Part II

Effect of various dental implant surfaces on the temporal expression of the osteoblastic phenotype *in vitro*

II/1 Introduction Part II

II/1.1 Introduction Study D

In recent years, the utilisation of endosseous implants for the rehabilitation of completely or partially edentulous patients has become a standard treatment modality in dentistry (Bornstein et al. 2003). Over the past two decades, numerous prospective long-term studies have documented a high efficacy and predictability of osseointegrated implants (Bornstein et al. 2003, Buser et al. 1997, Naert et al. 2002, Davarpanah et al. 2002, Sullivan et al. 2001, Friberg et al. 2000, Bahar 2000). Titanium is widely used as dental implant material, because direct contact occurs between bone and the implant surface (Brånemark et al. 1969, Schroeder et al. 1976, Ducheyne 1988, Davies 1996, 1998, 2000, Schenk & Buser 2000). Preferably, roughened surfaces like the titanium plasma-sprayed (TPS) surfaces have been used as the endosseous area of dental implants in order to increase the total surface area available for osseous apposition (Schenk & Buser 2000, Cochran 1999). In recent years, there has been the tendency to replace titanium plasma-sprayed surfaces by sandblasted and acid-etched surfaces in order to accelerate osseointegration (Bornstein et al. 2003, Davies 1998, Cochran 1999, Cochran & Buser 2000, Lazzara 2000).

Surface topography plays a critical role in the interaction of dental implants with adjacent tissues (Davies 1998, 2000, Schenk & Buser 2000). Many of the most important steps in the peri-implant healing cascade are profoundly influenced by implant surface microtopography (Davies 2000). *In vitro* studies have shown that microroughened sandblasted and/or acid-etched surfaces enhance platelet activation and aggregation and fibrin retention (Davies 1998, 2000, Park & Davies 2000). As a result a migratory pathway for the differentiating osteogenic cells to reach the implant surface is provided (Davies 1998, Park & Davies 2000). Furthermore, these microroughened surfaces enhance osteoblastic attachment, differentiation and matrix production and as well as their growth factor and cytokine production (Martin et al. 1995, Kieswetter et al. 1996, Boyan & Schwartz 2000, Lazzara 2000, Park & Davies 2000, Keller et al. 2003). Animal studies demonstrated an enhanced bone-implant contact and removal torque values with microroughened surfaces compared to smooth and TPS surfaces (Buser et al. 1998b, 1999b, Cochran & Schenk 1998, Cochran & Buser 2000, Cordioli et al. 2000, Lazzara 2000, Klokkevold et al. 2001). Clinical trials indicate high success rates after early loading (Lazzara et al. 1998, Testori et al. 2001, Bornstein et al. 2003). In addition, improved success rates have been reported in low quality bone, such as the posterior maxilla (Testori et al. 2001, Stach & Kohles 2003). Indeed, this aspect is important as, in general, clinical studies have demonstrated that these areas of the jaws where bone is extremely cancellous, such as the posterior maxilla, have a significantly lower success rate compared to areas of denser bone (Jaffin & Berman 1991, Hutton et al.

1995, Cune et al. 1996, Cochran & Buser 2000, Jansen et al. 2000). Therefore, another approach to improve osseous integration of dental implants in these anatomical regions has been the utilisation of calcium phosphate coated implants (Ducheyne 1988, Caulier et al. 1997, Keller 1998, Lacefield 1998, Vercaigne et al. 1998, Cochran 1999, Jansen et al. 2000), since these coatings have been found to accelerate initial stabilization of implants by enhancing bony ingrowth and stimulating osseous apposition to the implant surface (Ducheyne et al. 1980, 1990, 1992, Ducheyne 1987, 1988, Davies 1998, Caulier et al. 1997, de Groot et al. 1998, Keller 1998, Lacefield 1998, Vercaigne et al. 1998, Jansen et al. 2000, Ong & Chan 2000, Ong et al. 2002). Of the various calcium phosphates available, hydroxyapatite (HA) has been most commonly used for coating dental implants (Caulier et al. 1997, de Groot et al. 1998, Keller 1998, Lacefield 1998, Vercaigne et al. 1998, Jansen et al. 2000, Ong & Chan 2000, Ong et al. 2002). Although there are various techniques with attractive features available for producing HA coatings (Ong & Chan 2000), plasma-spraying has been studied most extensively and is still the most frequently applied technology for producing hydroxyapatite coatings on dental implants (Lacefield 1998, Cochran 1999, Ong & Chan 2000). Furthermore, recent clinical trials have shown higher long-term success rates for HA-coated implants compared to machined titanium implants (Jeffcoat et al. 2003) as well as an accelerated initial rate of osseointegration (Geurs et al. 2002). However, there are no clinical data available comparing dental implants with HA-coated surfaces to these with sandblasted and/or acid-etched titanium surfaces.

In order to cause more abundant and more expeditious bone formation at the bone-implant interface a dental implant surface has to possess the ability to enhance cell differentiation of osteogenic cells at it's surface. The use of *in vitro* osteogenic cell cultures has proven valuable for studying the biological reactions to dental implant surfaces (Davies 1996, Keller 1998). Quantitative evaluation of osteogenic proteins and their respective mRNAs in osteoblasts grown on different biomaterials facilitates gaining insight into the effect of endosseous implant materials on osteoblastic cell differentiation (Zreiqat & Howlett 1999). This is related to the *in situ* hybridization and immunocytochemical techniques which permit quantitative study of the expression of markers of the osteoblast phenotype (Zreiqat et al. 1996). Thus, valuable information concerning the osteogenic capacity of endosseous implant materials can be generated (Zreiqat & Howlett 1999).

Study D reports on the effect of two roughened titanium dental implant surfaces (an acid-etched and sandblasted surface and a TPS surface) on the osteoblastic phenotype of human bone-derived cells (HBDC) and compares these observations to those for cells on an HA-coated surface. Smooth machined titanium surfaces served as control.

II/1.2 Introduction Study E

Calcium phosphate coated titanium and titanium alloy are widely used as orthopaedic (Lemons 1988, Ducheyne et al. 1990, de Bruijn et al. 1994, Dhert 1994, Denissen et al. 1996, Ducheyne 1998, Keller & Dollase 2000, MacDonald et al. 2001, Geesink 2002, de Groot et al. 2002, Sun et al. 2002, Yan et al. 2003) and dental implant materials (Ducheyne 1988, Denissen et al. 1991, 1996, Kay 1992, Burke & Lucas 1998, Gross et al. 1998, Keller 1998, Burgess et al. 1999, Jansen et al. 2000, Ong et al. 2002). These coatings have been found to accelerate initial stabilization of implants by enhancing bony ingrowth and stimulating osseous apposition to the implant surface, promoting a rapid fixation of the devices to the skeleton (Ducheyne 1988, 1998, Lemons 1988, Ducheyne et al. 1990, Kay 1992, de Bruijn et al. 1994, Dhert 1994, Denissen et al. 1991, 1996, Burke & Lucas 1998, Gross et al. 1998, Keller 1998, Burgess et al. 1999, Gineste et al. 1999, Jansen et al. 2000, MacDonald et al. 2001, Sun et al. 2001, Geesink 2002, de Groot et al. 2002, Ong et al. 2002). Hence their use as coatings of the endosteal portions of implants. Of the various calcium phosphates available, hydroxyapatite (HA) has been most commonly used as coating for titanium and its alloy. Techniques for applying calcium phosphate coatings include plasma-spraying (Ducheyne 1988, 1998, Lemons 1988, Ducheyne et al. 1990, Denissen et al. 1991, 1996, Kay 1992, Radin & Ducheyne 1996, de Bruijn et al. 1994, Dhert 1994, Gross et al. 1998, Burgess et al. 1999, Gineste et al. 1999, Keller & Dollase 2000, MacDonald et al. 2001, 2002, Sun et al. 2001, Geesink 2002, de Groot et al. 2002, Ong et al. 2002, Yan et al. 2003) and biomimetic deposition techniques (Wen et al. 1998, de Groot et al. 2002, Barrere et al. 2003), electrophoretic deposition (Ducheyne et al. 1996), electrochemical deposition (Lin et al. 2003, Roessler et al. 2003), laser-pulsing (Garcia et al. 1998), magnetron sputtering (Jansen et al. 1993, 2000, Vercaigne et al. 2000a, 2000b), ion beam sputtering (Zeng et al. 1999) and sol-gel forming (Nguyen et al. 2004). Although all technologies have attractive features, plasma-spraying has been studied most extensively and is still the most frequently applied technology for producing hydroxyapatite coatings on metallic implants. However, a drawback of plasma-spraying is the thermal instability it subjects the HA powder to. The plasma-sprayed HA coatings may have different crystallinities (Radin & Ducheyne 1992, Burke & Lucas 1998, Gross et al. 1998, Jansen et al. 2000, Keller & Dollase 2000, Sun et al. 2001, 2002, Yan et al. 2003). This is directly related to their dissolution, this being higher for less crystallization (Radin & Ducheyne 1992, Burke & Lucas 1998, Jansen et al. 2000, Keller & Dollase 2000, Sun et al. 2001, 2002, Yan et al. 2003). Thus, heat treatments are often applied to increase the crystallinity (Burke & Lucas 1998).

This, however, may result in stresses between the coating and the underlying titanium alloy due to the mismatch in thermal expansion coefficient.

Novel calcium titanium and calcium titanium zirconia orthophosphates have been synthesized from $CaTi_4(PO_4)_6$ (CTP) and $CaZr_4(PO_4)_6$ (CZP) (Lugscheider et al. 1995, Berger et al. 1996, Ploska & Berger 1997). CTP and CZP are known to be biocompatible and to bond directly to bone (Smukler-Moncler et al. 1992). Recently novel compositions in this system $CaTi_xZr_{4-x}(PO_4)_6$ with x= 0-4 were described and characterized with regard to solubility (Lugscheider et al. 1995, Berger et al. 1996, Ploska & Berger 1997). These studies showed that these novel calcium titanium and calcium zirconium phosphate ceramics are less soluble than HA (Ploska & Berger 1997). Moreover, these compounds are suitable for plasma-spraying onto titanium substrata and have a thermal expansion coefficient similar to that of titanium and titanium alloy (Lugscheider et al. 1995, Berger et al. 1996, Ploska & Berger 1997). Thus heat-treatment of the sprayed coatings can be applied to increase the crystallinity without creating stresses between the coating and the underlying titanium substrata.

The use of *in vitro* osteogenic cell cultures has proven valuable for biological testing of endosseous implant materials (Davies 1996, Keller 1998). Quantitative evaluation of osteogenic markers in osteoblasts grown on different biomaterials facilitates gaining insight into the effect of endosseous implant materials on osteoblastic cell differentiation (Zreiqat & Howlett 1999). Thus, valuable information concerning the osteogenic capacity of candidate implant materials can be generated (Zreiqat et al. 1996, Zreiqat & Howlett 1999). These *in vitro* assays are useful for screening novel bioactive coatings, because bioactive calcium phosphate ceramics for use in bone regeneration ideally possess the ability to activate bone formation and, thus cause the differentiation of bone marrow stromal cells at their surfaces (Ohgushi et al. 1990, Davies 1996, Ducheyne & Qui 1999).

Study E reports on the effect of various novel bioactive calcium titanium and calcium titanium zirconium orthophosphates on the osteoblastic phenotype of human bone-derived cells (HBDC) and compares these observations to those for cells on implant materials already clinically used, i.e. HA-coated titanium and porous titanium surfaces.

II/2 Materials and Methods

II/2.1 Test Materials

II/2.1.1 Test Materials Study D

All titanium substrates were made from commercially pure titanium (cp Ti) grade 2 (ASTM-F67). The materials were titanium (Ti) discs of 10 mm in diameter and 2 mm thick. Four implant surfaces were examined in the present study. The first test material was a roughened titanium surface with deep profile structure (material denominated: Ti-DPS). This surface configuration was produced applying an acid-etch technique (combination of anorganic acids) to the grit-blasted surfaces. Blasting was performed using Al_2O_3 particles (grain size 300-600 µm). Two surfaces were created from Ti discs receiving a porous titanium plasma-sprayed coating (samples denominated: Ti-TPS), or a plasma-sprayed porous hydroxyapatite coating (surfaces denominated HA). Uncoated titanium samples with a machined surface served as control and were denominated Ti-ma. Surface roughness of the different specimens was measured using profilometry (UBM microfocus surface-measuring system, UBM Inc., Ettlingen, FRG). Parameters used to quantify surface roughness were: R_a (the