PhD Thesis

Osseo-Integration Assessment by means of Digital Panoramic Radiography and Cone Beam CT due to Platelet Rich Plasma Employment and Graft Placement in Jaw Bone Defects

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Patras, 2014, Hellas
ΠΑΝΕΠΙΣΤΗΜΙΟ ΠΑΤΡΩΝ
ΣΧΟΛΗ ΕΠΙΣΤΗΜΩΝ ΥΓΕΙΑΣ
ΤΜΗΜΑ ΙΑΤΡΙΚΗΣ

ΠΡΟΓΡΑΜΜΑ ΜΕΤΑΠΤΥΧΙΑΚΩΝ ΣΠΟΥΔΩΝ ΣΤΙΣ “ΚΛΙΝΙΚΕΣ & ΚΛΙΝΙΚΟΕΡΓΑΣΤΗΡΙΑΚΕΣ ΙΑΤΡΙΚΕΣ ΕΙΔΙΚΟΤΗΤΕΣ”

Διδακτορική Διατριβή

Εκτίμηση της Οστεοποίησης με Ψηφιακή Πανοραμική Φωτογραφία ή Υπολογιστική Τομογραφία μετά από τοποθέτηση Αυξητικών Παραγόντων και Μοσχευμάτων σε οστικές ελλείψεις των γνάθων

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Πλάτωνας (427-347 π.Χ.)
ACKNOWLEDGEMENTS

I wish to express my gratitude to my supervisor Professor T. Petsas for the assignment of this project and for his suggestions and guidance throughout this thesis.

I am also grateful to Professor G. Panagiotakis for his faith and confidence towards me and for his contribution in the fulfillment of this thesis. I would also like to thank him for his valuable guidelines in writing scientific articles.

I would like to thank Dr S. Tsantis for his selflessness support throughout this thesis, his proposition regarding the topic of this thesis and his valuable medical guidelines regarding growth factors.

Finally, I wish to express my gratitude to my family for their constant support and encouragement during the years of that work.
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ΕΙΣΑΓΩΓΗ

Στον πολιτισμό των Μάγιας έχει αποδειχθεί ότι έχουν χρησιμοποιηθεί τα πρώτα γνωστά οδοντιατρικά ενδοστικά εμφυτεύματα, (εμφυτεύματα ενσωματωμένα στο οστό) [1]. Σε αρχαιολογική ανασκαφή σε χώρους ταφής των Μάγιας στην Ονδούρα το 1931, οι αρχαιολόγοι βρήκαν ένα κομμάτι της κάτω γνάθου που χρονολογείται περίπου στο 600 μ.Χ. Αυτή η κάτω γνάθος, η οποία θεωρείται ότι ανήκει σε γυναίκα είκοσι ετών, είχε τρία εμφυτεύματα δοντιών στην κάτω γνάθο, κατασκευασμένα από κοράλλια. Για πολλά χρόνια υπήρχαν αμφιβολίες για το πότε τοποθετήθηκαν τα εμφυτεύματα αυτά. Το 1970 ο βραζιλιάνος οδοντίατρος και ακαδημαϊκός, ο καθηγητής Amadeo Bobbio, πήρε μια σειρά ακτινογραφίες οι οποίες φανέρωναν πλήρη οστεοενσωμάτωση μεταξύ εμφυτευμάτων και οστού. Τα ευρήματα αυτά τον οδήγησαν στο συμπέρασμα ότι τα εμφυτεύματα τοποθετήθηκαν κατά τη διάρκεια της ζωής της γυναίκας. Αυτό αποτελεί την πρώτη καταγεγραμμένη χρήση οδοντικών εμφυτευμάτων.

Το 1952 ο Σουηδός ορθοπεδικός χειρουργός, PI Branemark, ερευνώντας την οστική επούλωση και αναγέννηση πραγματοποίησε πειράματα σε κουνέλια χρησιμοποιώντας ειδικούς θαλάμους από τίτανιο στους οποίους εμφύτευε στα οστά των κουνέλιων. Μετά από αρκετούς μήνες μελέτης προσπάθησε να αφαιρέσει αυτούς τους θαλάμους αλλά διαπίστωσε ότι δεν ήταν σε θέση να αφαιρέθουν. O Branemark παρατήρησε ότι μέρος του οστού είχε προσκολληθεί στο τίτανιο. Ακολούθησαν πολλές μελέτες από τον ίδιο χειρουργό για το φαινόμενο αυτό, με τη χρήση του τίτανιού τόσο σε ζώα όσο και σε ανθρώπους, επιβεβαιώνοντας αυτή την μοναδική ιδιότητα του τίτανιου καθώς και τις επιμέρους ιδιότητες που έχει ως ολικό εμφυτεύματος [3].

Αν και αρχικά είχε θεωρηθεί ότι η χρήση του υλικού θα πρέπει να επικεντρωθεί στο γόνατο και το ισχίο, ο Branemark αποφάσισε τελικά ότι η γνάθος ήταν πιο προσιτή για τη συνέχιση των κλινικών παρατηρήσεων λόγω και του υψηλού νικτού ποσοστού στον γενικό πληθυσμό προσφέρονταν περισσότερα θέματα για εκτεταμένη μελέτη. Ονόμασε την ένωση του οστού με το τίτανιο ως «οστεοενσωμάτωση». Το 1965 πάλι o Branemark, ο οποίος ήταν τότε ο καθηγητής Ανατομίας στο Πανεπιστήμιο του Γκέτεμποργκ στη Σουηδία, τοποθέτησε το πρώτο οδοντικό εμφύτευμα από τίτανιο σε έναν Σουηδό εθελοντή ονόματι Gösta Larsson.

Κατά τα επόμενα δεκαετές χρόνια o Branemark δημιούργησε πολλές μελέτες για τη χρήση του τίτανιού για οδοντικά εμφυτεύματα (εμφυτευματολογία) μέχρι και το 1978 όπου και σύναψε μια εμπορική συνεργασία με τη σουηδική εταιρεία Αμινας, Bofors AB για την ανάπτυξη και την εμπορία των οδοντικών εμφυτευμάτων του. Η Bofors αργότερα ονομάστηκε Nobel Biocare και επικεντρώθηκε στην παραγωγή και έρευνα οδοντικών εμφυτευμάτων. Μέχρι και σήμερα πάνω από 7 εκατομμύρια
εμφυτευμάτων Branemark System έχουν τοποθετηθεί ενώ εκατοντάδες άλλες εταιρείες παράγουν οδοντικά εμφυτεύματα [3].

1. Οδοντικά Εμφυτεύματα

Τα οδοντικά εμφυτεύματα σήμερα είναι κατασκευασμένα από τιτάνιο ή χρυσό και αναπαράγουν μια μεγάλη ποικιλία εμφυτευμάτων δοντιών. Είναι κατασκευασμένα με τέτοιο τρόπο ώστε να αντιδρά με την ανθρώπινη δομή των οστών της γνάθου, έτσι ώστε μετά την επουλώση καθίσταται δύσκολο να διαχωριστεί από το φυσικό ιο. Η διαδικασία αυτή ονομάζεται οστεοενσωμάτωση. Η Οστεοενσωμάτωση επιρρέαζεται από έναν αριθμό μεταβλητών του ασθενείς και από την λειτουργία της μάσης [6]. Τα οδοντικά εμφυτεύματα μπορεί να είναι:

Ενδοστοιχία: Αυτό είναι ο συνηθέστερος χρησιμοποιούμενος τύπος εμφυτεύματος και τοποθετούνται εξολοκλήρου μέσα στο οστό. Είναι διαφόρων μορφών όπως: βίδες, κύλινδροι ή λεπίδες και τοποθετούνται χειρουργικά στη γνάθο. Σε κάθε εμφύτευμα τοποθετηθείται επιεμφυτευματική κατασκευή από ένα ή περισσότερα δόντια. Τα εμφυτεύματα αυτού του είδους χρησιμοποιούνται ως εναλλακτική λύση σε οδοντιατρικούς ασθενείς και χρησίζουν προσθετικής αποκατάστασης με γέφυρες ή αφαιρούμενες οδοντοστοιχίες.

Υποπεριοστική: Τα εμφυτεύματα αυτά όταν τοποθετηθούν στις γνάθους, ένα μέρος τους παραμένει εκτός της γνάθου. Το εξέχων τμήμα συγκρατεί την επιεμφυτευματική προσθετική αποκατάσταση. Αυτοί οι τύποι των εμφυτευμάτων χρησιμοποιούνται συνήθως σε ασθενείς με ελλειμματική στηθική ακρολοφία στους οποίους η συγκράτηση των τεχνιτών οδοντοστοιχίων είναι ελληνική ή αδύνατη. Τα οδοντικά εμφυτεύματα μπορούν να αποτελούν μια θεραπευτική επιλογή για τους ανθρώπους που έχουν χάσει ένα δόντι ή περισσότερα δόντια λόγω της περιοδοντικής νόσου, τραυματισμού, ή άλλης αίτησης. Είναι στην πραγματικότητα συνήθως μια μεταλλική κατασκευή σε μορφή σωλήνα που έχει έναν εσωτερικό κοχλία ή κλιπ (αντέρεισμα), όπου στηρίζεται το ψεύτικο δόντι (ή δόντια). Μετά από χειρουργική επέμβαση και διάνοιξη φρεατίου εισάγετε το εμφύτευμα στο ενδεδειγμένο σημείο της γνάθου όπου παραμένει συνήθως παθητικό για μερικές εβδομάδες έως ότου επέλθει πλήρη οστεοενσωμάτωση. Οδοντοστοιχίες, στεφάνες, γέφυρες άλλου τύπου επι-εμφυτευματικές προσθετικές κατασκευές μπορούν να συνδεθούν με το εμφύτευμα και να αντικαταστήσουν τα εκλιπόντα δόντια. Ένα ενιαίο εμφύτευμα μπορεί να αποκαταστήσει ένα ή περισσότερα δόντια. Μια πλήρης αντικατάσταση των δοντιών θα απαιτούσε τουλάχιστον έξι εμφυτεύματα στην άνω γνάθο και μεταξύ τεσσάρων και έξι στην κάτω γνάθο [8].
2. Διαδικασίες Οδοντικών Εμφυτευμάτων

Το πρώτο βήμα αυτών των διαδικασιών είναι η εκτίμηση των πανοραμικών ακτινογραφιών και των τομών αξονικής τομογραφίας (Cone Beam CT scan) προκειμένου να ελεγχτεί το πάχος και το πλάτος του οστού των γνάθων στην περιοχή όπου θα τοποθετηθούν τα εμφυτεύματα. Η επιτυχία ή η αποτυχία των εμφυτευμάτων εξαρτάται από την υγεία του ασθενούς, από φάρμακα που πιθανώς λαμβάνει και που μπορεί να επηρεάσουν τις διαδικασίες της οστεοενσωμάτωσης και την εν γένει υγεία των ιστών του στόματος. Οι δυνάμεις φόρτισης στα εμφύτευμα που εξασκούνται κατά τη διάρκεια της μασητικής λειτουργίας επίσης πρέπει να αξιολογούνται.

Το σχέδιο θεραπείας πρέπει να περιλαμβάνει τη θέση και τον αριθμό των εμφυτευμάτων που είναι πολύ σημαντικό για την μακροπρόθεσμη πρόγνωση της επιμεταβατικής προσθετικής. Η θέση των εμφυτευμάτων καθορίζεται από τη θέση και τη γωνία των γειτονικών δοντιών. Οι προσμοιώσεις στο εργαστήριο ή η χρήση υπολογιστικής τομογραφίας και CAD / CAM για κατασκευή χειρουργικών ναρθηκών οδηγών είναι επίσης πολύ σημαντικό παράμετρο στην ολή διαδικασία.

Οι προϋποθέσεις για τη μακροπρόθεσμη επιτυχία των οστεοενσωματούμενων οδοντικών εμφυτευμάτων είναι η κατάσταση των υγιών οστών των γνάθων και τα υγιή και τους μαλακούς μορίων που τα περιβάλουν. Δεδομένου ότι το οστό μπορεί να ατροφήσει μετά την εξαγωγή δοντιών, όπως για παράδειγμα στην πίσω άνω γνάθο στην περιοχή των ιμμορείων των αρσενικών ασθενών, χρειάζεται να προβούμε σε κατευθυνόμενη οστική και ιστική αναγέννηση (GBR, GTR) κάνοντας χρήση διαφόρων ειδών μοσχευμάτων. Η χρήση των μοσχευμάτων ενισχύεται με την πρόσμιξη αυτών με τους αυξητικούς παράγοντες για καλύτερη οστική και ιστική αναγέννηση [9].

Η τελική προσθετική αποκατάσταση μπορεί να είναι ακίνητη, συγκολλημένη, κοχλιωμένη ή τύπου Seeger ή και κινητή οδοντοστοιχία η οποία θα συγκρατείται στα τοποθετημένα εμφυτεύματα στο οστό της γνάθου. Η τελευταία προσέγγιση εφαρμόζεται συχνά σε ηλικιωμένα άτομα για αντιμετώπιση της κινητικότητας της κάτω ολικής οδοντοστοιχίας. Σε κάθε περίπτωση, επάνω στο οστεοενσωματούμενο εμφύτευμα στερεώνεται και κοχλιώνεται ένα άλλο τμήμα από τον οστεοενσωματούμενο abutment και οστεοενσωματωμένα εμφυτεύματα. Η εργασία αυτή μπορεί να είναι τύπου κορώνας- στεφάνης, γέφυρας, υβριδικής γέφυρας ή οδοντοστοιχίας. Η επιμεταβατική προσθετική εργασία στερεώνεται στα εμφυτεύματα με τρεις τρόπους, βίδες στήριξης, κόλα-τσιμέντο ή με δακτυλίους Seeger [9].

Οι κίνδυνοι και οι επιπλοκές που σχετίζονται με την θεραπεία των εμφυτευμάτων διαχωρίζονται σε αυτές που συμβαίνουν κατά τη διάρκεια της χειρουργικής επέμβασης (όπως υπερβολική αιμορραγία ή τραυματισμό νεύρων), σε αυτές που συμβαίνουν κατά τους πρώτους εξι μήνες (όπως μόλυνση και την
αποτυχία οστεο-ενσωμάτωσης) και σε αυτές που συμβαίνουν μακροχρόνια (όπως περιεμφυτευματιτίδας και μηχανικές βλάβες). Με την παρουσία των υγιών ιστών, ένα καλά ενσωματωμένο εμφύτευμα με κατάλληλα φορτία μπορεί να έχει μακροπρόθεσμα ποσοστά επιτυχίας από 93 μέχρι 98 τοις εκατό για το εξάρτημα [4-6] και 10 έως 15 ετών διάρκεια ζωής για τα προσθετικά δόντια [7].

Οι πιο γνώστες οδοντιατρικές τεχνικές εμφύτευσης είναι [8]:

Άμεση Φόρτιση Εμφυτευμάτων. Η άμεση φόρτιση είναι μια μέθοδος εμφύτευσης, όπου αμέσως μετά την τοποθέτηση των εμφυτευμάτων στην γνάθο ακολουθεί η φόρτιση τους με την επιεμφυτευματική προσθετική εργασία χωρίς να περιμένουμε να περάσει μεγάλο χρονικό διάστημα για την επίτευξη πλήρης οστεοενσωμάτωσης. Με τη χρήση αυτής της μεθόδου μειώνουμε τον χρόνο της θεραπείας από δύο στάδια σε ένα. Αυτό το είδος φόρτισης προτιμάται για εκείνους τους ασθενείς οι οποίοι δεν θέλουν να περιμένουν μήνες για την αποκατάσταση των δοντιών τους. Αυτή η νέα διαδικασία συμπληρώνει τα δύο στάδια των πρώην μεθόδων εμφύτευσης. Μόλις γίνει το εμφύτευμα, οι αποκαταστάσεις τοποθετούνται την ίδια ημέρα, έτσι ώστε η όλη διαδικασία να διαρκεί μόνο λίγες ώρες. Η άμεση φόρτιση αποτελεί μια σημαντική ανακάλυψη στον τομέα της οδοντιατρικής. Όπως σχεδόν όλες οι οδοντιατρικές θεραπείες έχει τα πλεονεκτήματά της αλλά και πιθανά προβλήματα και κινδύνους. Εν συντομία, η άμεση φόρτιση δεν επιτρέπει στα εμφυτεύματα να επουλώθουν τέλεια και η οστεοενσωμάτωση (η διαδικασία με την οποία το εμφύτευμα ενώνεται με το οστό της γνάθου σαν αναπόσπαστο κομμάτι) δεν μπορεί να πραγματοποιηθεί. Η Οστεοενσωμάτωση είναι ζωτικής σημασίας για τον προσδιορισμό της επιτυχίας της εμφύτευσης. Πρέπει να τονίσουμε ότι τα εμφυτεύματα πρέπει επουλωθούν πλήρως για να μπορέσουν να χρησιμεύσουν ως μια σταθερή βάση για τις προσθετικές αποκαταστάσεις.

Χρησιμοποιώντας την μέθοδο της άμεσης φόρτισης αυξάνεται ο κίνδυνος των αποτυχιών εμφύτευσης. Ωστόσο, το μεγάλο πλεονέκτημα αυτής της τεχνικής είναι ότι ο ασθενής δεν πρέπει να περιμένει για την πλήρη οστεοενσωμάτωση, και δεν θα παραμείνει για αρκετό διάστημα χωρίς δόντια. Παρά ταύτα, ο ασθενής θα χρειαστεί περισσότερα εμφυτεύματα. Εάν η παραδοσιακή διαδικασία απαιτεί ένα εμφύτευμα, μπορεί να χρειαστεί δύο, κ.λπ. Αυτό οφείλεται στον υψηλότερο κίνδυνο αποτυχίας εμφύτευσης. Η άμεση φόρτιση κοστίζει περισσότερο από ότι η κλασική θεραπευτική αντιμετώπιση με εμφυτεύματα που πραγματοποιείται σε δύο στάδια.

Μεθόδος Τοποθέτηση Εμφυτευμάτων. Η μεθόδος τοποθέτηση είναι ο όρος που χρησιμοποιείται για την θεραπευτική αποκατάσταση εμφυτευμάτων σε δύο στάδια. Το πρώτο στάδιο είναι η εμφύτευση εντός του οστού της γνάθου. Στη συνέχεια, μετά από μερικούς μήνες, το δεύτερο στάδιο
λαμβάνει χώρα, όπου τα δόντια ή οι μεγάλες προσθετικές αποκαταστάσεις συνδέονται με το εμφύτευμα. Το εμφύτευμα χρειάζεται περίπου τρεις έως εξί μήνες για να οστεοενσωματωθεί, έτσι οι δύο φάσεις χωρίζονται μεταξύ τους από ένα μεγάλο χρονικό διάστημα. Στην αρχή του πρώτου σταδίου, ο οδοντίατρος τοποθετεί το εμφύτευμα στην γνάθο του ασθενούς μετά από ανασθησία και δημιουργία κρημνού. Μετά την τοποθέτηση του εμφυτεύματος η τομή ράβεται και αναμένεται η πλήρης επούλωση και οστεοενσωμάτωση του εμφυτεύματος.

Κατά τη διάρκεια αυτής της διαδικασίας η χρήση μοσχευμάτων και αυξητικών παραγόντων βοηθά στην καλύτερη επούλωση και αναγέννηση των ιστών παρέχοντας μια ισχυρή βάση για το δεύτερο στάδιο όπου γίνεται η τοποθέτηση των προσθετικών αποκαταστάσεων των δοντιών. Τρεις έως εξί μήνες μετά την πρώτη φάση, ο οδοντίατρος τοποθετεί επί του εμφυτεύματος το προσθετικό abutment – κολόβωμα επί του οποίου θα στερεωθεί η όλη προσθετική εργασία.

Το εμφύτευμα είναι εντελώς αόρατο, αλλά το abutment-βάση όπου θα συνδεθούν τα προσθετικά μέρη είναι ορατό. Εάν το εμφύτευμα είναι καλά ενσωματωμένο στην γνάθο, οι αποκαταστάσεις μπορούν να χρησιμοποιηθούν ακριβώς όπως τα φυσικά δόντια. Σε αντίθεση με την άμεση φόρτιση, η μεθύστερη τοποθέτηση μειώνει τον κίνδυνο των αποτυχιών εμφύτευσης και ο ασθενής θα χρειαστεί λιγότερα εμφυτεύματα για την ίδια τύπου προσθετική αποκατάσταση. Μεταξύ του πρώτου και του δεύτερου σταδίου, η οστεοενσωμάτωση που λαμβάνει χώρα παρέχει μια ισχυρή και ιδανική βάση για τις προσθετικές αποκαταστάσεις. Η μεθύστερη τοποθέτηση είναι απλούστερη από ό, τι η άμεση φόρτιση, και κοστίζει λιγότερο. Το μόνο μειονέκτημα της μεθύστερης τοποθέτησης των εμφυτευμάτων είναι η καθυστέρηση: τα δύο στάδια της διάδικασίας χωρίζονται από τρεις έως εξί μήνες (ή, σε ορισμένες περιπτώσεις, ακόμη περισσότερο). Οι ασθενείς πρέπει να επιλέγουν την άμεση με ομολογία κατά τη διάρκεια της επούλωσης, καθώς αυτές θα παρέχουν την απαίτηση δόντια και το επίπεδο των δοντιών. Ως αποτέλεσμα, η επιλογή μετά την τοποθέτηση των εμφυτευμάτων είναι η καθημερινή ανάγκη της διαδικασίας και της περίπτωσης.

3. Οστικά Μοσχεύματα

Τα οστικά μοσχεύματα χρησιμοποιούνται από τους οδοντιάτρους σε περιπτώσεις μεγάλων ή μικρών οστικών ελλειμμάτων των γνάθων σε περιοχές όπου επιθυμούμε την τοποθέτηση των οδοντικών εμφυτευμάτων και όταν δεν υπάρχει ικανή ποσότητα οστών στις ακρολογίες των γνάθων για την τοποθέτηση εμφυτευμάτων. Ο ακτινογραφικός έλεγχος με την χρήση πανοραμικής ακτινογραφίας αλλά και αξονικής τομογραφίας κρίνεται απαραίτητος σε αυτές τις περιπτώσεις. Εάν διαπιστώσεις ότι δεν υπάρχει αρκετό οστό για τη στήριξη του εμφυτεύματος είναι απαραίτητη η κατευθυνόμενη οστική αναγέννηση με την χρήση μοσχευμάτων. Τα οστικά ελλείμματα στις γνάθους μπορεί να οφείλονται σε
φυσικές διεργασίες, όπως εξαγωγές η γήρανση αλλά και λόγο περιοδοτιτίδας η άλλων αιτιών. Σε αυτές τις περιπτώσεις το οστό των γνάθων συμπεριλαμβάνεται [15].

Εάν χαθούν αρκετά δόντια σε ένα άτομο θα επηρεαστεί αρνητικά και όλη η αισθητική εικόνα του προσώπου γιατί η γραμμή της κάτω γνάθου θα «βυθιστεί». Αυτή η εικόνα μπορεί να αποφευχθεί με την χρήση των οδοντικών εμφυτευμάτων. Ωστόσο, αν ο ασθενής έχει χάσει τα δόντια του πριν από αρκετά μεγάλο χρονικό διάστημα, είναι πιθανό ότι θα πρέπει να υποβληθεί σε χειρουργική επέμβαση με την χρήση οδοντικών εμφυτευμάτων. Αυτό πρέπει να γίνει κατά η πριν την τοποθέτηση των εμφυτευμάτων. Τα αποτελέσματα των οστικών μοσχευμάτων είναι πολύ ελπιδοφόρα και η χρήση τους συνεχώς επεκτείνεται διεθνώς.

Τα μοσχεύματα τοποθετούνται στην επιθυμητή περιοχή όπου σταθεροποιούνται με την χρήση διαφόρων τύπων μεμβράνων όπως την αυτόλογη βιολογική μεμβράνη με τους αυξητικούς παράγοντες η της μεμβράνες τιτανίου. Στα οστικού μοσχεύματος και των ούλων τοποθετείται μια μεμβράνη από βιοσυμβατό αυτοαπορροφούμενο υλικό που καλύπτει, σταθεροποιεί και προστατεύει το μόσχευμα. Παράλληλα αυτό το υλικό εμποδίζει την επέκταση των ούλων προς το χώρο του μοσχεύματος, οριοθετώντας την περιοχή όπου θα δημιουργηθεί σταδιακά το νέο οστό. Υπάρχουν τέσσερις τύποι οστικών μοσχευμάτων:

**Αυτογενή μοσχεύματα** που προέρχονται από το σώμα του ιδίου του ασθενή. Είναι η προτιμότερη επιλογή για λόγους βιοσυμβατότητας και ταχύτητας επούλωσης, αλλά έχουν το μειονέκτημα ότι απαιτείται να γίνει μια ακόμη χειρουργική επέμβαση σε άλλο σημείο του σώματος του ασθενή για τη λήψη του μοσχεύματος και μεταμόσχευσης στο επιθυμητό σημείο. Αποτελούν τα καλύτερα και τα πιο ασφαλή μοσχεύματα. Σε αυτές τις περιπτώσεις συχνά λαμβάνεται οστό από την λαγόνιο περιοχή και το γένιο. Η προσθήκη αυξητικών παραγόντων συμβάλει στην επεξεργασία της μεταμόσχευσης. Το οστικό μόσχευμα είναι η βάση πάνω και γύρω από την οποία θα δημιουργηθεί το νέο οστό. Μετά από 6-12 μήνες το μόσχευμα ή θα έχει ενσωματωθεί πλήρως ή θα έχει αντικατασταθεί από νέα οστικά κύτταρα που θα έχει παράγει το σώμα του ιδίου του ασθενή.

**Αλλογενή μοσχεύματα** τα οποία είναι μοσχεύματα ανθρώπινης προέλευσης αλλά από τρίτο δότη μέσα από μια τράπεζα οστού. Το ΕΚΕΦΕ Δημόκριτος δραστηριοποιείται σε αυτόν τον τομέα δημιουργώντας άλλα-μοσχεύματα από ζώντες δότες τα οποία λαμβάνονται κυρίως από αρθροπλαστικές επεμβάσεις και μετά από κατάλληλη επεξεργασία προσφέρονται στο εμπόριο. Τα άλλα-μοσχεύματα έχουν επιλεγεί προσεκτικά, ελέγχονται και αποστειρώνονται, έτσι ώστε η χρήση τους να είναι ασφαλής χωρίς κίνδυνο μετάδοσης ασθενειών.
Αλλοπλαστικά μοσχεύματα τα οποία είναι συνθετικά υλικά. Μπορούν να κατασκευαστούν από υδροξυαπατίτη, ένα φυσικό υλικό, που αποτελεί το κύριο συστατικό του οστού. Το φωσφορικό ασβέστιο χρησιμοποιείται σήμερα σε συνδυασμού με υδροξυαπατίτη. Πολυμερή και διάφορα άλλα ακρυλικά επικαλυμμένα με υδροξείδιο του ασβεστίου για την προσκόλληση, χρησιμοποιούνται, επίσης, ως αλλοπλαστικά μοσχεύματα, όπως και η Corallina officinalis.

Ξενογενή μοσχεύματα τα οποία είναι ζωικής προέλευσης. Τα μοσχέυματα (Xenografts) όπως υποδηλώνει και το όνομα, είναι ξενομοσχεύματα οστών που λαμβάνονται από ένα ζώο. Τα αλλογενή είναι ανθρώπινης προέλευσης. Οι οδοντίατροι μπορούν να χρησιμοποιήσουν οστά από ζώα όπως αγελάδες, άλογα ή χοιρινά.

Οστεομεταμόσχευση. Η μεταμόσχευση των οστών είναι μια συνήθης οδοντιατρική διαδικασία και όπως συμβαίνει με σχεδόν όλες τις ιατρικές επεμβάσεις, δεν είναι χωρίς κίνδυνο. Εάν συμβεί κάποια επιπλοκή η διαδικασία της μεταμόσχευσης μπορεί να επαναληφθεί. Η διαδικασία είναι αρκετά απλή: ο οδοντίατρος κάνει μια μικρή τομή για να αποκαλύψει ένα μέρος του σώματός της δότριας θέσης από όπου θα λάβει το οστό. Τέτοιες θέσεις είναι το ισχίο και το πηγούνι. Η διαδικασία μεταμόσχευσης αυτόλογου οστικού μοσχεύματος έχει μεγάλη επιτυχία χωρίς να υπάρχει μεγάλος κίνδυνος απόρριψης του μοσχεύματος. Μετά την επέμβαση, θα πρέπει να υπάρχει ένας χρόνος αναμονής 3 έως 12 μηνών πριν την τοποθέτηση των εμφυτευμάτων. Η όλη διαδικασία χρειάζεται αρκετούς μήνες για να ολοκληρωθεί. Γνωρίζουμε επίσης ότι όσοι αντιμετωπίζουν τέτοιου είδους προβλήματα επιθυμούν μια βραχύχρονη και όχι μακροχρόνια θεραπεία. Οι αυξητικοί παράγοντες και η Πλάσμα πλούσιο σε Αμιοπέταλα βοηθούν αφενός στην συντόμευση του χρόνου θεραπείας αλλά και στην μεγαλύτερη επιτυχία της.

Ανύψωση της Μεμβράνης του Ιγμορείου, συνήθως στο στόχο μέρος των άνω γνάθων δεν υπάρχει αρκετό οστό για την τοποθέτηση εμφυτευμάτων. Σε αυτές τις περιπτώσεις χρησιμοποιούμε την τεχνική της ανύψωσης της μεμβράνης του ιγμορείου. Το ιγμόρειο άντρο ή του Highmor είναι ένας μεγάλος, σε σχήμα πυραμίδας σχηματισμός. Ο καθένας άνθρωπος έχει δύο κόλπους, έναν στην αριστερή πλευρά και μία στη δεξιά πλευρά της μύτης σας, ακριβώς πάνω από τα δόντια του. Το οστό της άνω γνάθου, το οποίο διαχωρίζει τα ιγμόρεια από τα δόντια, σε ορισμένους ασθενείς δεν είναι αρκετά παχύ ώστε να μπορούν να τοποθετηθούν εμφυτεύματα. Εάν το οστό της άνω γνάθου είναι λεπτό πρέπει να ανύψωσε η μεμβράνη του ιγμορείου και ανάμεσα σε αυτή και το οστό να τοποθετηθεί οστικό μόσχευμα έτσι ώστε να δημιουργηθεί οστικό υπόβαθρο για την χρήση εμφυτευμάτων. Με αυτή την τεχνική, ο οδοντίατρος μπορεί να τοποθετήσει εμφυτεύματα ακόμη και σε περιπτώσεις όπου στην άνω γνάθο δεν υπάρχει αρκετό οστό.
Οδοντικά εμφυτεύματα. Τα οδοντικά εμφυτεύματα είναι η πιο καινοτόμος θεραπευτική διαδικασία στην οδοντιατρική η οποία χρησιμοποιείται ευρέως για την αποκατάσταση των δοντιών, της μασητικής λειτουργίας, της αισθητικής και της επικοινωνιακής λειτουργίας του ανθρώπου. Οι περισσότερες τεχνικές των οδοντικών εμφυτευμάτων είναι τεκμηριωμένες και προβλέψιμες. Ωστόσο, σε πολλές περιπτώσεις δεν είναι δυνατόν να τοποθετηθούν τα οδοντικά εμφυτεύματα στην επιθυμητή θέση λόγω της κακής ποιότητας του οστού ή της ανεπαρκής ποιότητας του. Μια ελλειμματική φατνιακή ακρολοφία συχνά σχετίζεται με την επιθυμητή θέση του εμφυτεύματος όπως στο οπίσθιο μήτη της άνω γνάθου. Για να εξερευνηθούν μερικά από αυτά τα προβλήματα, αυτογενή μοσχεύματα οστού λαμβάνονται από το γένιο, τον κλάδο της κάτω γνάθου, ή την λαγόνο ακρολοφία του ιδίου ασθενούς. Ωστόσο μπορεί να υπάρξουν ανεπιθύμητες ενέργειες και επιπλοκές, όπως μόλυνση, πόνος, σχηματισμός αματώματος και φλεγμονή στη δότρια περιοχή. Επιπλέον στη δότρια θέση δεν έχουμε πάντα την επιθυμητή ποσότητα οστού που χρειάζεται για την μεταμόσχευση. Για τον λόγο αυτό μπορούν να χρησιμοποιηθούν μοσχεύματα διαφορετικής προέλευσης. Τα μοσχεύματα αυτά υπόκεινται σε περιορισμούς.

Ένα ιδανικό μόσχευμα οστού στην οδοντιατρική εμφύτευμα θα πρέπει να έχει πολλαπλές ιδιότητες. Να είναι βιο-μιμητικό, θα πρέπει να έχει την ικανότητα να επάγει τη διαφοροποίηση των κατάλληλων κυττάρων (δηλαδή, ενδοθηλιακά και οστεοβλαστικά κύτταρα) για το σχηματισμό του νέου οστού, θα πρέπει εύκολα να συντίθεται και να παράγεται, αντί να προέρχεται από υλικά μοσχεύματα (για να εξαλειφθούν όλοι οι κίνδυνοι μετάδοσης της νόσου). Θα πρέπει εύκολα και γρήγορα να επαναπαρορροφάται όπως συμβαίνει στην οστεογονική απόκριση. Θα πρέπει να μεταφέρεται και να αποθηκεύεται εύκολα. Θα πρέπει να έχει λογικά οικονομικά αποδοτικό. Θα πρέπει να είναι ικανό να επιτύχει συνεπή και προβλέψιμα αποτελέσματα χωρίς να επηρεάζονται από το διαφορετικό επίπεδο τεχνικής ικανότητας του κλινικού.

Για να ανταποκριθεί σε αυτές τις απαιτήσεις, η οδοντιατρική έρευνα έχει επικεντρωθεί στη χρήση των βιοδραστικών μορίων τα οποία μπορούν να διεγέρουν τον τοπικό σχηματισμό οστού. Δεδομένου ότι οι διάφοροι αυξητικοί παράγοντες που έχουν επίδραση στην αναγέννηση των οστών έχουν ανακαλυφθεί, ο αριθμός των σχετικών μελετών έχει αυξηθεί σημαντικά. Ειδικότερα, οι παράγοντες ανασυνδυασμένη ανθρώπινη Μορφογενετική Πρωτεīνη-2 (rhBMP-2) [4-7] και ανασυνδυασμένο ανθρώπινο αμιοπεταλιακό αυξητικό παράγοντα (rhPDGF) [8], [9] και [10], έχει αποδειχθεί ότι έχουν σημαντική επίδραση στην οστεο-αναγεννητική διαδικασία. Αυτοί οι παράγοντες έχουν επίσης εγκριθεί από τον Αμερικανικό Οργανισμό Τροφίμων και Φαρμάκων (FDA) για χρήση στην οδοντιατρική.

Από αυξητικούς παράγοντες μπορεί να δημιουργηθεί επιθήλιο, ενδοθήλιο του μυοκαρδίου και του νευρικού ιστού, χόνδρος, οστό, όστειν και πιο ανεπτυγμένους τύπους του συνδετικού ιστού και του αίματος. Επίσης το κολλαγόνο και οι πρωτεογλυκάνες που περιβάλλουν άλλους πιο ανεπτυγμένους ιστούς και όργανα. Οι παράγοντες ανάπτυξης πολυπεπτιδίου και διαφοροποίησης (GDFs) ταξινομούνται ως βιολογικοί μεσολαβητές που έχουν επιδείξει ένα σημαντικό ρόλο στη διέγερση και τη ρύθμιση της διαδικασίας επούλωσης του τραύματος. Οι αυξητικοί παράγοντες που σχετίζονται με σημαντικές κυτταρικές διαδικασίες όπως: [11]

1. διέγερση και αναστολή της ανάπτυξης
2. Ανάπτυξη και διαφοροποίηση
3. αναγέννηση, επιδιόρθωση και επούλωση των πληγών
4. Μιτογένεση
5. Χημειοταξία
6. Μεταβολισμός
7. αγγειογένεση
8. Η απόπτωση ή ο προγραμματισμένος κυτταρικός θάνατος

Τα διαδοχικά βήματα που είναι κρίσιμες για περιοδοντική αναγέννηση εξαρτάται από τις διαδικασίες της οστεογένεσης, cementogenesis, και το σχηματισμό του συνδετικού ιστού. Διάφορα in vitro και in vivo μελέτες αποκάλυψαν ότι συγκεκριμένες αυξητικοί παράγοντες τροποποιούν φημισμένα στοιχεία της περιοδοντικής επούλωση τραύματος, επιτυγχάνοντας έτσι σημαντική περιοδοντική αναγέννηση σε ζώα. [11]

Το 1976, Melcher [34] πρότεινε την αρχική υπόθεση για το δυναμικό των περιοδοντικών αναγέννηση. Από τότε η ραγδαία αναπτυσσόμενο τομέα της αναγεννητικής έρευνα προσπάθησε να εντοπίσει τους
τρόπους θεραπείας που υποστηρίζουν την αναγέννηση των χαμένων περιοδοντικών ιστών. Ενσωματώνουν χρησιμοποιούνται σήμερα αναγεννητική τεχνική καθοδηγούμενη αναγέννηση ιστού (GTR) μόνο ή με βιοαπορροφήσιομο ή μη απορροφήσιμο μεμβράνες, αλλομοσχεύματα και αυτόγραφα των οστών, και παράγοντες όπως το κιτρικό οξύ ή τετρακυκλίνη χρησιμοποιούνται για να ρυθμίζουν τις ρίζες και να ενισχύσει την προσκόλληση των περιοδοντικών ιστών.

Αιμοπετάλια αυξητικούς παράγοντες-AA, -AB, -BB, Η κανονική καταμέτρηση των αιμοπεταλίων στην κυκλοφορία του αίματος είναι 150000-300000 / mm3. Προέρχονται από τη διαίρεση των μεγακαρυοκυττάρων στο μυελό των οστών. Στη συνέχεια εισέλθουν στην κυκλοφορία του αίματος, όπως από το κύτταρα και η διάρκεια ζωής τους κυμαίνεται από 7 έως 10 μηνείς. Τα αιμοπετάλια παράγουν παράγοντες ανάπτυξης, και να τους απελευθερώσουν ενεργά ανάλογα με τις ανάγκες της πηγής του αίματος [36]. Χαρακτηρίζονται από την παρουσία του ψευδοποδίου, προεξοχές της κυτταρικής μεμβράνης, και ενδοκυτταρικά κυστίδια ονομάζονται ενδοσώματα, τα οποία δρουν ως κόκκοι αποθήκευσης. Υπάρχουν τρεις τύποι των ενδοσώματων:

(α) Λυσσώματα: χρησιμοποιείται για την αποθήκευση υδρολυτικά ένζυμα
(β) Πυκνά ενδοσώματα: αποθηκεύονται και απελευθερώνουν ADP, το αιμοπεταλίων ενεργοποιητή
(γ) Άλφα (α) ενδοσώματα: ο μεγαλύτερος πληθυσμός των κόκκων και ο χώρος αποθήκευσης των αυξητικών παραγόντων, οι οποίοι είναι σε μια βιολογικά αδρανή μορφή. Οι α-κόκκοι είναι επίσης πλούσια σε βιτρονεκτίνη, ένα μόριο κυτταρικής προσκόλλησης αναγκαία osseoinduction και osseointergration (Rendu 2001).

Αιμοπεταλίων κυτόπλασμα περιέχει άλλους παράγοντες, όπως ινώδες, μία πρωτεΐνη που σχετίζονται με την πηγή του αίματος [36]. Η έναρξη του μηχανισμού πηγής σχετίζεται με τις διαρθρωτικές αλλαγές της υμένας κυττάρων αιμοπεταλίων. Τα αιμοπετάλια ενεργοποιούνται και απελευθερώνουν έτσι το περιεχόμενο των κόκκων. Οι α-κόκκοι κινούνται στη μεμβράνη των αιμοπεταλίων, όπου να καταπεί. Οι ελλείπεις και ανενεργοί αυξητικοί παράγοντες είναι πλήρως σχηματίζονται με την προσθήκη των ιστονών και καρβοξυλικών ομάδων. Ισχυρή αγωνιστική, όπως η θρομβίνη και το κολλαγόνο προκαλούν αυτή την κυκλοφορία, ακόμη και όταν οι συνθήκες δεν είναι ιδανικές για τη συσσώρευση των αιμοπεταλίων. Οι μηχανισμοί που καθορίζουν την κίνηση των ιόντων Ca2+ είναι επίσης απαραίτητο για την ενεργοποίηση των αιμοπεταλίων. Η διαδικασία επούλωσης τραύματος διαιρείται σε τρία στάδια:

- Βιοχημική ενεργοποίηση. Το τραύμα που μεταφράζεται σε ένα βιοχημικό σήμα για το κράτος να είναι σε θέση να εκτιμήσει τις συνθήκες. Αναστέλλοντας τη μικροκυκλοφορία, το πλάσμα έρχεται σε επαφή με τις πρωτεϊνές του ιστού, ενεργοποιούντας έτσι τον παράγοντα Hagemmann και τα κυκλοφορούντα αιμοπετάλια.
1. Η επίδραση του πλάσματος πλούσιο σε αιμοπετάλια στην οστεοαναγέννηση οδοντικών εμφυτευμάτων με την χρήση εικόνων οδοντιατρικής πανοραμικής ακτινογραφίας: Υπολογιστική Ανάλυση υφής.

Εισαγωγή
Σε αυτό την έρευνα για πρώτη φορά πραγματοποιήθηκε μια υπολογιστική μελέτη υφής για την εκτίμηση της διαφοροποίησης της υφής που συνδέεται με τις ιδιότητες του σχηματισμού των οστών, περιμετρικά του εμφυτεύματος μετά την χρήση πλάσματος πλούσιο σε αιμομετάλλια (Platelet-Rich-Plasma), σε πανοραμικές ακτινογραφίες. Ο κύριος στόχος ήταν να ποσοτικοποιηθεί οποιαδήποτε διαφοροποίηση της υφής σε εικόνες πανοραμικής ακτινογραφίας σε μια περίοδο παρακολούθησης 8 μηνών, στην διεπαφή οστών-εμφυτεύματος, μεταξύ των δύο κατηγοριών (0 & 8 μήνες) και κατά συνέπεια να αξιολογηθεί οποιαδήποτε στατιστική διαφορά στα χαρακτηριστικά υφής των ακτινογραφιών μεταξύ των ομάδων ελέγχου και δοκιμασίας που μπορεί να αποδοθεί στη θεραπεία PRP. Η ανάλυση υφής σε συνδυασμό με τις ιδιότητες του σχηματισμού των οστών γύρω περιμετρικά του εμφυτεύματος πραγματοποιήθηκε με ROC ανάλυση.

Υλικά και Μέθοδοι
Η μελέτη περιλαμβάνει 30 ασθενείς εκ των οποίων έγινε τυχαία επιλογή σε δύο ομάδες (πειραματική ομάδα – 15 ασθενείς, ομάδα ελέγχου– 15 ασθενείς). Οι ηλικίες των ασθενών κυμαίνονταν από 25 έως 65 χρόνια. Τα κριτήρια αποκλεισμού για τη συμμετοχή τους στη μελέτη ήταν κυρίως, διαβήτης, οστεοπόρωση, καρδιακές και θυρακικές ασθένειες, κάπνισμα και καρκινικοί ασθενείς. Όλοι οι ασθενείς που τελικά επελέγησαν είχαν απώλεια δοντιών όπως και κάτω γνάθου και είχαν επιλέξει το εμφύτευμα ως χειρουργική λύση, αν και είχαν ενημερωθεί για εναλλακτικές θεραπείες, όπως η προσθετική. Όλοι ήταν ενήμεροι για τις απαιτήσεις για τη συμμετοχή στη μελέτη και έχουν υπογράψει ένα έντυπο συγκατάθεσης. Η πειραματική ομάδα παραλαμβάνει την εφαρμογή PRP γύρω από τα νέα εμφυτεύματα κατά την χειρουργική επέμβαση, ενώ στην ομάδα ελέγχου τοποθετήθηκαν τα νέα εμφυτεύματα χωρίς θεραπεία PRP. 60 ψηφιοποιημένες πανοραμικές ακτινογραφίες, που αντιστοιχούν στους 30 ασθενείς λήφθηκαν αμέσως μετά από την εμφύτευση (κατηγορία Ι) και 8 μήνες αργότερα (κατηγορία II) και αναλύθηκαν με την βοήθεια αλγορίθμων ανάλυσης υφής.

Αποτελέσματα
Η υπολογιστική ανάλυση υφής που πραγματοποιήθηκε στην παρουσία μελέτη έδειξε σημαντική διαφορά μεταξύ των ομάδων ελέγχου και πειράματος. Όσον αφορά την ομάδα με θεραπεία PRP, τα τέσσερα χαρακτηριστικά υφής που ανάδειξαν την διαφοροποίηση συναρτήσει το χρόνου ήταν τα
Angular Second Moment (ASM), Correlation, Long Run Emphasis (LRE), Gray Level Non Uniformity (GLNU). Οι διαφορετικές τιμές του σε σχέση με την ομάδα ελέγχου απεικονίζονται στην εικόνα 1.

Εικόνα 1. Box plots των τεσσάρων χαρακτηριστικών για τις δύο ομάδες.

Συζήτηση
Η προτεινόμενη ανάλυση προσπαθεί για πρώτη φορά να συμβάλει στη σημερινή συζήτηση, κατά πόσον το PRP ωφελεί την αναγέννηση των οστών ή όχι, αξιοποιώντας την πανοραμική ακτινογραφική εικόνα. Το αποτέλεσμα της προτεινόμενης μεθόδου έχει σημαντικό κλινικό ενδιαφέρον επειδή ενισχύει την κυρίαρχη άποψη της οδοντιατρικής κοινότητας, ότι το PRP αυξάνει το οστεο-αναγεννητική δυναμική των γύρω ιστών μετά την οδοντιατρική εμφύτευση, προσανατολίζοντας την καθημερινή χειρουργική διαδικασία προς την χρήση του PRP.
2. Ενίσχυση των οστών στα ιγμόρεια χωρίς την ανόρθωση των ιγμορείων

Εισαγωγή
Η προτεινόμενη τεχνική που συνδυάζει τη χρήση των συμπυκνωμένων αυξητικών παραγόντων (CGF με βλαστοκύτταρα CD34 +), και του εμφυτεύματος, με έναν τέτοιο τρόπο ώστε το ιγμόρειο να μπορεί να προσαρμοστεί στις νέες συνθήκες και στην δημιουργία νέου οστού γύρω από τα εμφυτεύματα χωρίς την ανάγκη εκτέλεσης ανόρθωσης ιγμορείου. Τα εμφυτεύματα μπορούν να τοποθετηθούν είτε χρησιμοποιώντας μια χειρουργική προσέγγιση, ή χρησιμοποιώντας την τεχνική flapless. Στην παρούσα έρευνα επτά εμφυτεύματα τοποθετήθηκαν με την τεχνική flapless στα δύο ιγμόρεια, ακολουθούμενα από μια ακτινογραφική (πανοραμική ακτινογραφία και cone-beam αξονική τομογραφία – CBCT) καθώς και κλινική αξιολόγηση (από μετρήσεις Osstell) μετά από μία περίοδο 8 μηνών παρακολούθησης. Η προτεινόμενη τεχνική της εκ προθέσεως δεν έχει προηγουμένως αναφερθεί στη βιβλιογραφία.

Υλικά και μέθοδοι
Μία 50 ετών γυναίκα ασθενής με μερική οδοντοστοιχιά, μη καπνιστής σε κατάσταση καλής υγείας και χωρίς οποιαδήποτε χρόνια αρρώστηση επισκέφτηκε το ιατρείο ζητώντας μια αποκατάσταση της άνω γνάθου με μια μη-κινητή πρόσθεση. Με μόνο την πρόσθια οδοντοφυΐα παρούσα, η ασθενής είχε σοβαρές δυσκολίες μάσης της τροφής της. Δεδομένου ότι η ασθενής είχε ζητήσει μια μη-κινητή πρόσθεση, η επιλογή της τοποθέτησης 7 εμφυτευμάτων (4 στα αριστερά και 3 στην δεξιά πλευρά) προσφέρθηκε στην ασθενή. Μετά την λεπτομερή ενημέρωση της ασθενούς για την διαδικασία που επρόκειτο να πραγματοποιηθεί, υπογράφηκε μια γραπτή συγκατάθεση. Η καινοτομία της προτεινόμενης μεθόδου είναι ότι τα εμφυτεύματα τοποθετήθηκαν με μια flapless προσέγγιση με εκ προθέσεως διάτρηση της Schneiderian μεμβράνης. Επιπρόσθετα, συμπυκνωμένοι αυξητικοί παράγοντες (CGF), καθώς και υλικό μοσχεύματος από οστό χρησιμοποιήθηκαν σε ένα καινοτόμο πρωτόκολλο.

Αποτελέσματα
Οι ακτινογραφίες (πανοραμικές και Cone Beam CT) εξετάστηκαν σε διάφορα στάδια κατά τη διαδικασία της οστεοενσωμάτωσης προκειμένου να αξιολογηθεί η αύξηση και η ωρίμανση των αρρώστων που σχηματίζοντας γύρω από τα εμφυτεύματα και πέρα από το επιφάνεια της μεμβράνης. Ο νέος οστικός σχηματίζει στην κοιλότητα της μεμβράνης έδωσε την δυνατότητα στην μεμβράνη του ιγμορείου να ανοικοδομηθεί πάνω σε αυτό, γεγονός το οποίο επιβεβαιώνει πόσο καλά μας ο ανθρώπινος οργανισμός είναι ικανός να προσαρμόζεται σε νέες συνθήκες.

Συμπεράσματα
Τα αποτελέσματα της προτεινόμενης τεχνικής (flapless τεχνική τοποθέτησης εμφυτεύματος με εκ
προθέσεως διάτρηση της μεμβράνης του ιγμορείου σε περίπτωση που αυτή είναι πολύ λεπτή)
apέδειξαν ότι είναι δυνατή μια τέτοια προσέγγιση με εντυπωσιακά αποτελέσματα. Πρέπει να τονιστεί
ότι το χειρουργικό πρωτόκολλο θα πρέπει να ακολουθηθεί προσεκτικά και με ακρίβεια για να
υπάρχουν τα επιθυμητά αποτελέσματα.
Chapter 1 – Dental Implants

Summary
In this chapter an extensive review about dental implants history, technology and associate surgical procedures is introduced.

1.1 Dental Implant History

The Mayan civilization has been shown to have used the earliest known examples of Dental Implants, endosseous implants (implants embedded into bone) [1]. While excavating Mayan burial sites in Honduras in 1931, archaeologists found a fragment of mandible of Mayan origin, dating from about 600 AD. This mandible, which is considered to be that of a woman in her twenties, had three tooth-shaped pieces of shell placed into the sockets of three missing lower incisor teeth. For forty years the archaeological world considered that these shells were placed under the nose in a manner also observed in the ancient Egyptians [2]. However, in 1970 a Brazilian dental academic, Professor Amadeo Bobbio, studied the mandibular specimen and took a series of radiographs. He noted compact bone formation around two of the implants which led him to conclude that the implants were placed during life. This may be the first recorded use of Dental Implants. In the 1950s research was being conducted at Cambridge University in England to study blood flow in vivo. They devised a method of constructing a chamber of titanium which was then embedded into the soft tissue of the ears of rabbits. In 1952 the Swedish orthopaedic surgeon, P I Brånemark, was interested in studying bone healing and regeneration, and adopted the Cambridge designed ‘rabbit ear chamber’ for use in the rabbit femur. Following several months of study he attempted to retrieve these expensive chambers from the rabbits and found that he was unable to remove them. Per Brånemark observed that bone had grown into such close proximity with the titanium that it effectively adhered to the metal. Brånemark carried out many further studies into this phenomenon, using both animal and human subjects, which all confirmed this unique property of titanium and its unique potential for dental implants. Although he had originally considered that the first work should centre on knee and hip surgery, Brånemark finally decided that the mouth was more accessible for continued clinical observations and the high rate of edentulism in the general population offered more subjects for widespread study. He termed the clinically observed adherence of bone with titanium as ‘osseointegration’. In 1965 Brånemark, who was by then the Professor of Anatomy at Gothenburg University in Sweden, placed the first titanium dental implant into a human volunteer who was a Swede named Gösta Larrson.
Over the next fourteen years Brånemark published many studies on the use of titanium for dental implants (implantology) until in 1978 he entered into a commercial partnership with the Swedish defence company, Bofors AB for the development and marketing of his dental implants. With Bofors (later to become Nobel Industries) as the parent company, Nobelpharma AB (later to be renamed Nobel Biocare) was founded in 1981 to focus on dental implants and implantology. To the present day over 7 million Brånemark System implants have now been placed and hundreds of other companies produce dental implants [3].

1.2 Dental Implant overview

Dental implants are root devices used to replace one or more missing teeth in the human jaw. Dental implants are bolts placed in the human jaw to replace missing teeth. They are normally of metallic structure, usually titanium, made in a way to react with human jaw bone structure so that after healing it becomes difficult to separate from the natural tissue. The process by which this occurs is called osseointegration. If a dental implant becomes osseointegrated, the implant operation is considered a successful one. Osseointegration is affected by a number of patient and operation variables. Dental implants can be:

Endosteal (in the bone): This is the most commonly used type of implant. The various types include screws, cylinders or blades surgically placed into the jawbone. Each implant holds one or more prosthetic teeth. This type of implant is generally used as an alternative for patients with bridges or removable dentures and,

Subperiosteal (on the bone): These are placed on top of the jaw with the metal framework's posts protruding through the gum to hold the prosthesis. These types of implants are used for patients who are unable to wear conventional dentures and who have minimal bone height.

Dental implants may be an option for people who have lost a tooth or teeth due to periodontal disease, an injury, or some other reason. It is in fact a metal post that has an internal screw or clip (abutment) that holds a false tooth (or teeth) in place. Implants are usually made of titanium. After surgery to insert the implant the jawbone is fused with the titanium rod for several months. Dentures, crowns or bridges can be attached to the implant to replace your missing teeth. A single implant can support one or more replacement teeth. A full teeth replacement would require at least six implants in your upper jaw and between four and six in the lower jaw.
Initially, X-rays must be employed in order to inspect the shape and thickness of the jawbone. It’s also possible that you may need to have a CT scan if the X-rays don’t provide enough information. Success or failure of implants depends on the health of the person receiving it, drugs which impact the chances of osseointegration and the health of the tissues in the mouth. The amount of stress that will be put on the implant and fixture during normal function is also evaluated. Planning the position and number of implants is key to the long-term health of the prosthetic since biomechanical forces created during chewing can be significant. The position of implants is determined by the position and angle of adjacent teeth, lab simulations or by using computed tomography with CAD/CAM simulations and surgical guides called stents. The prerequisites to long-term success of osseointegrated dental implants are healthy bone and gingiva. Since both can atrophy after tooth extraction pre-prosthetic procedures, such as sinus lifts or gingival grafts, are sometimes required to recreate ideal bone and gingiva. The final prosthetic can be either fixed, where a person cannot remove the denture or teeth from their mouth or removable, where they can remove the prosthetic. In each case an abutment is attached to the implant fixture. Where the prosthetic is fixed, the crown, bridge or denture is fixed to the abutment with either lag-screws or cement. Where the prosthetic is removable, a corresponding adapter is placed in the prosthetic so that the two pieces can be secured together.
The risks and complications related to implant therapy are divided into those that occur during surgery (such as excessive bleeding or nerve injury), those that occur in the first six months (such as infection and failure to osseointegrate) and those that occur long-term (such as peri-implantitis and mechanical failures). In the presence of healthy tissues, a well integrated implant with appropriate biomechanical loads can have long term success rates of 93 to 98 percent for the fixture [4-6] and 10 to 15 year lifespans for the prosthetic teeth [7].

### 1.4 Dental Implant Techniques

The most acknowledged dental implant techniques are:

#### 1.4.1 Immediate Loading Implant

Immediate loading is an implantation method, where one does not have to wait before placing the tooth, teeth or other types of restorations. In other words, by using immediate loading implants, we can reduce the time and cost that a two-stage treatment (consisting of implantation and later restoration) needs. This immediate loading implant is the choice for those patients who don’t want to wait months for their new teeth. This new process condenses the two stages of former implantation methods. Once the implant is done, the restorations are attached on the same day, so the whole procedure takes only a few hours. The immediate loading implant sounds like a breakthrough in dentistry, and it indeed is, although it is not a miracle. Like almost all dental treatments, immediate loading has its advantages but it is not without potential problems and risks. In brief, immediate loading does not allow the implants to heal perfectly; and the osseointegration (the process whereby the implant grows into the jawbone, and becomes an inseparable part of it) cannot take place. Osseointegration is crucial for determining the success of implantation, because the implants have to render a solid foundation for the restorations. If the implant does not fuse properly with the jawbone, the implant may not be strong enough. We have to emphasize that the implants in normal cases do heal completely and provide a solid foundation for the restorations; but using immediate loading increases the risk of implant failures. The major advantage of this technique is that the patient does not have to wait for the osseointegration, and he or she does not have to wear a complete denture during those months (approximately three to six months) the osseointegration needs. However, the patient will need more implants. If the traditional process requires one implant, you may need two, etc. This is due to the higher risk of implant failures. The osseointegration cannot be completed in a day (it takes three to six months in normal cases), so the risk of failure increases and the patient’s jaw may even reject the implant – and the restoration with it. Immediate loading costs more than the traditional, two-stage placement.
1.4.2 Delayed Placement Implant

Delayed placement is the term used for two-stage placements. The first stage is the implantation; the implant is inserted into the jawbone. Then, after some months, the second stage takes place, when the tooth, teeth or major restorations are attached to the implant. The implant needs approximately three to six months to heal perfectly, so the two stages are divided by a rather long period. Thus it is for good reasons. In the beginning of the first stage, the dentist anaesthetizes the patient and exposes the jawbone by opening the gums. Once the gums are open, the implant or implants are placed, and the incision is closed. Opening and closing the gum seems to be a minor operation, though it is an operation, so, as in all other cases of oral surgery, the gum has to heal perfectly. The gum heals typically in three to six months. While the gum is healing, the implants are integrating to the jawbone; this is called osseointegration. During this process the implants and the jawbone become one firm structure, and provide a strong base for the second stage, the placement of the restorations, tooth or teeth. Three to six months after the first stage is done, the dentist opens the gum again so that he can have an access to the implants placed formerly. The dentist then uses an attachment, and fixes or screws it to the implant. The implant is completely invisible but it is the base where the visible parts, the restoration, can be attached. If the implant is well integrated with the jawbone, the restorations can be used exactly as natural teeth, and they also look the same. Contrary to immediate loading, delayed placement decreases the risk of implant failures. The patient will need fewer implants to provide the same stability his or her restorations need. Between the first and second stages, the osseointegration can take place (in fact we don’t start the second phase until the osseointegration is ready), so the implants provide a strong and perfect base for the restorations. Delayed placement is simpler than immediate loading, and costs less. The only disadvantage of delayed placement is the delay: the two stages of the process are separated by three to six months (or, in certain cases, even more). Some patients therefore choose immediate loading. The question is: “Should I choose immediate loading or delayed placement?” Immediate loading has more disadvantages then delayed placement, but that method also has its advantages. Try to gather as much information as you can, and feel free to ask our doctors too. You can choose whichever method you want; but do be sure that you know everything about both procedures, and make a decision which is based on facts, and not only on advertising hype and salesmanship.

1.4.3 Bone Grafts

Bone grafts are used by dentists when the patient’s jawbone is so thin or for some reason so shrunken that it cannot hold an implant. We use x-rays to get a picture of the overall state of your teeth and
jawbone; and if the bone cannot provide a firm base for the implant that will hold the restoration, a bone graft is necessary. The jawbone can be or become thin due to natural processes, like ageing; but it also happens due to gum disease, an accident, or other causes. If you lose a tooth, the gum will start to recede; and, for various reasons, the jawbone itself starts to shrink, too. While our full set of teeth is in its proper place, the gum and the jawbone are kept in healthy condition. If someone loses several teeth, the whole facial jaw line will “sink in”. This causes the person’s face to look aged and unattractive. We can prevent this by using dental implants; but if the patient lost their teeth quite a long time ago, it is likely that he or she will need bone grafts before the dentist may start the dental treatment. Bone grafts are new, but the results achieved by using them cannot be criticized or ignored. The method is quite simple: the dentist grafts bone from your body into the jawbone, thus making it firm and stable. To avoid all possible negative consequences in most cases, the patient’s own bone is grafted, though there are alternative methods, where an artificial material is grafted into the jawbone. The successful synthetic bone graft is just as good as the natural bone graft, but there may be complications when using the synthetic material.

There are four types of bone grafts. These are:

**Autografts:** The best and most secure way of bone grafting is without doubt using autografts. This means that bone is removed from the patient’s body and then it is grafted into the jawbone. We use bone taken from the patient’s hip in almost all cases. This is because the hip bone (technically speaking: the ilium) is rich in marrow, so bone cells can be produced at a high pace once the bone is grafted on the jawbone. The grafted bone then helps the jawbone heal; and because the grafted bone is removed from the patient and is full of marrow, the healing process is relatively quick and there is almost no risk of failure at all.

**Allografts:** Allografts and autografts differ in one important respect: if we use an autograft, it is taken from your own body; whereas allografts are taken from a donor’s body. This does not mean that the dentist has to find someone who can donate bone to you, because there are bone banks (like blood banks), and the dentist has merely to choose the bone suitable for you and then graft it into your jawbone. Allografts are completely safe; and if you don’t want to have an autogenous bone graft, allografts are the perfect solution. Of course these allografts are carefully chosen, checked and sterilized, so their use means no added infection risk at all. The bone is tested, then sterilized, and grafted only after proper preparation. The donor bone the will be assimilated by the jawbone of the patient.
Xenografts: As the name indicates, xenografts are bone taken from an animal. Allografts and autografts are from human donors, but there is another option: dentists can use bone from animals, mostly from cows. While at first glance it is a scary thought that the bone of a cow will be grafted into your jawbone, this method does not have higher risks than allografts. Cow bone is rigorously scrutinized and sterilized to avoid all possible negative consequences. On top of that, the cow bone will not remain in your jawbone forever, because it is slowly replaced by your own bone.

Alloplastic: Alloplastic grafts are synthetic bone. While the first three grafting methods require natural bone, this one requires only skill and technique. The alloplastic graft is made of calcium phosphate, and looks like your natural bone. As you have seen, xenografts are replaced by your own bone over the time, and this can happen to one type of alloplastic graft as well. These grafts are called resorbable (= reabsorbable) grafts. The other type is – of course – called a non-resorbable graft. Resorbable grafts are slowly replaced by your own bone, while non-resorbable grafts remain grafted into your jawbone forever. The result is guaranteed. it is the the patient’s choice which grafting method he or she wants.

1.4.4 Bone grafting

Bone grafting is a simple process, though, as with almost all medical interventions, it is not without risk. The procedure is quite simple: the dentist makes a small incision to reveal a part of your body that provides the bone to strengthen your jawbone. We harvest the bone for an autograft either from the hip or the chin. After this the dentist opens your gum and exposes the jawbone so that he can place the bone on and into your thin or shrunken jawbone. Thus the grafting process is ready, and in 99% of the cases you do not have to fear that your body will reject the “new” bone, because it is your own bone that will unite with the jawbone and make it stable and firm.

If required we can give you painkillers to smooth the negative effects of removing the bone from a part of your body and grafting it into another one. We have to inform you that although all grafting procedures are planned and meant to be totally safe and secure, we cannot guarantee that your body will receive the bone or synthetic graft without problem. If any complication occurs, the grafting process can be repeated. Once the intervention is done, you need to wait 3 months to a year to get your implants, and then another three to six months to get the required restoration. Although this is quite a long time, we remind you that there is no other method which reduces the waiting period. If you consider that your jawbone is in such a condition that it has to be “healed” and restored to its original form, three months (or even a year) does not seem so long. The most important thing is that we allow enough time for your jawbone to heal itself completely.
Of course we also know that if you want implants and restorations you want them as soon as possible. That’s why we use platelet rich fibrin, a type of glue, which speeds up the healing process of your jawbone. PRF helps new bone and tissue to grow, and helps nature do its job.

1.4.5 Sinus lift

A sinus lift (sometimes called a sinus graft) is the same process as jawbone grafting. The only difference is that it affects another part of your mouth, the upper jaw or maxilla. The upper jaw is not as thick as the lower, so it is more difficult to do a grafting in this area, though dentistry of course has a solution for this problem, too. The sinus (or maxillary sinus or antrum of Highmore) is a large, pyramid-shaped formation. Everyone has two sinuses, one on the left side and one on the right side of your nose, just above your teeth. The upper jaw, which separates the sinuses from your teeth, in certain patients is not thick enough to provide a firm base for implants that need to be screwed or fixed into the jaw. If your upper jaw is thin, your sinus has to be lifted – though this name is misleading, because in fact your sinus is not lifted, rather the bone beneath it is thickened. In other words, your sinus is made smaller so that your thickened upper jawbone can hold the implants. Previously, if your upper jaw was for some reason too thin, the only option was the use of a complete or partial denture. With this rather new technology, dentist can place implants even in these cases into your upper jawbone – if it was prepared in a proper way. The sinus wall is thickened through grafting bone onto it, and lifting the sinus membrane. Once osseointegration has taken place and your upper jaw has fused with the new bone, the base for placing implants is ready.

1.4.6 Onlay Graft

An onlay graft is a bone graft in which the transplanted bone tissue is not integrated into the jawbone but laid on the surface of the recipient bone. After the intervention you have to wait a few months to let the bone heal. Once the bone tissues are healed and healthy, they can hold the implants.

1.4.7 Ridge expansion

Ridge expansion means that the dentist splits the jaw to make it wider. Sometimes the jaw is not wide enough to provide a firm base for implants. In such cases the dentist has to intervene to make the jawbone appropriate for future implantation(s). Once the split has healed, the second and third phase of the treatment can take place, and the dentist can place the implants and the restorations. Some doctors say that you don’t have to wait until the split heals, and the implants can be placed immediately. We say
that it is safer and better if we let the wound heal, and move on to the next stages only if your jawbone is completely healthy.

1.4.8 Distraction osteogenesis

Distraction osteogenesis was a widely used medical treatment in the Soviet Union. Doctors broke the shorter leg of a patient whose legs were not of the same length, and then they distracted the broken bone. The bone’s middle ends tried to “catch each other” and stretch toward each other, just like when we give our hand to someone. Thus the bone was compelled to expand, and the shorter leg became more or less the same length as the other one.

A similar process is used in dentistry nowadays. A section of bone is divided from the jawbone, and a device prevents this section to contact with the rest of the bone. The space between the jaw and the jawbone is widened, thus the jaw is compelled to fill the space in order to unite with the separated part. We can say that we simply trick the jawbone to make it expand, just as when someone shows hay to a donkey to get it to take another step forward. Naturally we use titanium devices, not hay.

1.4.9 Nerve Repositioning

If the dentist repositions a nerve, he or she moves it to one side in order to make the area clear for future implanting interventions. Why? If someone lost a considerable amount of bone, the so called alveolar nerve can be near the surface. Thus the dentist would inevitably destroy or at least injure the nerve, and the patient would lose his or her sensation in the chin and the lower lip. To avoid this, the dentist moves the nerve to one side. This is a risky intervention as well, because even the reposition of this very sensitive nerve can damage it. If a dentist suggests to you to make this minor operation, it is because there is no other way to prepare your jaw for the implants, and because he pondered the risks and is completely confident in the success of this surgery.

1.4.10 Barrier Membranes

Barrier membranes are used in those cases when the patient has lost a serious amount of bone tissue, and other, weaker tissues started to take the place of the bone. The membranes can hold back the weaker tissues (connective and epithelial) and aid bone tissues to fill the area that once was theirs. The membranes help bone to regenerate and make a solid base for dental treatments. Barrier membranes, just like alloplastic grafts, can be either resorbable or non-resorbable. Nowadays we don’t use the latter, because resorbable membranes are natural and have the same effect as non-resorbable ones.
1.4.11 Cone Beam Volumetric Technology (CBVT)

CBVT is a cutting edge technology for craniofacial scanning. This method of computerised tomography creates 3D images, helping the dentist to gather as much information as he or she can before performing an implantation or another medical intervention, such as maxillofacial surgery or treatment of temporomandibular joint disorder (TMJD). CBVT technology uses multimodal imaging and provides high quality images showing soft tissue contrast. Using the new technology the patient is exposed to less radiation than with previous techniques. During the scan process the CBVT provides a continuous image. The image is created by an energy saving fluoroscopic tube. This method is remarkably faster than traditional CT mapping, and the patient is exposed to a lower dose of radiation. The new scanner allows the patient to sit upright (in contrast to a traditional CT scan, where the patient must lie horizontally) while the scanning process takes place. Both the x-ray tube and the detector is located around the patient’s head. There is an even better scanning device which provides more detailed 3D images. The images made with the Ultra Cone Beam CT scanner are extremely clear and detailed, and allow dentists to plan the dental treatment meticulously.

1.5 Advantages of Dental Implants

There are many advantages to dental implants, including:

− Improved appearance. Dental implants look and feel like human teeth. And because they are designed to fuse with bone, they become permanent.
− Improved speech. With poor-fitting dentures, the teeth can slip within the mouth causing word mumble. Dental implants also allow speaking without the worry that teeth might slip.
− Improved comfort. Because they become part of the patient’s mouth, implants eliminate the discomfort of removable dentures.
− Easier eating. Sliding dentures can make chewing difficult. Dental implants function allow eating with confidence and without pain.
− Improved self-esteem. Dental implants can give you back to the patient it’s smile and make him feel better about himself.
− Improved oral health. Dental implants don’t require reducing other teeth, as a tooth-supported bridge does. Because nearby teeth are not altered to support the implant, most human teeth are left intact, improving long-term oral health. Individual implants also allow easier access between teeth, improving oral hygiene.
Durability. Implants are very durable and will last many years. With good care, many implants last a lifetime.

Convenience. Removable dentures are just that; removable. Dental implants eliminate the embarrassing inconvenience of removing dentures, as well as the need for messy adhesives to keep them in place.

1.6 Side-effects

Side-effects are the unwanted but mostly temporary effects a patient may get after having the procedure. Some swelling and discomfort around the implant area may occur. Complications are when problems occur during or after the procedure. The possible complications of any operation include an unexpected reaction to the anaesthetic, or excessive bleeding. The nerve that runs to the patient’s face has branches that are in the lower jaw. These supply the feeling to the lower jaw, lower teeth and gums and bottom lip. If the nerves are damaged by the implant, permanent tingling or numbness may be felt. It may also be painful. X-rays and CT scans help your dentist to see the position of the nerves in the jawbone to minimise this risk.

Occasionally, the jawbone doesn't fuse with the implant properly and the implant can become loose. This isn't usually painful, but the implant won't be able to support false teeth. Another implant must be fitted. Regular check-ups must be attended to make sure that implants are still secure.
Chapter 2 – Growth Factors

Summary

2.1 Introduction

Dental implants are the most innovative and superior treatment in dentistry, and are widely used for a variety of cases. Most of the techniques that are used are evidence-based and predictable. However, in many cases, the intended implant site is inappropriate due to the poor bone quality or to an insufficient quantity of bone. An insufficient alveolar ridge height is often related to the proximity of the implant site to other anatomical structures, i.e., the maxillary sinus or the mandibular canal.

In order to overcome some of these difficulties, autogenous bone grafts taken from the chin, the ramus of the mandible, or the iliac crest of the same patient have historically been the standard for alveolar reconstruction, specifically, due to their osteoconductive, osteoinductive, and lack of immunogenic properties. However, the adverse events and complications, such as infection, pain, sensory loss, and hematoma formation at the donor site, occur frequently upon autogenous bone graft treatment. In addition, a donor site with a sufficient quantity of bone is not always available. Allograft bones, bones taken from a different person and processed and managed by a tissue bank or commercial supplier, have often been substituted. However, this method also has limitations, including an inconsistent osteoinductive activity, unfavorable host immune responses [20], a delayed resorption, and a risk for prion and virus transmissions [21] and [22].

An ideal bone graft in implant dentistry should have the following properties: it should be biomimetic; it should have the ability to induce differentiation of the appropriate cells (i.e., endothelial and osteoblastic cells) for the formation of new bone; it should be easily synthesized or produced, rather than extracted from allograft materials (to eliminate all risks of disease transmission); it should be easily and quickly resorbed as the osteogenic response occurs; it should have no immune-provoking properties; it should be easily transported and stored; it should be reasonably cost-effective; it should be capable of achieving consistent and predictable results without being affected by different level of technical ability of the clinician.

In order to meet these demands, dental research has focused on the use of bioactive molecules to induce local bone formation. Since the various growth factors that have an effect on the bone regeneration have been discovered, the number of related studies has increased substantially. In particular, the factors recombinant human Bone Morphogenetic Protein-2 (rhBMP-2) [23-26] and recombinant human Platelet-Derived Growth Factor (rhPDGF) [27], [28] and [29] have been shown to...
induce bone formation at the compromised sites in a variety of experimental and clinical situations. These factors have also been approved by the U.S. Food and Drug Administration (FDA) for use in dentistry.

2.2 Growth Factors

Growth factors are polypeptide hormones or biological factors. They mediate many cellular processes necessary for the various stages of growth, induction of phenotypic expression, and even the metamorphosis of totipotent germ cells into pluripotent stem cells. [30-31] A totipotent germ cell is a single cell that can recapitulate or repeat development, demonstrating the capacity to develop and differentiate into a complete organism [32-33]. A pluripotent stem cell is "capable of differentiating into several different final differentiated types." [34] When germ cells are cultured in certain polypeptide growth factors, they can be stimulated to transform into pluripotent stem cells. Growth factors are also known to influence the growth rate of certain cancers. [35]

Many tissues are mediated by growth factors: epithelium and endothelium; myocardium and neural tissue; cartilage, bone, and cementum; the more specialized types of connective tissue and blood: and the less specialized connective tissue replete with extracellular matrix, such as collagen and proteoglycans that surround other more highly developed tissues and organs.¹

Polypeptide growth and differentiation factors (GDFs) are classified as biological mediators that have demonstrated a significant role in stimulating and regulating the wound healing process. The growth factors associated with repair and regeneration coordinate important cellular processes such as:[36]

1. Stimulation and inhibition of growth
2. Development and differentiation
3. Regeneration, repair, and wound healing
4. Mitogenesis
5. Chemotaxis
6. Metabolism
7. Angiogenesis
8. Apoptosis or programmed cell death

The sequential steps that are critical for periodontal regeneration depend on the processes of osteogenesis, cementogenesis, and formation of connective tissue. Various in vitro and in vivo studies revealed that specific growth factors modify reputed elements of periodontal wound healing, thus achieving significant periodontal regeneration in animals. [37]
In 1976, Melcher [38] proposed the initial hypothesis on the potential of periodontal regeneration. Since then the rapidly growing sector of regenerative research has striven to identify modes of treatment that support regeneration of lost periodontal tissues. Currently utilized regenerative techniques incorporate guided tissue regeneration (GTR) alone or with bioabsorbable or nonresorbable membranes, allografts and bone autographs, and agents such as citric acid or tetracycline used to condition roots and enhance the attachment of periodontal tissues.[39]

The past decade has witnessed a concentrated effort by cell and molecular biologists to determine how polypeptide growth and differentiation factors (GDFs) effect the repair and regeneration of tissues. These molecules exist in nature and demonstrate pleiotrophic or multiple effects on wound repair in almost all tissues, including the periodontium. Growth and differentiation factors exert effects, both in vivo and in vitro, on soft tissue components of the periodontium such as the periodontal ligament (PDL) and gingival connective tissue as well as the hard tissue structures such as alveolar bone and cementum.[40]

As natural biological mediators, polypeptide growth factors modulate significant cellular events in tissue repair:1

1. Cell proliferation
2. Chemotaxis or directed migration
3. Differentiation
4. Matrix synthesis via binding to specific cell-surface receptors

The following nine growth and differentiation factors (GDFs) are found in bone, cementum, and healing wound tissues.[41]

1. Platelet-derived growth factor (PDGF)
2. Concentrated growth factors (CGF)
3. Bone morphogenetic proteins (bmps 1-12)
4. Vascular endothelial growth factor (VEGF)
5. Transforming growth factors (TGF-α and -β)
6. Acidic and basic fibroblast growth factors, (a- and bFGF)
7. Epidermal growth factor (EGF)
8. Insulin-like growth factors (IGF-I and -II)
9. Cementum-derived growth factor (CGF)
10. Parathyroid hormone-related protein (pthrp)
After an injury occurs, healing proceeds in a succession of “well orchestrated cell-cell and cell-macromolecular interactions”. In the process of normal wound healing, various growth factors act in conjunction to form a complex arrangement of molecules that regulate cellular activity within and bordering the wound.

When an acute injury of tissue extends into the level of subepithelial tissues, the interruption of wound vasculature results in the formation of fibrin and an aggregation of platelets that form a cellular plug. Platelets at the wound margins become activated and produce various growth factors such as PDGF, TGF-β, an epidermal growth factor (EGF)-like protein, and platelet-derived endothelial cell growth factor (PD-ECGF). Additionally plasma exudate produces insulin-like growth factors, an important source of these regulatory molecules.

Within a few hours after the injury occurs, cells bordering the site of injury produce growth factors such as IGF-I, PDGF, TGF-?? and TGF-?. Subsequent to tissue injury, neutrophils accumulate followed by migration of macrophages within the next several days into the area. Macrophages provide for the debridement of damaged tissue and also serve as another source of growth factors such as PDGF, TGF-α, and TGF-β1.

### 2.2.1 Platelet-Derived Growth Factor-AA,-AB,-BB

The normal count of platelets in blood circulation is 150000-300000 /mm3. They derive from the division of megakaryocytes in the bone marrow. They then enter the blood circulation as anucleated cells and their lifespan ranges from 7 to 10 days. Platelets produce growth factors, and actively release them according to blood clotting needs. They are characterized by the presence of pseudopodia, projections of the cell membrane, and intracellular vesicles called endosomes, which act as storage granules. There are three types of endosomes:

- a. **Lysosomes**: used to store hydrolytic enzymes
- b. **Dense endosomes**: they store and release ADP, the platelet activator
- c. **Alpha (α) endosomes**: the largest population of granules and the storage space of growth factors, which are in a biologically inactive form. The α-granules are also rich in vitronectin, a cell adhesion molecule necessary in osseoinduction and osseointegration (Rendu 2001).

Platelet cytoplasm contains other factors such as fibrin, a protein related to blood clotting. The initiation of the coagulation mechanism is related to structural changes of the platelet cell membrane. Platelets are activated and thus release content of the granules. The α-granules move to the platelet membrane, where they are being engulfed. The incomplete and inactive growth factors are completely formed with
the addition of histones and carboxylic groups. Strong agonists such as thrombine and collagen provoke this release even when conditions are not ideal for platelet accumulation. Mechanisms that determine the movement of Ca+ ions are also essential for the activation of platelets. The wound healing process is divided into three stages:

**Biochemical activation.** The trauma is translated into a biochemical signal for the host to be able to assess the conditions. By blocking the microcirculation, the plasma gets in contact with the tissue proteins, thus activating the Hagemann factor and the circulating platelets. The Hagemann factor is the one that initiates a chain reaction which ends with cellular activation. This chain reaction produces fibrin to achieve coagulation and thrombine to accomplish the maximum release of a-granules.

**Cellular activation.** Platelets responding to the initial trauma accumulate and act in a heamostatic and mediator manner. Following the exposure of sub-epithelial tissue to blood, accidentally or after surgical operation, platelets adhere to exposed collagen proteins and release granules containing ADP, serotonin and thromboxane, which in turn contribute to the coagulation and blood clotting mechanisms. In addition, platelets are attracted to the region participating in the clot formation, enhanced by fibrin. Subsequently, granulation tissue is produced, the collagen part of it being the structural base for growth factor activity. Their interaction with target cells activates the transcription of mRNA and the production of proteins necessary for the regenerating process. These proteins, combined with other transcriptive factors, activate an array of genes. The result of this process is the activation of mesenchymal and epidermal cells to migrate and proliferate and of collagen and glycoaminoglycans production, thus initiating the healing process. The aim of cellular activation is to produce sufficient activity in the host. This means that there is not adequate population of cells to promote healing, although there are some differentiated cells and a small number of progenitor stem cells with healing potential.

**Cellular response:** Monocytes are transforming into macrophages, accompanying neutrophils to host defence and producing growth factors that lead the healing process to the very end.
In vitro and in vivo, PDGF has been depicted as being the most thoroughly described growth factor associated with the periodontium. There are different forms of PDGF called isoforms and all of them have been shown to have a PDL fibroblast proliferative activity in vitro. An isoform is a protein having the same function and similar or identical sequence, but it is the product of a different gene and is usually specific to a particular tissue.

Since platelet-derived growth factor is chemotactic for fibroblasts in the periodontal ligament, it induces collagen and total protein synthesis. Platelet-derived growth factor stimulates gingival fibroblasts to synthesize hyaluronate, which is necessary for the formation of large groups of proteoglycans that supply the framework on which the extracellular matrix can develop. [46]

Lipoplysaccharide is a major constituent of the cell walls of gram-negative bacteria, i.e., periodontal pathogenic bacteria; it is associated with loss of alveolar bone in periodontal disease. Lipopolysaccharide inhibits the proliferation of gingival fibroblasts; and platelet-derived growth factor decreases this inhibitory effect.

Platelet-derived growth factor also increases the proliferation of fibroblasts under teflon membranes (polytetrafluoroethylene membranes [ePTFE]) in fenestration defects in dogs. Apparently, platelet-derived growth factor supports the healing in the periodontal soft tissue wound in a variety of ways. [47] PRF is a second-generation platelet aggregation fibrin-rich gel produced from the venous blood by single centrifugation. After centrifugation, the middle layer is obtained from the lowest level of red blood cells, and contains almost no platelets, while above there is a layer of plasma. PRF contains clotting factors that form a fibrin network that traps various cytokines in the PRF. It is not necessary to artificially delay PRF formation with an anticoagulant because it does not begin immediately. It is also not necessary to promote the natural blood-clotting process and platelet activation as the fibrin network structure is formed by centrifugation with large amounts of biological factors such as cytokines being captured. In
this study, blood was taken in exactly the same way as for PRP and put into two different tubes. The sample tubes were centrifuged with a PRF centrifuge (GYRO416, Gyrozen, Korea) at 3000 rpm for 10 min. During centrifugation, the hemostasis phenomenon divided the blood sample into layers, and one of these layers was PRF, fibrin layer containing platelets and plasma.

2.2.2 Concentrated growth factors (CGF)

Platelet concentrate such as platelet rich plasma (PRP) have been used to accelerate tissue healing for a long time. But the effect of PRP is controversial regarding hard tissue regeneration. Unlike PRP, CGF is well known to accelerate new bone formation. PRP uses complex protocols to prepare and chemical additives, concentrated growth factors (CGF) overcomes these disadvantages of PRP. The preparation of CGF is simple. Compared to PRF, CGF is attained by single centrifugation using special centrifuge. In addition CGF doesn’t require any chemical or allergenic additives, such as bovine thrombin or anticoagulants, so is free from viral transmission disease. CGF is 100% autologous fibrin. PRF was introduced by Choukroun in the first time. The protocol of CGF is very simple. The venous blood sample is taken without anticoagulants in1 sterile 10mL tube and immediately centrifuged in special centrifuge device (Medifuge, Silfradent srl, Sofia, Italy) for 12 minutes. (Fig 1)

![Special centrifuge for the preparation of CGF](image)

**Figure 2.3** Special centrifuge for the preparation of CGF (Medifuge, Silfradent srl, S.Sofia, Italy), one step protocol is needed to obtained CGF from blood sample unlike PRP

Concentrated growth factors are aggregated in the middle layer after 12 minutes special centrifugation. Red corpuscles is separated from fibrin clot with scissor before use Because anticoagulants are not used,
the blood sample should be centrifuged immediately after taking blood sample to best quality of CGF. After centrifugation, CGF is obtained between acelullar plasma at the top layer and red corpuscles at the bottom layer.

**Figure 2.4** Surgeons can use CGF as barrier membrane to accelerate soft tissue healing or be mixed with bone graft to accelerate new bone formation.

### 2.2.3 Bone Morphogenetic Proteins (BMPs)

The bone consists of hydroxyapatite and collagen, while being the storage place for the growth and differentiation factors. The bone morphogenetic proteins (BMPs) constitute strong growth factors that induce the differentiation of osteoblasts and bone formation. Therefore, they are important regulators of the healing process and are related to the homeostatic mechanisms of bone tissue. BMPs were discovered during research investigations for the identification of molecules responsible for the osseoinductive activity of decalcified bone [48-50].

BMPs are found in human and animal bones. Fifteen different types of BMPs have been identified [41-52]. They all belong to the wider family of TGF-β (transforming growth factor-β), apart from BMP-1. Their effect on human osteoblasts depends on the age of the individual. BMPs affect the phenotype, the increase and differentiation of osteocytes.

The bone morphogenetic proteins provoke the induction and the expression of many phenotypes in pluripotent undifferentiated mesenchymal cells, which are recruited from the surrounding tissues.
These proteins elicit an increase in the concentration of mesenchymal cells through chemotactic mechanisms on the above cells [53-55]. BMP-2 in particular, acts in an anabolic manner on human osteoblasts which derive from the mandible, through the differentiation of osteoblasts and the negative regulation of MMP-1 synthesis. It is related to the degradation of the extracellular matrix of bone tissue, collagen destruction and bone remodelling [56].

The osseoinductive activity of BMPs is affected by the transporter through which it is applied on the recipient site. This transporter must have the following properties:

A) to provide a net in order to facilitate the accumulation and adhesion of osteoblasts

B) to restrict the activity of the morphogenetic protein within its limits, in order to avoid the production of more bone tissue than needed

C) to be absorbed at a ratio similar to the that of bone tissue production

In case that the absorption of the transporter is faster, the protein might be lost or diffused and the outline of the implant could be damaged. In case that the absorption is delayed, an overproduction of bone tissue might be observed.

Due to the low concentrations of morphogenetic proteins observed in bones, higher quantities of bone tissue are needed if we want to obtain adequate concentration of protein for the biological applications.

In our days, taking advantage of the latest technologies of recombinant DNA, we can acquire vast quantities of recombinant human BMP (rhBMP) for several applications. These proteins have been successfully used in patients with extensive bone deficiencies, and also in periodontal regeneration techniques [57-59].

2.2.4 Transforming Growth Factor-β (TGF-β1)

Transforming growth factor-beta 1 strongly induces the production of extracellular matrix in periodontal ligament fibroblasts as well as many other cell types. Although transforming growth factor-beta [11] does not seem to be involved with the migration of periodontal ligament fibroblasts, it has a mild influence on the passage of periodontal ligament fibroblast cells through the cycle of cell division with resultant daughter cells. Transforming growth factor-beta 1, alone or in combination with platelet-derived growth factor-BB, induces periodontal ligament fibroblasts to proliferate at greater levels than gingival fibroblasts.[30] The PDL must be able to proliferate at a faster rate than gingival epithelium in order to achieve true new attachment.

Transforming growth factor-beta 1 increases the number of platelet-derived growth factor-b receptors, but at the same time decreases the number of PDGF-a subunits. A receptor is a molecular structure located within or on the surface of a cell; it selectively binds a specific substance followed by a specific
physiologic effect, for example, cell surface receptors for hormones. TGF-β1 hinders the reproduction of epithelial cells. Considering all of these facts together, TGF-β1 may participate in periodontal wound healing.

2.2.5 Basic Fibroblast Growth Factor (bFGF)

Basic fibroblast growth factor (bFGF) is a strong chemotactic agent (movement directed by a gradient) and mitogenic (cell division or transformation) agent for periodontal ligament fibroblasts. Dentin blocks coated with basic fibroblast growth factor induce the migration and multiplication of human endothelial cells. Exposure of type I collagen on dentinal surfaces increases the binding of basic fibroblast growth factor.

2.2.6 Epidermal Growth Factor (EGF)

Epidermal growth factor seems to exert only a minor effect on the promotion of mitogenesis, chemotaxis, or matrix synthesis in periodontal ligament fibroblasts. During differentiation, EGF and EGF receptors (EGF-R) are localized on periodontal ligament fibroblasts in rats. Epidermal growth factor receptors may stabilize the periodontal ligament fibroblast phenotype or cellular physical characteristics.

Stimulation of epidermal growth factor receptors and periodontal ligament fibroblasts is related to sustaining the cells in an undifferentiated state (with decreased alkaline phosphatase activity). Inhibition of epidermal growth factor receptors is associated with the differentiation of cells into osteoblasts or cementoblasts. In vivo testing is needed on the effects of epidermal growth factor on periodontal wound healing.

2.2.7 Insulin-Like Growth Factors (IGF-I and -II)

Insulin-like growth factor-1 is chemotactic for cells that come from the periodontal ligament and demonstrates significant effects on the mitogenesis of periodontal ligament fibroblasts and protein synthesis in vitro. Insulin-like growth factor-I receptors are present on the surface of periodontal ligament fibroblasts. However, the effect of insulin-like growth factor-II on the metabolism of gingival and periodontal ligament fibroblasts is uncertain.

2.2.8 Cementum-Derived Growth Factor (CGF)

Cementum-derived growth factor is a more recently characterized growth factor and is thought to be found only in cementum. It is mitogenic for gingival and periodontal ligament fibroblasts. Cementum-derived growth factor helps progenitor cells located in structures adjacent to the dentin matrix to differentiate into cementoblasts. It may also encourage their growth and migration.
Questions remain about the effects of cementum-derived growth factor on the synthesis of collagen by periodontal ligament fibroblasts. However, cementum-derived growth factor is responsible for more collagen synthesis in human lung fibroblasts than PDGF-AB or –BB. In vivo experimentation would be aided by producing larger quantities of cementum-derived growth factor via molecular cloning and expression.

2.2.9 Effects of Growth and Differentiation Factors on Bone and Cementum Regeneration

Growth factors are found in bone matrix and are thought to link the bone formation to bone resorption.

[32] Bone matrix is the bone tissue located in between bone cells; it consists of collagen fibers and ground substance, into which the inorganic salts (phosphate, carbonate, and some fluoride) are deposited in the form of an apatite. Hydroxyapatite is the calcium phosphate mineral that is a component of bone and dentin.

Many in vitro and in vivo model systems have been used to determine how polypeptide growth factors affect bone. Less data seems to be available on how growth factors affect alveolar bone metabolism. Various growth factors have been located in cementum matrix, but the specific effects that they exert on cementogenesis is less well understood. [30]

2.2.10 Transforming Growth Factor-β (TGF-β1)

Studies on stimulation or inhibition of osteoblast proliferation suggest that transforming growth factor-b is markedly dependent upon the source of bone cells, the dose applied, and the local environment. In addition to bone matrix deposition and chemotaxis, transforming growth factor-beta 1 enhances the biosynthesis of type I collagen, fibronectin, and osteonectin. Transforming growth factor-beta 1 decreases the destruction of connective tissue matrix by inhibiting the synthesis of metalloproteinases and plasminogen activator, which increases synthesis of tissue inhibitor of metalloproteinase (TIMP) and plasminogen activator inhibitor (PAI). While transforming growth factor-beta 1 seems to decrease formation of osteoclast-like cells, it may use a prostaglandin-mediated mechanism to increase bone resorption. Repeated injections of transforming growth factor-beta 1 leads to cartilage formation in long bones, and ultimately ossifies to bone through endochondral bone formation. During the normal healing process in human fractures, the body expresses genes for transforming growth factor-beta 1. Transforming growth factor-beta 1 is abundant in bone and exhibits multiple effects on the formation of bone matrix. As a result, it is thought to act as a bone coupling factor that links bone resorption to bone formation. [30]
2.2.11 Fibroblast Growth Factors (a- and b-FGF)

Both forms of fibroblast growth factors (acidic and basic) are present in bone matrix; they enhance synthesis of DNA and replication of cells in vitro. Not only do fibroblast growth factors fail to directly stimulate mature osteoblasts, but they inhibit the activity of alkaline phosphatase.[30] In an immortal cell line, basic fibroblast growth factors were reported to decrease mRNA levels of osteocalcin and type I collagen. However, researchers reported a significant finding that fibroblast growth factors were potent stimulators of angiogenesis, a process vital to the vascular invasion of bone.

2.2.12 Insulin-Like Growth Factors (IGF-I and IGF-II)

Although insulin-like growth factor-II is the most plentiful growth factor found in bone matrix, insulin-like growth factors-I and II both exist in abundant amounts in bone. Osteoblasts produce insulin-like growth factor-I, which stimulates cellular proliferation, differentiation, and type I collagen biosynthesis in order to form bone.[30]

Since high levels of insulin-like growth factor-I are synthesized and secreted by osteoblasts in bone, insulin-like growth factor-I might control the formation of bone in an autocrine manner. Autocrine secretion refers to the secretion of a substance, such as a growth factor, that stimulates the secretory cell itself. [30] It provides for independence from control of the rate of cellular division rather than of the size of an individual cell. Insulin-like growth factor-I has also been reported to increase the numbers of osteoclastic multinucleated cells. When insulin-like growth factor-I was applied to the root surfaces of rat teeth, it promoted cementogenesis within 8 days after the teeth were reimplanted. However, only small increases in new cementum and bone formation were reported when insulin-like growth factor-I was applied to naturally occurring periodontitis lesion in dogs.

When tested in vitro, insulin-like growth factor-II demonstrates effects similar to insulin-like growth factor-I. High levels of insulin-like growth factor-II can bind to the insulin-like growth factor-I receptor. Yet, results from studies imply that insulin-like growth factor-II is less potent than insulin-like growth factor-I in stimulating bone formation.

However, both insulin-like growth factor-I and insulin-like growth factor-II are equally potent in stimulating osteoblast chemotaxis in vitro. Researchers have also evaluated insulin-like growth factors in various combinations with other growth factors both in vitro and in vivo.

When insulin-like growth factor-I was combined with transforming growth factor-b 1 or with platelet-derived growth factor, either combination led to more bone matrix apposition in calvarial organ culture than with individual exposure to any of these 3 growth factors. This synergistic increase was also observed when osteoblast mitogenesis increased in bone culture cells after insulin-like growth factor-I
was combined with other growth factors such as basic fibroblast growth factor, platelet-derived growth factor, or transforming growth factor-β1. [30]

Cultured adult human osteoblasts responded with maximal proliferation to a mixture of insulin-like growth factor-I, platelet-derived growth factor, transforming growth factor-β1, and epidermal growth factor. Insulin-like growth factor-II in combination with transforming growth factor-β1 acted in synergy to increase new callus formation in tibial osteotomy defects beyond what could have been expected from the effects of the individual growth factors.

Therefore, results from various studies indicate that insulin-like growth factor-I or insulin-like growth factor-II, in combination with other growth factors, may enhance the wound healing process in bone.

2.2.13 Parathyroid Hormone-Related Protein (PTHrP)

Many cells including osteoblasts, activated lymphocytes, and keratinocytes produce parathyroid hormone-related protein which is a polypeptide growth factor. It is related to parathyroid hormone, produces bone resorption, and has a strong anabolic effect on bone. Studies have identified receptors for parathyroid hormone-related protein on PDL cells and evidence of expression of parathyroid hormone-related protein on cells resembling cementoblasts in the developing tooth. [30]

2.3 Future Perspectives

In earlier studies, the application of growth and differentiation factors provided differing degrees of success in stimulating wound healing in the periodontal and peri-implant areas. These studies did establish the need to further evaluate the biologic mechanisms that may be responsible for the promotion of tissue regeneration by growth and differentiation factors.

Researchers noticed a prolonged enhancement of new bone and attachment apparatus for several weeks to months following the application of growth and differentiation factors. Temporal changes in gene expression by cells within and adjacent to the wound site may be responsible for this regeneration. The delivery systems in the earlier studies permitted the periodontal tissues to be exposed to the growth and differentiation factors for a brief time of only a few hours. Longer exposure and improved delivery systems may enhance regeneration in future studies. Ultimately, these studies seek evidence to conclusively support the addition of growth and differentiation factor therapy to the protocol for reconstructive periodontal treatment.
Chapter 3 – Image Processing and Analysis Methods

Summary

This chapter discusses pattern recognition theory along with the implementation of Probabilistic Neural Network classifier employed in this thesis. In the first part of the chapter a detailed explanation is given regarding the feature generation employed as input in the classification system. Also the segmentation theory implemented is also given in details. In the second part the classification algorithms implemented in this thesis are presented.

3.1 Pattern Recognition Theory

Pattern recognition classifies objects into a number of categories or classes. This classification procedure is a two-folded process which at first generates a description of the object (i.e., the pattern) and then classifies it based on that description (i.e., the recognition). The object description involves feature generation techniques in order to produce certain attributes, whereas the classification task associates a predefined label with the object based on those attributes. The main goal of each pattern recognition system is to determine the most accurate label for each object analyzed. The pattern recognition procedure is accomplished with a training phase that configures the algorithms used in both the description and classification tasks based on a number of objects whose labels are known as the training set. During the training phase, a training set is analyzed to determine the attributes used to label the objects with the highest possible accuracy. Following the training phase, the classification takes place to an unlabeled object based on the attributes of that object. High coincidence between the known labels and those assigned by the pattern recognition system denotes high classification accuracy. The methodology for description and classification with known attributes is called supervised learning. In cases where the training set is not available the procedure employed is termed as unsupervised learning. A common step in the pattern recognition procedure that usually precedes the hierarchy presented before is the isolation of the object to be recognized from the surrounding environment. This step is prerequisite in order for the feature extraction to take place.

3.2 Object Isolation

It is very important to extract/isolate dental implants windings in order to assess the Osseo integration within for further processing. This isolation procedure employed the Marcov Random Filed segmentation method.
3.2.1 Markov Random Field (MRF)

Markov random field (MRF) theory provides a convenient and consistent way of modeling context dependent entities such as image pixels and other spatially correlated features. This is achieved through characterizing mutual influences among such entities using MRF probabilities. The practical use of MRF models is largely ascribed to the equivalence between MRFs and Gibbs distributions established by Hamersley and Clifford (1971) [81] and further developed by Besag (1974) [82] for the joint distribution of MRFs. This enables us to model vision problems by a \textit{mathematically} sound yet tractable means for the image analysis in the Bayesian framework Grenander 1983 [83], Geman and Geman 1984 [83]. From the \textit{computational} perspective, the local property of MRFs leads to algorithms which can be implemented in a local and massively parallel manner. Furthermore, MRF theory provides a foundation for multi-resolution computation Gidas 1989 [83].

For the above reasons, MRFs have been widely employed to solve vision problems at all levels. Most of the MRF models are for low level processing. These include image restoration and segmentation, surface reconstruction, edge detection, texture analysis, optical flow, shape from X, active contours, deformable templates, data fusion, visual integration, and perceptual organization. The use of MRFs in high level vision, such as for object matching and recognition, has also emerged in recent years.

MRF modeling combines conditional (local intensity distribution) with contextual (intensity similarity within small neighborhoods) information under the Bayesian framework in order to estimate the true intensities of the image rather than those based only on the conditional information [81]. It assumes that the class probability of a pixel is only dependent on class membership of its spatial neighbors (also called lattice) which in turn reduces the possible influence of noise and overlapping structures. The model assumption that the conditional distribution depends on the pixels in the near neighborhood is subject to the Bayesian framework which states that the decision rule for labeling an image pixel combines the conditional intensity distribution of an individual region with prior knowledge regarding that region [82].

Given the fact that the observed image $y$ is a realization of a random field $Y$, $x^*$ is the true unknown label of the observed pixel, and $\tilde{x}$ indicates the estimate of $x^*$, \textit{the main objective of the MRF segmentation model is to find $\tilde{x}$ given the observed image $y$}. Let’s assume that $P(X)$ is our prior knowledge and $P(Y|X)$ is the probability of realizing the observed image given the regions distribution in the image. $P(Y|X$ = Conditional Intensity Distribution) Then, in accordance to Bayes theorem

$$P(X | Y) = \frac{P(Y | X)P(X)}{P(Y)}$$  \hspace{1cm} (2.7)
where, $P(X|Y)$ is our posterior probability. The most widely used conditional intensity distribution is the Gaussian distribution, whose function, given the class $x_i$ is given by:

$$P(Y = y | X = x_i) = \frac{1}{\sqrt{2\pi\sigma^2}} \exp\left(-\frac{(y - \mu)}{\sigma^2}\right)$$  \hspace{1cm} (2.8)

Where, $\mu$ and $\sigma$ are the distribution parameters of class $x_i$. $\mu$ and $\sigma$ are the mean value and the standard deviation.

$P(\text{X- Contextual information})$ estimated by:

$$P(\text{X} = x) = \exp\left\{ \beta U(x) \right\}$$  \hspace{1cm} (2.9)

Then, $\hat{x}$ can be obtained by taking the posterior’s probability natural logarithm and minimizing its negative resultant:[81] (minimizing its negative resultant is faster from maximizing so for this reason it is used).

$$\hat{x} = \arg \min_x ( \log(P(X | Y))) =$$

$$\arg \min_x ( \log p(y | x) \log p(x)) =$$  \hspace{1cm} (2.10)

$$\arg \min_x ( \log p(y | x) \log p(x)) =$$  \hspace{1cm} (2.11)

Substituting equations 2.8 and 2.9 in equation 2.7, the final assessment of $\hat{x}$ obtained through the calculation of the Minimum a Posteriori (MAP) in the following equation:

$$\hat{x} = \arg \min_{x_i \in L} \left\{ \frac{y}{\sigma^2} + \frac{1}{2} \log(2\pi\sigma^2) + \beta U(x_i) \right\}$$  \hspace{1cm} (2.12)

Where, $U(x_i)$ is the number of pixels in the neighborhood that have color $x_i$. and $\beta$ is a positive constant that controls the interaction between the pixels within the neighborhood.

A number of approaches has been proposed to solve this difficult optimization problem. The solutions can, in general, be viewed under two categories: deterministic or stochastic. In the following sections, we will discuss Besag’s deterministic approach known as the iterated conditional modes (ICM algorithm) and the stochastic approach, Metropolis algorithm.

### 3.2.1.1 Icm Algorithm

The iterated conditional modes (ICM) algorithm, which is an approximate solution to the MAP estimate, was proposed by Besag [81]. The ICM solves the minimization problem by sequentially updating (i.e.,
raster scanning the image) labels by minimizing the following equation at each pixel \( s \):

\[
\hat{x} = \arg \min_{x_s \in L} \left\{ \frac{y - \mu_s}{\sigma_s} + \frac{1}{2} \log(2\pi\sigma_s^2) + \beta U(x_s) \right\}
\]  

(2.12)

we will refer to the expression inside the brackets as total energy, which is the sum of the negative log-likelihood and the Gibbs energy. \( \beta \) is a positive constant that controls the size of clustering or interaction between the sites. The energy function \( U(x) \), which we will also be referring to as Gibbs energy, has the following form:

\[
U(x) = \sum_{c \in C} V_c(x_c)
\]  

(2.13)

where \( C \) denotes the set of cliques* for \( N_s \) (\( N_s \) denote the neighborhood of \( s \)). Note that the sum runs over all clique configurations. \( V_c \) is the potential function associated with a clique. It is a function that maps a clique configuration to a real number, that is, \( V_c : I \rightarrow R \). The potential function is applicable to all the neighborhoods over the image space. A widely used two point clique potential can be given by:

\[
V_c(x) = \begin{cases} 
1 & \text{if } x_s = x_r, s, r \in C \\
1 & \text{if } x_s \neq x_r, s, r \in C
\end{cases}
\]  

(2.14)

In this formulation, the potential function will be equal to -1 if the pair have the same intensities and +1 otherwise.

\[
\begin{array}{ccc}
(i - 1, j - 1) & (i - 1, j) & (i - 1, j + 1) \\
(i, j - 1) & (i, j) & (i, j + 1) \\
(i + 1, j - 1) & (i + 1, j) & (i + 1, j + 1)
\end{array}
\]

*A clique is a subset of points \( cc \epsilon C \), which are all neighbors of each other.

The performance of the ICM algorithm depends heavily on the initial labeling. If a good initial labeling is possible, the ICM algorithm can quickly converge to a desired solution. If a reasonably good initial labeling is not possible, the stochastic algorithm, which will be discussed in the following sections, may be a better choice. Of course, if they can be executed in a reasonable time. For initial clustering can be used basic clustering algorithms, as k-means etc.
3.2.1.2 Simulated Annealing Algorithms

In this section, we will discuss a simulated annealing-(SA)-based algorithm that solve the MAP estimate in a manner similar to the physical annealing process that occurs in matters. The first simulated annealing algorithm was proposed by Metropolis et al. in 1953 [82]. It was motivated by simulating the physical process of annealing solids. In a physical annealing process, the matter is heated at a very high temperature and then gradually and very slowly cooled to reach the ground state. Inspired by the physical annealing, the SA-based solutions introduce a temperature variable, similar to the physical temperature in concept, into our energy functions. This variable will allow us to start our optimization process from a state in which all the configurations have equal probability, in other words, from a very hot state. Then, by gradually decreasing the temperature variable, we will be reaching to the global solution. Simulated annealing is a local search algorithm capable of escaping from local optima. The principal advantage of these approaches is that the performance of the optimization is no longer dependent on initial labeling. Kirkpatrick et al. were the first to introduce simulated annealing to optimization problems in 1982 [83]. Since then, simulated annealing has been widely used in combinatorial optimization problems and has achieved good results on a variety of problem instances.

We use \( E_n \) and \( E_c \) represent the new energy and current energy respectively. \( E_n \) is always accepted if it satisfies \( E_n < E_c \), but if \( E_n \geq E_c \) the new energy level is only accepted with a probability as specified by \( \exp\left(-\frac{(E_n-E_c)}{T}\right) \), where \( T \) is the current temperature. Hence, worse solutions are accepted based on the change in solution quality which allows the search to avoid becoming trapped at local minima. The temperature is then decreased gradually and the annealing process is repeated until no more improvement is reached or any termination criteria have been met.

3.2.1.3 Metropolis Algorithm

The method of Metropolis begins by choosing randomly a new value \( x' \) with uniform probability. If the energy is lowered by the replacement of \( x \) with \( x' \), the variable is set to this new value. If the energy is increased, then a random number \( u \) with uniform distribution between 0 and 1 is generated, and the variable is changed by \( x' \) only if \( \Delta E / T_k \) is greater than \( u \). Otherwise the variable retains its previous value \( x \). Note that the previous algorithm is in fact trying to minimize:

\[
(\log \sigma_s + (y_s - \mu_s) / \sigma_s + \beta U(x_s)) / T
\]

where \( T \) is the temperature parameter. Similar to the physical annealing process, we need to start with a high temperature and cool it down by decreasing the temperature very slowly. The most commonly
used cooling schedule is $T_{k+1} = C \cdot T_k$, where $C$ takes on values in the range $[0.97, 1)$. A value of 0.97 seems to give acceptable results. We will use $T_k = 4$ as the initial temperature. Note that this temperature has no physical relevance in terms of absolute value. To understand better how the algorithm works we present the pseudo code for 2-D images with two regions.

### 3.3 Feature Generation

The feature generation stage is the process of computing features from an image or from a region within this image to be used in the classification task. The generated features must encode this kind of information in order to enhance the classification accuracy. In US thyroid nodule image analysis, the computed features should exhibit high separability attributes between high and low risk cases. In the current project three categories of features were employed to assess the malignancy risk factor of thyroid nodules: (a) Textural features, (b) Shape and Geometrical features and (c) Wavelet Local Maxima features.

### 3.4 Textural Features

The textural information extracted from the thyroid nodule can be employed as criteria in assessing the risk factor of malignancy and can be of value in patient management, i.e. whether to recommend or not surgical operation. Textural features are divided in two main categories: First and second order statistical features.

#### 3.4.1 First Order Statistical Features

The 1\textsuperscript{st} order statistical features determine the distribution of grey level values within the thyroid nodule \cite{72}. The most important features are:

1. **Mean value** ($m$)

   \begin{equation}
   m = \frac{\sum_i \sum_j g(i, j)}{N} \tag{3.1}
   \end{equation}

   where $g(i,j)$ is the grey level value in the position $(i,j)$ and $N$ the number of pixels.

2. **Standard Deviation** (std)

   \begin{equation}
   std = \sqrt{\frac{\sum_i \sum_j (g(i, j) - m)^2}{N}} \tag{3.2}
   \end{equation}

   The standard deviation represents the variation of grey level value in comparison with the mean value $m$. 
3. **Skewness** ($sk$)

$$sk = \frac{1}{N} \sum_{i} \sum_{j} (g(i,j) - m)^3$$  

The skewness describes the degree of histogram asymmetry around the mean.

4. **Kurtosis** ($k$)

$$k = \frac{1}{N} \sum_{i} \sum_{j} (g(i,j) - m)^4$$  

Kurtosis describes the sharpness of the grey level histogram.

### 3.4.2 Second Order Statistical Features

Features resulting from the 2nd order statistics provide information regarding the spatial relationship between various grey level values within thyroid nodule. These textural features were derived from the co-occurrence and run-length matrices [84,85].

#### 3.4.2.1 Co-Occurrence Matrix Features

In the co-occurrence matrices, grey level pixels are considered in pairs with a relative distance ($d$) and orientation $\phi$ among them [84]. The orientation $\phi$ is quantized in four directions ($0^0, 45^0, 90^0, 135^0$). An example of co-occurrence matrix computation is depicted in Figure 3.1. Let an image array $I(m,n)$ with four grey levels ($N_g=4$) ranging from 0 to 3. Figure 3.1(b) depicts the general form of any grey tone co-occurrence matrix. For example, the element in the $(0,0)$ position of a distance $d=1$ is the total number of times two grey tones of value 0 and 0 occurred along the four quantized directions adjacent to each other. Figures 3.1(c) - 3.1(f) demonstrate all possible grey tones combinations with distance set to 1 along all four directions.
Figure 3.1 (a) Image array with four grey levels. (b) General form of any grey-tone co-occurrence matrix. (c)-(f) Computation of all four co-occurrence matrices with distance $d=1$.

The textural features that can be extracted from the co-occurrence matrices are presented below:

1. **Angular Second Moment (ASM)**

   \[
   ASM = \sum_{i=0}^{N_g-1} \sum_{j=0}^{N_g-1} (p(i,j))^2
   \]

   where $N_g$ is the number of grey levels in the image, $i,j=1,...,N_g$, and $p(i,j)$ is the co-occurrence matrix. The $ASM$ feature describes the degree of homogeneity within the thyroid nodule and takes small values in regions with no variability.

2. **Contrast (CON)**

   \[
   CON = \sum_{n=0}^{N_g-1} \left\{ \sum_{i=0}^{N_g-1} \sum_{j=0}^{N_g-1} (p(i,j))^3 \right\}^{1/3} = n
   \]

   $CON$ feature describes the amount of local variations present within the nodule and takes high values in regions of great variability. The factor $n^2$ enhances any possible existence of local variations.
3. **Inverse Different Moment (IDM)**

\[
IDM = \sum_{i=0}^{N_x-1} \sum_{j=0}^{N_y-1} \frac{p(i, j)}{1 + (i - j)^2}
\]

(IDM) feature takes high values for low-contrast images due to the inverse \((i-j)^2\) dependence.

4. **Entropy (ENT)**

\[
ENT = -\sum_{i=0}^{N_x-1} \sum_{j=0}^{N_y-1} p(i, j) \log(p(i, j))
\]

ENT feature describes the degree of randomness and takes low values for smooth images.

5. **Correlation (COR)**

\[
COR = \frac{\sum_{i=0}^{N_x-1} \sum_{j=0}^{N_y-1} (i - m_x)(j - m_y)}{\sigma_x \sigma_y}
\]

where \(m_x, m_y, \sigma_x, \sigma_y\) are the mean values and standard deviations of \(p_x\) and \(p_y\) (equations ...) respectively.

COR feature describes the spatial dependencies of the grey tones within the thyroid nodule.

\[
p_x(i) = \sum_{j=1}^{N_y} p(i, j)
\]

\[
p_y(j) = \sum_{i=1}^{N_x} p(i, j)
\]

Other features derived from the co-occurrence matrices are:

6. **Sum of Squares (SSQ)**

\[
SSQ = \sum_{i=0}^{N_x-1} \sum_{j=0}^{N_y-1} (1 - m)^2 p(i, j)
\]

7. **Sum Average (SAV)**

\[
SAV = \sum_{i=2}^{N_x \text{max}} ip_x(i)
\]

where \(p_{xy}\) is

\[
p_{xy}(k) = \sum_{i=1}^{N_x} \sum_{j=1}^{N_y} p(i, j), i + j = k, k = 2, 3, ..., 2N_x
\]

8. **Sum Entropy (SENT)**

\[
SENT = -\sum_{i=2}^{N_x \text{max}} p_{xy}(i) \log(p_{xy}(i))
\]

9. **Sum Variance (SVAR)**

\[
SVAR = -\sum_{i=2}^{N_x \text{max}} (i - SENT)^2 p_{xy}(i)
\]
10. **Difference Variance (DVAR)**

\[
DVAR = -\sum_{i=2}^{2^{N_g}} (i - SAV)^2 p_{xy}(i)
\]

where \( p_{xy} \) is

\[
p_{xy}(k) = \sum_{i=0}^{N_g-1} \sum_{j=0}^{N_g-1} p(i, j) \delta(i - k, j - k) \quad k = 2, 3, \ldots, N_g - 1
\]

11. **Different Entropy (DENT)**

\[
DENT = -\sum_{i=0}^{N_g-1} p_{xy}(i) \log(p_{xy}(i))
\]

12. **Information Measure of Correlation (ICM1)**

\[
ICM1 = \frac{HXY - HXY1}{\max\{HX, HY\}}
\]

13. **Information Measure of Correlation (ICM2)**

\[
ICM 2 = (1 - \exp(-2.0(HXY 2 - HXY)))^{1/2}
\]

\[
HXY = -\sum_{i=0}^{N-g-1} \sum_{j=0}^{N-g-1} p(i, j) \log(p(i, j))
\]

\[
HXY1 = -\sum_{i=0}^{N-g-1} \sum_{j=0}^{N-g-1} p(i, j) \log(p(i)p(j))
\]

\[
HXY2 = -\sum_{i=0}^{N-g-1} \sum_{j=0}^{N-g-1} p(i)p(j) \log(p(i)p(j))
\]

### 3.4.2.2 Run-Length Matrix Features

The run length matrix encodes textural information based on the number each grey level appears in the image by itself [85]. Let an image array \( I(m,n) \) with four grey levels \( (N_g=4) \) ranging from 0 to 3 (Figure 3.2(a)). For each direction \( (0^\circ, 45^\circ, 90^\circ, 135^\circ) \) the corresponding run length matrix is computed (Figure 3.2(b) - Figure 3.2(e)).
Figure 3.2 (a) Image array with four grey levels. (b)-(e) Computation of all four run length matrices for texture analysis.

Each matrix element specifies the number of times that the picture contains a run of length (0...3) in the given direction. The first element of the first row of the matrix is the number of times grey level ‘0’ appears by itself, the second element is the number of times it appears in pairs and so on.

The textural features that can be extracted from the run length matrices are presented below:

1. **Short Run Emphasis (SRE)**

   \[
   SRE = \frac{\sum_{i=1}^{N_g} \sum_{j=1}^{N_r} r(i,j)}{N_g N_r \sum_{i=1}^{N_g} \sum_{j=1}^{N_r} j^2}
   \]

   where \( r(i,j) \) is the run length matrix, \( N_g \) is the number of gray values in the image, \( N_r \) is the largest possible run, \( i=1,...,N_g, j=1,...,N_r \). \( SRE \) tends to emphasize short runs due to the division with \( j^2 \). It takes large values for nodules with high variability.
2. **Long Run Emphasis (LRE)**

\[
LRE = \frac{\sum_{i=1}^{N_x} \sum_{j=1}^{N_y} j^2 r(i, j)}{\sum_{i=1}^{N_x} \sum_{j=1}^{N_y} r(i, j)}
\]

$LRE$ tends to emphasize long runs. It takes large values for nodules with low variability.

3. **Grey Level Non Uniformity (GLNU)**

\[
GLNU = \frac{\sum_{i=1}^{N_x} \left( \sum_{j=1}^{N_y} r(i, j) \right)^2}{\sum_{i=1}^{N_x} \sum_{j=1}^{N_y} r(i, j)}
\]

$GLNU$ is proportional with large run length values that are uniformly distributed. It takes large values for nodules with high variability.

4. **Run Length Non Uniformity (RLNU)**

\[
RLNU = \frac{\sum_{i=1}^{N_x} \left( \sum_{j=1}^{N_y} r(i, j) \right)^2}{\sum_{i=1}^{N_x} \sum_{j=1}^{N_y} r(i, j)}
\]

$RLNU$ encodes long runs that are non-uniformly distributed. It takes small values for nodules with high variability.

5. **Run Percentage (RP)**

\[
RP = \frac{\sum_{i=1}^{N_x} \sum_{j=1}^{N_y} r(i, j)}{P}
\]

where $P$ is the total possible number of runs in the nucleus image. This feature takes its lowest value in nodules with low variability.

### 3.4.3 Data Normalization

In most cases the features values have different dynamic ranges. In order to overcome this problem all features values are normalized so that they lie within similar dynamic ranges. The normalization technique used in this thesis is made via the mean and variance of the feature values [72,81].

\[
\hat{x}_{ik} = \frac{x_{ik} - \bar{x}_{ik}}{\sigma_k}
\]
where \( x_{ik} \) and \( \tilde{x}_{ik} \) are the \( k \)th feature values before and after the normalization. \( \bar{x}_k \) and \( \sigma_k \) are the mean value and standard deviation of the \( k \)th feature.
Chapter 4 – The Impact of Platelet Rich Plasma (PRP) in Osseo integration of Oral Implants in Dental Panoramic Radiography: Texture Based Evaluation

Summary

In this chapter the temporal texture differentiation associated with the bone formation properties, around loaded oral implants after Platelet-Rich-Plasma (PRP) employment, was investigated in Panoramic Radiographs. At first, an extensive review of the literature regarding PRP computerized analysis towards Osseo integration is presented. Afterwards the study made in order to quantify the effect of PRP is presented. In the Results section a strong hypothesis indicating the significant temporal textural differentiation is made. In the discussion section an extensive discussion regarding the current study is given.

4.1 Review of the literature

Implantology has been the key factor in dentistry effectiveness throughout the last few decades. Although a number of restorative options for the treatment of missing teeth still exist, none have proven to be as functionally effective and robust as implants. In many cases, dental implants may be the only consistent and successive choice for the restoration of all necessary functionality of the teeth and supporting structures. The majority of dental implants in use today are made from titanium or titanium alloy [93]. Studies have shown a five-year success rate of 95% for lower jaw implants and a 90% success rate for upper jaw implants. The success rate for upper jaw implants is slightly lower because the upper jaw (especially the posterior section) is less dense than the lower jaw, making a successful implantation and osseointegration potentially more difficult to achieve [94].

Despite the high success rate of dental implants, the osteo-regenerative potential of the surrounding tissues (soft and hard) that substantially affect the outcome of the overall surgical procedure still remains an important issue. Several studies have shown that the surgical placement of implants in sites with poor osteo-regenerative properties has a decreased probability of success. A high degree of primary implant stability (high value of insertion torque) seems to be one of the prerequisites for a successful procedure [94]. Dental implants may break or become infected (like natural teeth) and crowns may become loose. In order to promote healing of end-osseous implants and bone grafts, various procedures have been proposed. The majority of these proposals concentrate on strengthening the bone-to-implant contact area so as to accelerate the osseous healing. These, include the application of PRP, bone morphogenetic proteins (BMPs) and growth factors [95].
Platelet-rich plasma is an autologous concentration of human platelets in a small volume of plasma. Due to its concentration of platelets, PRP consists of the 7 fundamental protein growth factors proved to be actively secreted by platelets. These growth factors include the three isomers of platelet-derived growth factor (PDGFαα, PDGFββ, and PDGFαβ), two of the numerous transforming growth factors-β (TGFβ1 and TGFβ2), vascular endothelial growth factor, and an epithelial growth factor. All the aforementioned growth factors possess anti-inflammatory and pro-regenerative properties that permit tissue wounds to heal faster and more efficiently. Additionally they can stimulate cell proliferation, matrix remodeling and angiogenesis [96,97].

The use of these growth factors in order to enhance healing is of a great interest to a large number of researchers and clinicians. Their main approach is the PRP effect in bone regeneration analyzing histological and histomorphometric data of animal and human tissue. The majority of these studies report a significant enhancement of healing when PRP is used with positive results in either or both bone and soft tissue healing. In these studies an enhancement of bone regeneration is also observed [98-99].

However, there have also been studies that concluded that there was little or no benefit from PRP treatment [100,101]. The commercial exploitation of these growth factors has led to the development of a wide range of preparation protocols, kits and centrifuges. Most of these products were called PRP, the same name as the original transfusion platelet concentrates, which does not allow distinction between the different systems and protocols employed [102-104]. Only a few studies have attempted to quantify dental bone density and the PRP effect in dental implantations [105-109]. “Gatti et al [105] investigated the bone density alteration, based on the gray level values, around the implant in three different types of dental implants which were placed in dogs. Youssif et al [106] presented a new approach of applying computer algorithms to radiographic images of dental bone implantation used for bone regeneration. This study is applied to two dental cases at different follow up times so as to assess the rate of bone density alterations by comparing the baseline radiograph to the follow up images. The density changes at the intra-bony affected area are assessed by means of a statistical analysis of parameters from the gray-level histogram and have shown a 37.32% and a 63.37% increase in the bone density by the end of the follow up period of about 12 months.

Barone et al [107] analyzed the bone density around the loaded oral implants by using a new volumetric CT scan and compared it to unloaded implants. Four patients were selected for this study. A total of 12 oral implants were placed. In these patients six of these implants were loaded immediately, while six were left unloaded. Six months after the placement of immediately loaded and unloaded oral implants,
they were analyzed by a volumetric CT scan by means of the densitometric profile from para-axial slices (mean and standard deviation values). The differences observed between the two groups of the oral implants (immediately loaded and unloaded) were statistically significant (p<0.05).

Wilding et al [108] also investigated the possibility that fractal dimensions of bone images taken from routine orthopantomograms could be used to observe and to monitor bone remodelling in response to dental implants. This study involved 18 patients who had received fixed implant-supported prostheses. A window of bone adjacent and distal to the most posterior implant was defined as the region of interest from which the fractal dimension of the image was calculated in order to evaluate whether there were any significant shifts in fractal dimension during the recall period after implantation. A significant increase in fractal dimension was found during the period up to 2 years after implantation (p < 0.001).

Wojtowicza et al [109] studied the regeneration of the maxillary alveolar process after PRP use in a 17-year-old patient who had lost the upper central incisors together with the alveolar bone as a result of a car accident. The regenerated bone was analyzed after 10 months and compared to the intact bone using the Fourier and fractal analysis of radiograms. Fractal analysis of intact and regenerated bone showed a higher fractal dimension for the intact bone in comparison with the regenerated bone, confirming a lower complexity of the newly formed trabecular structures.

For the first time a randomized experimental case control study, in terms of a computerized texture analysis, of platelet-rich plasma (PRP) as a promoter of bone healing in dental implants is carried out. The main objective was to quantify any texture differentiation that occurred during the 8 month follow up period, into the bone-to-implant contact region, between the two classes (0 & 8 month follow up period) and consequently to evaluate any statistical difference between test and control groups that can be attributed to the PRP treatment. The texture analysis in association with bone formation properties around the loaded oral implants was employed in the panoramic radiographs by means of first order, co-occurrence, run length textural features and Receiver Operating Characteristic (ROC) curve analysis.

4.2 Material and Methods

4.2.1 Clinical Dataset and Surgical Procedures

This study has undergone an ethics review by the University of Patras Ethics Board. A clinical dataset of 30 patients was selected for the study that was randomly assigned to two groups (test group–15 patients, control group–15 patients). Ages within the clinical dataset ranged from 25 – 65 years. Exclusion criteria for participating in to the study were mainly diabetes, osteoporosis, cardiac and
thoracic diseases, non-smoking and cancerous patients. All patients that were finally chosen had maxillary and mandibular tooth loss and had chosen the surgical implant solution although they had been advised of an alternative treatment plan such as prosthetics. They had all been informed of the requirements for participation in the study and had a consent form signed. The test group received the PRP application around new implants and within the surgical site whereas in the control group the new implants were placed without PRP treatment. A follow up clinical sample of 60 digitized panoramic radiographs, corresponding to the 30 patients imaged immediately after implant loading (Class I) and 8 months later (Class II) were analyzed.

All surgical procedures were performed under local anesthesia (Ubistesin forte – 1.7 ml). A number of 2 – 6 implants were placed in each patient. The bone surface was exposed by of type –H– Incision. Bone defects of 3.60 mm were created. Implants 11.50 mm in length and 3.75 mm in diameter were then placed (MF7-11375, MIS Implants Technologies Ltd, Israel) after been immersed in PRP. PRP was also placed within each surgical site before implant loading.

PRP was derived in the test group with the following procedure: a 40 ml of autologous blood was drawn from each patient the sample initially centrifuged at 2400 rpm for 10 min in order to separate the PRP and the platelet-poor plasma (PPP) from the red blood cells. Following both the PRP and PPP were again centrifuged at 3600 rpm for 15 min to further separate the PRP from the PPP. Platelet counts were then done for each patient. Subsequently, the PRP was activated just before application with a 10% calcium chloride solution [110].

An image dataset consisting of two panoramic radiographs for both groups, the first one (Class I, 76 implants) was acquired immediately after surgery (baseline panoramic radiograph) and the second one (Class II, 76 implants), eight months later [111] leading to a total of 60 radiographs, is analyzed. All (baseline) radiographs had a one-to-one correlation to those obtained after the 8 month follow up period.

Regarding the standardization of the panoramic radiographs all images were taken by the same technician according to a standardized protocol for patient positioning and exposure parameter setting. In addition, in for each patient a registration method (Matlab built-in intensity based algorithm that employed as input the output from the MRF-based segmentation method introduced in this study) utilized the mean square error metric derived from the segmented implants in order to compensate any minor geometrical misalignment between the two panoramic radiographs of each patient (0 & 8 months). The majority of the images (baseline and follow up) produced a mean square error close to
zero. As a result, all (baseline) radiographs had a one-to-one geometrical correlation to those obtained after the 8 month period.

The follow up period is within the time range of (6 to 12 months) as already proposed in most of the aforementioned studies (clinical and quantitative) and has been considered adequate for bone regeneration evaluation by the expert dentist participated in this study. Figure 4.1 highlights the region from an implant in which the texture analysis has been employed along with relevant zoomed area indicating a radiological appearance of the patterns that were considered.

![Figure 4.1](image)

**Figure 4.1.** Oral implant of a dental panoramic radiograph. Zoomed image part at arrow locations indicate the bone-to-implant regions in which the texture analysis is applied. The differentiations in radiologic appearance patterns (increased gray tone illumination and variability) of the bone-to-implant area are been considered towards efficient segmentation.
The panoramic x-ray equipment that utilized was the Orthophos C (Siemens co, AG Wittelsbacherplatz 2, 80333 Munich, Germany) and the radiographic parameters were set at 66-69 kVp and 16 mA. All panoramic radiographs were digitized by the MicrotekScanMaker i800 (Positive film, 600 dpi, 16 bit grayscale) scanner (Microtec Industries, Silicon valley, Taiwan). The software employed for digitization and storage was the MicrotekScanwizard Pro V7.11. The provided digitized images were in TIFF format (Tagged-Image File Format). The x-ray equipment as well as the film digitizer performance were evaluated, employing appropriate quality control protocols, so as to ensure proper functioning of the medical equipment and the high quality of the digitized images [112,113].

4.2.2 Identification of bone-to-implant contact region (ROIs)

A specifically designed detection algorithm was targeted to identify ROIs from panoramic radiographs so as to sample the bone to implant contact region. This region encompasses tissue in between and adjacent to implant windings, candidate for bone regeneration. Accurate ROI identification is critical, as over-segmentation (inclusion of an implant border) or under-segmentation (loss of valuable information within the implant’s windings) would compromise subsequent texture analysis.

At first, the dental implant is extracted from the surrounding tissue by means of the Markov Random Field (MRF) method [114]. MRF modeling combines conditional (local intensity distribution) with contextual (intensity similarity within small neighborhoods) information under the Bayesian framework in order to estimate the true intensities of the image rather than those based only on the conditional information [115]. It assumes that the class probability of a pixel is only dependent on class membership of its spatial neighbors (also called lattice) which in turn reduces the possible influence of noise and overlapping structures. The model assumption that the conditional distribution depends on the pixels in the near neighborhood is subject to the Bayesian framework which states that the decision rule for labeling an image pixel combines the conditional intensity distribution of an individual region with prior knowledge regarding that region.

Let us assume that \( P(X) \) is our prior knowledge and \( P(Y|X) \) is the probability of realizing the observed image given the distribution of regions in the image. The most widely used conditional intensity distribution is the Gaussian distribution, whose function, given the class \( x_s \), is given by:

\[
P(Y = y | X = x_s) = \frac{1}{\sqrt{2\pi}\sigma_s^2} e^{-\frac{(y-\mu_s)^2}{\sigma_s^2}}
\]

where \( \mu_s \) and \( \sigma_s \) are the parameters of the distribution of the class \( x_s \).

Then, by Bayes' theorem
\[
P(X | Y) = \frac{P(Y | X)P(X)}{P(Y)} \quad (4.2)
\]
where \( P(X|Y) \) is our posterior.

Then \( \hat{x} \) can be obtained by taking the negative of the posterior estimate natural logarithm and minimizing it: \[ \hat{x} = \arg\min_x (-\log(P(X \mid Y))) \] \quad (3)

Simulated annealing and iterated conditional modes are the two main approaches in order to solve the optimization problem. In this study the stochastic approach, simulated annealing (SA) was implemented [116].

The SA solves the minimization problem by sequentially updating (i.e raster scanning the image) labels by minimizing the following equation at each pixel \( s \):

\[
\hat{x} = \arg\min_{x_s \in L} \left\{ \frac{(y - \mu_s)}{\sigma_s^2} + \frac{1}{2} \log \left( 2\pi\sigma_s^2 \right) + \beta U(x_s) \right\} \quad (4.3)
\]
where \( U(x_s) \) is the number of pixels in the neighborhood that have the color \( x_s \).

After the dental implant boundary extraction, the particular interface region (i.e. tissue in between and adjacent to the implant windings) in the binary images was sampled by circular Regions Of Interest (ROIs) that were derived from the specifically designed detection method based in morphological closing by means of circular closing elements of varying size. Finally, two ROI classes for both groups were created corresponding to the radiographs acquired immediately after the implant and 8 months later.

### 4.2.3 Texture and Statistical Analysis

Forty-one (41) textural features were automatically calculated from each segmented ROI. The textural features are related to the gray-tone structure for the bone-to-implant interface and possess information relevant to the osseo-integration potential in that region. Four (4) features were computed from the gray-tone histogram, while the mean and range of Thirteen (13) second order statistic features, extracted from the co-occurrence matrices and Five (5) features extracted from the run-length matrices over four directions \((0^0, 45^0, 90^0 \text{ and } 135^0)\) and a distance of \(d=1\) pixel, resulting in 26 and 10 features respectively [117,118]. Additionally, the fractal dimension feature was also computed carrying information regarding the degree of gray level complexity (Table 1) [119]. The selected feature set, provides significant information, regarding the evaluation of a possible change throughout the 8 month period of spatial and intensity dependencies of the pixels within the bone-to-implant region that can be attributed to the osseo-regeneration procedure. Moreover, the generated features must encode this
kind of information in order exhibit high separability attributes between the two classes.

**Table 4.1**

<table>
<thead>
<tr>
<th>Textural Feature</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gray-Tone Histogram Features</strong></td>
</tr>
<tr>
<td>1 Mean value(m)</td>
</tr>
<tr>
<td>2 Standard Deviation (std)</td>
</tr>
<tr>
<td>3 Skewness (sk)</td>
</tr>
<tr>
<td>4 Kurtosis (k)</td>
</tr>
<tr>
<td><strong>Co-Occurrence Features</strong></td>
</tr>
<tr>
<td>5 Angular Second Moment (ASM)</td>
</tr>
<tr>
<td>6 Contrast (CON)</td>
</tr>
<tr>
<td>7 Inverse Different Moment (IDM)</td>
</tr>
<tr>
<td>8 Entropy(ENT)</td>
</tr>
<tr>
<td>9 Correlation(COR)</td>
</tr>
<tr>
<td>10 Sum of Squares (SSQ)</td>
</tr>
<tr>
<td>11 Sum Average (SAV)</td>
</tr>
<tr>
<td>12 Sum Entropy (SENT)</td>
</tr>
<tr>
<td>13 Sum Variance (SVAR)</td>
</tr>
<tr>
<td>14 Difference Variance (DVAR)</td>
</tr>
<tr>
<td>15 Different Entropy (DENT)</td>
</tr>
<tr>
<td>16 Information Measure of Correlation (ICM1)</td>
</tr>
<tr>
<td>17 Information Measure of Correlation (ICM2)</td>
</tr>
<tr>
<td><strong>Run-Length Features</strong></td>
</tr>
<tr>
<td>18 Short Run Emphasis(SRE)</td>
</tr>
<tr>
<td>19 Long Run Emphasis(LRE)</td>
</tr>
<tr>
<td>20 Grey Level Non Uniformity (GLNU)</td>
</tr>
<tr>
<td>21 Run Length Non Uniformity (RLNU)</td>
</tr>
<tr>
<td>22 Run Percentage (RP)</td>
</tr>
<tr>
<td>23 Fractal Dimension (FD)</td>
</tr>
</tbody>
</table>

Ideally, all 41 features at hand should be utilized, but since a number of them may be redundant due to mutual correlations, an optimum number of them had to be selected to achieve the highest discrimination. A common technique in achieving this is the Stepwise Regression Analysis (SRA) that led to a subset of features that is significant different and carries the aforementioned discriminant properties [120]. SRA is a sequential feature selection technique specifically designed for least-squares fitting in a multiple regression model. It is based on an add/remove features scheme from a multilinear model based on their statistical significance in a regression. The stepwise regression procedure starts off by choosing an equation containing the single best feature and then attempts to build up with
subsequent additions of other features one by one as long as these features are ‘statistically significant’ or highly correlated with that feature.

The order of addition is determined by using the partial F-test values in order to select which feature should enter next. The highest partial F-value is compared to a selected F-to-enter value. A feature is considered significant and is added provided that its highest partial F-value is less to the selected F-to-enter value. After a feature is added, the regression equation is examined to see if any feature already added in the earlier stages of the procedure should be removed because of the relationship between it and other new added variables now in the regression model. A feature is removed if its highest partial F-value is greater than the selected F-to-remove value. Stepwise regression has the advantage of removing features that have been added or adding features that have been removed during the regression process and follows the “minimum redundancy – maximum relevance” principle as adopted by several recently proposed feature selection methods [121]. The filter-based approaches for feature selection such as SRA provide the identification of the discriminant features, instead of the so called wrapper approaches such as Principal Component Analysis (PCA) which can only estimate a good feature subset [122].

Statistical differentiation for the selected subset was exploited by means of ROC curve analysis. The ROC curve is a plot of the true positive rate (sensitivity) versus the false positive rate (1-specificity) for different thresholds over the entire range of each feature values and it is independent of class distribution or error costs. The AUC can be statistically interpreted as the probability that a random (class I, Class II) pair of feature values will be correctly discriminated [123]. ROC analysis has been utilized to assess the discriminant power of each independent feature from the selected subset. Features achieving the highest Area Under the Curve (AUC) are capable of capturing the osseo integration enhancement due to the PRP employment. The AUC values were obtained by the binormal parametric method in order to approximate the area [124]. It computes the AUC by fitting two normal distributions to the data. Compared to the empirical ROC approach, the binormal ROC is computationally more affordable and robust in small sample size cases [125].

Prior to calculation of textural features, normalization in each ROI was implemented so as to compensate for the possibility that a segmented ROI might include some pixels from the dental implant, which might in turn create outliers and an imbalanced influence on features values. Each ROI intensity values were normalized between $\mu \pm 3\sigma$ where $\mu$ was the mean value of the gray levels inside the ROI and $\sigma$ the standard deviation. The gray levels that were located outside the range $[\mu-3\sigma, \mu+3\sigma]$ were excluded from further analysis.
4.2.4 Algorithm Implementation

The ROI segmentation and registration methods as well as the feature extraction computations and stepwise regression analysis were all implemented in Matlab R2012 (MathWorks, 3 Apple Hill Drive Natick, Massachusetts 01760, USA). The ROC analysis was performed with the NCSS, PASS and GESS software package (NCSS, 329 North 1000 East, Kaysville, Utah 84037, USA). The computer used for processing had a Dual Core AMD 64 Athlon processor running at 2.8 GHz and 4 GB of RAM.

4.3 Results

Regarding the dental implant boundary extraction, the proposed MRF model consists of two components: a random region labeling component (Figure 4.2a) which serves as the initialization step of the model and a combination of two textural features that serve as input to the model. Image gray level and local entropy values that represent textural information for each pixel were employed as input to the MRF segmentation algorithm in order to fit the image data into the two final clusters. The MRF algorithm provided as an output, a binary image presenting two final clusters (i.e., one cluster concerning the area that presents with a high x-ray reflectance (implant) and a second cluster concerning the areas in the image with a high x-ray absorbance (surrounding tissue and bone – Figure 4.2b)). The segmented implant border is depicted in Figure 4.2c.

The main challenge in estimating the implant segmentation performance is “ground truth”, i.e. defining implant borders. In this study an expert observer, a dentist with seventeen years of experience, has defined the ground truth by generating manual outlines of the implant. The degree of overlap between the two segmented areas, as derived by the observer–“ground truth” and the computer, was used to assess accuracy. Overlap is defined as the ratio of intersection over the union of the two segmented areas, the ground truth and the computer-generated one [33]. The value of overlap is bound between zero (no overlap) and one (exact overlap). To assess the difference in the segmented border shape, mean ($d_{mean}$), root mean square ($d_{rms}$) and maximum ($d_{max}$) distance between the computer- and manually-defined borders were calculated for the 96 implants included in the dataset. The proposed implant segmentation method demonstrated a high segmentation accuracy, corresponding to overlap=0.934 ± 0.010, $d_{mean}$=2.172 ± 0.345 pixels, $d_{rms}$=3.826 ± 0.301 pixels, and $d_{max}$=7.867± 3.047 pixels.

To identify circular ROIs from bone-to-implant contact region, sampling the tissue in between and adjacent to the implant windings, the cluster corresponding to the implant boundary was subjected to morphological closing using a circular closing element of varying size (7-10 pixels), from whom the
original MRF implant cluster was subtracted. This result in the area segments (Figure 4.2d) of the bone to implant contact region to which the circular sampling ROIs sizes were adapted (Figure 4.2e).

Figure 4.2. (a) Random image initialization, (b) MRF clusters (c) implant segmentation (black outline), (d) Areas of bone to implant contact, (e) fitted circular sampling ROIs (black color) and (f) radiologists’ selected ROI (gray color).
To improve ROI detection rate further, a home developed Graphical User Interface (GUI) is introduced, on top of the automated ROI identification system proposed, allowing for manual placing of circular ROIs in the bone-to-implant contact regions missed by the automated step (Figure 4.2f). The radiologist exploring the GUI places 2 control points one corresponding to the center of bone-to-implant contact region and the second one used to define the radius of the ROI. The application of the proposed bone-to-implant contact region identification system resulted in 573 circular ROIs of Class I and 573 ROIs of Class II (mean ROI area= 209.00 ±81.49 pixels and pixel size=23.6 μm).

The stepwise regression analysis procedure in the feature set (41 textural features) towards feature reduction was performed utilizing the threshold of probability to enter and the probability to remove 0.05 & 0.10 respectively, leading to a subset of 4 features (Angular Second Moment, Correlation, Long Run Emphasis and Gray Level Non Uniformity). Table 2 provide the results of the ROC analysis employed at both (PRP and test) groups respectively for the selected feature set. All features selected from the stepwise regression analyses procedure achieved AUC values greater than 0.77 in the PRP group, yielding increased differentiation capability for the selected features. On the contrary, in the control group the corresponding AUC values were smaller ranging from 0.56 – 0.68 (Table 2) indicating lesser osseo-integration activity in the bone-to-implant regions compared with the PRP group.

<table>
<thead>
<tr>
<th>Textural Feature</th>
<th>PRP GROUP</th>
<th>CONTROL GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC</td>
<td>AUC</td>
</tr>
<tr>
<td></td>
<td>(Lower – Upper 95.0%</td>
<td>(Lower – Upper 95.0%</td>
</tr>
<tr>
<td></td>
<td>Confidence Limit)</td>
<td>Confidence Limit)</td>
</tr>
<tr>
<td>Angular Second Moment</td>
<td>0.80</td>
<td>0.66</td>
</tr>
<tr>
<td>(Average)</td>
<td>(0.69 – 0.87)</td>
<td>(0.55 – 0.69)</td>
</tr>
<tr>
<td>Correlation</td>
<td>0.78</td>
<td>0.61</td>
</tr>
<tr>
<td>(Average)</td>
<td>(0.74 – 0.82)</td>
<td>(0.58 – 0.66)</td>
</tr>
<tr>
<td>Long Run Emphasis</td>
<td>0.77</td>
<td>0.56</td>
</tr>
<tr>
<td>(Average)</td>
<td>(0.68 – 0.83)</td>
<td>(0.49 – 0.61)</td>
</tr>
<tr>
<td>Gray Level Non Uniformity</td>
<td>0.81</td>
<td>0.68</td>
</tr>
<tr>
<td>(Range)</td>
<td>(0.76 – 0.85)</td>
<td>(0.59 – 0.73)</td>
</tr>
</tbody>
</table>

The corresponding box plots of each feature from the selected subset are depicted in Figure 4.3.
(a) ASM

(b) GLNU
4.4 Discussion

To our knowledge the only texture-based study that investigated the bone regeneration properties in Dental implants after the PRP immersion was held by Wojtowicza et al [109] by means of Fourier and fractal analysis. That study, considered of a very limited dataset (1 patient), and had shown that the fractal dimension feature exhibits a high performance in the bone regeneration procedure evaluation. However, in our clinical dataset this particular feature exhibited poor differentiation performance (AUC=0.57). The other aforementioned methods [105-108] studied bone remodeling in dental implants in radiographs or CT scans without the use of PRP.

Texture analysis carried out in the present study has demonstrated a significant difference in the selected texture subset between the two groups. Regarding the PRP group, the four selected image texture features in this study are capable of capturing the increased temporal texture differentiation, compared to the control group, in the bone to implant contact regions that can be attributed to PRP effect to bone remodeling. The presented results are in total accordance with previous studies [6-8] that analyzed histological and histomorphometric data of animal and human tissue in the same contact
regions. Angular Second Moment (ASM), Correlation (COR) and Gray Level Non Uniformity (GLNU) features describe the spatial dependencies, the degree of homogeneity and the presence of large run lengths within the bone-to-implant region and take high values in regions with great variability. Long Run Emphasis (LRE) feature is indicative of long runs and in contrast with the other features it takes low values for areas with high variability.

All features in the selected subset from the PRP group carry the temporal alteration of the gray value profile within the bone-to-implant interface. The co-occurrence based features (Angular Second Moment and Correlation) had a tendency toward higher values in the PRP-treated cases, which in turn suggest that a newly bone-regeneration procedure is active within the implant windings. The run-length features (Long Run Emphasis and Gray Level Non Uniformity) also demonstrate the lack of the gray level uniformity during this time period which also proves the variable state of the gray values due to the osteo-regenerative procedure that takes place within the bone-to-implant interface. In particular, GLNU presented relatively higher values in the cases where PRP was applied, whereas LRE presented lower values due to high variability in the bone-to-implant region (Figure 4.3a,b,c).

In the control group, the ASM, COR and GLNU features presented lower values compared to PRP group which in turn suggest that the osteo-regenerative procedure is less active. In the same context the LRE values in control group exhibited higher values due to less variability of the gray value profile within the bone-to-implant interface (Figure 4.3d).

The proposed image analysis scheme attempts for the first time to contribute to the current debate, whether PRP benefits the bone regeneration or not, exploiting radiographic image appearance. The present study exploits within a clinical dataset (PRP and control group) an extended computerised feature set in dealing with image appearance differentiation between immediate implant loading (Class I) and after 8 month follow up period (Class II) representing Osseo integration or not. This effect is investigated by means of the ROC analysis providing a more robust measure of differentiation.

Despite the fact that bone formation evaluation is carried out mostly in Computed Tomography dental images, it is not the first time that X-rays are employed in computerized analysis towards either bone or other structure temporal alteration. Even in a single plane (such as panoramic radiographs) the sophisticated 2nd order textural features (co-occurrence and run-length features) employed in this study have the potential to reveal any changes in the absorption of x-rays throughout time. This implies that the quantification of any absorption change could be attributed to bone density alteration. We strongly believe that this study could provide new knowledge regarding the debate around PRP.

The outcome of the proposed method is of a significant clinical interest because it reinforces the
prevailing view of the dental community, that PRP augments the osteo-regenerative potential of surrounding tissues after dental implanting, which in turn orients the daily surgical procedure towards PRP employment.

4.5 Conclusion

The temporal texture differentiation associated with the bone regeneration properties, around loaded oral implants, after Platelets Rich Plasma (PRP) application, was investigated in a follow up clinical sample of panoramic radiographs by means of the differentiation of the image texture. The results of the ROC analysis demonstrated that the addition of PRP had a significantly positive effect on bone formation as captured by dental panoramic radiographs.

In terms of analyzing the information in widely used panoramic radiographs, the proposed approach in PRP effect quantification and evaluation, highlights the potential of appropriately designed image analysis methods, such as segmentation and texture analysis, as essential auxiliary tools, in an image based tissue parameterization and quantification.
Chapter 5 – The new simplified clinical protocol, “IPG” Dentist Edu technique, for bone augmentation in the sinuses without performing a sinus elevation: A case-study of seven implants placed in the upper jaw with intentional perforation of the sinus membrane

Summary

In this chapter a new and innovative technique named “IPG” utilized for the placement of seven (7) implants in the posterior areas of the maxilla in a 50 year old female patient, is presented. The novelty of the proposed method is that the implants – that were placed in a flapless approach – entered both the sinus cavities with intentional perforation of the Schneiderian membrane. Concentrated growth factors (CGF), as well as Combioss – alloplastic bone grafting material – were employed in this study, following an innovative protocol. Radiographs were examined at various stages during the process of osseointegration in order to assess the increase and maturation of bone structure formed around the implants and over the sinus floor. New bone formation in the sinus cavity allowed for the sinus membrane to reorganize and reconstruct over it, which constitute another proof of how well our organism is capable of adapting under new conditions.

5.1 Introduction

Partially, or completely edentulous patients have a preference to either a tooth-supported, or an implant-supported fixed partial denture. With removable dentures becoming less acceptable nowadays, dental practitioners need considerably less effort to convince patients to receive treatment with dental implants than several years ago. In many occasions, during treatment planning, various procedures such as bone augmentation, bone transplantation, or both are considered necessary in order to acquire the desired alveolar ridge dimensions so as to achieve implant stability and long term aesthetic results.[126]

In cases of vertical alveolar ridge deficiencies of the posterior areas of the upper jaw, either extensive bone transplantation techniques are utilized, or in most cases sinus floor elevation procedures are undertaken in order to create the necessary bone height for implant stability to occur.[127] Previous investigations have reported maxillary sinusitis in up to 20% of patients following Sinus Floor Elevation procedures (SFE).[128] The most common complications of SFE procedures include disturbed and delayed wound healing, followed by haematoma, sequestration of bone, and transient maxillary sinusitis.[129,130] In addition, postoperative acute maxillary sinusitis could even cause implant and graft failures. The aforementioned limitations of the SFE procedures necessitate the implementation of new
techniques that could provide us and the patient with more stable and predictable results and relieve the latter from a painful and expensive surgical experience.

The rapid placement of implants in the sinus cavity with intentional perforation of the sinus membrane following a certain protocol – called the “IPG” DentistEdu technique – is introduced in this article. The proposed technique combines the use of concentrated growth factors (CGF with stem cells CD34+), bone grafting and implant placement, in such a manner that the sinus can adapt to the new conditions and form new bone around the implants without the need to perform an SFA procedure. Implants can be placed either using a surgical approach, or by utilizing the flapless technique which is greatly advocated by the authors and was also utilized for the patient presented in this article.[131] In this case-study presentation, seven implants were placed in both sinuses followed by a radiographic (Panoramic radiography and Cone Bean Computed Tomography – CBCT scans) and clinical evaluation (by Osstell measurements) after an 8 month follow-up period showing good implant stability. To the best of our knowledge, the proposed technique of intentional direct implant placement into the sinus with intentional sinus perforation has not been previously reported in the literature.

5.2 Materials and Methods

A 50 year old partially dentate, non-smoker female patient in good health condition and without any chronic diseases visited the Dentist Education Institute postgraduate center in Athens–Greece, requesting an upper jaw rehabilitation with a non-removable prosthesis. With only the anterior dentition present, the patient was having serious difficulties chewing her food. Since the patient had requested a non-removable prosthesis, the option of placing a total of 7 implants (4 in the left and 3 in the right side) was offered to the patient. After informing the patient in details the procedure that was going to be performed, a written consent was signed. Cone Beam Computed Tomography (CBCT) scans confirmed the alveolar ridge deficiency in both sides with a highly resorbed and short ridge of 1–2 mm in height in the area of the upper right 1st molar (#16), and 2–3 mm in the area of the upper left 1st molar (#26), (Figure 5.1).

Since no pathology was found in the posterior segments of the maxilla, the assessment of the CBCT scan allowed for a precise planning of the sites for implant placement. These sites were decided to be at tooth area #14 (upper right first premolar), #15 (upper right second premolar), #16 (upper right first molar), #24 (upper left first premolar), #25 (upper left second premolar), #26 (upper left first molar), and #27 (upper left second molar). Implant placement was planned to be performed atraumatically
using the flapless technique which was preferred over the traditional surgical approach in order to eliminate postoperative infections and provide less discomfort to the patient.[132]

**Figure 5.1** CT scan of both sinuses in which the bilateral alveolar ridge deficiency is obvious

### 5.3 Surgical Procedure and concentrated growth factors CGF

As part of the authors' everyday clinical practice for all surgical procedures, concentrated growth factors (CGF) with stem cells CD34+, in all its various forms was prepared.[133] At first blood was drawn from the patient utilizing eight sterile tubes (9 ml each) and centrifuged in a special centrifuge device (Medifuge, Silfradent srl, St. Sofia, Italy) for approximately 13 minutes (Figure 5.2).

For optimum quality of CGF matrices the blood samples were centrifuged immediately after the blood was drawn. After centrifugation, in each sterile tube four components can be easily identified from top to bottom: (a) a superior phase represented by the serum (blood plasma without fibrinogen and coagulation factors), (b) an interim phase represented by a very large and dense polymerised fibrin buffy coat, (c) a liquid phase containing the white blood cells and (d) the lower red blood cell portion, a viscous and dense platelet-rich coagulation mass (Figure 5.3a).[134] A large number of growth factors and stem cells CD34+ are aggregated in the middle layer (between the dense polymerized fibrin buffy coat and the upper 3-4 mm of red blood corpuscles mass of the bottom layer – Figure 5.3a). This growth factors-rich segment is separated from the rest of the red corpuscles using scissors (Figure 5.3b) in order to obtain the CGF-CD34+ matrix (Figure 5.3c).
Installing the drilling device in the preparation site, Povidine-iodine solution (Betadine) was first employed extra-orally for disinfection of the surgical site in order to reduce the probability of microbial contamination, and then infiltration was performed using a 2% lidocaine solution containing a ratio of 1:100,000 epinephrine. In each predetermined site, the osteotomy was extended all the way through the whole bone height available. Drilling did not stop only until the sinus membrane was intentionally perforated. A CGF matrix, created in the previous process of blood centrifugation, was then cut in half approximately.

**Figure 5.2** Special centrifuge for the preparation of CGF (Medifuge, Silfradent, Italy)
Figure 5.3 (a) Sterile tubes after centrifugation, (b) Separation of the dense platelet-rich coagulation sample from the CGF matrix using scissors, (c) the CGF-CD34+ matrix
One half of the matrix was inserted through the osteotomy site and into the sinus through the membrane perforation using the fibrin injector (Silfradent-Italy – Figure 4a), which proved to be a great tool for the swift insertion of the fibrin gel block (Figure 5.4b).

![Image of fibrin injector and osteotomy site](image)

**Figure 5.4:** (a) Fibrin injector, Silfradent-Italy, (b) Insertion of the fibrin gel block within the osteotomy site.

The remaining half of CGF matrix (highly concentrated growth factors and stem cells) was then cut into small pieces and mixed with a small quantity of the alloplastic bone grafting material combioss (0.5ml, by Silfradent-Italy – Figure 5.5a). This mixture is then placed within the osteotomy site (Figure 5.5b).
Figure 5.5: (a) A mixture of highly concentrated growth factors, stem cells CD34+ and bone grafting material, (b) placement of the aforementioned mixture in the osteotomy site.
Figure 5.6: (a) Process of LPCGF with CD34+ production utilizing the CGF-forceps, (b) Implant immersions into LPCGF, towards the creation of a bioactive membrane around it.
For faster osseointegration of the implants, each implant was immersed into a Liquid Phase of the Concentrated Growth Factors (LPCGF) in order to create a "bioactive" membrane around it. The LPCGF was prepared by squeezing some of the remaining seven CGF-CD34+ matrices by means of the CGF-forceps (Silfradent, Italy – Figure 5.6a) and was collected in a sterilized container. Each implant was carefully and fully immersed into the liquid phase CGF (Figure 5.6b).

All implants were then placed using a hand wrench and the insertion torque was measured to be between 20-25 N/cm[127]. The low insertion torque values are expected due to the small bone heights at all the implant sites.

5.4 Results

All the implants in-situ 8 months later are depicted in Figure 5.7.

Figure 5.7: Surgical site of all placed implants 8 months after.

The proposed clinical protocol was evaluated radiographically by means of Panoramic radiography and CBCT scans and clinically in terms of osstell readings and stability values.
5.5 Radiographic Evaluation

Figure 5.8: Panoramic radiograph before (a) and after the implant placement (b).
The panoramic radiographs in figure 5.8 shows the patient’s mouth before and after the implants placement following the proposed clinical protocol, whereas figure 5.9 shows some of the CT scans showing new bone formation around the implants.

![CT scan of the surgical site 8 months after the procedure](image)

**Figure 5.9:** CT scan of the surgical site 8 months after the procedure

The new bone formation within the sinus cavity and around the implant in tooth area #15 (middle implant in the right sinus) can be seen in figure 5.10.
Figure 5.10: (a) Implant in tooth area #15 in which the ridge height did not exceed 2mm. (b) The same site 8 months after the implant placement following the proposed protocol where the ridge now nearly covers the full length of the implant.
5.6 Clinical Evaluation

Following implant placement, the primary stability of each implant was investigated by means of Resonance Frequency Analysis (RFA) using the Osstell device.[135] The RFA technique is essentially a bending test of the bone-implant interface in which an extremely small bending force is applied by stimulating a transducer. It can provide valuable and reliable clinical information regarding the state of the bone-implant –interface since the use of the Osstell device provides the dental practitioner an Implant Stability Quotient (ISQ) value. The measurements can range from 0 to 100 ISQ units, where the higher the ISQ values the more stable the implant. To perform the RFA test, a metal rod is first attached to the implant with a screw connection. The rod has a small magnet incorporated to its top that is stimulated by magnetic pulses from a handheld electronic device. Analysis of the resonance frequency of the rod is then automatically performed by the device and an ISQ measurement is provided (Figure 5.11).

![Osstell measurement underway](image)

**Figure 5.11:** Osstell measurement underway

For all seven implants placed using the IPG-Dentist Edu technique, the ISQ range of values was between 61 and 69, which shows high stability for all implants placed..


5.7 Discussion

The aesthetics and functional integrity of the periodontal tissues, as well as the vertical and horizontal dimensions of the alveolar processes are usually compromised following tooth loss. In such cases, various bone regenerative techniques are employed in order to restore the alveolar processes back to their original shape, allowing for a more predictable long term esthetic and functional success of the implants placed.

For the posterior segments of the maxilla, a regenerative technique called the “sinus floor elevation procedure” (SFA), has spread widely and is taught extensively. A “sinus floor elevation procedure” can be carried out before, or in the same day with implant placement depending on each case, but nevertheless, it constitutes a more complex treatment plan and an unpleasant and longer surgical procedure for both the surgeon and the patient. Moreover, the predictability of the treatment outcome also depends on the operator’s experience performing this technically demanding surgical procedure. Sinus elevation procedures also increase both the cost and time required for completion of each case. Despite the profound drawbacks, this procedure is generally accepted by patients when they are informed that it is the only way for the posterior areas of the maxilla to be restored with a functional and easily adaptable non-removable prosthesis. Without doubt, patients do not consider SFA as a “minor procedure” and probably would have chosen an alternative non-surgical, non-invasive and painless option if it was offered to them.

The IPG DentistEdu technique described in this study, involves the utilization of bone grafting material, implant placement and concentrated growth factors-CGF (with stem cells CD34+) into the intentionally perforated sinus membranes. This allowed for all implants to be placed atraumatically in both sides of the maxilla and with no sinus elevation procedure. This protocol has demonstrated stable and reliable results with very high implant success rates. The IPG DentistEdu technique has proven to be an absolutely safe procedure without any what-so-ever post-operative complications. Neither of the sinuses presented any signs of infection that affected the well-being of the patient.

Anchorage of the CGF matrix in the sinuses is achieved by platelets released after the penetration and slight hemorrhage of the sinus membranes. Platelets also found in the CGF matrix allow for anchorage on the surface that they are placed on, or at the area where there is trauma. Therefore, when the CGF matrix is placed in the sinus cavities it will not be displaced away from where it is originally placed, forcing the bone to regenerate locally and around the implants. During new bone formation in the sinus
cavities following sinus membrane penetration, it is believed by the authors, that the sinus membrane slowly repairs itself and covers the former, while any parts of the sinus membrane under the bone grafting material slowly resorbs.

A metal-acrylic fixed partial denture (with an acrylic masticatory surface) was fabricated, and was preferred over a metal-ceramic because the masticatory forces are generally absorbed better. The fixed partial denture was inserted about 9 months after implant placement in order to allow enough time for new bone growth to occur around the implants. It is believed that a shorter osseointegration period before implant loading could be equally successful in similar cases. Future case studies and research will provide us with the important information of the minimum amount of time that must be allowed before uncovering the implants. Future studies are also required to determine whether the observed augmentation in bone height will be maintained over the long term or, if there will be bone loss due to remodelling.

5.8 Conclusion

The results of the proposed IPG DentistEdu technique support the concept of a one-stage, flapless - whenever possible- implant placement with intentional sinus membrane perforation whenever there is ridge-height deficiency. It must be emphasized that the protocol should be carefully and precisely executed if the desired results are to be expected. Therefore, it is the authors’ belief, that adequate training on how to perform this technique has been completed first, before any attempt is made in utilizing this technique on patients.
Chapter 6 – Conclusions

Numerous studies have demonstrated the healing and regenerative properties of PRP, such as diabetic foot ulcers that cannot be treated with other methods and PRP injection or membrane placement provided significant results. Fresh platelet concentrates are also used in many centers to treat recalcitrant wounds. Research results have shown that all PRP preparations increased granulation tissue formation as assessed by surface coverage, thickness, and angiogenic response, when compared with untreated wounds. In addition, wounds treated with FD PRP, and biochemically stabilized FD PRP, exhibited higher proliferative levels. The possibility to deliver growth factors using platelets, and the potential to extend the shelf-life of platelet concentrates makes freeze-drying methods particularly suitable for enhanced wound care.

In addition, one of the most popular methods used to biologically enhance healing in the fields of orthopaedic surgery and sports medicine is the use of autologous blood products, namely, platelet rich plasma (PRP). PRP is commonly used in orthopaedic practice to augment healing in sports-related injuries of skeletal muscle, tendons, and ligaments. Despite its pervasive use, the clinical efficacy of PrP therapy and varying mechanisms of action have yet to be established. Basic science research has revealed that PRP exerts its effects through many downstream events secondary to release of growth factors and other bioactive factors from its alpha granules. These effects may vary depending on the location of injury and the concentration of important growth factors involved in various soft tissue healing responses. This review focuses on the effects of PrP and its associated bioactive factors as elucidated in basic science research. Current findings in PRP basic science research, which have shed light on its proposed mechanisms of action, have opened doors for future areas of PrP research.

In dentistry, the PRP and PRF employment throughout recent years extends in many applications involving the oral surgery, implantology and the treatment of periodontitis and periemfytefmatitidas. Nowadays most clinical research efforts are concentrated not only into healing and tissue regeneration GTR (Guided Tissue Regeneration) but also in guided bone regeneration (GBR). Bone regeneration is the desired goal of dentists in those cases where the patient's jaw bone has receded and there is enough bone to support the implant. CGF (Concentrated Growth Factors) containing growth factors and mesenchymal stem cells CD34 positive type is currently utilized for these purposes.
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