



**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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By

Ahmed Mohamed Elkady

*B.D.S., Faculty of Oral and Dental Medicine,
Misr International University, (2009)*

Under Supervision of

Ass. Prof. Dr. Mohamed El Mofty

Associate Professor of Oral Medicine, Periodontology and Oral Diagnosis
Faculty of Dentistry - Ain Shams University

Ass. Prof. Dr. Ahmed Abd El Aziz

Associate Professor of Oral Medicine, Periodontology and Oral Diagnosis
Faculty of Dentistry - Ain Shams University

**Faculty of Dentistry
Ain Shams University
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Dedication

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LIST OF CONTENTS

List of Tables	II
List of Figures	III
List of Abbreviations	VII
Review of Literature	1
Aim of the Study	23
Subjects and Methods	24
Results	34
Cases Presentation	53
Discussion	75
Conclusions	82
Recommendations	83
Summary	84
References	86
Arabic Summary	-

LIST OF TABLES

Table No.	Title	Page
1	The mean and standard deviation (SD) of osstell readings in group I (plasma group) at different follow up periods	36
2	The mean and standard deviation of the osstell readings for group II (PRGF group) at different follow-up periods	38
3	The mean and standard deviation (SD) of osstell readings for group III (Plasma and PRGF group) at different follow-up periods.....	40
4	The mean and standard deviation of the osstell readings for group IV (control group) at different follow-up periods	42
5	The mean and standard deviation (SD) of osstell readings in the four groups	44
6	The mean and standard deviation (SD) of density before and after 6 months in group I (Plasma group).....	46
7	The mean and standard deviation (SD) of density before and after 6 months in group II (PRGF group).....	47
8	The mean and standard deviation (SD) of density before and after 6 months in group III (Plasma and PRGF group)....	48
9	The mean, standard deviation (SD) of density before and after 6 months in group IV (Control group)	49
10	The mean and standard deviation (SD) of the change in density in the four groups	50
11	The mean and standard deviation (SD) of bone loss in the four groups	52

LIST OF FIGURES

Figure No.	Title	Page
1	The cold plasma device, the piezo brush PZ2	28
2	Implant treatment with cold plasma prior to placement	28
3	10 ml vaccumed tube.....	30
4	Blood sampling	30
5 & 6	Sodium citrate 1 ml. mixed with 9 ml. blood	30
7	Centrifuge set at the required speed and time.....	31
8	Different plasma fractions after centrifugation.....	31
9	Bar chart showing the implant stability for group I (plasma group) at different follow-up periods.....	36
10	Bar chart showing implant stability for group II (PRGF group) at different follow-up periods.....	38
11	Bar chart showing implant stability for group III (Plasma and PRGF group) at different follow-up periods.....	40
12	Bar chart showing implant stability for group IV (control group) at different follow-up periods.....	42
13	Bar chart showing the difference between the four groups in all follow-up periods.....	45
14	Bar chart showing the difference between the four groups in each follow-up period	45
15	Bar chart showing the density around implants before and after 6 months in group I (plasma group)	46
16	Bar chart showing density around implants before and after 6 months in group II (PRGF group).....	47
17	Bar chart showing the density around implants before and after 6 months in group III (Plasma & PRGF group)	48
18	Bar chart showing the density around implants before and after 6 months in group IV (control group)	49

Figure No.	Title	Page
19	Bar chart showing the change in density in the four groups...	50
20	Bar chart showing the difference in bone loss between the four groups after 6 months.....	52
21&22	Preoperative photographs of the surgical site for missing upper left first premolar	53
23	Preoperative cone beam CT showing bone measurements.....	53
24	Preoperative Panoramic view	53
25	Midcrestal incision and sulcular incisions	54
26	Flap elevation	54
27	Initial drilling with pilot drill	54
28	Parallelism checking with parallel pin.....	54
29	Before plasma treatment	55
30	After plasma treatment.....	55
31	Implant placement.....	55
32	Primary stability measurement	56
33	Baseline osstell reading	56
34	Primary closure	56
35	Follow-up measurement	57
36&37 &38	Osstell readings for 1 month, 3 month and 6 month follow up periods.....	57
39	Postoperative Conebeam CT after 6 months	58
40	Postoperative panoramic view after 6 months	58
41	Final restoration lateral view	58
42	Final restoration occlusal view	58
43&44	Preoperative photographs for the surgical site for upper left second preomlar	59
45	Preoperative cone beam CT showing bone measurements.....	59
46	Peroperative panoramic view.....	59

Figure No.	Title	Page
47	Midcrestal incision and sulcular incisions	60
48	Flap elevation	60
49	Initial drilling with pilot drill	60
50	Implant humidified with PRGF	60
51&52	Implant placement.....	61
53	Measuring primary stability.....	61
54	Baseline osstell reading	61
55	Primary closure	62
56	One-month healing	62
57	Follow-up measurements.....	63
58&59 &60	Osstell readings for 1 month, 3 month and 6 month follow up periods.....	63
61	Post-operative conebeam CT after 6 months	64
62	Post-operative panoramic view after 6 months	64
63	Final restoration occlusal view	64
64	Final restoration lateral view	64
65	Preoperative cone beam CT showing bone measurements.....	65
66	Peroperative panoramic view.....	65
67	Preoperative photograph for the surgical site	65
68	Midcrestal incision and sulcular insicons	66
69	Flap elevation	66
70	Implant after plasma and PRGF treatment	66
71	Implant placement.....	66
72	Primary stability measurement	67
73	Baseline osstell reading	67
74	Primary closure	67
75	One month healing.....	67

Figure No.	Title	Page
76	Follow up measurements	68
77&78 &79	Osstell readings for 1 month, 3 month and 6 month follow up periods.....	68
80	Post-operative conebeam CT after 6 months	69
81	Post-operative panoramic view after 6 months	69
82	Final restoration occlusal view	69
83	Final restoration lateral view	69
84&85	Preoperative photographs for the surgical site for upper left second premolar	70
86	Preoperative cone beam CT showing bone measurements.....	70
87	Preoperative panoramic view.....	70
88	Midcrestal incision and sulcular incisions	71
89	Flap elevation.....	71
90	Implant with no chairside treatment	71
91	Implant replacement	71
92	Primary stability measurement	72
93	Baseline osstell reading	72
94	Primary closure	72
95	One month healing.....	72
96	Follow up measurements	73
97&98 &99	Osstell readings for 1 month, 3 month and 6 month follow up periods.....	73
100	Post-operative conebeam CT after 6 months	74
101	Post-operative panoramic view after 6 months	74
102	Final restoration occlusal view	74
103	Final restoration lateral view	74

LIST OF ABBREVIATIONS

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 permanent 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 important to initiate 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 endosseous implants. Blood cells include red cells, platelets, and inflammatory cells such as polymorphonuclear granulocytes and monocytes emigrate from post-capillary venules, 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 et al. 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 biomimetic and biocompatible (*Ozcan and Hammerle 2012*).