Biofunctionalization of Patient Specific Implants in Titanium with Nano Hydroxyapatite and other Nano Calcium Phosphate Coatings in Vivo

A Systematic Review

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Alexander Bral

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Promotor: Prof. Dr. M. Mommaerts
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1. Abstract and keywords

Objective: To delineate the best procedures for increasing osseointegration with nano calcium phosphate coating on titanium patient specific implants in cranio-maxillo-facial surgery.

Materials and methods: A multi-database single-reviewer systematic review model was chosen.

Results: Twenty-eight papers met the selection criteria of which twenty-five animal studies and three human studies. Titanium implants coated with nano calcium phosphate and hydroxyapatite improve osseointegration and implant fixation. Not all coating techniques are proven to improve biofunctionalization. Important contributing factors are implant micro-roughness, calcium phosphate solubility and nanotopography. Additional data derived from clinical studies are imperative to support this statement, especially regarding the influence of the routine autoclaving procedure.

Keywords: Implant, Hydroxyapatite, Calcium Phosphate, Titanium, Nanoparticles

2. Introduction

Coatings of calcium phosphates (CaP) on titanium implants have shown to improve biofunctionalization, and as such to facilitate osseointegration and functional longevity.[1] Failure of osseointegration is the result of insufficient bone formation on the implant surface and results in incomplete fixation of the implant. [2] The philosophy behind nano CaP and hydroxyapatite (HA) coatings is that the closer the structure of human bone is mimicked, the higher the chance of biological integration.[3] Important inorganic components of bone are nanoscale CaP particles with a size of 20-40nm. This would imply that nano HA coatings and possibly other calcium phosphate coatings create biocompatible surfaces and therefore unite with bone tissue.[4] Hydroxyapatite is part of the calciumorthophosphates and is composed of calcium, phosphorus and oxygen and as formula Ca10(PO4)6(OH)2 with a CaP ratio of 1.67, it is the least soluble and most stable calcium orthophosphate. The various range of CaP differ in solubility and stability, these characteristics alter biocompatibility. [5] The nano scale reaches from 0.1nm to 100nm (Fig.1). If the dimensions of the particles range from 100nm to 1micron, they are called submicron particles. [6] Nanoparticles smaller than 100nm are most effective in cellular integration and it is suggested that they induce a different response than submicron structures.[7] Difficulties are met when trying to characterize and investigate nanostructures on implant surfaces. There is no quantitative technique available to accurately assess the nanotopography on a micro-texturised surface due to interference between both. Only field emission scanning electron microscopy (FE-SEM) allows accurate examination.[6] The majority of the included publications used scanning electron microscopy (SEM) to investigate the coating. For the examination of the coating roughness, atomic force microscopy (AFM) and optical interferometry (OI) were used.
Different techniques exist to create nano CaP coatings, such as the dip coating process [8], the sol gel method [9], the electrochemical method [10], electrophoretic deposition process [11], the biomimetic deposition process [12], the hydrothermal treatment method [13] and ion beam assisted deposition (IBAD) [14]. They have their different advantages and disadvantages in terms of processing and results.

![Figure 1 Size of known objects in relation to their size.](image)

The effectiveness of the coatings can be compared in a hierarchical order on the basis of in-vitro studies, in-vivo studies and clinical studies. In-vitro research of surface coatings aims mainly to evaluate biocompatibility and assess cytotoxicity. In-vivo studies apply principally histomorphometrical analysis and removal torque (RTQ) testing [15]. The histomorphometrical measurements are capable of showing biocompatibility, osseoconductivity and the general tissue response of implants. The biomechanical tests measure the force or torque that is needed to induce failure in the bone and implant surface bond.[16]

This paper will structurally review which technique(s) can produce a calcium phosphate coating that yields good in vivo results and compare these results to micro CaP-coated and uncoated titanium implants. Furthermore, this paper will systematically review CaP coatings on titanium implants by histomorphometrical analysis and RTQ testing.
3. Material and Methods

Animal as well as human experiments were included to examine nano CaP coatings compared against uncoated titanium implants. Papers on testing implants with submicron coatings were excluded. Coatings were compared by two criteria. The first criterion is the use of histomorphometrical analysis to obtain the percentage of bone-to-implant contact (BIC). The second was the use of data regarding RTQ values to test mechanical stability and implant fixation.

Six search-engines were used, the first being Pubmed (http://www.ncbi.nlm.nih.gov/pubmed/), with the MeSH terms “nano hydroxyapatite” OR “nano calcium phosphate”. 974 articles were identified. Web of Science (https://webofknowledge.com/) with TOPIC: (nano hydroxyapatite titanium) OR TOPIC: (nano hydroxyapatite coating) OR TOPIC: (nano calcium phosphate coating) OR TOPIC: (nano calcium phosphate titanium) yielded 1431 article titles. In the Directory of Open Access Journals, MESH terms “nano hydroxyapatite” OR “nano calcium phosphate” generated 19 articles. SAGE premier 2011 (http://online.sagepub.com/) with the following search terms “nano hydroxyapatite” OR “nano calcium phosphate” amounted to 93 articles. The Wiley Online Library (http://onlinelibrary.wiley.com/) was searched. MESH terms were “nano hydroxyapatite titanium” in FullText OR “nano hydroxyapatite coating” in FullText OR “nano calcium phosphate coating” in FullText OR “nano calcium phosphate titanium” in FullText. This search provided a total of 4389 titles. Lastly, the Cochrane Library (http://www.cochranelibrary.com/) was searched with MESH terms “nano calcium phosphate” OR “nano hydroxyapatite”. This searched yielded 18 articles.

Inclusion criteria were animal or human studies presenting data regarding bone-to-implant contact or RTQ testing for nano-coated cpTi or Ti6Al4V implants.

Databases were searched for by screening all abstract using before mentioned inclusion criteria. If the abstract met the inclusion criteria, the full article was checked with the inclusion criteria. Additional articles were searched for in the reference lists of the previously included articles. Only articles written in English, German, French and Dutch were included. The publication dates of articles were restricted to before September 1st 2014.

After application of inclusion criteria, Pubmed yielded 13 articles, Web Of Science database 10, Open Access Journals 0 articles, SAGE premier 1, The Wiley Online Library 18 articles, and the Cochrane Database another 18 articles. After removal of the duplicates, 19 articles remained. Another 8 articles were found by manual search of the reference lists in previously found articles, which yielded a total of 27 articles (Fig. 2).
4. Results

4.1) nHA promimic method

Method
The nHA promimic method is a technique that uses an aqueous solution composed of calcium, phosphor and a surfactant. The solution is placed in an ammonia atmosphere, to induce the formation of nano-sized crystals after which the treated liquid is diluted by a hydrophobic organic solvent, to obtain a solvent of nano-sized crystals in water. An oxide coated substrate is immersed into the nanocrystal solvent. After the removal from the solvent, the organic solvent and surfactant are removed from the surface coating.[17] This technique is able to produce a coating with nano HA particles 10-15nm wide and 100-200nm in length. [23,25]
In vivo animal experiments

The osseointegration of electropolished titanium implants was compared with electropolished titanium implants modified with the nHA promimic method in an animal model was studied. The implants, 10 uncoated and 10 coated, were fixated and stabilized by 2 screws corticomedullary in the tibiae of 10 rabbits. The experiment showed significantly higher BIC values after 4 weeks for nHA implants (9%) compared to uncoated implants (3.2%). OI surface roughness parameters showed similar density of summits, whereas AFM showed increased average height deviation in the nanometre levels for the nHA coated implants. The difference measured by AFM could be due to an increased surface porosity (%) and number of pores for nHA coated implants. Implant surface modification was applied so that microstructures were removed and would not interact. With this information the authors concluded that the difference in osseointegration is only explained by a difference in nanotopography and surface chemical composition.[18] Another study used the same rabbit model previously explained but increased the implantation site by 0.35mm in radius to induce a gap healing model. They examined electropolished titanium implants and nHA coated titanium implants. After 4 weeks average BIC percentages and bone area percentages were 2.0%/82.7% for the electropolished implants and 1.7%/74.7% for the nHA coated implants. This difference was not statistically significant. The data of this experiment does not support the observations of the previous study. It was hypothesized that the gap design of 0.35mm might have disrupted osseointegration and that a more precise surgical fit is needed. [19] In another experiment it was evaluated if there was enhanced osseointegration with nHA coatings compared to a nano-titani covered surface. Nano-titania implants were coated with the MetAlvive method, this is a dip-coating technique. The coating technique produces a coating layer composed of titanium particles with a diameter of 23 nm. Nano-titania coated implants had a higher feature density and coverage with 45% of the implant covered compared to nHA with 23% of the
implants surface. AFM images showed the average diameter of nano-titania particles to lower compared to the nHA particles. The histomorphometrical analysis showed BIC values of 17% for nHA and 21% for nano-titania coated implants measured on the lateral wall, which was an insignificant difference. At the apical region measurements showed a significant increase in osseointegration with BIC values of 19% compared to 7% for nHA. But the results at the apical region might be influenced by increased developed surface ratio (Sdr) values at the apical region with nano-titania implants. This difference was not found at the lateral wall. These results showed no support for the enhancement of bone-formation with bioactive HA. The bone area measurements exhibited similar values for both coatings with 48% for each. It was concluded that the bone healing events in this study were only dependable of the nano feature size and distribution after a 4 week healing period.[20] In another study, Meirelles et al. 2008b found that implants chemically modified with nHA how enhanced osseointegration compared to uncoated implants with similar microtopography. They evaluated osseointegration after 4 weeks in the proximal rabbit tibia. OI microscopy showed similar average height deviation for blasted implants (1.42 µm) and for nHA coated implants (1.36 µm). At 4 weeks significantly higher BIC was observed for the nHA coated implants (35.8%) compared to blasted implants (21.5%). Removal torque testing showed similar values with 37Ncm for nHA compared to 29Ncm for TiO₂.[21]

A study evaluated bone apposition onto coated and uncoated heat treated pure titanium implants in a rabbit model. OI images showed no significant difference in average height deviation and surface roughness. In 18 rabbits two implants were inserted bilaterally in the femur. Half were sacrificed after 2 weeks of healing and the other half after 4 weeks. After 2 weeks of healing, the mean percentage of newly formed bone contact with nHA coating was 30.2% compared with 24.7% for uncoated implants. This is a statistically significant difference. But after 2 weeks of healing, the uncoated implants seemed to have reached a plateau of BIC. The BIC after 4 weeks was 33.4%, which is significantly higher than 24.3% for uncoated implants.[22] Additionally it was investigated if early osseointegration of nHA coated and sandblasted, acid etched and heat treated Ti6Al4V implants. SEM showed that the nHA coating fully covered the substrate surface, and is composed of rod shaped HA particles of 10-15nm in width and 100-200nm in length, without altering the surface roughness at the microlevel. (Fig. 3). The authors evaluated bone adhesion in the rabbit tibia. After 3 weeks, the animals were sacrificed and no significant difference was observed for the nHA coating (35.7%) in relation to the uncoated implants (32.1%). The nano-indentation testing showed a significant difference between uncoated implants and nHA implants. They concluded that there is better bone quality around the nHA coated implants.[23] Another study compared, in a rabbit femur model, two implant surfaces divided into 3 groups at 2,4 and 9 weeks. The implant surfaces were composed of sand blasted and acid etched surfaces, uncoated or modified by the nanoHA promimic method. Interferometer characterization showed similar nano- and microtopography between coatings. XRD analysis proved the presence of crystalline HA on the nHA modified surface. Arithmetic average
height deviation for uncoated implants was 114nm and for the nHA coated implants 119nm. There was no significant difference in removal torque analysis or bone area contact. At the time of implant retrieval all implants were stable. They concluded that nanocrystals deposited onto blasted and acid etched titanium implants did not enhance the osseointegration after 2, 4 or 9 weeks in comparison to the control implants. [24]. Implants were sand blasted and acid etched and afterwards 1 group was heat treated and another group coated with nHA particles. All implants were inserted in the femur of rabbits, and retrieved after 12 weeks of implantation time for histomorphometrical evaluation. The nHA coating was fully covered with rod-shaped HA particles approximately 10-15nm wide and 100-200nm in length. AFM did not find any differences between heat treated and nHA implants. Interferometer analysis revealed significant differences of arithmetic average height deviation from a mean plane with a smoother surface for the nHA coated implants (0.93µm) relative to the control ones (1.026µm). The heat treated implants had improved BIC (57.1%) compared to nHA coated implants (38.7%). The data show that after a healing period of 12 weeks, non-coated surfaces with increased microroughness had improved osseointegration in comparison to nHA coated surfaces. This could suggest that the microtopography is more important at longer healing periods, while the nHA coating has an higher impact on the early bone-healing period.[25]

4.2) Nanotite

Method
The Nanotite coating technique is a colloidal sol-gel process. The procedure starts with the dipping of samples in an alcohol-based solution containing CaP nanoparticles. After withdrawal of the substrate, the sample is dried at 100°C. The procedure is able to create a discrete crystalline deposition (DCD) composed of nano-structured CaP particles that cover 50% of the surface with CaP nanocrystals and the remaining surface is covered with a TiO2 layer. The CaP particles have a nominal size of 20-100nm and a crystallinity of over 95%. [25,27]
In vivo animal experiments

The interfacial bonding strength of Nanotite coated cpTi and Ti6Al4V implants were compared with uncoated cpTi and Ti6Al4V implants. In the rat femur, implants were unscrewed with RTQ devices after a 9-day healing period. The mean RTQ showed that uncoated cpTi and Ti6Al4V implants had significantly lower values than coated implants. The mean RTQ values are 7.2Ncm for coated cpTi and 11.3Ncm for coated Ti6Al4V, the difference between these two coatings is also significantly higher for the Ti6Al4V implants. The authors concluded that the nHA deposits are able to promote bone bonding on cpTi and Ti6Al4V surfaces. They assumed that the increased nanosurface complexity was able to induce the bone bonding process, and not so the CaP chemistry. [26] The same group,
tested in the same animal model bone growth by implantation of “ingrowth chambers”. Miniature bone ingrowth chambers were made of commercially pure titanium or titanium alloy, altered by dual acid etched (DAE) and coated by the Nanotite method or only acid etched by a DAE method (Fig. 4). Nine days after implantation a statistically significant difference existed between Nanotite coated implants which had higher BIC values compared to only acid etched implants. No statistically significant difference was found between DCD commercially pure titanium coated and DCD titanium alloy implant chambers. The authors concluded that deposition of discrete CaP nanocrystals on a complex microtopography significantly enhanced osseointegration.[28] Dual acid etched implants and implants coated by DCD nanoparticles were compared. Implants were inserted in fresh extraction sockets of beagle dogs. Harvesting was after 2 weeks, 4 weeks and 8 weeks. Higher numerical BIC percentages were seen at early healing phases (2 weeks-4 weeks), however no statistically significant differences were found. The amount of animals used in each group could have been too low to show any significant difference as each group was composed of only 3 dogs.[29] Another study compared in premolar dog sockets, the BIC of grit blasted and acid etched implants with grit blasted, acid etched and nanoscale HA surface-modified implants. Implantation was in perfectly sized implant beds and in a non-submerged position. The degree of bone-to-implant contact was equal for both implants at all healing times periods of 2 weeks, 4 weeks and 8 weeks. On the other hand, the authors observed increased BIC for both implant groups during implantation time between 2 and 8 weeks. They proved that both implant surfaces are able to induce osseointegration and stimulate crestal bone formation. The authors did not confirm that nanoscale calcium phosphate coatings resulted in an increased bone response at 2, 4 and 8 weeks.[30] In the dog mandibular model, osseointegration was evaluated by histomorphometrical analysis of Nanotite implants versus DAE mplants. Insertion was in a no gap model, a 1mm, 1.5mm and 2mm gap model. After 8 weeks of healing, BIC was not significantly higher for Nanotite coated implants although micro-roughened implants showed better numerical values. The authors could not show any benefit for the DCD CaP coating. [31] Additionally a study examined bone healing to dual acid-etched implants with and without a nano CaP coating. The BIC was significantly higher for the uncoated implants compared to nano calcium phosphate coated implants at 2 and 4 weeks after implantation. They theorized that the additional coating of dual-acid-etched implants does not improve the early bone healing properties. [32]

In vivo human experiments
A study compared titanium implants with dual etched surfaces implants to those coated by a DCD Nanotite coating. Implantation was in the posterior maxilla for an average of 8 weeks. Comparison of both coating surfaces was done by SEM. Average BIC values of nano HA coated implants was 32.2%, which is significantly higher than the BIC values of the control groups 19.0%. Their results suggested that the coating of implants could shorten osseointegration time of implants. This could be clinically advantageous by minimizing micromotions and decrease the healing period. [33]
In another experiment, DAE implants were compared in the posterior maxillary bone with Nanotite implants. Implants were harvested after a 4 and 8 week healing period. Histomorphometrical analysis showed enhanced BIC at 4 and 8 weeks for Nanotite of 44,5% and 45,3% respectively, compared to Osseotite with 15,5% and 18,3% respectively. These data support an increased early osseointegration for Nanotite compared to DAE implants. [34] Another group investigated the histomorphometrical healing properties of dual acid-etched mini-implant surfaces and implants coated with nHA particles. The study design was to fix iliac crest bone grafts onto maxillary bone with coated or uncoated DAE-implants. Harvesting and analysis was after 3 months. Histomorphometrical measurements showed higher BIC and bone area values for coated implants compared to uncoated DAE implants in the maxillary bone. The authors concluded that implants coated with nano HA particles improved the osseointegration in the maxillary bone in comparison to only DAE treated titanium implants. Nanotite did not improve healing properties at the graft area, which might be due to the lower remodelling of bone in the graft. [35]

4.3) Sol-gel method

The method used in these experiments is very similar to the one used by Nanotite. The coating is made with nanoparticles between 25-35nm for the first experiment discussed and particles around 25nm in the second. [36,37]

In vivo animal experiments
One study compared bioresorbable crews, uncoated Ti6Al4V screws and Ti6Al4V screws coated with nano HA or micro HA. [35] Bioresorbable screws were composed of lactic acid and trimethylene carbonate. In a sheep model, screws were implanted in the tibial bone and harvested after 8 weeks by axial pull out testing. Highest fixation strength was achieved by nanoscale HA coated screws (713N), which was significantly higher than microscale coated implants (594N) and the uncoated implants (403N). The authors concluded that nHA coated screws provide good and stable retention during the bone healing process, without inducing any adverse effects. [36] Another experiment compared intramedullary nails that were uncoated or coated with nHA or a micro-scale HA (mHA). The titanium implants were placed endomedullary in a rabbit model for 45 days. Testing showed that the nano HA group had a statistically higher tensile strength than implants with a mHA coated and the control group, with 14.8, 14.37 and 9.1 MPa respectively. Axial pull-out tests showed an increase in tensile resistance of 58% for the mHA group and 63% for the NHA group compared to the control group, which was statistically significant. [37]
4.4) Biomimetically deposited calcium phosphate and electrochemically deposited hydroxyapatite coating

The biomimetically deposited Calcium phosphate coating (BCaP) and electrochemical deposited hydroxyapatite (EDHA) coatings were researched and compared by a single experimental group. For this reason these two different coatings are grouped.

Method

The biomimetrically deposited CaP coating is produced in two steps. At first the implants are soaked in a supersaturated calcium and phosphate solution with a high salt concentration. Inhibitors of crystal growth are added in order to aid heterogeneous nucleation. Implants are soaked for 24 hours, and afterwards soaked in another solution of supersaturated CaP solution with another composition of salts. This technique yields a sharp crystal flake of approximately 6 to 8 micrometre length and 100nm thickness composed of octacalcium phosphate, which covered the entire surface (Fig. 5B.). The electrochemical deposition hydroxyapatite uses the implant as working electrode, and a platinum plate as counter electrode. The electrolyte solution contains Ca(NO3)2 and NH4H2PO4 with a CaP ratio of 1.67. A direct current induces the deposition of CaP nanocrystals on the implant surface. The process is finished within 2 hours. The coating homogenously covers the entire surface with oriented and dense rodlike crystals with a diameter of 70-80nm (Fig. 5C). [38,39]

Fig 5. A) Roughened group, B) BDCaP coated surface C) EDHA coated surface

In vivo animal experiments

The interfacial biomechanical properties of biomimetically and electrochemically deposited nano HA coatings were compared with roughened implants (Fig. 5A) in an animal experiment. In the rabbit femur model, implants were unscrewed with RTQ devices after healing periods of 2, 4 and 8 weeks. The mean RTQ of implants showed significantly greater values for the EDHA coating at all healing periods compared with roughened and biomimetically deposited calcium phosphate coatings. The biomimetically deposition did not increase implant fixation compared to roughened implants.
authors concluded that EDHA coatings improve fixation between implant and bone compared to the roughened surface, but biomimetical CaP coatings have little effect on fixation. [38]

To assess osseous healing of biomimetically and electrochemically deposited nHA coatings, the same research group, performed a rabbit experiment. Implants were inserted intra-cortical in the tibial bone. The animals were divided into two groups. The first group was sacrificed at 6 weeks and the second group at 12 weeks. Histomorphometrical measurements for electrochemically deposited nano HA coatings showed significantly greater values at both healing periods compared to BDCaP implants. No significant differences were found between BDCaP and roughened implants. At 6 weeks EDHA implants showed significantly higher BIC values compared to roughened implants. This was not found after a 12 weeks healing period. This study showed that the thinner EDHA coating is more favourable for bone integration. This could ensure long-term stable bony fixation of a porous implant.[39]

Additionally a study tested an electrochemically deposited nano HA coating to assess osseointegration in an ovariectomized rat model. The animals were sacrificed after 12 weeks, after which removal torque testing and histomorphometrical analysis were conducted. Mean surface roughness of the roughened titanium implants (control group), and the HA-coated implants (experimental group) were 1.185 and 1.167 respectively. No significant difference was found between both groups. The histomorphometrical results showed significantly greater BIC and bone area percentages for nano HA coated implants compared to roughened implants by large corundum grit blasting. They also found significantly greater removal torque values for the nano HA coated implants. The authors demonstrated that the thin nano-HA coating is able to increase bone bonding strenght and improve osteogenesis around the implants. [40]

### 4.5) Ion beam-assisted deposition

In vivo animal experiments

In a dog experiment, the performance of a nano-thick ion beam assisted deposition (IBAD) coating was evaluated after 3 and 5 weeks. Four different groups of alumina blasted/acid-etched Ti6Al4V implants were compared, uncoated (control group), coated by either one of two ion beam-assisted deposition (IBAD) setups and plasma sprayed HA(PSHA). Difference between both IBAD coating techniques was duration of coating procedure, giving a thickness of 30-50nm thickness for IBAD II and a submicron coating of 300-500nm for IBAD I. (Fig. 6). AFM showed surface roughness parameters of 0.66 µm for control implants compared to IBAD I 0.54µm, IBAD II 0.48µm and PSHA 1.8µm. In vivo testing showed significantly higher torque to interface fracture values for PSHA and IBAD II. Significantly higher BIC values were also found for PSHA (71.86%), for IBAD I (68.97%) and IBAD II (68.22%) coatings compared to the control group (62.84%). The nanocoating yielded inferior results for bone anchorage compared to the micron and submicron coating. [41]
4.6) Other techniques

In the proximal tibial metaphysis and rabbit femur, BIC percentage as well as the bone anchorage was compared for CaP coated implants with porous oxide surface coated implants. Coating procedure comprised of dip coating in a solution of water, surfactant and nano CaP particles. Afterwards the
implants were dried and heat treated. Coating comprised of nano CaP particles with a nominal size of 10nm and a thickness of 200nm, both implant surfaces had a surface roughness of 1.3µm. At healing periods of 2, 4 and 9 weeks no significant difference was found for BIC and removal torque values. The authors couldn't find an improvement for interfacial strength or bone apposition with nano CaP coated implants, and even found that it decreased during the 2 to 9 week healing period.[43] In the femoral condyles of rats, grit-blasted/acid-etched surface Ti-implants with hydrothermally treated implants were compared. The produced CaP coating consisted of a 100nm thick CaP layer, with the surface containing micron, submicron and nano features. After 6 weeks, harvested implants showed a significantly greater osseointegration for CaP coated implants with 77.8% compared to grit-blasted/acid-etched implants with 64.1%. [44]

5. Discussion

Eight studies were identified that applied the nHA promimic technique, all comparing titanium implants with or without nano HA modification. No clinical studies were identified. One study demonstrated that the nHA coating could improve osseointegration on smooth implants. However, this improved biofunctionality did not correlate to a gap model, which would suggest that the nHA coating isn’t able to enhance osseointegration without a tight fit. They also compared nanotitania coatings with nano HA coatings and established that there was no difference in BIC values. In addition they concluded that the topographical composition was more important than the chemical alteration. Though, this conclusion however cannot be made based on the available data, due to dissimilarities in surface coverage and particle size. Considering the smaller surface coverage of the nHA coating compared to nano-titania coating, the findings could be biased. Two experiments using roughened titanium implants with similar roughness for nHA coated and control implants, showed improved osseointegration and bone anchorage for nHA coated implants. Another study with similar microroughness showed a non-significant difference in BIC values, but BIC values at four weeks were numerically higher than those of the control group. The study demonstrated improved bone strength quality with a nano-indenter. One study showed improved RTQ and another showed similar results as well as improved BIC values These studies show similar results, and show improved osseointegration and higher fixation strength on a smooth surface and on a microroughened surface. One study comparing uncoated implants with a higher micro-roughness during a 12 week healing span showed worse BIC values for the nano HA coated implants compared to sand blasted and acid-etched implants. This indicates that micro-roughness has a higher impact on long term healing periods compared to nanotopography, which has been shown in previous studies to be of great importance at early healing periods. The early enhancement of osseointegration is hypothesized to be due to dissolution of nano HA particles from the surface coating. Hence the nano HA particles accounted for
improved bone formation and support the differentiation and proliferation of osteoblast cells. [38] The micro-roughness has a higher effect after longer healing periods, when no more dissolution of nHA particles occurs. It is equally or more important for osseointegration to have ideal micro-roughness values. The data found suggests that the nHA promimic coating technique is capable of improving osseointegration by altering the surface’s nanotopography and chemical composition. Therefore this coating assists in improving the micro-roughened implants fixation and shorten the required healing period. No long-term survival rates were reported, indicating the need for testing in long-term clinical studies.

Six animal studies and three human studies were included for Nanotite implants. Two animal studies reported an increase in osseointegration and bone anchorage over a short-term healing period of nine days. One animal study found numerically higher values of BIC, though it should be noted that the rather small sample size . Two studies found no improved osseointegration after a long term healing period, and one of those studies did not establish any enhancement of BIC at early implantation. One study reported worse results for nHA coating of dual acid-etched implants, where the histomorphometrical results were significantly lower for test implants. All three human studies demonstrated increased BIC values after four to eight weeks of implantation. These data suggest improved osseointegration and bone anchorage for Nanotite implants compared to DAE implants. Furthermore, two clinical studies published by the same group evaluated immediate loading implants coated with the Nanotite coating. Both studies found high survival rates within a 1 year follow up period. The 2 clinical studies established that Nanotite implant have a good viability, if the implants have a adequate primary stability. Further research is warranted to substantiate these findings and a longer implantation time should be considered for future studies. [45,46]

Two animal studies were found with different sol gel coating techniques. One study provided better load-to-failure results compared to microscale coated and uncoated screws. The second experiment showed higher tensile strength of nHA coated implants compared to mHA coated implants and uncoated implants. These two coating techniques show promising results, which is in line with the sol gel method used for Nanotite implants. Though this requires confirmation on long term clinical studies.

Both studies using BDCaP coatings failed to show an improvement on porous titanium implants with regard to osseointegration and bone anchorage. The EDHA coatings showed an increased bone anchorage and osseointegration compared to roughened implants. Two studies demonstrated improved bone anchorage at short term healing periods. Histomorphometrical analysis in 2 studies showed increased BIC values after six weeks and the other showed enhanced integration after 12 weeks. These results show that the EDHA coating is a better coating in the short term healing period up.
results demonstrate the superiority of the EDHA coating compared to the BDCaP coating at a short term healing period. This can be attributed to the composition of the BDCaP coating, which is composed of octacalciumphosphate making it more dissolvable in vivo compared to the HA of the EDHA coating. [47] The difference in solubility is presumed to be partially responsible for the variable bone formation. The Ca-P dissolution is essential for ideal bone growth and strong anchorage during early implantation. [48] Dissolution should only decrease remodelling after the CaP coating has fully dissolved, which defines that no more detachment can occur. Yet the resorption of CaP coatings will prevent loosening or fractures at the interfacial bonding between the coating and the titanium implant. It could also be the surface chemistry and surface roughness of the HA coating which improve osseointegration compared to the CaP coating. It has already been proven that nHA particles improve osteoblast proliferation, adhesion and calcium deposition. [49] A study demonstrated based on FE-SEM that bone failure appeared in the bone trabeculae for the EDHA coated implants, whereas in the BDCaP-coated implants this occurred at the interface between implant and bone. [39] Based on all of these findings, we can conclude that BDCaP-coated implants produce inferior bone anchorage and integration compared to the EDHA coated implants.

The nHA IBAD coatings showed inferior results in osseointegration by comparison of histomorphometric measurements, compared to PSHA coated and submicron IBAD coated implants. We can conclude that the IBAD nano-coating produces inferior results than submicron IBAD and PSHA coatings and does not improve osseointegration and fixation in short term healing periods.

Only one study evaluated titanium implants dip coated with CaP and no difference in implant fixation or osseointegration within a short healing period was demonstrated. This can be attributed to the faster dissolution of the CaP particles as they dissolve faster than other nHA coated implants. The produced coating in this study has a faster dissolution than other nHA coated implants. Another article created a 100nm thick CaP coating with nano-, submicron- and micron scale surface structures using the hydrothermal treatment and this coating showed improved integration after six weeks.

Bontoux et al. studied the impact of roughness dimensions on the biocompatibility. Microroughness ranges between 0-10 µm were investigated, to see which results in maximized interlocking. The optimal microroughness has been established to be 1,5 micrometer with a Sdr of 50%. [50] The found studies show that some CaP nano-coatings superposed to a micro-roughened surface enhance biocompatibility, though it should be noted that the optimal nanoroughness and particle size is not known. The optimal nanotopography has not yet been established as then number of studies that compare the different nanocoatings is scarce. Therefore, no comparison can be made based on the current data between the available CaP nano-coatings, even though the majority of these coatings
showed good results with regard to enhancement of osseointegration and implant fixation. The majority of studies investigated the nHA promimic and the Nanotite coatings, and both coatings have shown enhancement in vivo. Additionally, the Nanotite demonstrated positive clinical data.

A limitation of this study is that the majority of studies that were included investigated implants over a short healing period (i.e. between two and 12 weeks), and the rather small sample sizes made them prone to bias. The various study designs make it difficult to compare the different coatings. Since the 27 studies that were included consisted of small sample sizes and studied different coatings, they provided insufficient evidence to draw a solid conclusion. Yet it can be noted that the IBAD and BDCaP coatings yielded no enhancement compared to the roughened implants, whereas the Nanotite, nHA promimic method, hydrothermal treatment and other sol gel nHA coatings showed favorable results. These coatings are able to minimize the problems with current implants conform slow osseo-integration and implant failures.

6. Conclusion

Various available studies show that nano-CaP coatings improve biocompatibility and implant fixation, however not all nano-CaP coatings yield the same results. Coating techniques produce differing CaP ratio’s, thickness, nanoroughness and particle size, all these factors influence the reaction of bone. The ideal implant surface nanotopography is not known and remains to be elucidated. The CaP solubility is an important factor in biocompatibility and should be further investigated. However, data shows that the sol gel method (e.g. Nanotite), the nHA promimic method, EDHA and hydrothermal treatment show good results. Nanotite has also proven to be effective for the survival rate of oral implants in clinical studies. The BDCaP and IBAD I coating have not shown to aid the osseointegration of implants in animal studies.
7. References

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8. Appendix

Figure 1: Size of known objects in relation to their size. [51]
Figure 2: Prisma diagram: selection procedure of included articles.
Figure 3: A) Heat treated surface B) Nano-coated surface with nHA promimic method at micron magnification C) Heat treated and the D) Nano-coated surface at a 200nm magnification [23]
Figure 4: A-C) cpTi surfaces, D-F) Ti6Al4V G-I) DCD coated cpTI surfaces J-L) Ti6Al4V DCD coated [26]
Figure 5: A) Roughened group, B) BDCaP coated surface C) EDHA coated surface [38]
Figure 6: SEM: A) Alumin-blasted and acid-etched surface B) IBAD coated implant with 20-50nm thickness Ca-P coating. [42]