery. Biological fixation of the implant involves biophysical conditions such as primary stability which is implant mechanical fixation, bio-mimetic implant surface and right distance between the implant and the bone, that is clearly observed at rough implant surfaces ***(Franchi et al. 2005)***. Next, woven bone is progressively remodeled and substituted by lamellar bone that may reach a high degree of mineralization. At three months post-operative, a mixed bone types of woven and lamellar bone can be found around different types of titanium implants ***(Chappard et al. 1999)***.

Bone in contact with the implant surface undergoes morphological remodeling as adaptation to stress and mechanical loading. The turnover of peri-implant mature bone is confirmed by the presence of marrow spaces containing osteoclasts, osteoblasts, mesenchymal cells and lymphatic/blood vessels next to the implant surface. During the remodeling of the peri-implant bone, new osteons circle around the implant with their long axis parallel to the implant surface and perpendicular to the long axis of the implants. Osteoid tissue is produced by osteoblasts suggesting that osteogenesis is ongoing, the remodeled bone can extend up to 1 mm from the implant surface ***(Franchi et al. 2005)***.

After better understanding of the osseointegration process it was found that many factors can influence the path of this process, ***Albrektsson et al.*** suggested six factors that are very important for establishment of reliable osseointegration as; implant material, implant design, surface conditions, status of the bone, surgical technique and implant loading conditions ***(Albrektsson et al. 1981)***

Pure titanium and its alloys are firmly established materials in implant dentistry because of their combination of favorable characteristics as mechanical stability, chemical stability and biocompatibility ***(Brunette et al. 2001).*** Several studies have shown that titanium has a biocompatible nature and less foreign body reactions compared to other materials ***(Eisenbarth et al. 2004, Hallab etal. 2003).*** Although commercially pure titanium and its alloys show these remarkable advantages, recent trends in dental biomaterials are still applied on it to make it more biomemitic and biocompatible ***(Ozcan and Hammerle 2012)***.