47. Schmitt A, Zarb GA, The longitudinal clinical effectiveness of osseointegrated dental implants for single-tooth replacement. Int J Prosthodont (1993), 6, p.197-202
48.Kasemo:Metallauswahlundoberflachenbeschaffenheit. Gewebeintegrierter Zahnersatz. Quintessenz, Berlin (1985), p.110
49. Strunz: Enossale implantatmaterialien in der Mund- und kieferchirurgie. Hanser, Munchen (1985), p.111
50. Schmalz G, Garhammer P, Biological interactions of dental cast alloys with oral tissues.Dent Mater (2002), 18, p.396-406
51. Tschernitschek H, Borchers L, Geurtsen W Nonalloyed titanium as a bioinert metal—a review. Quintessence Int (2005), 36, p.523-530
52. Buser, D., RK Schenk, S. Steinenann, J.P. Fiorellini, C.Fox, H.Stich: Influence of surface characteristics of bone integration of titanium implants.A histomorphometric study in miniature pigs. Journal of Biomed, (1991), p.889-902
53. Ban. S Maruno S, Arimoto N, Harada A, Hasegawa J. Effect of electrochemically deposited apatite coating on bonding of bone to the HA-G-Ti composit and Titanium. J Biomed Mat Res (1997), 36, p.9-15
54. Yang CY, Wang BC, Lee TM, Chang E, Chang GL. Intramedullary implant of plasma-sprayed hydroxyapatite coating: An interface study. J Biomed Mat Res (1997), 36, p.39-48
55. B.D. Boyan, T.W. Hummert, D.D. Dean and Z. Schwartz.Biomatherials (1996), 17, p.137
56. P.F.Chauvy, C. Madore and D. Landolt.Surf.Coat.Technol. (1998), 110, p.48

Charles University in Prague, Faculty of Medicine
in Pilsen, Department of Stomatology

Bone Augmentation Materials Evaluation of Implant Osteointegration

DISSERTATION THESIS

MUDr.Omid Moztarzadeh
Pilsen 2009

Dizertační práce byla vypracována v rámci postgraduálního doktorandského studia na Stomatologické klinice LF UK v Plzni.

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Prof. MUDr. Jindřich Pazdera, CSc., Klinika ústní, čelistní a obličejové chirurgie LF UP, Olomouc

Stanovisko k dizertační práci vypracovala Stomatologická klinika LF UK a FN v Plzni.

Autoreferát byl rozeslán dne: ……

Obhajoba disertační práce se koná dne 7.05.09 v 13:00 před komisí pro obhajoby dizertačních prací v doktorském studijním programu stomatologie.

S disertační prací je možno se seznámit na děkanátě LF UK, Husová 3, Plzeň

Doc. MUDr. Antonín Zicha, CSc.
Předseda komise pro obhajoby dizertačních prací v doktorském studijním programu stomatologie

34. Dressmann H. Ueber Knochenplombierung bei Hohlenformigen Defekten des Knochens. Beitr Klin Chir (1892), 9, p.804-10
35. Aderriotis D, Sàndor GKB.Outcomes of vicryl rapide fast-absorbing suture in 80 oral and 42 scalp wounds. J Can Dent Assoc (1999), 65, p.345-347
36. Suuronen R, Haer PE, Lindqvist C, Sailer HF.Update on resorbable plates in maxillofacial surgery. Facial Plast Surg (1999), 15, p.61-72
37. Suuronen R, Kallela I, Lindqvist C. Bioabsorble plates and screws: Current state of the art in facial fracture repair. J Craniofac Trauma (2000), 6, p.19-27
38. Tallgren A. The continuing reduction of the residual alveolar ridges in complete denture wearers: a mixed longitudinal study covering 25 years. J Prosthet Dent (1972), 27, p.120-32
39. Samuel B. Linhart, James J. Kennelly : Fluorescent Bone Labeling of Coyotes with Demethylchlortetracycline The Journal of Wildlife Management, (1967),Apr, Vol.31, No.2, p. 317-321
40. Milch RA, Rall DP, Tobie JE Bone localization of the tetracyclines. J. Natl Cancer Inst. (1957), 19, p.87–93
41. Tallgren A. The continuing reduction of the residual alveolar ridges in complete denture wearers: a mixed longitudinal study covering 25 years. J Prosthet Dent (1972), 27, p.120-32
42. Atwood DA. Some clinical factors related to the rate of resorption of residual ridges. J Prosthet Dent (1962) 12, p.441-50
43. Esposito M, Hirsch JM, Lekholm U, Thomsen P .Biological factors contributing to failures of osseointegrated oral implants.(I).Success criteria and epidemiology. Eur J Oral Sci (1998), 106, p.527-551
44. Albrektsson T, Zarb G, Worthington P, Eriksson AR ,The long-term efficacy of currently used dental implants: a review and proposed criteria of success. Int J Oral Maxillofac Implants (1986), 1, p.11-25
45. Albrektsson T, Sennerby L, State of the art in oral implants. J Clin Periodontol (1991), 18, p.474-481
46. Shulman LB, Rogoff GS, Savitt ED, Kent RL Evaluation in recosntructive implantology.Dent Clin North Am (1986), 30, p.327-349

22. Van Den Bogaerde J, White DJ. Xenogeneic transplantation.Brit Med Bull (1997), 53, p.904-20
23. Hammer C, Linke R, Wagner F, Diefenbeck M. Organs from animals for man. Int Arch Allergy Immunol (1998), 116, p.5-21
24. Block JE, Poser J. Does xenogeneic demineralized bone matrix have clinical utility as a bone graft substitute? Med Hypotheses (1995),45, p.27-32
25. Jensen SS, Aaboe M, Pinholt EM, Hjorting-Hansen E, Melsen F, Ruyter IE.Tissue reaction and material characteristics of four bone substitutes.Int J Oral Maxillofac Impl (1996), 11, p.55-66.
26. Poumarat G, Squire P, (1993) Biomaterials 14, p.337-340
27. Byrd HS, Hobar PC, Shewmake K. Augmentation of the craniofacial skeleton with porous hydroxyapatite granules. Plast Reconstr Surg (1993), 91, p.15-22
28.Mercier P, Failures in ridge reconstruction with hydroxyapatite.Analysis of cases and methods for surgical revision. Oral Surg Oral Med Oral Path Oral Radiol Endod (1996), 81, p. 376-384
29. Prousaefs P, Lozada J, Valencia G, Rohrer MD. Histologic evaluation of a hydroxyapatite onlay bone graft retrieved after 9 years: A clinical report. J Prosthet Dent (2002), 87, p.481-484
30. Mors W, Kaminski E. Osteogenic replacement of tricalcium phosphate ceramic implants in the dog palate. Arch Oral Biol (1975), 20, p.365-367
31. Hollinger JO, Schmitz JP, Mizgala JW, Hassler C.An evaluation of two configurations of tricalcium phosphate for treating craniotomies. J Biomed Mater Res (1989), 23, p.17-29
32. Goran Knežević, Marijana Rinčić, Dinko Knežević. Radiological Evaluation of the Healing of Bone Defects Filled with Tricalcium Phosphate (Bioresorb) after Cystectomy of the Mandible.Acta Stomatol Croat. (2007), 41(1), p.66-73
33. Ziffe D, Moroni A, Pezzuto V. Histological and Physico-Chemical Analyses on Transformations of Some Bioactive Glasses Implanted in Long Bones of Rabbit and Sheep. Vincenzini P, editor.Ceramics in Substitute and Reconstructive Surgery. Amsterdam: Elsevier Science Publishers (1991), p.115-132

Table of Contents

13. Giannobile WV, Hernandez RA, Finkelman RD, Ryan S, Kiritsy CP, D'Andrea M, Lynch SE.Comparative effects of platelet-derived growth factor-BB and insulin-like growth factor-I, individually and in combination, on periodontal regeneration in Macaca fascicularis. J Periodontal Res (1996), 31(5), p.301-312

14. Giannobile WV, Whitson SW, Lynch SE. Non-coordinate control of bone formation displayed by growth factor combinations with IGF-I. J Dent Res (1997),76, p.1569-1578

15. Howell TH, Fiorellini JP, Paquette DW, Offenbacher S, Giannobile WV, Lynch SE. A phase I/II clinical trial to evaluate a combination of recombinant human platelet-derived growth factor-BB and recombinant human insulin-like growth factor-I in patients with periodontal disease. J Periodontal (1997)b, 68, p.1186-1193

16. Bonutti PM, Cremens MJ, Miller BG. Formation of structural grafts from cancellous bone fragments. Am J Orthop (1998), 27, p.499-502

17. Keller EE, Tolman D, Eckert S. Endosseous implant and autogenous bone graft reconstruction of mandibular discontinuity: A 12-year longitudinal study of 31 patients. Int J Oral Maxillofacial Implants (1998), 13: p.767-780

18. Vinzenz KG, Holle J, Wuringer E, Kulenkampff KJ, Plenk H Jr. Revascularized composite grafts with inserted implants for reconstructing the maxilla: Improved flap design and flap prefabrication. Brit J Oral Maxillofac Surg (1998), 36, p.346-352

19. Brooks DB, Heiple KG, Herndon CH, et al: Immunological factors in homogenous bone transplantation. IV. The effect of various methods of preparation and irradiation on antigenicity. J Bone Joint Surg (1963) Am 45, p.1617–1626

20. Friedlaender GE, Strong DM, Sell KW: Studies on the antigen city of bone. II. Donor-specific anti-HLA antibodies in human recipients of freeze-dried allografts. J Bone Joint Surg (1984) Am 66, p.107–112

21. Herron LD, Newman MH: The failure of ethylene oxide gas sterilized freeze-dried bone graft for thoracic and lumbar spinal fusion. (1989) Spine 14, p.496–500

Literature

1. Buser, D., Ch. Dahlin and R.K. Schenk , Guided bone regeneration in Implant Dentristry,Quintessence Publishing Co, Inc., Chicago, Berlin, London, Tokyo, Moscow, Prague, Sofia, and Warsaw,(1994), p.69
2. Prolo DJ: Biology of bone fusion. Clin Neurosurg, (1990), 36: p.135–146
3. Buchardt H. The biology of bone graft repair. Clinical Orthop (1983), 174, p.28-42
4. Joyce ME, Jingushi S, Bolander ME.Transforming growth factor- in the regulation of fracture repair. Orthop Clin North Am (1990), 21, p.199-209
5. Bourque WT, Gross M, Hall BK.Expression of four growth factors during fracture repair.Int J Dev Biol (1993), 37, p.573-79
6. Nakamura T, Hara Y, Tagawa M, Tamura M, Yuge T, Fukuda H, et al. Recombinant human basic fibroblast growth factor accelerates fracture healing by enhancing callus remodeling in experimental dog tibial fracture. J Bone Miner Res (1998), 13, p.942-9
7. Trippel SB. Potential role of insulinlike growth factors in fracture healing. Clin Orthop (1998), 355S, p. 301-13
8. Nash TJ, Howlett CR, Martin C, Steele J, Johnson KA, Kicklin DJ.Effect of platelet-derived growth factor on tibial osteotomies in rabbits. Bone (1994), 15, p.203-8
9. Parikh SN. Bone graft substitutes: past, present, future. J Postgrad Med. (2002), 48, p.142-8
10. Wozney JM.Bone morphogenetic proteins.Prog in Growth Factor Res (1989), 1, p.267-280
11. Wozney JM, Rosen V, Byrne M, Celeste AJ, Moutsatsos I, Wang EA. Growth factors influencing bone development. J Cell Science Suppl (1990), 13, p.149-156
12. Celeste AJ, Iannazzi JA, Taylor RC, Hewick RM, Rosen V, Wang EA, Wozney JM. Identification of transforming growth factor beta family members present in bone-inductive protein purified from bovine bone. Proc Nat Acad Sci USA (1990), 87, p.9843-9847

Bone graft substitute materials

Introduction

The filling of a bone defect is a significant issue in each day of clinical work. Restitutio ad integrum is achieved in minor defects through biological self healing. Self healing is limited by the size of the defect. In larger defects where the organism is unable to heal itself, the restoration of the original condition must be the objective of the therapy. Bone tissue is unable to regenerate defects larger than 1 mm in a single step. Weeks or even months are required to heal bone defects larger than 3 mm [1].

Bone grafts are necessary to provide support, fill voids, and enhance the biologic repair of skeletal defects. They are used by orthopaedic surgeons, neurosurgeons, craniofacial surgeons and periodontists.

There are several physiological properties of bone grafts which can directly affect the success or failure of graft incorporation. These properties are osteogenesis, osteoconduction, and osteoinduction [2].

Osteogenesis is the ability of the graft to produce new bone, and this process is dependent on the presence of living bone cells in the graft.

Osteoconduction is the physical property of the graft to serve as a scaffold for viable bone healing [3].

Osteoinduction is the ability of graft material to induce stem cell differentiation into mature bone cells, or in more detail to induce differentiation of undifferentiated pluripotential cells toward an osteoblastic phenotype. Several osteoinductive agents which are generally proteins, have been identified. Among these compounds are transforming growth factor (TGF-β) [4], fibroblast growth factors (FGFs) [5,6], insulin-like growth factors (IGFs) [7], and platelet-derived growth factors (PDGFs) [8].

Bone Morphogenetic Proteins (BMP), are low-molecular-weight non-collagenous glycoproteins that belong to an expanding TGF-β super family of at least 15 growth and differentiation factors [9]. They are known for their ability to induce the formation of bone and cartilage [10,11].

Transforming Growth Factor (TGF-β) is a osteopromotor protein agent which participates in all phases of bone healing [12].

Platelet-Derived Growth Factor (PDGF) is dimeric glycoprotein growth factor that regulates cell growth and division by playing a role in embryonic

Publications:

1. Moztarzadeh O: Le fort fracture, new classification. Prague Medical report, Multidisciplinary Biomedical Journal of the First Faculty of Medicine, Charles University in Prague, Vol.105 (2004) No. 2, p. 180-181

2. O. Moztarzadeh, D. Hrusak, P. Andrle, L. Hauer, L. Hosticka: Ectopic Chondroosteoid tissue formed by tricalcium phosphate. Journal of Cranio-Maxillofacial Surgery, Vol. 36, Suplement 1, September 2008, p. 272

3. L. Hosticka, O. Moztarzadeh, D. Hrusak, P. Andrle, L. Hauer: A comparison of imaging methods for solivary gland ducts. Journal of Cranio-Maxillofacial Surgery, Vol. 36, Suplement 1, September 2008, p. 202)

4. D. Hrusak[1], L. Dluhos[1], C. Arnold[2], O. Moztarzadeh[3], L. Hosticka[3], P. Andrle[3]: Immediate load of nanostructured Ti implants. Journal of Cranio-Maxillofacial Surgery, Vol. 36, Suplement 1, September 2008, p. 262

5. L. Dluhos[1], J. Petruzelka[2], R. Valiev[3], O. Moztarzadeh[4]: Biological properties of nanostructured titanium made by SPD. Journal of Cranio-Maxillofacial Surgery, Vol. 36, Suplement 1, September 2008, p. 260

6. L. Hauer, D. Hrusak, P. Andrle, L. Hosticka, O. Moztarzadeh: Bisphosphonate-associated osteonecrosis of the jaws. Journal of Cranio-Maxillofacial Surgery, Vol. 36, Suplement 1, September 2008, p. 182-183

7. P. Andrle, L. Hauer, L. Hosticka, D. Hrusak, O. Moztarzadeh: Glandular odontogenic cyst: a case of early recurrence. Journal of Cranio-Maxillofacial Surgery, Vol. 36, Suplement 1, September 2008, p. 178

8. Šafářová M., Moztarzadeh O. : Liposarkom jazyka. Čes. Stomat., roč. 108, 2008, č. 6, s. 149-151.

Based on these results, we can conclude that the surface of the implant has a very important role in osteointegration, and that mechanically added roughness to these surfaces significantly increases the contact area between the implant surface and the peri-implant bone.

development, cell proliferation, cell migration and angiogenesis.

Insulin-like growth factor (IGF) is a polypeptide that is secreted from many different cells. Their designation as "insulin-like" is due to the fact that they have high sequence similarity to insulin. Due to their growth promoting activity, they were formerly called somatomedins. PDGF and IGF have shown an ability to work together during the reparative stages of bone healing in defects associated with dental implants and teeth [13,14,15].

Platelet rich plasma (PRP) is a modification of fibrin glue made from autologous blood and is used to deliver growth factors in high concentration to sites requiring osseous grafting.

Basic fibroblast growth factor (bFGF) in normal tissue is present in basement membranes and in the sub endothelial extracellular matrix of blood vessels. Hyaluronic acid (Hy) is a viscoelastic polymer found throughout the body that cushions and protects soft tissues. The synergistic combination of bFGF and Hy appears to accelerate the fracture healing process.

Classification of bone grafting materials based on source

Autograft: refers to a transplant of viable cortical or cancellous bone [16,17,18] from one location to another within the same patient. The disadvantages of autografts include the need for a separate incision for harvesting, increased operating time and blood loss, the risk of donor-site complications, post-operative pain and the frequent insufficient quantity of bone graft.

Allograft: refers to a transplant of non-vital osseous tissue from genetically no identical members of the same species. It has low or no osteogenicity, increased immunogenic activity and resorbs more rapidly than autogenously bone. The disadvantages of allograft include delayed vascular penetration, slow bone formation, accelerated bone resorption, and delayed or incomplete graft incorporation [19,20,21].

Xenograft: also called heterograft refers to a skeletal tissue that is harvested from an animal of one species and transferred to the recipient site of another species [22,23], such as the use of inorganic bovine bone or bovine collagen in human subjects [24,25]. Xenograft bone could be processed to be safe for transplantation in a human host [26].

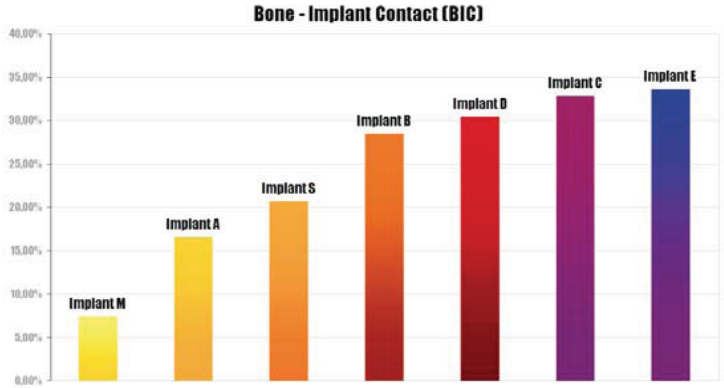
Conclusion

We found out that the different types of laser treated and sandblasted implant surfaces had the highest percentage of Bone-Implant Contact, whilst the machined surface had the lowest, as expected. However in comparison between laser treated and sandblasted surfaces, the laser treated surfaces almost had a higher BIC % than the sandblasted implant. The results show that laser treatment produced the highest BIC%, and is therefore the method of choice when treating the surface of the implant. This may be due to the laser source used for manufacturing the implants, which was provided by Synthegra Technology. It was able to produce surfaces with biometric characteristics without altering the characteristics of the implant material itself, in this case, titanium. The use of the laser allowed the creation of a highly controlled surface which helped to improve the integration of the implant with the surrounding tissue, as it allowed the creation of a vast number of highly accurate niches for each osteoblast. The laser source resulted in the ability to micro-roughen the titanium surface in a controlled and uniform manner without chemically altering it, and also assisted in stimulating adhesion, proliferation and differentiation of the osteogenic cells in contact with the implant surface.

Results

Average BIC (%) for each type of implant:
M = 7.36
A = 16.54
S = 20.64
B = 28.44
D = 30.36
C = 32.80
E = 33.58

A = laser 5 µm pores, pitch 15 µm; **B** = laser 10 µm pores, pitch 20 µm; **C** = laser 20 µm pores, pitch 30 µm; **D** = laser 5 µm pores, pitch 5 µm; **E** = laser 10 µm pores, pitch 10 µm; **F** = laser 20 µm pores, pitch 20 µm; **S** = sandblasted; **M** = machined



BIO-GEN® is a natural bone conductive material without collagen, deantigenised and derived from equine bone, which is completely absorbable. It elevates level of bone genesis thanks to the absence of the calcification process within the production process, that occurs through physical and chemical processes at a maximum temperature of 130°C in a humid environment. The final bone tissue, constituted of bone mineral matrix which is unmodified at the atomic structural level. The absorption time of spongy granular is 4-6 month and cortical granular type is 8-12 month. Granular mix is made up of a calibrated mixture of cortical and spongy bone tissue with cortical grains of 0.5 mm and spongy grains of 1 mm.

Alloplast: refers to implantation of synthetic materials which are available in different forms with variable density, porosity and crystallinity, all of which are dependent on the manufacturing process.

Used alone as a single graft material, these materials can result clinically in improved bone density, and can lead to more complete bone fill of defects via osteoconduction.

Hydroxyapatite (HA) ($Ca_{10}(PO_4)_6(OH)_2$) is a primary inorganic, natural component of bone, comprising about 65% of the calcified skeleton and 98% of dental enamel.

HA is brittle and its strength decreases exponentially with increase in porosity therefore implants can not be placed in a HA-treated ridge. Granular migration and incomplete resorption are other dissadvantages of HA [27,28,29].

Tricalcium Phosphate (TCP) ($Ca_3(PO4)_2$) like hydroxyapatite is a synthetic calcium phosphate ceramic with a different stoichiometric profile [30,31]. TCP is bioabsorbable and biocompatible material which is used in a variety of orthopaedic and dental applications since 1981. The mechanism of the effect of tricalcium phosphate is explained by an enrichment of the microenvironment by the biodegradable material and release of ions of calcium and phosphor which stimulates the activity and proliferation of cells [32].

Bioglass or Bioactive glass is an amorphous synthetic material composed of calcium phosphate, sodium, and silicon. These materials have the ability to chemically bond with bone [33] and been used in dentistry as restorative materials such as glass ionomer cement.

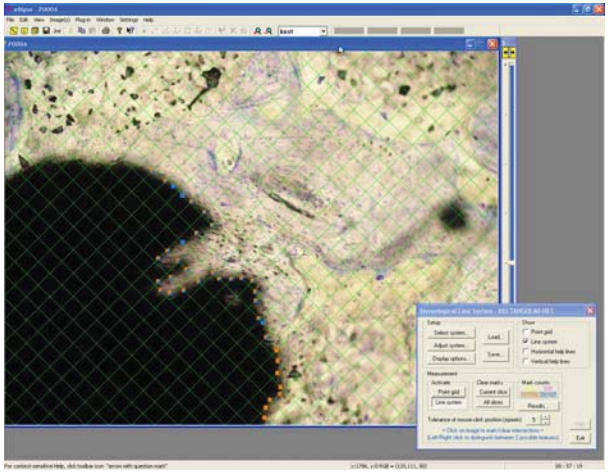
Stereologically there are 200 evaluation points for the 1 histological preparation. In our experiment this point refers to the meeting point between each rectangular network with the margin of the implant.

For our experiement we evaluated the meeting of network-margin of implant by the following formula:

Contact of network-Bone implant contact / network-margin of implant x 100

Or alternatively: **Bone/Implant x 100**

(See page 52 for the results of BIC)



Green rectangular network can be seen. The meeting point from which the formula can be used can be seen as small orange squares (margin of implant contact) and small blue squares (Bone contact with implant)

Introduction To Bone-Implant Contact

Osteointegration is defined in histological implant studies as the direct contact between living bone and implant, on the level of a light microscope. One of the most used variables in histomorphometric analysis of implants is the fraction of surface areas of mineralized bone in contact with the implant surface (BIC).

Histomorphometry is an established method to determine the extent of osteointegration and the rate of healing of dental implants. We can use this to measure the percentage of bone implant contact (BIC).

We evaluate the osteointegration of different types of surface treated implants by measuring of bone-implant contact (BIC) % according to the Elipse programme.

Protocol for BIC measurement, in order to obtain results for the evaluation of osteointegration

This was done by the use of the programme Elipse produced by ViDiTo Košice org. module line system.

The BIC was evaluated in a rectangular network with each rectangle being 75 x 75 px to 150 x 150 px.

The size of the network allows a great number of evaluation points from only one preparation.

Calcium Sulphate (CaSO4) also called Plaster of Paris or Gypsum, is a biologically inert, resorbable osteoconductive material. Their first internal use to fill bony defects was reported in 1892 by Dressmann [34].

Polymer is a large molecule composed of many smaller repeating units (the monomers) bonded together. They are non-biologic materials and have the advantage of the ability to control all aspects of the matrix, avoidance of immunologic reaction, and excellent biocompatibility. Polylactic acid (PLA) and Polyglycolic acid (PGA) polymers have been used extensively as suture materials [35], and biodegradable fracture fixation implants [36,37].

Property requirements of bone augmentation materials

The ideal bone substitute material should have properties similar to that of human bone. Bone augmentation materials used to support the biological bone regeneration process must fulfil various functions in the different phases of regeneration.

<u>In the early inflammatory phase the bone substitute materials must have the following properties</u>:

Biocompatibility of bone augmentation: displays a very high integration within the natural bone without any soft tissue encapsulation or pathological reactions.

In addition, the biomaterial must not be carcenogenic, immunogenic, antileukotactic or mutagenic. In turn, the environment should not cause degradation or corrosion of the biomaterial that would result in loss of physical and mechanical properties.

Materials should not:

- Cause thrombus formations

- Destroy or sensitize the cellular elements of blood

- Alter plasma proteins (including enzymes), so as to trigger undesirable reactions

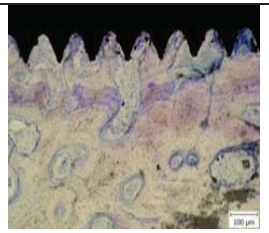
- Cause adverse immune responses

- Cause cancer

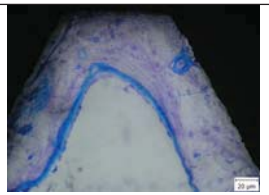
- Cause teratological effects

- Produce toxic and allergic responses

Microphotographic images obtained by light microscope

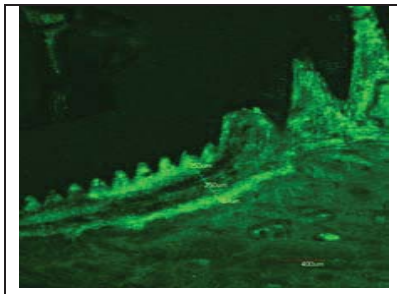


Implant A, right ulna, pig 3 (longitudinal section).

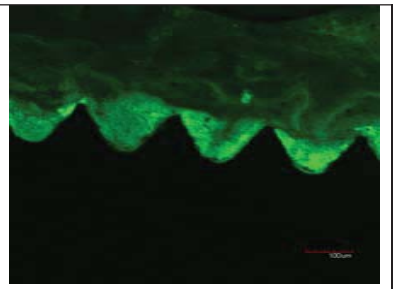


Implant C, left tibia, pig 1 osteointegration between two threads (longitudinal section).

Microphotographic images obtained by CLSM



Implant E, right mandible, pig 1.(Two lines of tetracycline labelled bone).



Implant A, left mandible, pig 3.

- **Staining and Mounting**

	
Staining.	Preparation is ready for light microscope.

After bone processing and staining for eventual histological analysis, microphotographic images were taken under both light and CLSM showing the bone-implant interface.

Images from CLSM reveal that during the period of application, the TTC accumulated and labelled the newly formed bone demonstrated two levels of bone formation labelled as fluorescent lines. (See images on page 49).

- Deplete electrolytes

- Be affected by sterilization

At present date, there are no known materials which totally satisfy these criteria so when a foreign material is placed into a biological environment, inevitable reactions occur which are detrimental to both host and material.

Materials all possess inherent morphological, chemical, and electrical surface qualities which elicit reactionary responses from the surrounding biological environment. In fact, biocompatibility can be described as multifactorial in that simultaneous stimuli from any of these material properties can affect the host response.

- *Osteoconductivity:* allows bone to directly grow into the interconnecting pores prior to the resorption process.

- *Haemostatic effects*: Refers to the ability of the augmentation to prevent or absorb blood from the surrounding tissue.

-*Porosity*: Bone knitting and tissue attachment to the material is encouraged by employing structures containing sufficient, correctly sized porosity to provide

a penetrable host for the infusion and growth of new tissue and bone material. From a biomedical point of view, a bone augmentation material must feature porous connectivity to ensure progressive angiogenesis (penetration of the bone augmentation material by blood vessels).

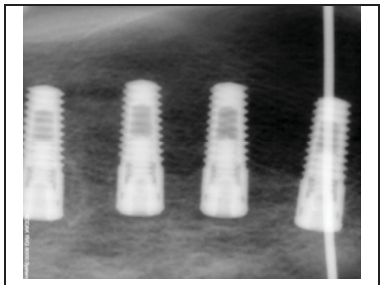
- *Encouragement of angiogenesis*: Angiogenesis is a physiological process involving the growth of new blood vessels from pre-existing vessels.

- *Physical stability*: strength needs to be high enough to resist fragmentation before the cells synthesizes their own extracellular matrix.

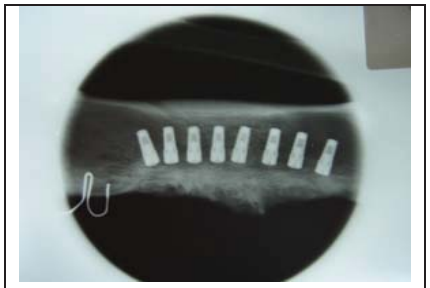
Premature disintegration into micro particles provokes the activity of phagocytozing macrophages and polymorphic polynuclear cells and thus intensifies the nonspecific immune defense reaction which is detrimental to regeneration.

So in conclusion the augmentation material must be hard enough to withstand forces from surrounding structures and at the same time be porous enough to allow the growth of bone in to it, this is achieved by many different methods. For example by the use of material consisting of different layers, all having

Radiographic Results



right tibia



right tibia

Bone processing for eventual histological analysis

- **Embedding in Epon 812**

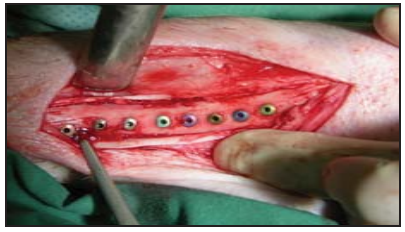
Cured blocks ready for cutting.

The glued on bone-implant section is ready for grinding.

Materials and Methods

Different types of surface treated Implants inserted into the long bones (ulna and tibia) and mandible of 4 piglets under general anesthesia. Two doses of antibiotic Tetracycline (TTC) were applied in different periods of time to demonstrate regions of active bone formation, mineralisation and to demonstrate the quantity of newly formed bone at the implant interface with the help of a confocal microscope due to its fluorescent property.

The pigs were sacrificed after the periods of 52 and 64 days. A Series of radiographical images were taken and revealed that opposition of bone occurred above the implant head, where the largest part of the implant remained inside the bone marrow cavity due to the osteoclastic activity of osteoclasts. Note: For performing a similar experiment, the author recommends the use of older animals or miniature pigs that have a slower rate of growth.



Implants inserted into the tibia

different rolls in the process of bone growth. The material used must have slight elastic properties that prevent it from cracking when under stress.

There are various methods of strengthening and stabilizing a bone augmentation. An altogether different technique for enhancing bone density at the region of the implant, involves effectively transferring loading stress from an implant to the surrounding bone through the use of an implant having a tapered body shape.

- *No stimulation of immune reaction*: Another factor of great importance when selecting the bone substitution materials is that the material cannot stimulate the immune system to react to its presence. If the immune system reacts to the foreign bodies presence as something that should not be there it will reject it preventing any interaction between the material and the bone.

- *No pathological concentration of chemical substances.*

In the reparative phase of bone healing, the substitute materials must have the following properties:

- *Simultaneous resorption for the purpose of bone regeneration*: excessively rapid degradation rate does not allow the proper regenerative processes to occur. If too slow however, degradation rate interferes with remodelling.

- *No pathological pH values*

-*Physiological supplementation with growth factor* (Please refer back to´osteoinduction´on pg 6).

- *Osteoinductivity*

In the biological remodelling phase the bone substitute materials must have the following properties:

- *Complete resorption of the material:* An ideal bone augmentation material adapts its resorption rate to the surrounding conditions. The time required for final resorption is also the crucial quality criterion of the bone augmentation material. It should fulfil its space holder function only until the bone can regenerate itself, so that it can react to changing biomechanical loads freely and undisturbed by remodelling.

- *No disturbing influence on biological remodelling*

traditional method, however, our method was significantly less time consuming, more cost efficient and easier to carry out.

Experiment 6 - Experimental study in pigs with the purpose to evaluate and examine osteointegration of dental implants with differently treated surfaces.

Aim

Examination and evaluation of osteointegration of dental implants with different laser treated surfaces, and the comparison of those results with sandblasted and machine treated implant surfaces.

Our aim is also to apply tetracycline (TTC) in order to demonstrate regions of active bone formation, mineralisation and to demonstrate the quantity of newly formed bone at the implant interface with the help of confocal microscope due to its fluorescent property. During the period of application, the TTC accumulates and labels the newly formed bone. Our expectation is to demonstrate two levels of bone formation labelled as fluorescent lines.

Conclusion

Experiment 4**:** We ascertained that the traditional method of producing histological specimens of bone and implant is very time consuming and complicated.

The experimental method that was developed utilizes the confocal laser microscope, allowing accurate results and more rapid specimen preparation time whilst being easier to perform.

Experiment 5: The results were highly successful and provided an excellent view of the bone-implant interface. The images made by laser confocal microscope showed osteointegration between the bone in red, and the dark black implant structure.

Evaluating the radiographical images, we can establish that there are no spaces between bone and implant surface, showing good osteointegration.

Based on our results, in both the nonliving and living tissues, we can deduce that the alternative method is a very successful way in preparing and evaluating histological specimens in order to show the osteointegration between the bone and implant surface. Our results were similar to those provided by the

Indications and usage of bone substitute materials in dentistry

- Augmentation or reconstruction of the alveolar ridge
- Elevation of the maxillary sinus floor (sinus lift)
- Filling of extraction sockets to enhance preservation of the alveolar ridge: Extraction of teeth may result in 40% to 60% alveolar bone loss in a period of two to three years [38].
- Filling of periodontal defects
- Filling of peri-implant defects
- Filling of defects after root resection, apicectomy and cystectomy.

Experiment 1 - study of statistical data of augmentation materials

Aim

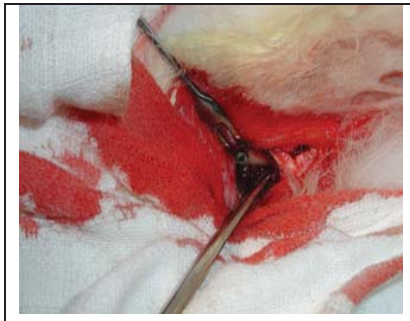
To understand what the most common indications of using augmentation materials are and to obtain some statistical data concerning 3 different bone substitute materials.

Materials and Methods

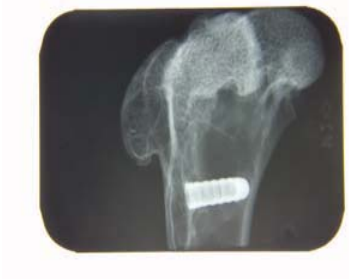
This study is based on the statistical data of different augmentation materials as used when indicated in patients of the Maxillofacial Surgery Department, Faculty Hospital in Pilsen, during the period of 2003-2007.

During this period we treated 19 patients with 3 different augmentation materials; Biogen, Bioresorb and Cerasorb. Regarding this fact that one patient could have been treated by different augmentation materials or by one augmentation material in different regions of the jaws, we record the results of indicated material as a case which were a total of 30.

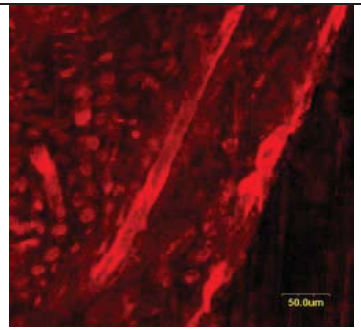
After histological preparation the specimens were observed under CLSM. The results were highly successful and provided an excellent view of the bone-implant interface for evaluation of osteointegration. We confirmed the results obtained from CLSM pictures by performing the traditional method and analysed our results under light microscope.



Inserted implant can be seen.



Radiographic image of implant in femur of rabbit after sacrifice.



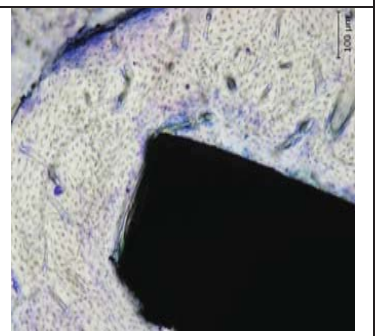
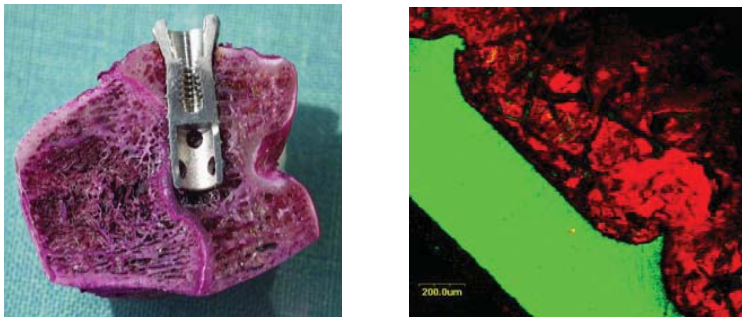


Image from CLSM and Histological section of Rabbit Femur (Longitudinal Section)

expected, no osteintegration had taken place due to implantation into nonliving tissue, the bone-implant interface can clearly be seen when carrying out this implantation in a living animal, we will expect to see these dark spaces replaced by area of osteoblast activity with subsequent bone formation and osteointegration. The preparation of the histological specimens of the bone-implant surface for evaluation with a Light Microscope was complex and required a large proportion of time to complete.

A B



A: Finished Grinding of bone and implant **B:** Bone-implant interface seen with green implant structure and red surrounding bone. Please make note of black space area between surface of implant and bone.

Experiment 5: Having established the success of the experimental method, we decided to repeat our experiment but with the use of a living rabbit.

Results

1. Of all 19 patients, 10(53%) were treated by Bioresorb, 5(26%) by Cerasorb and 4(21%) patients by Biogen.

2. Of all 30 cases, 16(53%) Bioresorb, 9(30%) Cerasorb and 5(17%) Biogen were used.

3. Of all 19 patients, 10(53%) were male, and 9(47%) were women.

4. Of all 30 cases of indication for treatment using augmentation materials 8(28%) were for sinus lift, 7(23%) for augmentation of bone after extraction, 5(17%) after cystectomy, 4(13%) for augmentation of alveolar ridge, 4(13%) after root resection, 1(3%) for augmentation of periimplant defect and 1(3%) for augmentation of alveolar ridge with distraction.

5. Of all 9 cases treated by Cerasorb, 4 cases were indicated for sinus lift, 3 for augmentation of alveolar ridge, 1 for augmentation of bone after extraction and 1 after extraction.

6. Of all 16 cases teated by Bioresorb, 4 cases were indicated for sinus lift, 4 for augmentation after cystectomy, 4 for augmentation of bone after extraction, 2 after root resection, 1 for augmentation of

periimplant defect and 1 for augmentation of alveolar ridge.

7. Of all 5 cases treated by Biogen, 2 cases were indicated for augmentation of bone after extraction, 1 after root resection and 1 for augmentation of alveolar ridge.

8. In total 5 augmentation materials were used for augmentation of alveolar ridge, among them in 3 cases Cerasorb were used and 1 case for Bioresorb and Biogen each.

9. In total 4 augmentation materials were used after root resection, among them in 2 cases were used Bioresorb and 1 case for Cearsorb and Biogen each.

10. In total 5 augmentation materials were used after cystectomy, among them in 4 cases were used Bioresorb and for 1 case Cerasorb.

11. In total 8 augmentation materials were used for sinus lift operation, among them in 4 cases were used Bioresorb and 4 cases were used Cerasorb.

acquired point-by-point and reconstructed with a computer, allowing three-dimensional reconstructions of topologically-complex objects.

Materials and Methods

Experiment 4: In the first step we tried to determine the best staining method for staining of different sections of various bone by different staining methods and materials.

We inserted the titanium implant and screw in to the femur of the dead rabbit. In the next step, grinding of the bone and implant was done until the bone-implant interface was seen. This was then stained with basic fuchsin ready for histological evaluation under the confocal laser scanning microscope (CLSM).

Experiment 5: Under general anesthesia titanium implant was surgically inserted into the femur of live rabbit.

After 45 days the rabbit was sacrificed and a series of radiographical images were taken.

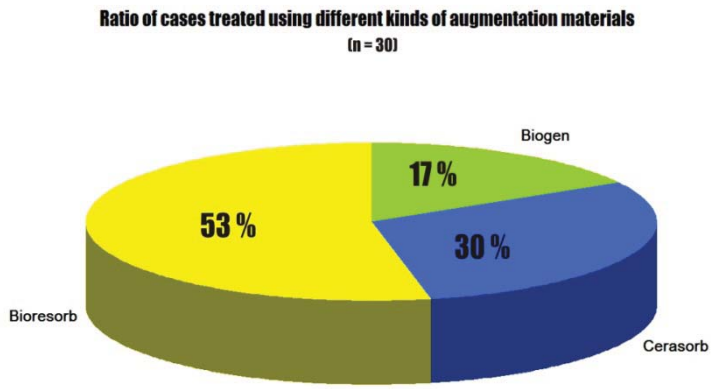
Results

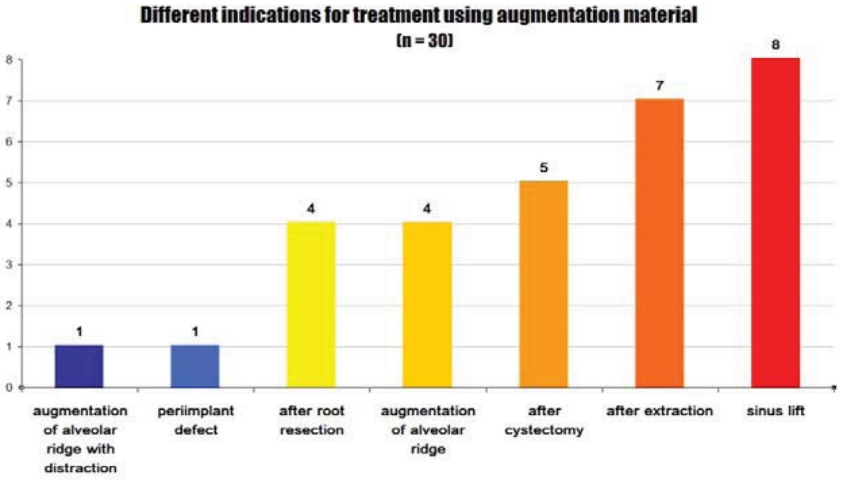
Experiment 4: The results, as can be seen under a confocal laser microscope are very positive. Although, as

Experiment 4 and 5 - to establish the most efficient evaluation method of a bone-implant interface in a dead rabbit (experiment 4), and if successful to use this Method to evaluate the actual osteointegration in a living rabbit (experiment 5).

Aim

For evaluation of osteointegration between bone and dental implants, the preparation time can exceed 7 months when following the traditional method of preparation of the histological specimens. The main difficulty in the traditional method is to get a very thin section of bone-implant, without any displacement and micro-movements of osteointegrated implants from the bone, to be able to observe true bone-implant interface. Our aim in these experiments was to find an alternative method for preparation of the bone-implant interface for evaluation, by using a confocal laser microscope in order to improve cost efficiency and reduce the preparation time whilst obtaining reliable and useful results. The advantage of CLSM is that speciment should not be sectioned as thin as for light microscope and its ability to produce in-focus images of thick specimens, a process known as *optical sectioning*. Images are





Conclusion

The use of augmentation materials were slightly more common in treatments carried out on males than in females.

Bioresorb was generally the most commonly used augmentation material.

The principal indicators for use of augmentation materials were concluded to be primarily sinus lifts followed closely by their use in post-extraction cases. It is also indicated in other minor surgical procedures, such as following root resections and cystectomy.

It was established that in cases of sinus lift, Bioresorb and Cerasorb were most commonly used. Bioresorb was also the principal material used after root resection and cystectomy, whereas Cerasorb was preferred in alveolar ridge augmentation.

Indications for use of Biogen were in post extraction cases.

behavior, one can verify that there is bone apposition onto the implant surface independant of whether it is polished or rough, made of titanium or ceramic. Roughness is not necessary for bone apposition. Osteoblasts have higher probability to adhere to a rough titanium surface while fibroblasts and epithelial cells adhere mainly to very smooth surfaces[55,56]. However, it has been shown that roughness may play an important role in the percentage of bone apposition as well as in the velocity of apposition. Roughness or acid conditioning of the surfaces can therefore significantly improve shear strength. Besides optimizing the procedure, these surface characteristics may allow for an earlier loading of the implant and extend the indications for implants in low-density alveolar bone and in regenerated bone.

coating. The layer is typically 20-30µm thick with a roughness of approximately 15µm.

Plasma coating is economically compatible, and its biological compatibility is at least as good as that of normal titanium.

Acid-etching

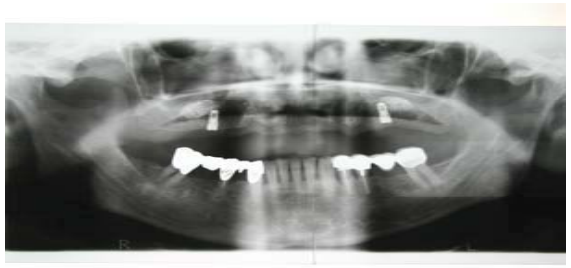
Acid etching of titanium is of particular interest because it creates a microtextured surface (fine rough surface with micro pits of 1-3µm and larger pits of approximately 6-10µm) that appears to enhance early endosseous integration and the stability of the implant [126].

Sand-blasted

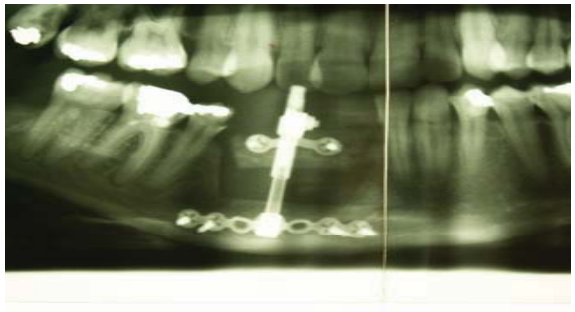
In this case the sand-blasting roughens the surface of the implant achieving both microretentive topography and increased surface area [53,54].

Sandblasting treatment consists of mechanical abrasion of surfaces using oxide particles shot against the implant.

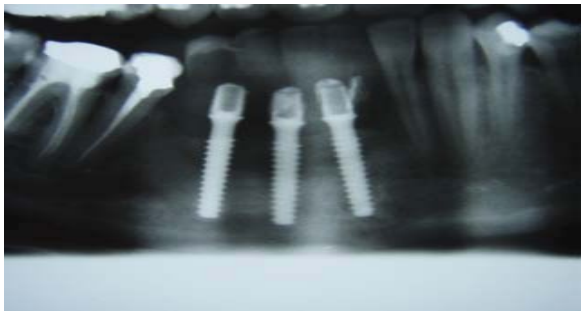
Texture was the most remarkable isolated feature, regarded as an osteointegration promoter. In a review of the effects of implant surface topography on cell



Panoramic radiograph taken after sinus lift using Cerasorb, and insertion of two implants at (26, 15).



Augmentation of alveolar ridge, using Distractor and Biogen.



Inserted 3 nanoimplants. 2 years after augmentation.

Experiment 2- Experimental study in pigs with the purpose of evaluating the possible osteogenic activity of bone augmentation materials

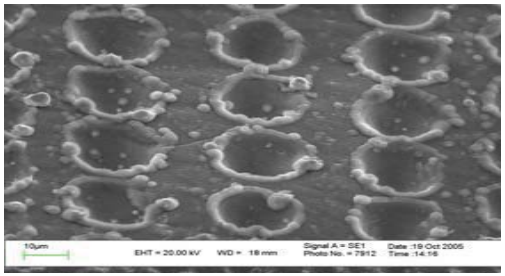
Aim

To evaluate in vivo, the osteoinduction potential and osteogenic activity of two different bone augmentation materials, and if ectopic bone formation could be induced when implanted subcutaneously to the extremities of the pigs.

Materials and Methods

The experiment was performed under general anaesthesia, using aseptic techniques. Two bone augmentation materials: microporous granules (Ø 500-1000 µm) of pure phase ß-tricalcium phosphate ceramic Cerasorb® and granular mix (Ø 0.5-1 mm) derived from equine bone BIOGEN® were applied at the ulnar region between muscular and cutaneus tissue. We performed an intramuscular application of the antibiotic Tetracycline 16 days after operation. Tetracycline is deposited where bone or cartilage matrix is mineralizing and can therefore demonstrate regions of active bone formation and mineralization. After 52 days the pigs were terminated. At the site of application of Cerasorb

smoothly polished or finely structured titanium implants which had values of slightly over 20%[52].



Laser treated implant surface

Plasma-coated spray

Coating is to produce a rough implant surface that significantly improves the anchorage of the implant in bone. This process can be used for both metal and ceramics.

Plasma coating works by blowing an inert gas through an intense electric arc. Down the arc the coating material is introduced in the form of a extremely hot gas. The inert gas is broken down into ions and electrons in the arc. This state is known as plasma. The titanium hydride (coating material), decomposes in the gas stream forming droplets of molten metal that are projected on to the implant surface to build up a

3. Transosseous implants: were designed to be used in people who had very little bone in their lower jaws and who had no lower teeth.

Most clinicians nowadays however, prefer to use bone grafts and one of the other endosseous implant methods described earlier.

Implant surfaces:

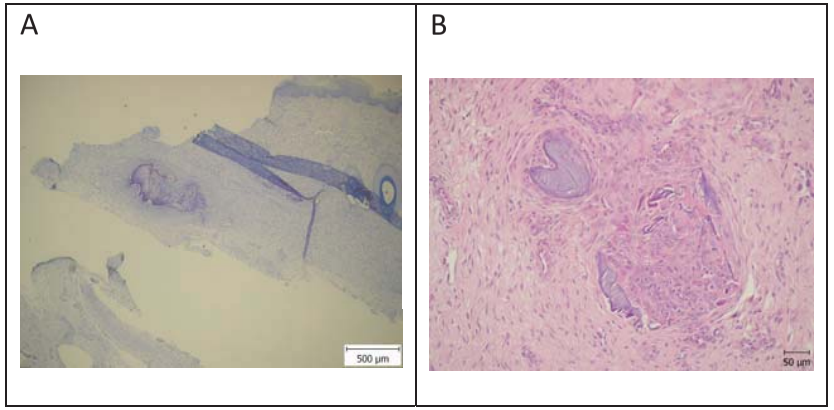
Future dental implant requirements should include proper surface preparation and surface quality maintenance of the implants themselves. Types of surface categories include:

Laser treated

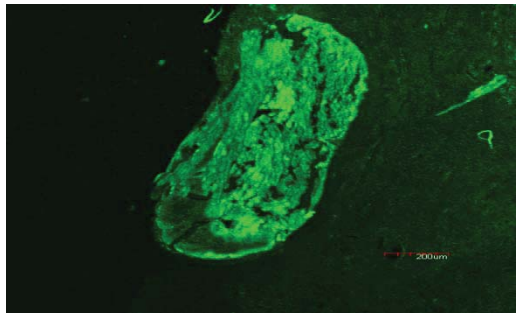
There are different laser sources used in implantology. In our experiment we used Nd:YAG diode pumped laser operating in Q-switching for treatment of the implant surface. This laser radiates electromagnetic energy which interacts with the titanium taking it to a plasma state from its solid state. An important goal of laser treatment of implant surface is to produce a surface with thousands of hemispheric pores for bone apposition.Tianium implants with coatings have an average bone-implant contact in cancellous bone of nearly 40%, which was significantly higher than

some hard tissue material was found (none at BIOGEN®).

The specimens were observed by optical and confocal laser microscope after histological processing.



A: Partially calcified tissue in the dermis was located approximately 1.2-1.44 mm below the epidermis. Alizarin red and toluidine blue stain. **B:** Incompletely mineralized osteoid tissue in the dermis. H.E stain.



Labelling of newly formed chondroosteoid structure with tetracycline.

Results

The observation revealed incompletely mineralized osteoid material and layers of chondroid tissue mass.

Bright fluorescent zones showed the deposition of tetracycline-induced fluorescence limited to area of active hard tissues formation.

Conclusion

Based on our results, it is possible to use this sequential methodology in soft tissues, with the objective to verify the osteoinduction potential of different bone augmentation materials. With respect to this original method, we can analyse different augmentation materials to establish the most successful one with the best osteinductive properties.

We also established that our results from the labelling of the tissue by tetracycline exhibited the same results as that shown in previous publication by Mitch et al [39,40].

These results highlight the potential future use of augmentation materials in creation of hard tissues, in areas where soft tissues are normally present. This would be invaluable, for example, in the clinical setting of cosmetic surgery or maxillofacial surgery where bone

prosthetic crowns if required. They are usually used when there is insufficient live bone structure because the jawbone is too narrow or not deep enough or certain vital anatomical structures prevent conventional implants from being placed.

1c. Ramus Frame Implants: belong in the category of endosseous implants. They are designed for the toothless lower jaw only and are surgically inserted into the jaw bone in three different areas: the left and right back area of the jaw (the approximate area of the wisdom teeth), and the chin area in the front of the mouth.

This type of implant is usually indicated for a severely resorbed, toothless lower jaw bone, which does not offer enough bone height to accommodate root form implant as anchoring device and when the jaws are even resorbed to the point where subperiosteal implant will not suffice anymore.

2. Subperiosteal Implants: are designed to sit on top of the bone, but under the gums, fusion takes place between the jawbone and the subperiosteal implant. They are an option used in cases of advanced bone loss where the condition of the jawbone is such that an insert is not possible and a bone graft is not feasible.

the main factor: Some jawbones can be wide and deep and others narrow and shallow with many variations in between.

In general, Oral Implants can be categorized into three main groups:

1. Endosseous Implants: are implants that are surgically inserted into the jawbone. They are moulded or shaped to fit in a cavity in the jaw rather than sit on top of the jaw. These implants are the most frequently used implants today and based on their shape, function, surgical placement and surface treatment could be further categorized into several sub-categories:

1a. Root Form Implants: also called cylindrical or screw type implants, resemble in shape the natural root of a tooth with a surface area designed to promote good attachment to the bone.

They are the most widely used design and are the most popular type of dental implants.

1b. Plate Form Implants: also known as blade form implants, they are flat rectangles which take the form of a long narrow strip of titanium which is inserted between the jawbone and the gum and will fuse with the jawbone giving a foundation for a number of new

reconstruction must take place following oncological or traumatic destruction of bone.

Experiment 3 - experimental study in pigs to evaluate the ability of Cerasorb as a bone augmentation material for healing of bone defects

Aim

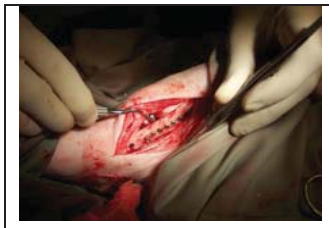
To evaluate the efficiency of Cerasorb bone augmentation material in bone healing when it is applied into the artificial hole made in the extremities of the pigs.

Materials and Methods

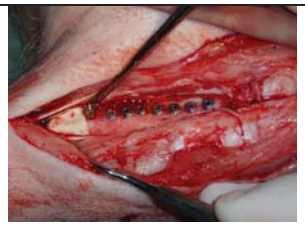
The experiment was performed under general anaesthesia, using aseptic techniques. Two artificial holes were made at the tibia of two pigs. In each tibia one hole was filled by microporous granules (Ø 500-1000 µm) of Cerasorb and the second hole was kept empty as a control hole for further comparison. Two holes are covered by Biocollagen membrane to prevent outside influence.

After 52 days the pigs were terminated and radiograms were obtained from the sites of artificial holes.

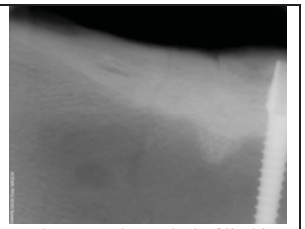
The specimens were observed by optical microscope after histological processing.



Proximal hole filled by Cerasorb, distal hole kept empty as a control hole.



Two holes are covered by Biocollagen membrane.



Radiogram shows hole filled by Cerasorb, control hole (radioluscent) and part of nanoimplant.

Results and Discussion

Based on careful analysis of the radiographic and histological images, we can conclude that Cerasorb gradually resorbs and is taken over by new bone formation. The brown roundish microporous granules of Cerasorb show different stages of peripheral penetration by vascularized connective tissue, leading to new bone formation. Histolological findings revealed that Cerasorb granules were surrounded by connective tissue and osteoblasts and new bone formation is replaced by gradual resorption of cerasorb mainly from the peripheral site. Therefore we can conclude that Cerasorb has very good bone augmentating properties and can be readily used for healing of bone defects.

which prevents direct contact of the metal with the surrounding tissue.

Titanium does not simply behave passively in tissue and bone. Bone grows into the rough surface and bonds to the metal, a reaction which is normally only attributed to so-called bioactive materials.

This ankylotic anchoring forms the best possible basis for a functional dental implant, as it can withstand all possible load types, E.g. tensile, compressive and shear forces. This ankylotic anchorage, also termed osteointegration, is accepted today as the most promising method of stabilizing endosteal implants and endoprostheses.

High-resolution electron microscopy to study the bone-titanium interface shows a layer or matrix material about 20 nm thick, and then, at a distance of about 100 nm, massively mineralized collagen fibers.

Types of oral implants:

Many clinicians designed implants to fit certain needs and properties. There are several types of implants available which are chosen for a particular patient depending upon a number of factors. The size and condition of the patient's natural jawbone is probably

Titanium, Tantalum and other metals (e.g., niobium) as well as aluminum oxide ceramics are described as being bioinert.

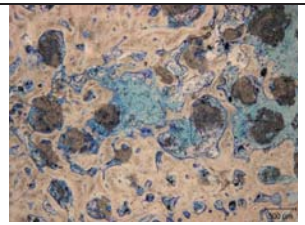
When an optimal fit is achieved between the implant bed and the implant, new bone formation and bone remodeling occurs up to the implant surface this is termed contact osteogenesis.

A full bond osteogenesis, in which a chemical reaction plays an important role, is characteristic of the so-called bioactive materials these are described in literature under the generic term glass ceramics.

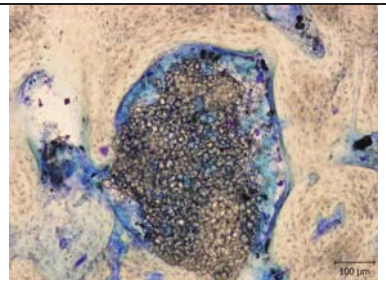
Titanium and response of bone:

Titanium alloys of interest to dentistry exist in three forms: alpha, beta and alpha-beta. The most commonly used alloys for dental implants are of the alpha-beta variation contain 6% aluminum and 4% vanadium (Ti 6Al 4V). Compared with Co-Cr-Mo alloys, titanium alloy is almost twice as strong and has half the elastic modulus. Corrosion leads to the release of compounds into biological environments. Corrosion resistance is, therefore, a prerequisite for biocompatibility [50,51].

Pure titanium is one of the bioinert implant materials. This biocompatibility is due to a surface oxide layer,



Histological image of dark brown roundish Cerasorb granules surrounded by blue stained connective tissue and osteoblasts demonstrating new bone formation.There is also a surrounding presence of light brown lamellar bone. (Please note that the smaller the foci of Cerasorb the greater the bone formation).



Higher magnification view of microporous Cerasorb granules surrounded by connective tissue with osteoblasts causing peripheral resorption of Cerasorb, with new bone formation. There is also some central penetration of Cerasorb granules by connective tissue.

Dental Implantology

Introduction

A dental or oral implant is defined as a device which is surgically placed into the jaw bone to replace one or several lost roots of teeth. It can also be defined as a device which is placed onto the bone, replacing several, if not all roots of lost teeth. Missing teeth can lead to speech difficulties, an unattractive smile embarrassment from loose dentures and pain or difficulty with eating. Atrophy of the jaw bone is another problem associated

with the loss of teeth. Extraction of teeth may result in 40 to 60% alveolar bone loss in a period of two to three years [41,42]. Oral implants have increased the treatment possibilities for patients and improved the functional results of their treatment. Patients who had to compromise with their aesthetic appearance, chewing and nutritional intake due to complete or partial loss of teeth can now be restored back to various degrees of normal aesthetics and function to improve the quality of their life. Research efforts from many different disciplines such as material science, physics, medicine, biochemistry and others, form a foundation for continual improvements in the field of Oral Implantology.

The criteria required for osteointegration of oral implants into bone

To be considered successful, an Osteointegrated oral implant has to meet certain criteria in terms of function, tissue physiology, and user satisfaction [43]. The following include certain requirements: **1)** immobility in any direction; **2)** the average radiographic marginal bone loss should be less than 1.5 mm during the first year of function and less than 0.2 mm annually following thereafter; **3)** the radiograph should not demonstrate any evidence of periimplant radiolucency; and **4)**The

individual implant performance must be characterized by absence of signs and symptoms such as pain, infection, paresthesia, or violation of the mandibular canal [44,45,46]. Esthetic requirements, patient's and dentist's satisfaction with the implant prosthesis should also be considered as criteria of success [47]. For osteointegration of dental implants the correct surface modifications, shape and materials are vital.

Biocompatibility can be defined as the compatibility of any (foreign) material with a living organism

Factors which can influence biocompatibility include chemical, mechanical, electrical and the surface-specific properties [48] .

Classification of materials with respect to their compatibility in bone according to Strunz: Metals, such as stainless steel, Co-Cr-Mo alloys, noble metal alloys, polymethylmethacrylate (PMMA), and other polymers are tolerated by bone to a certain extent, but cannot be said to integrate with it. Histological appearance interface shows that the bone keeps its distance and in this case the term distant osteogenesis is used to describe the situation in which there is a thick and fibrous layer of connective tissue between the implant and the bone. [49]