BONE-BONDING TO ALKALI/HEAT TREATED TITANIUM

By

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A thesis submitted in conformity with the requirements for the degree of Master of Science
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ABSTRACT

BONE BONDING TO ALKALI/HEAT MODIFIED TITANIUM


Graduate Department of Dentistry, Institute of Biomaterials and Biomedical Engineering,
University of Toronto

This study tests the hypothesis that surface topographical changes induced by alkali/heat treatment of cpTi metal implants will cause bone to mechanically bond with the surface of the implants. Therefore, the changes of the surface features of machined and acid-etched cpTi metal were characterized both prior to and after alkali/heat treatment. Such surface features were also analyzed with respect to the biological responses of both osteoconduction and bone-bonding.

It was found that alkali/heat treatment of both machined and acid-etched surfaces 1) renders the surface oxide microporous, 2) significantly increases Ra, Rq, SA, SAI values, and 3) significantly increases surface wettability. The level of the osteoconductivity was found to be significantly higher on untreated or treated acid-etched vs. untreated-machined samples. Conducting the detachment tests it was found that considerable forces were required to detach the bone from alkali/heat treated surfaces and the cement line of the newly formed bone was interdigitating with the microporosity of the alkali/heat treated surfaces.
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DEDICATORY

To my father, my brothers Javad and Mahmood, my sister Talat. To my husband Oveis Nayeri.
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1. General Introduction

Metals have been used as endosseous implants in the orthopedic and dental fields with considerable success for over three decades. Currently, the majority of such implants are made from titanium and its alloys and, in the case of uncemented prostheses, rely on gross surface topographical features such as porous coatings and screw threads for their anchorage in bone tissue. Such surface geometries are essential since bone tissue has no inherent capacity to bond to titanium oxide (titania) surfaces of titanium and its alloys.

However, there is another class of materials generally known as 'bioactive' or 'bone bonding' materials to which bone will bond, and thus diminish the need for gross surface design geometries. These materials are all based on either calcium phosphates or other materials that have a capacity to generate, in vivo, a surface reactive layer of calcium phosphate that comprises the material surface contributing to the biological interface.

Interestingly, irrefutable evidence has emerged from the work of Kokubo and his colleagues (Miyaji, et al. 1994; Kim, et al. 1996; Yan, et al. 1997a,b; Nishiguchi, et al. 1997 and Nishiguchi, et al. 1999) that titanium and its alloys can be rendered 'bioactive' by simple chemical protocols involving strong alkalis. Since it is generally believed that 'bioactive' materials are 'bone bonding' as a result of chemical bonding between bone and the calcium phosphate surface of the implant, this chemical bonding theory has also been adopted to explain bonding to alkali treated titania. However, despite the plethora of publications concerning bioactive materials, no experimental evidence has emerged to unequivocally demonstrate that chemical bonding is indeed the mechanism of the bone bonding to bioactive materials. On the contrary, recent evidence has been published
(Dziedzic, et al. 1996) to demonstrate that surface microporosity is essential to render calcium phosphates bone bonding. These authors demonstrated that bonding comprised micromechanical interdigitation of the interfacial biological matrix formed during de novo bone formation (the cement line) with the surface microporosity of the implant material.

Given this dichotomy between conventional wisdom and emerging experimental evidence supporting the micromechanical mechanism of bone bonding, the purpose of the present work was to study the mechanism by which bone interacts, and in particular bonds, with alkali treated titania.

The healing of bone around endosseous implants is a cascade of three distinct phenomena, which can each be addressed experimentally and have been recently reviewed (Davies, 1998). The first, osteoconduction, involves the migration of populations of osteogenic cells to the implant surface. The second, bone formation relies on the differentiation of the osteogenic cells to fully functional osteoblasts at the implant surface where they initiate bone matrix elaboration. The combination of osteoconduction and the resultant formation of matrix by cells which have migrated to the implant surface results in the histological consequence of osteoconduction in which bone apparently grows over the implant surface and has also been described as contact osteogenesis (Osborne and Newesley, 1980). Osteoconduction is therefore an essential prerequisite for bone formation to occur at the implant surface. The third phenomenon is the long term peri-implant bone reorganization due to the phenomenon of bone remodeling.

Therefore, while bone bonding requires that the phenomena of osteoconduction and bone formation to occur it will, itself, be dependent upon the surface characteristics
of the implant material employed. Thus the work described herein examines both osteoconduction and the phenomena of bone bonding to custom-made commercially pure titanium implants, and the changes in these biological phenomena brought about by surface modifying the titanium using strong alkali treatments.

To arrive at the specific aims of the work reported we first review, in more detail, the biological cascade of bone healing around endosseous implants and the published reports concerning the materials characteristics of bonding and non-bonding materials.
1.1 Peri-implant bone healing

The process of bone healing around an implant can be compared to the normal bone healing found at fracture site (Glowacki, et al. 1991 and Jingushi, et al. 1991). The first event after the initial physical trauma of implantation is bleeding. The accumulated blood clot and the coagulation cascade is followed by an acute inflammatory response. Granulation tissue forms at the site of the clot, and with this a population of mesenchymal cells invades the granulation tissue that is thought to participate in the bone healing process (Rosen and Thies, 1995; Sennerby, et al. 1991).

Bone formation around an implant is generally defined as two processes of distance and contact osteogenesis (Osborn and Newesley 1980). Distance osteogenesis is a process by which new bone is formed on the existing bone at the peri-implant site. This is similar to normal appositional bone growth. Here the existent bone surface provides a population of osteogenic cells, which lays down a new matrix (reviewed by Davies 1998). As the bone grows towards the implant, the osteoblasts that were depositing bone matrix, to fill the gap between the bone and the implant, will be cut off from their blood supply and die. Subsequently they will be eliminated from the interface (Clokie et al. 1995) and then during the remodeling process the new bone tissue will gradually replace the dead tissues, occupying the spaces between implant surface and the surrounding bone. During contact osteogenesis, new bone is formed for the first time on the implant surface. Davies (1996) employed the term de novo bone formation to describe this event and distinguish it from appositional growth of bone where the already differentiated osteoblasts, lining the margins of the bone defect, are responsible for bone matrix production.
Both forms of osteogenesis occur during peri-implant healing. Thus, it is important to recognize the existence of these phenomena so that the individual mechanisms can be understood.

Sennerby, et al. (1991) and Piattelli, et al. (1996) reported the two types of bone formation during the healing process around cpTi implants. Both groups acknowledged that there are differences in the mechanisms of initiating either of these forms of osteogeneses. Piattelli, et al. (1996) also reported that surface features like roughness might alter the type of bone growth at peri-implant site.

Davies (1996) reviewed the way bone tissue interfaces with endosseous implants. He proposed that the constituents of implant/bone space would vary as a function of implant design. For example, with screw-threaded implants, the tips of the implant screw threads will compress the neighboring bone tissue, and the space between two threads will be occupied by blood together with cells and tissue remnants. Where the implant transverses the medullary compartment, both endosteal cells of osteogenic phenotype and undifferentiated mesenchymal cells with the capacity to differentiate into osteogenic cells will have the opportunity to migrate to the surface of the implant and initiate the process of the bone formation. However, in the transcortical portion, migration of endothelial and mesenchymal cells to the implant surface might be limited due to their availability in the space between implant surface and the margins of the bone defect. In this case blood volume occupying those spaces will clot and prevent the blood flow in the neighboring bone which, in turn, will diminish the availability of perivascular cells which could provide new bone formation. Therefore, the immediately surrounding bone tissue will suffer from low nutrient availability, leading to bone necrosis. Then there will be gradual
replacement of the peri-implant bone by bone remodeling. In contrast, the pore volumes of porous implants potentially provide space for blood flow and the establishment of a new capillary network and perivascular osteogenesis.

Thus, it can be concluded that implant design and its surface features play important role in the processes of peri-implant bone healing. In this process osteogenic cell migration to the implant surface, osteoconduction, is the prerequisite step for bone formation.

1.2 Osteoconduction

Davies (1998) reviewed the phenomenon of osteoconduction, the migration of differentiating osteogenic cells towards the implant surface, and its importance in bone formation over an implant surface. He suggested that during peri-implant healing, fibrin, the reaction product of thrombin and fibrinogen released into the healing site, could be expected to adhere to almost all surfaces, and facilitate osteogenic cell migration towards any implant. Potentially, cell migration in this network will cause retraction of the fibrin scaffold away from the implant. Therefore, for a material to be osteoconductive, it needs to withstand the retraction forces produced by the cells migrating towards the implant surface. Certain surface characteristics might facilitate these processes. Others interpret the phenomenon of osteoconduction somewhat differently. Glowacki, et al. (1991) defined osteoconduction as "the creeping substitution process of bony ingrowths and resorption of dead bone." Furthermore, this group and Einhorn (1995) characterized osteoconductive materials as those, which support the ingrowths of sprouting capillaries, perivascular tissues, and osteoprogenitor cells from the recipient host bed into an implant.
or graft. However, what is common to all these studies on osteoconduction is the importance of the migration of osteogenic cells to the peri-implant site.

The significance of the size of the bony defect, cellularity, and vascularity of the recipient bed (Glowacki, et al. 1991 and Einhorn, 1995) in the process of osteoconduction are evident. However, the emphasis of the present study is on implant surface features, which would facilitate such cell migration.

Surface chemical composition is regarded to be one of the important factors in determining the material’s osteoconductivity. Davies (1998) suggested that the chemistry of some implant surfaces might increase both the adsorption and retention of macromolecular species, from the biologic milieu, which would potentiate osteoconduction. Calcium phosphate-based materials and coatings have been thought to exhibit such characteristics, since these materials have been reported to demonstrate higher osteoconductivity compared with other materials such as alumina (Ono, et al. 1990) or titanium (Ricci, et al. 1991).

It is thought that the formation of a reactive surface (apatite like) layer on the bone-interfacing biomaterials is positively correlated to their osteoconductivity (Ono, et al. 1990). In view of this, several investigators have focused on developing materials that could form a surface apatite layer. They have used solutions with similar ionic contents as biological fluid and no protein species to examine apatite formation on their experimental surfaces.

Hanawa, et al (1997) and Murakami, et al. (1995) examined the effect of surface modifications, such as treatment of the titanium with calcium ion containing solution or calcium ion implantation, on improving bone conductivity of the material. They found
undetected on unmodified titanium. Therefore, they concluded that the surface modification of titanium might improve bone conductivity.

In addition to surface chemistry, surface topography also plays a significant role in osteoconductivity of the material. Albrektsson, et al. (1981) suggested that rough-featured surfaces on titanium implants might promote greater bony contact (an indication of osteoconduction) than smooth ones. Buser, et al. (1991) and Piatteli, et al. (1998) had confirmed that and reported such observation during their studies. Davies and Yan (1998) also reported that topographical changes created on the roughened surface, prior to and after the chemical treatment, increased osteoconduction when compared with the machined surface. These observations could be the result of an increase in surface area 1) for primitive connective tissue or fibrin network attachment (Davies 1998), or 2) for the adsorption of the cellular and molecular constituents of the biological fluid surrounding the implant which all in turn could enhance osteogenic cell migration.

An increase in surface roughness is not always associated with increased bone formation. Buser, et al. (1991) reported that the percentage of direct bone contact with rough-blasted/acid-etched surfaces was higher than that of titanium plasma-sprayed surfaces, which were apparently even rougher. Vercaigne, et al. (1998) also reported similar results and suggested that an increase in surface roughness might cause an increase in Ti ion release, which could have a negative effect on osteogenesis. Murray, et al. (1989) suggested another negative effect of rough surfaces. They showed that macrophages adhering to an irregular surface release more bone resorbing factors than those adhering to a smooth surface. In view of that, Dziedzic, et al. (1996) suggested that
the integrity of the implant surface was essential for bone mineralization to avoid a chronic inflammatory response, which may enhance material biodegradation.

From these reports, it can be concluded that both osteoconduction and bone formation depend on the surface characteristics of the implanted material. Furthermore, the occurrence of these phenomena is a prerequisite step in the process of bone bonding.

1.3 Bone-Bonding

Currently used biomaterials are stabilized in bone through different mechanisms. However, the phenomenon of the bone-bonding is only reported with materials such as bioactive glass and hydroxyapatite which are thought to act by means of specific chemical properties (Bagambisa, et al. 1993 and Hench 1988), so they are referred to as bone-bonding or bioactive materials.

It is believed that the common feature of the interface between bone and bioactive materials is the presence of an intermediate calcium phosphate layer through which the materials bond to bone (Hench, et al. 1982; Anderson, et al. 1988 and Kitsugi, et al. 1989). Hench (1991) suggested that the index of bioactivity ($I_B$) for bioactive implants depends on (a) the rate of calcium phosphate film formation and time of crystallization to hydroxylcarbonate apatite (HCA) and (b) the selective adsorption on the growing HCA layer of extracellular proteins that control cellular attachment, differentiation, and growth. It is suggested (LeGeros, et al. 1991) that collagenous fiber matrix is laid down directly at the implant surface, subsequently mineralizes and is incorporated within the chemically active surface of the implant. Davies (1998) stated that this mechanism is
inconceivable if the first extracellular matrix elaborated by bone cells at the implant surface is a collagen-free cement line. In this process, the noncollagenous proteinaceous matrix, which includes osteopontin and bone-sialoprotein, produced by differentiating osteogenic cells precedes mineralization. The cement line, which is so formed, is not specific to bone-bonding biomaterials.

It is important to consider that the type of bone formation (contact vs. distance osteogenesis), occurring at the interface between bone and implant materials, determines the constituents of the bonding zone. If new bone formation is the result of contact osteogenesis, the bonding zone is described as an “afibrillar cement-like” (Davies, et al. 1991 and Davies 1996) layer of about 0.5 um. In the case of distance osteogenesis, old bone, injured during the implantation, will remodel and grow towards the implant surface. The mineralized matrix would then be separated from the implant surface by a 20 nm electron-dense, proteoglycan layer (Steflik et al. 1998). The matrix immediate to the implant in these cases is different. Therefore the underlying mechanisms for bone bonding could not be the same in both cases.

The chemical bone-bonding hypothesis, repeatedly reported as the underlying mechanism for bone attachment to bioactive materials, disregarded the type of bone formation occurring. In fact there has been no evidence presented to support this hypothesis. The observation reported by Dziedzic, et al. (1996) on interdigitation of the newly formed bone’s cement line with the microporosity of the hydroxyapatite ceramics, and lack of any type of bone bonding with nonporous ceramics, sheds the light on the subject that bone-bonding phenomenon of bioactive materials might depend solely on mechanical interlocking.
With regards to non-bone-bonding materials such as titanium (Pilliar 1986) and alumina ceramics (Takagi, et al. 1989) it is shown that they are influenced by their surface features such as porosity, which would allow bone ingrowth and promote mechanical interlock. In these cases, it is shown that the surface macroporosity plays an important role in the mechanism underlying the non-bone-bonding implant material stabilization in the bone tissue.

In view of these observations it can be concluded that mechanical interlocking in either micro- or macro-scale can occur to secure both bioactive and non-bonding materials in the bone tissue.

1.4 Bone-Remodeling

Bone remodeling is one of the primary phenomena in determining both the short- and long-term structure of the bone-biomaterial interface (Brunski 1991). It is the process of localized removal of old bone by bone resorbing cells, osteoclasts, and replacement with newly formed bone tissue by bone producing cells, osteoblasts. So it requires interactive cellular activity, which is regulated by a variety of biochemical (Mundy 1993) and biomechanical (Kimmel 1993) factors.

Loading of the implant is shown to increase the extent of bone remodeling at peri-implant site (Hoshaw, et al. 1994), and it is demonstrated to be even more pronounced in the case of non-axial loading (Barbier and Schepers 1997). Therefore, the loading of an implant should be done when bone has regained its biomechanical stability. To control the implant load effects, different implant material properties (Weinans, et al. 1992), designs, and surface structures (Bobyn, et al. 1987; Suzuki, et al. 1997; Oka, et al. 1997)
have been studied. It is suggested that these features would affect stress transfer between implant and bone, and subsequently influence the rate and extent of the bone tissue remodeling at a peri-implant site.

Stress shielding (reduced stresses) of bone surrounding the implant is shown to decrease by increasing the implant material elasticity (Weinans, et al. 1992). However, there is a limit to the beneficial effect of increased elasticity of the implant material on bone remodeling (Harvey, et al. 1999). Another implant material property that can alter the outcome of the bone remodeling at the bone-implant interface is its chemical composition. Some calcium phosphate materials (reviewed in Davies 1998) can be resorbed during bone remodeling at the interface and can be replaced by bone tissue. This would perhaps influence the design of endosseous implants.

1.5 Bone-substitute materials

Bone-substitute materials are generally divided into two groups; those to which bone tissue bonds, so-called bioactive or bone-bonding materials, and those to which bone tissue does not bond (Hench and Wilson 1984). Metals such as titanium are non-bonding and calcium phosphate materials are considered bone-bonding.

1.5.1 Bone-Bonding Materials

Ducheyne, et al. (1992) characterized the bone bioactive materials as those that can enhance bone tissue forming reactions and formation of a continuous transition from tissue to implant material. Calcium phosphates and materials that have a capacity to
generate in vivo, a surface reactive layer of calcium phosphate that comprises the material surface contributing to the biological interface, are classified as such materials. Therefore it has been proposed that the prerequisite for bone to bond with implants is the formation of a biologically active bone-like apatite layer on the surfaces of implants.

The mechanism underlying the bond between bone and calcium phosphate materials such as hydroxyapatite (HA) was suggested by Le Geros, et al. (1991) to be the result of partial dissolution of the crystals of the Ca-P which in turn cause an increase in the levels of calcium and phosphate ions in the biological fluids. Subsequently, apatite crystals precipitate with ions such as Ca$^{2+}$, Mg$^{2+}$, Na$^+$, CO$_3^{2-}$ as well as organic molecules present in the fluid to form a biologically similar hydroxyapatite layer. This process is suggested to be followed by epitaxial crystal growth that is a thermodynamically controlled process, in which guest crystals (in the present case bone crystals) use a host crystal surface (here, HA) as nucleation site and/or template for the deposition and perpetuation ("growth") of their own phase (Bagambisa, et al. 1993).

Bioactive glasses (BG) and glass-ceramics (BGC) are also considered bone-bonding materials. Shirkhanzadeh (1995) suggested that silicon and calcium ions leach out of the bulk glassy material and lead to the formation of hydrated silicon subsequently, providing optimal sites for apatite crystal nucleation. In view of this, the presence of hydroxyl groups on the surface is thought to play an important role in initiating apatite formation, and provides the basis for the bone-bonding potential of bioactive glasses and glass-ceramics. Earlier investigations of the importance of hydroxyl group by Li, et al. (1994) had also demonstrated that apatite induction not only depends on presence of hydroxyl groups but also on the type of the hydroxyl containing compounds on the
surface. They showed that whereas glass ceramics and titanium, with Si(OH) and Ti(OH) groups respectively, could induce apatite formation; Al₂O₃ material and the Al(OH) groups are incapable of initiating apatite nucleation. Ohtsuki et al. (1997) proposed that basic titanium hydroxyl groups have the ability to induce formation of a biologically active bone-like apatite layer on the surface.

In addition to hydroxyl groups the point of zero charge (pzc) is also suggested as an important factor responsible for apatite nucleation. Pzc is defined as the pH at which the surface charge of oxide is zero. The pzc of anatase is at pH 6.8 and Al₂O₃ at pH 7.8. So it can be expected that alumina gel will be charged positively at the surface in the simulated body fluid with pH 7.4, while anatase is negatively charged (Li, et al. 1994).

Since apatite formation is shown to occur on titanium oxide not aluminum oxide, it is suggested that successful apatite inducers may be those materials, which possess and/or develop both negatively charged surfaces and abundant hydroxyl groups in physiologically related fluid. These materials are thought to be candidates to serve as bone-bonding biomaterials.

Taking this into consideration different chemical treatments have been employed to increase the rate and extent of the formation of hydroxyl containing groups on the surface of titanium and its alloys.

1.5.2 Non-Bone-Bonding Materials

Given that formation of an apatite-like layer on the surface of endosseous implants is thought to be associated with their bone-bonding potential, metallic implants are often
considered non-bone-bonding materials. There have been some controversial reports regarding the formation of hydroxyapatite-like layer on the surface of metals either in vivo, or in vitro. Takatsuka, et al. (1995), and Kitsugi, et al. (1996), in a series of in vitro and in vivo experiments, showed no Ca-P rich layer forming on titanium implant surfaces. In contrast, Ducheyne, et al. (1991) and Hanawa (1991), in a series of in vitro experiments, found that titanium and its alloys have the capability of forming a hydroxyapatite-like layer on their surfaces. However, the rate of precipitation of such layers on those surfaces was found to be several orders of magnitude slower than that on bioactive ceramics and bioactive glass materials.

What is repeatedly reported in the literature is that non-bone-bonding materials depend on their surface macrotopographical characteristics for their stabilization in bone tissue. Special implant designs like screw-threaded implants (Johansson, et al. 1991) in addition to surface features such as porosity of non-bonding materials of alumina (Takagi, et al. 1989) and titanium alloy (Pilliar, et al. 1986) have been demonstrated to allow bone ingrowth and promote mechanical interlocking.

1.6 Titanium-Based Implants

Titanium and its alloys have been used as endosseous implants in the orthopedic and dental fields with considerable success for over three decades. These materials are usually preferred over other metallic materials due to their good mechanical strength, biocompatibility, and corrosion resistance. The last two features are due to the formation of a surface oxide layer, which is shown to be mainly TiO₂ with a minor amount of
TiO, TiO and Ti nitride (Lausmaa, et al. 1990). Since the reaction energy of oxidation of titanium is greater than that necessary to decompose water, the oxide-forming reaction is always spontaneous and it is shown to be stable through a wide range of pH variations (Steinemann, 1996). Munuera, et al. (1971) also reported that when titanium is placed in aqueous solution, for every water molecule split two hydroxyl groups are produced. Subsequently, these groups would be positioned differently on the surface and produce an acid or base property in their vicinity.

Surface macro- and micro-features in addition to surface chemical compositions are thought to influence the bony response to these implants. Features of screw-threaded and porous-beaded implants, for example, provide surface macro-roughness, that can affect the processes of bone healing around the implant (Davies 1996). Macro-implant designs also affect the level of biomechanical stress and strain transfer between the implant and bone and subsequently the level of bone remodeling around the implant. Using techniques such as micromachining, sand blasting, acid etching, or plasma spraying provide the surface with micro-roughness that is reported to affect the cell-implant interactions (Boyan, et al. 1998 and Chehroudi, et al. 1992). As a result, surface micro-roughness was reported to influence bone formation in relation to the implant surface (Buser, et al. 1991; Piattelli, et al. 1998; Wennerberg, et al. 1997; Li, et al. 1997; Vercaigne, et al. 1998).

Surface chemical composition is also reported to influence surface ability in adsorption of water, ions, and proteinaceous macromolecules. These are the constituents of the first molecular events that occur at the surface of a material placed in a biological environment (Davies 1998). Therefore, the developing biological film on the surface of
the implant would have an indirect effect on the cellular activity and subsequent bone formation.

Considering these factors, many researchers have investigated implant designs with different physical or chemical modifications of the titanium to control and optimize the bone healing response at the peri-implant site.

1.6.1 Chemical & Physical Modification of Titanium-Based Implants

The response of bone to titanium-based implants can be influenced by the physical or chemical modifications of the surface. Titanium plasma spraying (TPS), bead coating, sand blasting, and acid etching or the combination of the last two are generally the methods used to physically modify the surface of the material. Since the chemistry of the surface may remain unchanged, the surface topographical features produced by using these methods usually determine the bony reactions at peri-implant site. Although researchers (Buser, et al. 1991 and Ricci, et al. 1991) have demonstrated the importance of surface topography, they have failed to show that these changes have an equally positive effect on bone response as seen with bioactive glass or calcium phosphate materials. As a result, the bioactivity of the materials has been often thought to be associated with the presence of an intermediate apatite layer on the surface of the implant material (Hench, et al. 1982; Anderson, et al. 1988 and Kitsugi, et al. 1989). Since synthetic hydroxyapatite (HA) is similar to the natural apatite component of the bone tissue, different methods have been studied to combine this material with the surface of the implant. Physical application techniques are such as plasma spraying, dip coating,
electrophoretic deposition, electrocodeposition, hydrothermal reaction, physical vapor deposition, and ion-beam or radiofrequency sputtering. Each of these methods has its own technical limitations such as controlling the composition and structure of HA, which is often altered due to high degrees of heat involved in using those techniques. The bond between HA coatings and the core material is also often not stable and would result in releasing debris at implantation site. To overcome some of these problems, researchers have recently focused on methods to chemically induce HA surfaces either by *in vitro* or *in vivo* procedures. An example of such is biomimetic preparation techniques, a process of immersion of the titanium material in a supersaturated calcification solution at low temperatures to mimic the natural process of apatite formation. This method has drawn considerable attention since 1) conformal coatings can be produced onto complex-shaped and/or microporous implants, 2) no adverse effect of heat on substrates occurs, 3) the biomimetic Ca-P coating is expected to show higher bone-bonding ability, and 4) it is a simple and cost effective way (reviewed by Wen, et al. 1997).

Although Ducheyne, et al. (1991) and Li, et al. (1994) have shown that the titanium metal is capable of inducing HA formation on its surface, the formation of this layer is reported to be very slow. Hanawa (1991) reported that calcium phosphate layers are formed on the passive oxide films of titanium in neutral electrolyte solution, which is similar to apatite, in 30 days or more. Therefore, pretreatment of titanium metal has been investigated to increase the rate of calcium phosphate deposition on its surface when using a biomimetic method.

metal and reported that pretreatment of this material prior to its immersion in solutions having similar ionic contents to biological fluid had facilitated the deposition of a HA layer on the surface. Some of these investigators speculated on the mechanism underlying their chemical treatment that led to HA formation. Ohtsuki, et al. (1997) suggested that providing the titanium metal surface with basic Ti-OH groups would influence the rate of a biologically active bone-like apatite layer formation on the surface. Wen, et al. (1998) speculated that providing the surface of titanium with negatively charged microporosity might have a function similar to biological macromolecular matrices with negatively charged nanosized spaces for natural apatite formation.

While investigations of different pretreatment of titanium metal and its alloys for calcium phosphate induction in some type of physiological fluid in vitro were carried out, Yan, et al. (1996) demonstrated the HA layer can directly form on alkali/heat-treated titanium when implanted in vivo and can mediate bone-bonding. They hypothesized that alkali and heat treatment of titanium covers the surface with TiO₂ hydrogel, which in turn can initiate apatite nucleation on itself in a biological environment. Once apatite nucleation occurs, it spontaneously grows by taking calcium and phosphate ions from the surrounding body fluid and then bonds to the apatite of bone tissue.

Most of the investigators using chemical treatments have paid limited attention to the changes in surface morphology due to the chemical treatments. They have also often disregarded the influence of surface morphology on bony reactions, which they observed.

Wen, et al. (1998), Nishiguchi, et al. (1999) and Yan, et al. (1998) are of those groups who noticed that alkali/heat treated titanium metal has porous structure. Here again the first two groups suggested that the importance of surface microporosity was not
as significant as its chemistry in determining the bioactivity of the material. However, Yan and Davies in 1998 suggested that the surface topography produced after alkali/heat treatment, not the surface chemistry, increased osteoconduction.

In view of the latter observations, in this study we have focused on the importance of the surface topographical changes due to alkali/heat treatments on bony reactions specially bone-bonding at peri-implant sites. The bone-bonding phenomenon requires osteoconduction as a preceding step, so the influence of topographical changes on the phenomenon of the osteoconduction was also studied.
1.5 Statement of the Hypothesis

Alkali and heat treatment of the machined or acid-etched surfaces will alter the surface topography such that the osteoconduction on the surface will increase and the newly formed bone at peri-implant sites will mechanically bond with the surface.

1.6 Objectives

The objectives of this study were:

1. To characterize machined and acid-etched cpTi metal surfaces before and after alkali/heat treatment by using Scanning Electron Microscopy, Optical Profilometry, Contact Angle measurements and X-ray Photoelectron Spectroscopy.

2. To quantify osteoconduction on machined and acid-etched surfaces with and without alkali/heat treatment.

3. To examine the mechanism of, and quantify bone-bonding to those surfaces with or without alkali/heat treatment.
2. Materials Characterization

The variation observed in bone tissue response around different endosseous implant materials raises the question: How does the material surface influence biological response? To address this question, researchers have recognized the importance and necessity of surface characterization of candidate biomaterials.

Surface properties that are investigated include both chemical and structural. The latter include both macro- and microscopic length scales. An example is the case of titanium-based endosseous implants, in which the surface chemical composition is generally that of TiO₂, which is considered to contribute to corrosion resistance and biocompatibility. However, bone tissue has no inherent capacity to bond biologically to titanium oxide. Thus, researchers have employed different chemical treatments (Murakami, et al. 1995; Kim, et al. 1996; Hanawa, et al. 1997; Ohtsuki, et al. 1997 and Wen, et al. 1998) to render titanium bone-bonding. Li, et al. (1994) and Ohtsuki, et al. (1997) suggested that bony response was influenced by chemical treatment which would incorporate surface functional groups such as hydroxyl ions in titania. However changing the surface chemistry may also change its surface energy and charge (Lopes, et al. 1999 and Ohtsuki, et al. 1997), which also have been suggested to indirectly affect cell-biomaterial interactions.

Surface microstructure is another factor that is implicated in the interactions of the biomaterial with its surrounding biological environment. Boyan, et al. (1998) reported that MG63 osteoblast-like cells when cultured on rougher surfaces tended to exhibit characteristics of more differentiated osteoblasts than did those cells cultured on
In the studies mentioned in the former paragraphs and similar investigations, researchers have used a number of techniques to understand how the surface chemical, physical and morphological properties of the biomaterials correlate with their in vivo performance.

Techniques such as x-ray photoelectron spectroscopy (XPS), auger electron spectroscopy (AES), and secondary ion mass spectroscopy (SIMS) have been used to analyze the chemical composition of the surface. Contact-angle measurements are usually used to determine the surface energy of the material. Variations in surface texture or microtopography can be analyzed by techniques such as scanning electron microscopy (SEM), optical profilometry (OP), atomic force microscopy (AFM), and scanning tunneling microscopy (STM).

In addition to proper surface characterization methodology, a systematic approach and an appropriate experimental design can also play an important role in determining the significance of a surface feature on a particular bony reaction. In a study conducted by Buser, et al (1998), the removal torque values (RTV) of titanium implants in the maxilla of miniature pigs were compared. Two types of implants were compared and the difference in RTV attributed to the observed differences in surface topography. However differences in the overall implant design were disregarded. In another study conducted by Boyan, et al. (1998), surface roughness achieved by different techniques was reported to influence differentiation of the osteoblast like cells. The authors suggested that it was possible that some of the differences attributed to surface roughness might be due to differences in surface chemistry. Therefore, it is evident that an inappropriate experimental design would lead to equivocal or inconclusive results.
In this study, we have focused on the investigations which were conducted by Kokubos' group (Yan, et al. 1997 a,b; Nishiguchi, et al. 1997; Skriptiz, et al. 1998 and Nishiguchi, et al. 1999). Those authors used chemical changes in the surface to explain biological bonding to alkali/heat treated titanium samples and disregarded the physical and topographical changes that occurred on the surface.

Therefore, we characterized surface features of alkali/heat treated and untreated titanium samples with either machined or acid-etched primary surface topography. We have examined the changes in surface topography using SEM and quantified surface profile features by OP. To determine surface chemical composition and its wettability, we have used XPS and contact-angle measurement, respectively.

2.1 Materials and Methods

2.1.1 Sample Preparation

All the experimental samples were commercially pure titanium (99.5%), provided by Implant Innovations Inc. (3i). They were either machined or dual acid-etched, according to the procedure employed for Osseotite™ dental implants.

Both surface types were cleaned by sonicating three times in a 2% Decon solution for 5 minutes and dried at 40°C for one hour. Specimens were then divided into two groups and received one of the following treatments:

- Control group: No treatment
- NaOH/heat treated group: Soaked in 10M NaOH aqueous solution at 60 °C for 24 hours, washed with DDH_{2}O and air-dried at 40 °C for one hour. Samples were placed in an aluminum boat, and heated approximately at 24
°C/min to 600 °C in an electric furnace. They were kept at given temperature for one hour and cooled to room temperature.

The following three different sample designs were used in these experiments:

- Disc, 1.5 cm in diameter (either machined or acid-etched)
- Cylindrical mini implant, 1 mm diameter x 3 mm height (either machined or acid-etched)
- Tapered dental implant A, top collar 6 mm diameter x 18 mm height (machined at the top and acid-etched at the base)
- Tapered dental implant B, top collar 4 mm diameter x 4 mm height (machined, acid-etched, or titanium plasma-sprayed)

2.1.2 Surface Topography

Scanning Electron Microscopy (SEM - Hitachi S-570, 15K) was used to examine surface topography of the entire different sample designs that received treatments mentioned in section 2.1.1. The surfaces of the specimens were examined at the various magnifications of x100, x500, x1K, x2K, x5K and x7K.

SEM was also employed to compare the surface topographies of dual acid-etched discs and implants. Images at x1K and x7K magnifications of the three discs and three mini-implants with acid-etched surfaces were used to estimate the size of the etching pits. Two areas on each sample were chosen randomly to prepare SEM images at both magnifications. The etching pit sizes were estimated by using an intercept method, described as follows. Straight lines all with the same length were drawn through photomicrographs prepared from each sample. The array of pits intersected by each line segment were counted. To obtain pit size, the line length then was divided by the number
of pits intersecting that line subsequently, the average pit size was calculated (method used was adapted from the method used in quantifying crystal grain size of a metal or ceramic (Callister, 1994). All the measurements were done using the image analyzer (National Institute of Health, NIH Image 1.61).

2.1.3. **Surface Profile Analysis**

Optical Profilometry (OP- WYKO NT-2000, USA) was used to conduct a quantitative analysis of the surface profile of five discs (prepared as treatment groups mentioned in section 2.1.1) and three tapered dental implant B (no treatment) samples.

Disc specimens were placed flat on the sample board and the OP apparatus was set on Vertical Scanning Interferometer (VSI) mode and sampling was done on four quarterly regions of each sample. A fixture held dental implant B specimens such that the incident light beam was vertical to the surface of the implant, and three regions on each of the first three thread peaks and thread valleys were examined using VSI mode at x100 magnification. The lateral resolution of the OP system analyzing both sample designs was ~90 nm.

Surface profile data collected by OP was used in quantifying four parameters of 1) average surface roughness (Ra); 2) root mean square (rms) of average surface roughness (Rq); 3) surface area (SA); and 4) surface area index (SA/- exposed SA/lateral A).

*Ra* is the most reported parameter in the literature and it is the mean height as calculated over the entire measured array. *Rq* is the rms averages of the measured height deviations taken within the evaluation length or area and is measured from the mean linear surface. Because height values are squared in the calculation, *Rq* is more sensitive
to peaks and valleys than $Ra$. This makes it a better parameter for discriminating between different types of surfaces.

$S4$ is the total exposed surface area being analyzed, including peaks and valleys. $SAI$ is the ratio of exposed surface area to lateral surface area and it is a measure of relative flatness.

2.1.4. **Surface Wettability Analysis**

Contact angles (CA-goniometer, Rame-Hart, USA) were determined by dropping equal volume (5 μl) of distilled water to the surface of disc samples (six samples of each surface type were prepared as in section 2.1.1) and advancing angle was measured with the assistance of a goniometer.

2.1.5. **Surface Chemistry Analysis**

X-ray Photoelectron Spectrometer (XPS- Leybold Max 200) with a monochromatic (AlKα) X-ray source was used 1) to determine surface chemical composition on tapered dental implant A samples (four samples were prepared as in section 2.1.1) using survey spectra and 2) to determine and quantify chemical components of the surface using high resolution spectrum of each element.

Sampling was done on an area of $2.5 \times 2.5$ mm on three regions of top (machined), middle (acid-etched), and base (acid-etched).
2.2 Results

2.2.1 Surface topography

The electron micrographs prepared at different magnifications from the disc and mini implant samples demonstrated that the machined and acid-etched surfaces were markedly different. The machined surface exhibited distinct ridges and grooves, and the topographical features of the acid-etched surface included etching pits with average of $13.6 \pm 1.96$ micron scale, which were also characterized with $1.5 \pm 0.25$ micron scale pits within them (figure 2.2.1.a).

Similar results were observed with tapered dental implant B samples. Machined regions exhibited mainly unidirectional machining grooves, which seemed more pronounced on thread peaks. Acid-etched implants displayed two types of topography. First, an etching profile of two different scales (micropits with average of $1.14 \pm 0.17 \mu m$ and larger features of approximately $18.3 \pm 4 \mu m$ were determined on uniformly etched regions). Second, unetched areas seen only on the thread peaks, which appeared as irregularly sized plateaus standing above the surrounding etched surface. Thread valleys were always uniformly etched and showed no unetched areas. Titanium-plasma sprayed (tapered dental implant B) samples displayed a complex three-dimensional surface with a particulate size in the range of 1-25 $\mu m$. The particle density appeared similar in both the valleys and thread peaks (figure 2.2.1.b). Examining each surface at higher magnification of x10K showed similar smooth surface on both machined and TPS samples in contrast to microscale roughness seen with acid-etched surface (figure 2.2.1.c).

Comparing the etching pits of both scale ranges on disc samples with implant samples, it was found that the sizes of the large pits were significantly ($p < 0.05$) greater
on implant surfaces, while the sizes of the small pits were significantly (p < 0.05) smaller (t-test was used for statistical analysis and power of test was 94%). Summary of the data obtained is shown in table 2.2.1.a.

Table 2.2.1.a Summary of data obtained from etched pit size determination.

<table>
<thead>
<tr>
<th>Etching Pit Type</th>
<th>Large Pit</th>
<th>Small Pit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Disc</td>
<td>Implant</td>
</tr>
<tr>
<td>1 (area 1)</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>1 (area 2)</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>2 (area 1)</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>2 (area 2)</td>
<td>16</td>
<td>19.2</td>
</tr>
<tr>
<td>3 (area 1)</td>
<td>12</td>
<td>19.2</td>
</tr>
<tr>
<td>3 (area 2)</td>
<td>13.7</td>
<td>19.2</td>
</tr>
<tr>
<td>Average</td>
<td>13.6</td>
<td>18.3</td>
</tr>
<tr>
<td>Sd</td>
<td>1.96</td>
<td>4</td>
</tr>
</tbody>
</table>

The topography of the machined and acid-etched surface types (using disc and mini-implant samples) was dramatically changed following an alkali/heat treatment. While there was still some evidence of both the machining and larger scale topography features respectively, the smaller scale etched topography was completely obliterated. In all cases, the oxide surface comprised a reticular appearance with scattered cracked regions (figure 2.2.1.d).

2.2.2 Surface Profile

3-D reconstruction of the experimental surfaces using the Optical Profilometer (OP) confirmed our observations by SEM (figure 2.2.2.a). The quantitative data obtained from disc samples are shown in Table (2.2.2.a). All the parameters showed an
increasing trend (figure 2.2.2.b, c, d, e) in the order of $M < M + \text{NaOH} < A < A + \text{NaOH}$ ($M$ = machined and $A$ = acid-etched).

One-way analysis of variance (ANOVA) and pairwise multiple comparison procedures (Student-Newman method) were used to compare $Ra$, $Rq$, $SA$, and $SAI$ parameters between the experimental groups (the power of test was above 75%).

There was a significant difference ($p < 0.05$) between $Ra$ and $Rq$ parameters for $M$, $A$, $M + \text{NaOH}$, $A + \text{NaOH}$ groups, except for $A$ vs. $A + \text{NaOH}$ ($p > 0.05$). There was also a significant difference between $SA$ and $SAI$ parameters for $M$, $M + \text{NaOH}$, $A$, $A + \text{NaOH}$ groups ($p < 0.05$), except for $A$ vs. $M + \text{NaOH}$ ($p > 0.05$).

### Table 2.2.2.a: Surface profile parameters measured on the disc samples

<table>
<thead>
<tr>
<th>Number of samples</th>
<th>$Ra$ (nm)</th>
<th>$Rq$ (nm)</th>
<th>$SA$ (nm$^2$)</th>
<th>$SAI$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Machined</td>
<td>208 ± 57</td>
<td>254 ± 69</td>
<td>566 ± 28</td>
<td>1.12 ± 0.06</td>
</tr>
<tr>
<td>Machined + NaOH/heat</td>
<td>259 ± 66</td>
<td>342 ± 81</td>
<td>849 ± 119</td>
<td>1.69 ± 0.23</td>
</tr>
<tr>
<td>Acid-etched</td>
<td>432 ± 93</td>
<td>539 ± 109</td>
<td>893 ± 59</td>
<td>1.77 ± 0.12</td>
</tr>
<tr>
<td>Acid-etched + NaOH/heat</td>
<td>460 ± 79</td>
<td>589 ± 92</td>
<td>1054 ± 80</td>
<td>2.1 ± 0.16</td>
</tr>
</tbody>
</table>

The optical profilometry 3-D reconstructs of machined and acid-etched surfaces of tapered dental implant B samples, also confirmed our observations by SEM (figure 2.2.2.f). However, the OP analysis of TPS surfaces was unsuccessful since the complex surface topography prevented a complete incident beam reflection. Thus, TPS results are not included.
The quantitative data obtained from the tapered dental implant B samples are shown in Table (2.2.2.b). All the parameters, for both of the thread peak and valley, showed an increase from the machined to acid-etched surface.

One-way analysis of variance (ANOVA) and pairwise multiple-comparison procedures (Student-Newman method) were used to compare Ra, Rq, SA, and SAI parameters of thread peaks and valleys within and between each group (the power of test was > 80%). It was found that there is a significant difference (p < 0.05) between the experimental surfaces. Within each group all the measured parameters were significantly different (p < 0.05) between thread peaks and valleys of the acid-etched implants. The machined surface showed no significant difference for parameters of Ra and Rq of thread peaks and valleys. However, the SA and SAI of the thread peaks were significantly greater than the valleys (p < 0.0001).

Table 2.2.2.b Surface profile parameters measured on the thread peak (TP) and thread valley (TV) of tapered dental implant B samples.

<table>
<thead>
<tr>
<th></th>
<th>Ra (nm)</th>
<th>Rq (nm)</th>
<th>SA (nm²)</th>
<th>SAI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TP (n=27)</td>
<td>TV (n=27)</td>
<td>TP (n=27)</td>
<td>TV (n=27)</td>
</tr>
<tr>
<td>Machined</td>
<td>185 ± 69</td>
<td>200 ± 45</td>
<td>241 ± 94</td>
<td>246 ± 85</td>
</tr>
<tr>
<td></td>
<td>1.05 ± 0.03</td>
<td>1.01 ± 0.02</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Acid-etched</td>
<td>494 ± 145</td>
<td>380 ± 75</td>
<td>629 ± 243</td>
<td>485 ± 92</td>
</tr>
<tr>
<td></td>
<td>0.0006</td>
<td>0.0057</td>
<td>0.0087</td>
<td>0.0088</td>
</tr>
</tbody>
</table>
2.2.3 Surface Wettability

The advancing Contact Angle (CA) values are reported in Table (2.2.3.a). A decrease in advancing CA corresponds to an increase in surface wettability. The one-way analysis of variance (ANOVA) was used to find whether there was a statistical difference between the experimental groups. It was found that there was a significant decrease \( (p < 0.05) \) in advancing CA after alkali/heat treatment with both surface types. Untreated acid-etched samples also demonstrated significantly smaller \( (p < 0.05) \) advancing CA compared with untreated, machined samples (the power of the test was > 80\%)(figure 2.2.3.a).

Table 2.2.3.a The mean values for advancing contact angle of each treatment group.

<table>
<thead>
<tr>
<th>Number of samples</th>
<th>Machined NaOH</th>
<th>Machined NaOH +</th>
<th>Acid-etched NaOH</th>
<th>Acid-etched NaOH +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advancing Contact Angle</td>
<td>58 ± 5</td>
<td>&lt; 10</td>
<td>48 ± 5</td>
<td>&lt; 10</td>
</tr>
</tbody>
</table>

2.2.4 Surface Chemistry

The survey spectrum of control (tapered dental implant A, untreated) samples was characterized by peaks for O1s, Ti2p, and C1s (figure 2.2.4.a). Titanium and oxygen signals were representative of an oxide layer at the surface. The carbon signal was from adventitious hydrocarbon, which was introduced from the laboratory environment or from oil vapors coming from the pumps into the spectrometer.
High-resolution spectra for the above elements revealed the nature of the chemical compositions in which they were present. TiO₂ made up a major part of the titanium spectrum where TiO and Ti made up the rest (figure 2.2.4.c). There was no significant difference (ANOVA was used, n = 3) between the percentages of these compounds present on machined or acid-etched regions of the implants.

Metal oxide was the major component of oxygen spectrum and basic or acidic hydroxyl ions were the other constituents of the spectrum (figure 2.2.4.e). Although hydroxyl ions percentage values seemed to be lower, here again there was no significant difference (ANOVA was used, n = 3) between the percentages of these compounds present on machined or acid-etched regions of the implants.

The spectrum of NaOH/heat treated (dental implant A) sample was characterized by peaks for O1s, Ti2p, C1s, and NaKLL (figure 2.2.4.b). It was evident that during the treatment, Na is incorporated into the surface of the sample. Titanium high-resolution spectra demonstrated an increase in the TiO₂ level. The signal received from Ti metal almost disappeared, which indicated an increase in the oxide layer thickness (figure 2.2.4.d). The oxygen spectrum revealed that relative percentage of the metal oxide species were increased after the NaOH/heat treatment of the implants on both machined and acid-etched regions (figure 2.2.4.f).

### 2.3. Discussion and Conclusion

Scanning electron microscopy is one of the most common methods used to examine surface microstructure. Comparing the acid-etched surfaces of discs and threaded implants revealed that the etching pits were significantly different in size (large pits 13.6 ± 1.96 vs. 18.3 ± 4 and small pits 1.5 ± 0.24 vs. 1.13 ± 0.16). This emphasizes
the importance of employing model surfaces, which reflect the surface characteristics of real implants. Examining the images of machined and acid-etched surfaces prior to, and after, alkali/heat treatment demonstrated that alkali/heat treatment would render the surface microporous (defined in this study as a surface with submicron reticular appearance) with scattered cracked regions, independent of the underlying surface microtopography. Surface microporosity has been shown (Dziedzic, et al. 1996) to allow mechanical bone/implant interlocking. Therefore, it can be anticipated that the surface porosity produced by alkali/heat treatment of titanium also would participate in mechanical interlocking with bone tissue at peri-implant site. The cracked regions of the alkali/heat treated surfaces might also provide the opportunity for the bone tissue to bond with the implant surface.

The surface average roughness is the most reported parameter to characterize surface profile of a biomaterial. However, unlike SEM, which is an accepted method for surface imaging, there is no accepted standard system to quantify surface roughness. This is perhaps due to the limitations of the technologies available. In addition, the parameter itself carries some limitations in characterizing the surface. Average roughness is the mean of height deviations from the middle line. A surface could have small peaks and valleys frequently repeated or large peaks and valleys less frequently repeated and Ra values of both surface types could be very similar. However, the surface area parameter can distinguish between those surface types. The results of this study show that surface area (SA) is a better measurement parameter than surface roughness (Ra) to define surface profile of a material.

In this study we examined the machined and acid-etched surfaces with Atomic Force Microscopy (AFM) prior to using Optical Profilometry (OP). Examining the
profile created by AFM revealed this system is suitable for the machined surface but not for the acid-etched surface. The triangular cantilever tip of the AFM probe, which was placed in contact with the acid-etched sample, produced artifacts in the generated surface profile. However, the profile generated by the OP system corresponded with the electron micrographs produced by SEM; hence, it was chosen as the more suitable system for the characterization of both machined and acid-etched surfaces before and after alkali/heat treatment. Seeking the appropriate technique has not always been the case in surface characterization studies. Wennerberg, et al. (1997) used a confocal laser scanning profilometer to characterize surface profile of machined and blasted surfaces. Examining the computer-generated images of those surfaces clearly demonstrates that the system had generated artifacts and therefore it is not a suitable technique. However, the authors still report the surface roughness values provided by that system and they also suggest the best bone response based on those values. It is clear such studies would be misleading in developing the implant surface designs and understanding the bone response at peri-implant site.

Several researchers (Buser, et al. 1991a; Piatteli, et al. 1998) have associated the increase in surface roughness with the increase in bony responses such as osteoconduction. On the contrary, Buser, et al. (1991b) and Vercaigne, et al. (1998) reject such a correlation. It is evident there are conflicting reports regarding the effects of surface roughness on bony response at peri-implant site. Since researchers use different techniques on different surfaces, the level of optimum roughness also varies among their reports. Another important factor that was considered along with surface roughness was the surface morphology. In this study, although it was shown that there was a trend in surface roughness these differences were not significantly different. The
fine microporosity produced on the surface was perhaps smaller than the OP apparatus spatial resolution (~90 nm), therefore was not detected by that system. However, great changes in surface morphology were accompanied by significant increases in surface area. These may lead to different bony responses.

Contact Angle (CA) measurements are often conducted to measure surface wettability. Van Kooten, et al. (1992) reported that cells adhere more firmly to substrata with high wettability than to substrata with low wettability. Web, et al. (1998) also reported similar results and demonstrated that hydrophilic surfaces support significantly greater cell attachment, cell spreading, and cytoskeletal organization relative to hydrophobic surfaces.

In this study we compared machined and acid-etched surfaces, and observed that the latter surface demonstrated significantly lower advancing CA. Since the XPS results showed that surface chemical composition of those surfaces are the same, it can be concluded that surface roughness may increase surface wettability. However, we found a significant decrease in the measurements on the acid-etched surfaces after alkali/heat treatment. These changes in contact angle measurements would seem to contradict the optical profilometer results, which showed no significant difference in roughness for these two surfaces. However, it should be noticed that the OP measurements did not reflect the obvious morphological changes seen in the SEM which indicates the limitations of OP as a mean of recording the submicron changes that had occurred in the surface topography. Thus it is not possible to determine from these results, which surface topographical or chemical changes are responsible of the changes in CA measurements.
These observations led us to believe that alkali/heat treatment of cpTi not only changes its surface chemistry as reported by Kokubos' group, but also creates extensive physical and topographical changes on the metal surface that may participate in bone-bonding.
Figure 2.2.1.a  Scanning electron micrographs of machined and acid-etched disc surfaces prior to alkali/heat treatment.

1) Machined surface with distinct ridges and grooves.

2) Acid-etched surface with etching pits at ~13 micron scale, which were characterized with ~1.5 micron scale pits within them.
Figure 2.2.1.b Scanning electron micrographs of the machined, acid-etched and titanium plasma sprayed, tapered dental implant A surfaces.

1, 2, and 3) Machined, acid-etched and titanium plasma sprayed (TPS) implants at x40 magnification.

4, 5, and 6) the above implant surfaces at x100 magnification, demonstrating two thread peaks and valley between them.

7, 8, and 9) the above implant surfaces at x1K magnification at thread peak region. Machined surface demonstrating pronounced machining features. Acid-etched surface demonstrating unetched areas. TPS surface demonstrating similar particle density as thread valley.

10, 11, and 12) Machined surface demonstrating smoother surface than thread peak. Acid-etched surface demonstrating uniformly etched surface. TPS surface demonstrating similar particle density as thread peaks.
Figure 2.2.1.c Scanning electron micrographs of the machined, acid-etched and titanium plasma sprayed surfaces of tapered dental implant B at x10K magnification.

1) Machined surface

2) Acid-etched surface

3) Titanium plasma sprayed surface

Machined and TPS surfaces are similarly smooth unlike acid-etched surface which demonstrates micro scale roughness.
Figure 2.2.1.c continued
Figure 2.2.1.e Scanning electron micrographs of the machined and acid-etched disc surfaces after alkali/heat treatment.

1) Machined surface demonstrating some evidence of machining, underlying the reticular surface oxide layer.

2) Acid-etched surface demonstrating only 13-micron topography features, the 1.5-micron topographies were completely obliterated by the reticular surface oxide layer.
Figure 2.2.2.a  3-D reconstruction of surface profile of the machined and acid-etched disc samples.

1) Machined
2) Acid-etched
Figure 2.2.2. Bar-graphs representing Ra, Rq, SA, SAI parameters for machined and acid-etched disc samples prior to and after alkali/heat treatment.

b) Average surface roughness values (Ra)

c) Root mean square of surface average roughness (Rq)

d) Surface area (SA)

e) Surface area index (SAI)
Figure 2.2.2.f

The 3-D reconstruction of surface profile of the machined and acid-etched tapered dental implant B samples on thread peak and valley regions.

1) Machined, thread peak
2) Machined, thread valley
3) Acid-etched, thread peak
4) Acid-etched, thread valley
Figure 2.2.3.a  Bar-graph representing advancing contact angle values for machined and acid-etched disc samples prior to and after alkali/heat treatment.
Figure 2.2.4 The X-ray photoelectron spectroscopy survey spectrum of tapered dental implant A surface,
a) Untreated sample
b) Alkali/heat treated sample
The high-resolution spectra of,
c) Ti2p of the untreated sample
d) Ti2p of the alkali/heat treated sample
e) O1s of the untreated sample
f) O1s of the alkali/heat treated sample
Metal Oxides

TiO$_2$(H$_2$O)

Ti-O-H

Intensity

Binding Energy (eV)

CP-TI[NAOH]

Metal Oxides

TiO$_2$(H$_2$O)

Ti-O-H

Intensity

Binding Energy (eV)
3. **Osteoconduction**

Osteoconduction is an essential prerequisite for bone formation to occur at the implant surface. This phenomenon is reported to rely on migration of the osteogenic cells to the implant site (Glowacki, et al. 1991; Einhorn 1995 and Davies 1998). However, the exact mechanism that mediates cell migration is still unclear.

It has been suggested that calcium phosphate-based materials (eg. Hydroxyapatite, HA) provide the required chemical composition for adsorption of the proteins that can mediate cell migration to the implant surface (Davies 1998). These materials have been shown repeatedly to be more osteoconductive than metallic biomaterials (Buser, et al. 1991; Wong, et al. 1995 and Hayshi et al. 1999). However, calcium phosphate-based materials are brittle and mechanically weak. There are also complications associated with HA coated implants, such as weak bond between the HA coating and the substrata, which would lead to release of coating fragments in the peri-implant site. Therefore, modifying the surface topography of metallic biomaterials, such as titanium and its alloys, which are biocompatible and biomechanically strong, has been examined as a means of improving their osteoconductivity. Buser, et al. (1991); Yan and Davies (1998), and Piatteli, et al. (1998) reported an increase in bone-implant contact (an indication of osteoconduction) with an increase in surface roughness. On the contrary, Wong, et al. (1995), and Vercaigne, et al. (1998) reported no significant difference in bone-implant contact between rough and less rough surfaces. The latter observation could be the result of the implant topography roughness exceeding a certain (unknown) threshold which, if excessive, has been suggested to have a negative effect on osteoconduction (Murray, et al. 1989, Vercaigne, et al. 1998).
Recently different chemical treatments of metallic implants have been used to improve bony response around endosseous implants. Examples of such work are the studies conducted by Kokubo and his colleagues (Miyaji, et al. 1994; Kim, et al. 1996; Yan, et al. 1997 and Wen, et al. 1998). Their main objective was to use alkali/heat treatment on the titanium and its alloys to render their surfaces bioactive or bone bonding. They reported that the alkali/heat treated titanium metal has different surface chemistry (Miyaji, et al. 1994 and Kim, et al. 1996) and topography (Wen, et al. 1998; Yan, et al. 1998 and Nishiguchi, et al. 1999) compared with the untreated material. As a result of these changes the treated implants showed strong bonding with bone tissue, furthermore histological observations by this group consistently revealed (Yan, et al 1997 and Nishiguchi, et al. 1999) that bone juxtaposition to such modified titanium surfaces was increased with respect to non-treated controls.

Focusing on their last observation, I hypothesize that alkali/heat treatment of the titanium metal provides the surface with features that promote osteoconduction and, thus, bone formation on the implant. However, Yan and Davies (1998) evaluated the bony reactions to alkali/heat treated Ti implants vs. untreated controls, and showed, qualitatively, by examination of histological sections of the bone/implant interface, that topographical changes on implant surfaces, rather than changes in surface chemistry were responsible for the increase in observed osteoconduction.

The purpose of the present study was to confirm the observations reported by Yan and Davies (1998) by conducting a quantitative comparison of the bone-implant contact percentage between alkali/heat treated titanium implants vs. untreated, and to determine
whether there is a correlation between surface roughness and osteoconductivity of these materials.

3.1 Materials and Methods

3.1.1 Implant Model:

Custom-made, miniature implants were manufactured from commercially pure Ti (99.5%). They were supplied by 3i as machined, or dual acid-etched according to the procedure employed for Osseotite™ dental implants. The implants were designed with a medullary component and a threaded trans-cortical portion. The head of the implant contained a hexagonal opening to accept an Allen key to facilitate engaging the cortex (figure 3.1.1.a).

3.1.2 Sample Preparation:

12 implants (6 machined and 6 acid-etched) were sonicated in a 2% Decon solution for 5 minutes and dried at 40°C for 1 hour. This procedure was performed three times; the samples were then divided into two groups and received one of the following treatments:

1. Control group: No treatment

2. NaOH/heat-treated group: Soaked individually in 2 ml of 10M NaOH aqueous solution at 60°C for 24 hours, washed with DDH2O and air-dried at 40°C for one hour. Machined and acid-etched samples were separated in a two-compartment aluminum boat and heated approximately at 24
°C/min to 600°C in an electric furnace. They were kept at the given temperature for 1 hour and cooled to room temperature.

3.1.3 Animal Model and Implant Site:

Implants were placed aseptically into the mid-lateral aspect of both femora of 250-gram male Wistar rats. The top threaded part of the implants was screwed into the cortical portion of the mid-diaphyses by an appropriate sized Allen-key.

3.1.4 Surgical Procedure:

Sterile surgical conditions and inhalation anaesthesia (fluorothane in nitrogen and oxygen 500 ml flow rate; 5% induction, 2% maintenance) were used on rats for all surgeries. All implants were sterilized by γ-irradiation prior to use.

Each rat leg was shaved and cleaned (10% Betadine). An incision was made along the long axis of each lower limb and the underlying skin was freed from the muscle. The muscles were gently separated and the periosteum of the lateral femur was incised and reflected. An end-cutting bur (supplied by 3i) was used to prepare the hole (1 mm) for the custom-made implants. All drilling was done using a right-angle handpiece (supplied by 3i) with copious saline irrigation. The implants were pressed into place and secured by engaging the threads in the cortical bone using an Allen-key. A total of 12 implants were used in 6 animals. The muscle and skin layers were closed using tapered needle 3/0 Vicryl and surgical staples, respectively.

Following a two-week experimental period, the animals were killed and the femoral bones were dissected. Samples were trimmed with a 2-3 mm margin distal and
proximal to the implant and were immediately fixed in 10% formalin solution for 24 hrs., followed by processing for histological analysis.

3.1.5 Histological Preparations:

Samples were dehydrated through increasing concentrations of ethanol (70%, 95%, and 100%) and embedded according to the Osteobed resin embedding protocol.

Blocks were sectioned into two halves approximately perpendicular to the longitudinal axis of the implant with a diamond wheel (1A1-3X, Norton; USA). Sections were ground to 1200 grit and stained with Toluidine blue (1:1, 0.3% toluidine blue and 2% sodium borate) and Van Gieson picro-fuchsin (10% of 1% acid fuchsin in picric acid) solutions for light microscopy observations. Light microscopy images were prepared at x40 and x60 magnifications for qualitative analysis.

3.1.6 Quantification Method:

Histological sections were carbon-coated for Backscattered Electron Image (BSEI-SEM) observations on the scanning Electron Microscope (Hitachi Model S-570, accelerating voltage 15-20 KV).

Backscattered images (20x magnification) were used with a computer assisted morphometric (National Institute of Health, NIH Image 1.61) system to quantify the length of bone-implant contact percentage. The area considered for analysis was limited to the medullary portion of the implant, then the total length of the bone-implant contact length was traced and the obtained value was recorded. The total perimeter of the implant at the medullary portion of the bone was also traced and the corresponding value
was recorded (figure 3.1.6.a). The percentage ratio of the last two values was calculated and reported. If there were more than two samples from the same treatment groups, the value reported was the mean of the all percentage contact lengths for that group.

3.2 Results

3.2.1 Qualitative Analysis:

Eight samples were successfully retrieved and four samples were lost due to fracture of the femoral bone during the experimental period. LM images (figure 3.2.1.a, b) of the histological sections (one per sample) of the retrieved samples were ranked by eye from the most to the least bone-implant contact. Samples were then divided into two groups of most and least osteoconductive. Two machined and two acid-etched samples were placed in the most vs. three machined and one acid-etched in the least osteoconductive group respectively. Three alkali/heat treated samples were placed in the most vs. two in the least osteoconductive group (Table 3.2.1.a)

<table>
<thead>
<tr>
<th>Samples</th>
<th>First 50th percentile</th>
<th>Second 50th percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone-implant Contact</td>
<td>CM, 1M, 1E, 1E, CE, CM, 1M, 1M</td>
<td></td>
</tr>
</tbody>
</table>

CM = control machined, CE = control acid-etched, 1M = machined + alkali/heat,
1E = acid-etched + alkali/heat
3.2.2 Quantitative Analysis

One control machined sample was lost during the preparation. Mean bone-implant contact percentage (B/I %) values for the rest of the samples are summarized in Table 3.2.2.a.

Table 3.2.2.a. Mean bone-implant contact percentage values

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CM</th>
<th>CE</th>
<th>IM</th>
<th>IE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Samples</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Mean B/I %</td>
<td>53</td>
<td>40</td>
<td>53 ± 8</td>
<td>52 ± 15</td>
</tr>
</tbody>
</table>

3.3 Discussion and Conclusion

In this study, the osteoconduction phenomenon was examined on alkali/heat treated titanium, custom-made implants. Two surface types of machined and acid-etched were used to distinguish the effect of surface topography from surface chemistry. These surface types had different primary surface roughness that would affect the level of roughness after alkali/heat treatment.

Qualitative analysis of histological sections, indicated a small increase in osteoconduction on alkali/heat treated samples vs. untreated. There was also an indication of an increase in acid-etched samples vs. machined. However, quantitative analysis of the same sections failed to show these differences. The problems identified by this pilot study were as follows:

- Variable sample number in each group due to
(a) Small initial sample number

(b) Fracture of the femoral bone during the surgery

- Inappropriate implantation of some samples, such that implant was adjacent to the cortical bone on one side and bone marrow on the other side
- Inappropriate sectioning of the resin blocks containing histological sections, such that the samples were not all cut identical (figure 3.2.2.a).
Figure 3.1.1.a  Custom-made cylindrical miniature implant model used in the osteoconduction experiment. (a) parallel-sided medullary component, (b) threaded trans-cortical component, (c) hexagonal opening contained in the head of the implant to accept an Allen key.
Figure 3.1.6.a Example of backscattered images used with a computer assisted image analyzer (NIH). $l_n = n$th length of bone/implant contact, $L =$ total length of implant perimeter.

Bone/implant Contact $\% = \frac{l_1 + l_2 + l_3 + \ldots + l_n}{L} \times 100$
Figure 3.2.1.a  Light microscopy image prepared from the histological section of one of the most osteoconductive ranked implant surface (alkali/heat treated acid-etched) at

1) x 40 magnification.

2) x 60 magnification. Arrow is pointing to the arrangement of the osteogenic cells on the implant surface.
Figure 3.2.2.a Backscattered images of the sections cut for qualitative and quantitative analysis. Off-centric implantation, cortical bone is in contact with the implant on one side and bone marrow on the other side and off-centric sectioning are evident in this images.
3.4 Materials and Methods (Main Study)

3.4.1 Implant Model:

Custom-made, miniature implants were manufactured from commercially pure Ti (99.5%). They were supplied by 3i as machined, or dual acid-etched according to the procedure employed for Osseotite™ dental implants. The implants were designed with a medullary component and a threaded trans-cortical portion. The head of the implant contained a hexagonal opening to accept an Allen key to facilitate engaging the cortex.

3.4.2 Sample Preparation:

30 implants (10 machined and 20 acid-etched) were sonicated in a 2% Decon solution for 5 minutes and dried at 40°C for 1 hour. This procedure was performed three times; the machined samples were used as controls (no treatment) and acid-etched samples were divided into two groups and received one of the following treatments:

1. Control group: No treatment
2. NaOH/heat-treated group: Soaked individually in 2 ml of 10M NaOH aqueous solution at 60°C for 24 hours, washed with DDH2O and air-dried at 40°C for one hour. Machined and acid-etched samples were separated in a two-compartment aluminum boat and heated approximately at 24 °C/min to 600°C in an electric furnace. They were kept at the given temperature for 1 hour and cooled to room temperature.
3.4.3 *Animal Model and Implant Site:*

Implants were placed aseptically into the mid-lateral aspect of both femora of 425-450 gram male Wistar rats. The implants were press fit into the bone tissue.

3.4.4 *Surgical Procedure:*

Sterile surgical conditions and inhalation anaesthesia (fluorothane in nitrogen and oxygen 500 ml flow rate; 5% induction, 2% maintenance) were used on rats for all surgeries. All implants were sterilized by γ-irradiation prior to use.

Each rat leg was shaved and cleaned (10% Betadine). An incision was made along the long axis of each lower limb and the underlying skin was freed from the muscle. The muscles were gently separated to expose the femur. An end/side-cutting bur (supplied by 3i) was used to prepare the hole (1 mm) for the custom-made implants. All drilling was done using a right-angle handpiece (supplied by 3i) with copious saline irrigation. The implants were pressed into place and in this experiment the implants were not secured by engaging the threads in the cortical bone using an allen key. It was found during the process of screwing the implant in the cortical bone, the accepting hole was getting larger and therefore implant was left loose. A total of 30 implants were used in 15 animals. The muscle and skin layers were closed using tapered needle 3/0 Vicryl and surgical staples, respectively.

Following a two-week experimental period, the animals were killed and the femoral bones were dissected. Samples were trimmed with a 2-3 mm margin distal and proximal to the implant and were immediately fixed in 10% formalin solution for 24 hrs. followed by processing for histological analysis.
3.4.5 **Histological Preparations:**

Samples were dehydrated through increasing concentrations of ethanol (70%, 95%, and 100%) according to the Osteobed resin embedding protocol.

Blocks were sectioned close to the head part of the implants approximately parallel to the head of the implant with a diamond wheel (1A1-3X, Norton; USA). Then the blocks were ground to the level just below the cortex with silicon carbide papers of 320-1200 grits. Prepared blocks were used for backscattered electron microscope imaging (BSI) at x60 and x70 magnifications for quantitative analysis. After preparing the BSIs the blocks were stained with Toluidine blue (1:1, 0.3% toluidine blue and 2% sodium borate) and Van Gieson picro-fuchsin (10% of 1% acid fuchsin in picric acid) solutions for light microscopy observations. The first section of the blocks were cut with a diamond wheel (1A1-3X, Norton; USA) to approximately 200 microns and were ground down to about 10 microns using silicon papers of 320-1200 grits. Light Microscopy (LM) images of the thin sections were prepared at x50 and x120 magnifications for qualitative analysis. The blocks were again ground using silicon papers of 320-1200 grits and BSIs were prepared at x60 magnification for the second quantification analysis.

3.4.6 **Quantification Method:**

The Backscattered Electron Imaging (BSEI-SEM) was done on the scanning Electron Microscope (Hitachi Model S-570, accelerating voltage 20 KV). Backscattered images (60x magnification) were used with a computer assisted morphometric (National Institute of Health, NIH Image 1.61) system to quantify the length of bone-implant
contact percentage. The area considered for analysis was limited to the medullary portion of the implant, then the total length of the bone-implant contact length was traced and the obtained value was recorded. The total perimeter of the implant was also traced and the corresponding value was recorded. The percentage ratio of the last two values was calculated and reported.

3.5 Results

3.5.1 Qualitative analysis

Light microscope images of the histological sections of the retrieved samples were examined by eye. It was observed that the bone tissue surrounding the implant surface was separated from the implant surface at most regions of the machined or acid-etched implant perimeter. However, bone tissue surrounding the alkali/heat treated implant retained its contact at most regions of the implant surface perimeter (figure 3.5.1.a).

Comparing the LM images of the samples with their corresponding BSI demonstrated that the separation of the bone tissue surrounding the machined or acid-etched implant could be partly due to the retraction of the tissue during the histological preparations (figure 3.5.1.b).

3.5.2 Quantification analysis

Two cross sections (upper and lower) were cut from each of the 30 implants used in this study. Of a total of 60 sections only 47 sections were used for histomorphometric analysis. Of these sections 26 were from the upper section of each implant while the
remaining 21 were from the lower section. Thus of the 30 implants, 21 provided 2 sections and 5 provided only 1 section (summary of the total number of sections prepared for each treatment group is reported in table 3.5.1.a). The rest of the samples were lost during the preparation procedures. Mean bone/implant contact percentage (B/I %) values of first and second sections of each treatment group are summarized in Table 3.5.2.a and the mean values of all treatment groups were also graphed (figure 3.5.2.b).

The one-way analysis of variance (ANOVA) and Student-Newman-Keuls methods were used to find whether there was a significant difference between the experimental groups. The machined surface demonstrated to have significantly (p < 0.05) lower B/I % than the acid-etched or alkali/heat treated acid-etched surface. However, there was no significant (p > 0.05) difference found between the B/I % of the latter two experimental groups.

Table 3.5.1.a  Mean and median values of bone/implant contact percentages (B/I %) for both first and second sections. CM = control machined, CA = control acid-etched, A + NaOH/Heat = NaOH/Heat treated acid-etched surface.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CM</th>
<th>CA</th>
<th>A + NaOH/heat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples</td>
<td>14</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>Mean B/I % + Sd</td>
<td>30 ± 16.9</td>
<td>57.9 ± 22.7</td>
<td>52.3 ± 27</td>
</tr>
<tr>
<td>Median B/I %</td>
<td>35.2</td>
<td>53</td>
<td>59</td>
</tr>
</tbody>
</table>
3.6 Discussion and Conclusion

It has been suggested that implant surface chemistry (Davies 1998) and topography (Piattelli, et al. 1998) influence the biological phenomenon of osteoconductivity. Calcium phosphate-based materials are thought to represent the most suitable chemistry for potentiating osteoconduction. Based on that line of thought Yan et al. (1997) and Nishiguchi, et al. (1999) hypothesized that the increase of bone juxtaposition to alkali/heat treated titanium implant surfaces vs. non-treated controls was because of their ability to induce calcium phosphate formation on their surfaces. Those authors had investigated the bony response on alkali/heat treated vs. untreated machined surfaces. We have shown that the machined surface before alkali/heat treatment has considerably lower surface roughness and surface area compared to after treatment. Therefore, there were physical changes that were necessary to be considered along with changes of the surface chemistry.

Examining the three surface types, machined, acid-etched and alkali/heat treated acid-etched employed in this experiment revealed that the physical changes such as average surface roughness (Ra) and surface area (SA) are important factors in determining the osteoconductivity of a surface. The acid-etched surface with higher Ra and SA values demonstrated to be more osteoconductive than machined surface, it is important to notice that there was no significant difference in the chemical constituents of those surfaces. An increase in surface roughness can provide the surface with the ability of sustaining its relation with fibrin network during cell migration and subsequently facilitating bone formation on the implant surface (Davies, 1998). Comparing the acid-etched surface before and after alkali/heat treatment it was found that the Ra value did
not change significantly however the SA value was significantly increased after the treatment. An increase in surface area was expected to provide more sites for possible interactions of the surface with its biological surroundings. However, it was found that osteoconductivity of the acid-etched surface did not change after the alkali/heat treatment. Considering the latter results and conflicting reports in the literature with regard to the importance of surface roughness in increasing osteoconduction (Buser, et al. 1991a and Piatteli, et al. 1998) or it is not (Buser, et al. 1991b and Vercaigne, et al. 1998), it can be suggested that there are optimum levels for Ra and SA values to increase so that they can make a difference in osteoconductivity of the material.

Wennerberg, et al. (1997) reported better bone response at surfaces with average roughness of 1.5 μm. However, the system used by those authors in determining the surface profile parameters such as average roughness was not accurate (discussed in section 2.3). In this study we demonstrated that the optical profilometry was a suitable method for determining the parameters of the surface profile of the machined and acid-etched samples. Therefore, the optimum suggested values for Ra and SA were found to be approximately 0.4 μm and 0.9 μm² respectively.

The results of this study were in agreement with the observation reported by Yan and Davies (1998) that the chemical changes of the surface after alkali/heat treatment do not play any role in its osteoconductivity and demonstrated that the acid-etched surfaces can provide a suitable surface for facilitating the migration of the osteogenic cells to the implant surface.
Figure 3.5.1.a Light microscope images of the histological sections of the retrieved samples. The bone tissue surrounding the machined implant was separated from the implant surface at most regions of the implant perimeter. The bone tissue surrounding the alkali/heat treated implant retained its contact at most regions of the implant surface perimeter.

1) Machined implant at x50 mag.

2) Machined implant at x120 mag.

3) Alkali/heat treated acid-etched implant at x50 mag.

4) Alkali/heat treated acid-etched implant at x120 mag.
Comparing the LM images of the acid-etched samples with their corresponding BSI demonstrated that the separation of the bone tissue surrounding the machined or implant could be partly due to the retraction of the tissue during the histological preparations.

1) LM image of the acid-etched sample

2) BSI of the acid-etched sample
Figure 3.5.2.a. The bar graph representing the mean values of B/I contact percentage of all treatment groups.
$M = \text{Machined}$

$A = \text{Acid-etched}$

$A + \text{NaOH} = \text{Acid-etched + Alkali/Heat}$
4. Bone Bonding

Currently, the majority of implants in the orthopedic and dental fields are made from titanium and its alloys. Different methods such as push-out (Wong, et al. 1995 and Li, et al. 1997) and torque removal (Carlsson, et al. 1988; Branemark, et al. 1997; Buser, et al. 1998 and Buser, et al. 1999) measurements have been used to demonstrate that implant surface topographical features play an important role in their anchorage in bone.

These tests are considered to be representative of shear strength at the bone-implant interface. However, if the surface has rough features, these test results would be influenced by surface roughness and not represent the true bonding at the interface (Lin, et al. 1998). Tensile testing is suggested to overcome the effect of surface roughness and represent the bonding strength at the bone-implant interface (Lin, et al. 1998). Tensile tests have been used to measure bond strength between a variety of materials and the bone tissue. Examples include different kinds of hydroxyapatite materials and bone (Chung, et al. 1997) or plasma-sprayed HA coated-titanium and bone (Lin, et al. 1998). Kokubo's group have extensively used this method in determining the bond strength of alkali-treated titanium implant and bone (Nakamura, et al. 1995; Yan, et al. 1997; Nishiguchi, et al. 1997; Yan, et al. 1997 and Nishiguchi, et al. 1999). They found 1) a significantly greater force was required to detach bone from alkali/heat treated titanium compared with an untreated controls, and 2) a calcium phosphate rich layer was formed on treated surfaces either when soaked in simulated body fluid (SBF) or when they were implanted directly in vivo.

Since it has been thought that 'bioactive' materials are 'bone bonding' as a result of chemical bonding between bone and calcium phosphate surface of the implant, this
chemical bonding theory has been also adapted to explain biological bonding to alkali treated titania. However, little attention has been paid to changes in implant surface topography created during the alkali/heat treatment or to how these features influence bone-bonding ability. Some authors (Wen, et al. 1998 and Nishiguchi, et al. 1999) did report that a microporous surface was formed on alkali/heat treated titanium implants. However, they dismissed the importance of surface topographical features in light of the prevailing chemical bonding theories of bone-bonding.

Nevertheless surface features, such as macroporosity in titanium based implants (Pilliar 1986) or microporosity in bioactive ceramic materials such as hydroxyapatite, have been shown to influence the bone-bonding properties of the materials (Ripamonti 1991 and Dziedzic, et al. 1996). Thus, the purpose of the present work was to study the mechanism by which bone interacts and bonds with alkali treated titania.

4.1 Materials and Methods (Pilot Study)

4.1.1 Implant Model:

Custom-made, miniature rectangular plate implants (5 x 3 x 1 mm) manufactured from commercially pure titanium (cpTi 99.5%) were supplied by 3i as machined samples. Two notches were made on both ends of each plate using a straight handpiece drill. The plate was secured at the implant site using a circumferential femoral ligature while engaged in the implant notches (figure 4.1.1.a).
4.1.2 Sample Preparation:

Eight implants were sonicated three times in a 2% Decon solution for 5 minutes and dried at 40°C for 1 hour. The samples were then divided into two groups and received one of the following treatments:

1. Control group; no treatment
2. NaOH/heat-treated group; soaked individually in 2 ml of 10M NaOH aqueous solution at 60°C for 24 hours, washed with DDH2O and air-dried at 40°C for one hour. Samples were placed in an aluminum boat and heated at 24 C/min to 600°C in an electric furnace. They were kept at the given temperature for 1 hour and cooled to room temperature.

All implants were individually placed in sterilization bags, sealed and were sterilized by \(\gamma\)-irradiation (2.5 MRads).

4.1.3 Animal Model and Implant Site:

Implants were placed transcutically (anterior/posterior) into the mid-diaphyses of both femora of 300-gram male Wistar rats. Approximately 1 mm of the implant extruded out of the bone anteriorly and posteriorly to enable engagement of the circumferential ligature.

4.1.4 Surgical Procedure:

Sterile surgical conditions and inhalation anaesthesia (fluorothane in nitrogen and oxygen 500 ml flow rate; 5% induction, 2% maintenance) were used on rats for all surgeries. All implants were sterilized by \(\gamma\)-irradiation prior to use.
Each rat leg was shaved and cleaned (10% Bentadine). An incision was made along the long axis of each lower limb and the underlying skin was freed from the muscle. The muscles were gently separated and the periosteum of the anterior femur was incised and reflected. The plates were implanted using the drilling guide (designed by Dr. J. Davies and fabricated by 3i) and end/side-cutting bur (supplied by 3i) in both mid-diaphyses of rat femora. All drilling was done using a right-angle hand-piece drill with copious saline irrigation. The implants were pressed into place and secured by tying the resorbable gut ligature around the bone and implant through the implant notches. The muscle and skin layers were closed using tapered needle 3/0 Vicryl and surgical staples respectively. Following a two-week experimental period, the animals were killed and the femoral bones were dissected.

4.1.5 Sample Preparation for Mechanical Testing

Samples were trimmed to the width of the plate such that only the plate was separating the anterior wall from the posterior wall of the diaphysis (figure 4.1.5.a). The trimmed specimens were stored in buffered solution overnight and were subjected to detachment testing using uniaxial tensile testing machine (Instron 8501 Servo-Hydraulic). The second halves of the bone with the attached plates left from the detachment test were processed for SEM. They were fixed in 10% formaline aldehyde for 24 hrs and dehydrated in graded ethanol solutions (70%, 95%, and 100%). Samples were platinum-coated to prevent charging.
4.1.6 Sample Assembly for Mechanical Testing

A fixture attached to the uniaxial tensile testing machine (Instron 8501 Servo-Hydraulic) held the plate implants. Trimmed specimens had a loop of nylon fishing line passed through the space between plate and cortical bone of one side (the nylon line was placed as centered as possible through either anterior or posterior side), and were hung on a hook which also was attached to the testing machine (figure 4.1.6.a). Traction, through the nylon line looped around the bone was applied vertically to the implant surface at a crosshead speed of 30 mm/min. The detaching failure load was recorded once the bone became separated from the plate. If the plate was loose in the bone prior to the test, the failure load was defined as 0 kilogram force (kgf).

4.2 Results

Two alkali/heat treated samples and two of the control samples were excluded from the experiment due to either fracture or inappropriate (off-center) placement during implantation. The rest of the control samples, which were included in the test, were loose in the bone prior to processing for the detachment test. Therefore, their failure load was defined as 0 kgf. The forces required to separate the bone tissue from the two remaining alkali/heat treated plates were 1.938 kgf and 1.772 kgf, respectively (or estimated about 1.6 and 1.5 N/mm²).

The alkali/heat treated implant surfaces were examined after the detachment test, using Scanning Electron Microscopy (SEM). It was demonstrated that 1) cohesive failure was occurring within bone tissue; 2) medullary surface of the implant was covered by new bone, clearly identified by the interwoven collagen matrix containing osteocyte
lacunae; 3) in the central area there was evidence of considerable resorption of the new bone, which seemed to expose the underlying implant surface; 4) it was also evident that the collagen compartment of the bone was separated from the implant surface by a continuous sub-micron thick layer comprised of individual, fused globules of the cement line matrix and 5) the globules of the forming cement line were seen to interdigitate with the microtopography of the metal oxide surface (figures 4.2.a,b,c,d,e).

4.3 Discussion and Conclusion

In this study, the bony reaction to the machined titanium implants with and without alkali/heat treatment was observed. The detachment test clearly demonstrated that bone bonds to alkali/heat treated samples. This confirms the observations reported by Kokubo’s group (Yan, et al. 1997; Nishiguchi, et al. 1997; Yan, et al. 1997; Nishiguchi, et al. 1999). However, these groups did not examine the surface of the implants after the detachment test. Our examination of the implant surfaces after the detachment test using SEM revealed, for the first time, that the cement line (Davies, et al. 1991a,b) matrix of the newly formed bone interdigitates with the microporosity of the alkali/heat treated surfaces. This observation does not support the theory, proposed by earlier studies, of chemical bonding between bone and alkali/heat treated implants.

Detachment testing was used by Kokubo's group and in this study. It is defined as a measure of “bond strength” between two materials (Steinemann 1996). “Bond strength” is reported to be influenced by either the basic adhesion phenomena related to atomic and molecular structure of the material at the interface (in other words the chemical interactions of the two bonding matrices), or the structural characteristics of the
bonding surfaces, and sometimes both (Mittal 1976). Therefore, the detachment force measured by tensile testing for bone-bonding phenomenon between alkali/heat treated titanium implant and bone cannot solely rely on chemical bonding as suggested by Kokubo’s group. A change in surface structure and its influence on bone bonding, which was clearly demonstrated in this study, also has to be taken into consideration.

4.4 Recommendations for the Main Study

A large number of implants (7 out of 12) were excluded prior to detachment testing either due to femoral fracture or inappropriate (off-centre) implantation site. To overcome these problems following revisions were considered:

1) Larger rat size, so its femoral bones can withstand the stress caused by the implantation

2) Small implant size and different design, so to minimize the possibility of femoral fracture and can hold a longitudinal hole within to facilitate implant stabilization

3) Shorter implantation period, so less bone remodeling would occur
Figure 4.1.1.a Diagram of the custom-made, miniature rectangular plate implant model used for pilot bone-bonding experiment. Arrow is pointed to the notches made on both ends of the plate to hold securing ligature.
Figure 4.1.5.a  Diagram of the prepared sample for the detachment test. Bone tissue was trimmed to the width of the plate such that only plate was separating the anterior wall from the posterior wall of the diaphysis.

[Diagram with labels: Posterior wall of diaphysis, Anterior wall of diaphysis, Plate implant, Notches]
Figure 4.1.6.a  Diagram of the fixture used to hold the trimmed samples for the detachment test.
Figure 4.2 Scanning electron micrographs of alkali/heat treated implant surfaces after the detachment test in pilot study demonstrating,

a) The cohesive failure occurring within the bone tissue attached to the implant surface.

b) The newly formed bone covering the medullary surface of the implant. Arrow is pointing to the interwoven collagen matrix.

c) The considerable resorption of the new bone formed on implant surface. Arrow is pointing to the intact underlying implant surface topography.

d) The collagen compartment of the new bone formed was separated from the implant surface by a continuous sub-micron thick layer of the cement line matrix.

e) The globules of the forming cement line were interdigitating with the microtopography of the implant surface.
Collagenous bone matrix
Figure 4.2 continued
Figure 4.2 continued
4.5 Materials and Methods (Main study)

4.5.1 Implant Model

Custom-designed rectangular cpTi (99.5%) plate implants (4.0 x 2.5 x 1.3 mm, containing a 0.70 mm longitudinal hole, figure 4.5.1.a) were designed in the lab and supplied by Implant Innovations Inc. (3i), FL. These implants were smaller than the ones in the pilot study (4.0 x 2.5 mm vs. 5.0 x 3.0 mm) to minimize the possibility of femoral fractures. However, the thickness of the implants was increased slightly to enable a longitudinal hole to be included in order to facilitate implant stabilization during the surgery (figure 4.5.1.a). Half of the implants were acid-etched, according to the procedure employed for Osseotite™ dental implants and the rest were machined.

4.5.2 Sample Preparation

Twenty-four implants were sonicated in a 2% Decon solution for 5 minutes and dried at 40 °C for 1 hr. Each surface type was divided into two groups as follows:

1. Control group; no treatment
2. NaOH/heat-treated group; soaked individually in 2 ml of 10M NaOH aqueous solution at 60°C for 24 hours, washed with DDH2O and air-dried at 40°C for one hour. Samples were placed in an aluminum boat and heated at 24 C/min to 600°C in an electric furnace. They were kept at the given temperature for 1 hour and cooled to room temperature.

All implants were individually placed in sterilization bags, sealed and were sterilized by γ- irradiation (2.5 MRads).
4.5.3 Animal Model and Implant Site:

To find the appropriate rat size, the femoral bones of 45 male Wistar rats, of different weight groups were dissected and measured at two sites of, 1) about 6mm from the knee joint and 2) mid femur. At each site two diameters of lateral-medial and anterior-posterior were measured (Table 4.5.3.a). Mean values for each weight group were calculated and plotted vs. the corresponding weight group (figure 4.5.3.a).

There were no significant variations in the obtained mean values. Keeping in mind the unequal number of femoral bones analyzed in each weight group (due to availability), the diameters of femoral bones at both analyzed sites remained very much the same through the weight range examined.

Male Wistar rat (325-350 grm) was selected since these animals were young for bone-formation activity but also had an appropriate femoral size with A-P mid-femur diameter of 3.33 ± 0.25 mm. Implants were placed transcortically (anterior/posterior) into the mid-diaphyses of both femora.
Table 4.5.3.a. Mean values for Wistar rat femoral bone diameter at two different sites of Lateral-Medial (L-M), and Anterior-Posterior (A-P).

<table>
<thead>
<tr>
<th>Weight</th>
<th>Sample Number</th>
<th>6 mm from knee joint</th>
<th>Mid-femur</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>L-M ± sd (mm)</td>
<td>A-P ± sd (mm)</td>
</tr>
<tr>
<td>301-325</td>
<td>2</td>
<td>5.4</td>
<td>4.0</td>
</tr>
<tr>
<td>326-350</td>
<td>2</td>
<td>5.5 ± 0.07</td>
<td>3.95 ± 0.21</td>
</tr>
<tr>
<td>351-375</td>
<td>10</td>
<td>5.8 ± 0.25</td>
<td>4.3 ± 0.22</td>
</tr>
<tr>
<td>376-400</td>
<td>10</td>
<td>5.9 ± 0.3</td>
<td>4.6 ± 0.33</td>
</tr>
<tr>
<td>401-425</td>
<td>4</td>
<td>5.7 ± 0.1</td>
<td>4.35 ± 0.24</td>
</tr>
<tr>
<td>426-450</td>
<td>2</td>
<td>6.05 ± 0.21</td>
<td>4.4 ± 0.25</td>
</tr>
<tr>
<td>451-475</td>
<td>4</td>
<td>6.43 ± 0.22</td>
<td>4.65 ± 0.21</td>
</tr>
<tr>
<td>476-500</td>
<td>10</td>
<td>5.9 ± 0.24</td>
<td>4.05 ± 0.23</td>
</tr>
<tr>
<td>501-525</td>
<td>10</td>
<td>6.05 ± 0.2</td>
<td>4.4 ± 0.19</td>
</tr>
<tr>
<td>526-550</td>
<td>10</td>
<td>6.05 ± 0.26</td>
<td>4.23 ± 0.23</td>
</tr>
<tr>
<td>551-575</td>
<td>10</td>
<td>6.35 ± 0.2</td>
<td>4.43 ± 0.16</td>
</tr>
<tr>
<td>576-600</td>
<td>10</td>
<td>6.45 ± 0.3</td>
<td>4.63 ± 0.37</td>
</tr>
<tr>
<td>601-625</td>
<td>4</td>
<td>6.6 ± 0.5</td>
<td>4.83 ± 0.38</td>
</tr>
</tbody>
</table>
4.5.4 Surgical Procedure:

Sterile surgical conditions and inhalation anesthesia (fluorothane in nitrogen and oxygen 500 ml flow rate; 5% induction, 2% maintenance) were used on the rats for all surgeries.

Each rat leg was shaved and cleaned (10% Betadine). An incision was made along the long axis of each lower limb and the underlying skin was freed from the muscle. The muscles were gently separated and the periosteum of the anterior femur was incised and reflected. An end/side cutting bur (1.4 mm diam., supplied by Brasseler) was used to prepare an anterior-posterior channel in the mid-diaphyses of the rat femora. All drilling was done using a right-angle handpiece drill with copious saline irrigation. The implants were pressed into place and secured in the bone by passing a degradable suture through the hole within the implant and tying it around the bone. The muscle and skin layers were closed using tapered needle 3/0 Vicryl and surgical staples, respectively (figures 4.5.4.a,b).

The animals were killed following a ten-day experimental period and the femoral bones were dissected.

4.5.5 Sample Preparation for Mechanical Detachment Test

Since considerable resorption of newly formed bone was observed in the pilot study, implants were retrieved from animals after a shorter experimental period (10 vs. 14 day). Samples were trimmed to the width of the plate such that only the plate was separating the anterior wall from the posterior wall of the diaphysis. The trimmed
specimens were stored in buffered solution for a short time (about 3-4 hrs) and were immediately subjected to the detachment test. The surface from which bone was separated was prepared for SEM observations.

4.5.6 Sample Assembly for Mechanical Detachment Test

A fixture attached to the uniaxial tensile testing machine (Instron 8501 Servo-Hydraulic) held the plate implants. Trimmed specimens had a loop of fishing line passed through the space between the plate and cortical bone of one side (the nylon line was placed as centered as possible through either anterior or posterior side), and were hung on a hook which also was attached to the testing machine. Traction through the fishing line looped around the bone was applied vertically to the implant surface at a crosshead speed of 30 mm/min. The detaching failure load was recorded once the bone separated from the plate. If the plate was loose in the bone prior to the test, the failure load was defined as 0-kilogram force (kgf).

4.6 Results

The results of this experiment are shown in Table 4.6.a. There was no force required to detach the bone from the machined or acid-etched implants. The difference between these groups was in the number of the implants, which were loose prior to processing for the detachment test, and the number of implants which became loose during processing. The machined samples demonstrated a higher percentage of loosening prior to processing when compared to the acid-etched samples (57 % vs. 33%). The detachment force required increased significantly after the alkali/heat treatment of the
samples in both surface types. The acid-etched samples required an average of 1.17 kgf vs. 0.9 kgf (or estimated about 1.5 and 1.2 N/mm²) for machined samples.

Table 4.6.a The results obtained for main detachment test.

<table>
<thead>
<tr>
<th></th>
<th>Machined</th>
<th>Acid-etched</th>
<th>Machined NaOH</th>
<th>Acid-etched NaOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (implant)</td>
<td>8</td>
<td>5</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>N (fractured)</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>N (recovered without fracture)</td>
<td>7</td>
<td>3</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>N (loose in bone)</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N (loosen during processing)</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>N (lost during processing)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>N (tested)</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Range of forces (Kgf) required for detachment</td>
<td>0</td>
<td>0</td>
<td>0.74 – 1.07</td>
<td>0.82 – 1.38</td>
</tr>
<tr>
<td>Mean kgf required to detach the bone from the implant</td>
<td>0</td>
<td>0</td>
<td>0.91 ± 0.14</td>
<td>1.17 ± 0.3</td>
</tr>
</tbody>
</table>

SEM images prepared from the alkali/heat treated implant surfaces after detachment demonstrated similar results to those observed in the pilot study. It was shown that 1) cohesive failure had occurred within the bone tissue; 2) cement line of the newly formed bone had interdigitated with surface microporosity; and 3) resorption of new bone had started. It also seemed that in scattered areas of the implant surface the oxide layer was separated from the implant surface. That may occur due to different events such as cohesive failure within the microporous oxide layer, perhaps it could originate from the cracked regions, or its detachment from the implant surface. However,
to determine the reason for such observation requires further investigations (figure 4.6.a, b, c, d, e).

SEM images of the untreated implant surfaces after the detachment test demonstrated that adhesive failure had occurred at the bone-implant interface (figure 4.6.f).

4.7 Discussion and Conclusion

The result of this experiment confirmed the earlier observations from the pilot study. Alkali/heat treated surfaces required significantly higher detachment forces compared with untreated surfaces. However, the values obtained for the forces required to detach the bone from the implant surface were comparatively lower than the values obtained in the pilot study. That may occur due to reasons such as 1) shorter experimental time period, 2) smaller implants, or 3) non-vertical loading (the nylon line could be placed off-center). It was also shown that the cement line of newly formed bone had interdigitated with microporosity of treated surfaces.

The acid-etched surface was included in this study to examine the effect of primary surface roughness and increased surface area on bone-bonding phenomena. Implant design and dimension were kept the same between different surface treatments to limit discriminating factors between the experimental groups. Push-out force (Wong, et al. 1995; Li, et al. 1997) and torque removal force (Carlsson, et al. 1988; Branemark, et al. 1997; Klokkevold, et al. 1997; Buser, et al. 1998a,b) measurements have been used repeatedly to demonstrate integration of roughened surfaces in bone compared with less rough, smooth surfaces. The acid-etched surface also has been shown to have a significant increase in torque removal value compared with the machined surface
(Klokkevold et al. 1997). However, in this study using a tensile test we demonstrated that there was no significant increase in the force required for detaching bone from these surfaces compared with machined surfaces.

These results led us to conclude that surface roughness is not a factor in the bone bonding property of a material, but it is the microporosity on the surface that participates in the micromechanical interlocking phenomenon with bone tissue.

SEM images prepared in this study from implants subjected to a detachment test also demonstrated isolated areas where the surface oxide layer was separated from the implant surface. The reason for such observation could be due to different events such as the cohesive failure within microporous oxide layer or its detachment from the primary underlying titanium oxide layer on the implant. What is important to consider is the long-term surface stability of alkali/heat treated cpTi as an endosseous implant material.
Figure 4.5.1.a Scanning electron micrograph of the custom-designed rectangular cpTi implant designed for the main detachment test. The arrow is pointing to the longitudinal hole within the plate.
Longitudinal hole
Figure 4.5.3.a  Bar-graphs representing the mean values for mid-femur region (anterior-posterior) of 45 male Wistar rats vs. their weight group.
Figure 4.5.4  Photographs of the plate implant placement during the main bone bonding experiment.

a) Plate implant was placed anterior-posteriorly in mid-diaphysis of the rat femur.

b) Plate implant was secured in place by passing a degradable suture through the hole within the implant and tying it around the bone.
Figure 4.6  Scanning electron micrographs of the alkali/heat treated implant surface after the main detachment test demonstrated,

a) The cohesive failure had occurred within the bone tissue attached to the implant surface.

b) The cement line of the newly formed bone was separating the collagen matrix from the implant surface.

c) The cement line of the newly formed bone had interdigitated with the surface microporosity of the treated implant surface.

d) The resorption of the new bone formed on the treated implant surface had started.

e) The corrosion of the oxide layer formed after alkali/heat treatment had occurred is scattered regions of the implant surface.
B = bone tissue, P = plate implant surface, C = collagenous matrix, CL = cement line matrix
B = bone tissue, CL = cement line matrix, R = resorption region

Figure 4.6 continued
Figure 4.6 continued
Scanning electron micrographs of the untreated plate implant surface after the main detachment test demonstrated,

f) The adhesive failure had occurred at the bone-implant interface.
5. **General Discussions**

In this study we examined both the phenomena of osteoconduction and bone bonding to custom-made commercially pure titanium implants, and the changes in these biological phenomena brought about by surface modifying the titanium using strong alkali/heat treatments. In the following sections we will discuss the topographical, physical and chemical features of untreated and treated surfaces which were analyzed in this study in relation to the results of the in vivo studies conducted for osteoconduction and bone-bonding properties of those surfaces.

5.1 **Osteoconduction**

Scanning Electron Micrographs of machined and acid-etched surfaces before and after alkali/heat treatment demonstrated the machined surface to be less rough than the acid-etched surface. The SEM observations were verified by using optical profilometry where the acid-etched surface showed a significantly higher surface roughness value. An increase in surface roughness is repeatedly suggested (Albrektsson, 1981; Buser, et al. 1991; Piatteli, et al. 1998) to influence bone-implant contact relation or in other words osteoconduction. That is in agreement with our results from the in vivo experiments, where the acid-etched surface demonstrated significantly higher osteoconduction than the machined surface.

A microporous layer covered both surface types after alkali/heat treatment as also reported by Wen, et al. (1998) and Nishiguchi, et al. (1999). The treated surfaces appeared rougher than untreated. However, the surface roughness values obtained by OP demonstrated a significant increase for only treated vs. untreated machined surfaces and
not for acid-etched surfaces. This might be the result of the lateral resolution limitations of the OP apparatus. However, the results of our osteoconduction experiment also revealed that there was no significant difference in osteoconductivity on untreated vs. alkali/heat treated acid-etched surfaces. It is obvious, from the SEM results, that there is an increase in surface roughness at submicron level after alkali/heat treatment of the acid-etched surfaces although, as mentioned above, not detected by optical profilometry. Indeed, Murray, et al. (1989) and Vercaigne, et al. (1998) suggested that increase in surface roughness might have a negative effect on osteoconduction. These seemingly conflicting results may point to a threshold level of surface roughness up to which osteoconduction is enhanced, and beyond which osteoconduction is diminished.

There was a significant increase in both parameters of surface area and surface wettability after alkali/heat treatment of the machined and acid-etched surfaces. It was reported that increase in surface area might increase the attachment of primitive connective tissue or fibrin (Davies 1998), which would promote osteoconduction. Increase in wettability had also been reported (Van Kooten, et al. 1992 and Wen, et al. 1998) to favor cell attachment, which again would facilitate osteoconduction. However in both cases the optimum values had never been defined. In this study we observed no significant increase in osteoconductivity of the acid-etched surfaces after alkali/heat treatment. Here again these results may point to a threshold level of surface area which osteoconduction is enhanced and beyond which osteoconduction is diminished. There may also be a limit to usefulness of increasing surface wettability level.

In addition to changes in surface topography, the chemical nature of both machined and acid-etched surfaces prior to, and after, alkali/heat treatment were analyzed
using the X-ray Photoelectron Spectroscopy (XPS). The results showed that there was no significant difference in the chemical constituents of the machined and acid-etched surfaces. However both of those surfaces were changed after alkali/heat treatment. In addition to sodium ion inclusion into the surface oxide layer, the relative amount of the metal oxide species also was increased. It was reported (Yan, et al. 1997 and Nishiguchi, et al. 1999) that bone juxtaposition to such titanium surfaces was increased due to the chemical treatment that induces the formation of bone-like apatite layer on titanium implants. It is also thought that the formation of an apatite layer on the implant surface is positively correlated to osteoconduction (Ono, et al. 1990). However, Yan and Davies (1998) conducted a qualitative examination of the bone/implant interface of alkali/heat treated and untreated cpTi implants and reported that topographical changes on implant surfaces, rather than changes in surface chemistry were responsible for the increase in observed osteoconduction. The results of the osteoconduction experiment in this study were in agreement with the importance of surface topography rather than chemistry of alkali/heat treated surface. However those authors did not show whether or not the type of topography or the morphology of the surface would influence this biological response. In this study we demonstrated that the type of the implant surface topography was not important as long as the surface features are such that it can facilitate osteogenic cell migration towards the implant surface. The acid-etched surface in both untreated and treated form proved to be equally osteoconductive despite the changes in its surface topography and chemistry.
5.2 Bone-bonding

Recently many researchers (Nakamura, et al. 1995; Chung, et al. 1997; Yan, et al. 1997; Nishiguchi, et al. 1997; Lin, et al. 1998 and Nishiguchi, et al. 1999) have favored the tensile test over torque removal or push out test to measure the bond strength between different biomaterials and its adjacent bone tissue. Where bone tissue can grow into irregularities of the rough surface and resist the force of the torque removal or push out test and surpass the true bond strength of the interface the tensile testing is suggested not to be influenced by surface roughness (Lin, et al. 1998). Therefore, a tensile test was also chosen in this study to quantify the bond strength of alkali/heat treated and untreated cpTi implants. In addition the detached surfaces of the implants were examined to understand the mechanism underlying bone bonding.

It was reported by Kokubo’s group (Miyaji, et al. 1994; Kim, et al. 1996; Yan, et al. 1997 and Wen, et al. 1998) that alkali/heat treatment of the titanium based implants render their surfaces bone bonding. In this study also it was shown that a significant force was required to detach the bone tissue from alkali/heat treated where no force was required for untreated titanium implant surfaces. As it was anticipated, the surface roughness present on the acid-etched surface did not play any role in bonding the bone tissue to the implant surface (since there are no undercuts in the surface) and did not influence the result of the tensile test. It has been reported that the acid-etched surface requires a significant torque removal force compared to machined surface (Klokkevold, et al. 1997).

Kokubo and his colleagues had also associated the bond strength, measured at alkali/heat treated titanium implant/bone interface, to the formation of an apatite layer on
the surface of the implant. Formation of such an active layer is thought by many researchers to be responsible for the chemical bonding of the material to the bone tissue (Hench, et al. 1982; Anderson, et al. 1988; Kitsugi, et al. 1989 and Hench, et al. 1991). Tensile tests are a measure of the “bond strength” between two materials (Steinemann 1996) and “bond strength” is influenced by chemical interactions of the two bonding matrices or the structural features of the bonding surfaces and sometimes both (Mittal 1976). Although in this study we were unable to demonstrate whether there was a chemical bonding between alkali/heat treated implant surfaces and bone tissue, we showed that the microporosity of the surface produced due to such treatment provides the possibility for bone tissue to bond to the surface of the implant by mechanical interlocking.

Examining the chemically treated implant surface that was detached from the bone tissue it was clearly evident that the cement line of the newly formed bone was interdigitating with the microporosity of the implant surface. Dziedzic et al. (1996) also reported such observation where they showed the cement line of the bone tissue was interdigitated with the microporosity the calcium phosphate ceramic employed, causing a cohesive failure in the material. They also reported an adhesive failure occurring with the dense calcium phosphate ceramic material, which represented the lack of bonding between the material and the bone tissue.

Considering the results of this study and what was reported by Dziedzic, et al. (1996), in addition to the fact that there has been no evidence presented to support the chemical bone-bonding hypothesis of calcium phosphate materials, we can strongly
suggest that the mechanism underlying the bone-bonding of the alkali/heat treated titanium implants is based on mechanical interlocking.
6. **Conclusions**

Several conclusions that can be drawn from the work described:

- Alkali/heat treatment of cpTi implant surfaces changes the physical, topographical and chemical features of that surface.

- Topographical features of the alkali/heat treated cpTi implant surfaces are the most important factor in rendering the implant surface osteoconductive and bone-bonding.

- The bone-bonding property of alkali/heat treated cpTi implant surfaces is based on mechanical interlocking of the newly formed bone with the microporosity of the surface.
7. **Future Directions**

The Optical Profilometer was shown in this study to be suitable for determining the profile parameters of the machined and acid-etched surfaces, however alkali/heat treatment provides the surface with features of a few nanometers in scale, which is lower than OP system’s highest lateral resolution. Therefore, for more accurate quantifications of the alkali/heat treated implant surface profile parameters, a system with higher resolution would be required.

Custom-made cylindrical mini-implants were used for osteoconduction experiment. They were cut in sagittal plane during the pilot study and coronal plane in the main study. In both cases we encountered the difficulty of sectioning. In future studies a more controlled method of embedding and sectioning of the samples would be required to keep the quantification system more consistence.

A study including dense and microporous calcium phosphate coated titanium implants, which provides a surface chemistry that is constantly reported to support chemical bonding with the bone tissue, would complement the findings of this study on the effect of surface microporosity on bone bonding regardless of the effect of surface chemistry.
References:


