

**SURFACE CHARACTERISTICS AND  
IN VITRO BIO-ACCEPTABILITY OF  
MACHINED AND CAST  
PURE TITANIUM  
AND TITANIUM ALLOY**

BY

**LORNA CELIA CARNEIRO**

Thesis submitted in fulfillment for the requirements  
of the degree

**Philosophiae Doctor**

In the School of Dentistry  
Faculty of Health Sciences  
University of Pretoria

**Promotor**

Prof Dr SJ Botha  
Centre of Stomatological Research  
School of Dentistry, University of Pretoria.

**Co-Promotor**

Prof Dr PL Kemp  
Department of Prosthetics  
School of Dentistry, University of Pretoria

January~2003

## DECLARATION

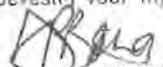
I, Lorna Celia Carneiro, declare that this thesis entitled:

**“Surface Characteristics and *In Vitro* Bio-acceptability  
of Machined and Cast Pure Titanium and Titanium Alloy”**

which I herewith submit to the University of Pretoria for the Degree of Philosophiae Doctor in Dentistry, is my own original work, and has never been submitted for any academic award to any other tertiary institution for any degree.

2003  
Date

  
Lorna Celia Carneiro

<p>Die verklaarder erken dat hy/sy ten volle op hoogte is van die inhoud van hierdie verklaring en dit begryp. Hierdie verklaring was beëdig/bevestig voor my.</p> <p></p> <p>Landdrostdistrik/Magisterial District Kom. van Ede/Com. of Oaths PRETORIA</p>	<p>The deponent has acknowledged that he/she knows and understands the contents of this affidavit which was sworn to/affirmed before me.</p> <p>DANIEL PIETER BOTHA Asst. Registrateur/Registrar Univ. van/of Pretoria</p> <p>Datum <u>28/01/03</u> Date</p>
--	--

## **DEDICATION**

My late brother-in-law

**Agnelo**

**My parents**

My husband

**Primo**

Our children

**Ryan, Elton and Michael**

*Commit to the LORD whatever you do, and your plans  
will succeed*

**Proverbs 16:3**

## ACKNOWLEDGEMENTS

The Training Fund for Tanzanian Women for the partial scholarship grant.

The Muhimbili National Hospital/Muhimbili University College of Health Sciences for study leave and partial scholarship grant.

Prof SJ Botha for his untimely effort in planning, constant encouragement and many other aspects for which there are no words just gratitude.

Prof PL Kemp for his profound contribution to unlimited knowledge, constant assistance and guidance.

Colleagues in the Centre of Stomatological Research and Department of Prosthetic Dentistry.

Southern Implants - Graham Blackbeard and Hein van Heerden for all machined samples and the enhancement of cast samples.

J & D Chrome, Deon Botha and Johan Voss for the fabrication of Cast Titanium samples.

Department of Materials Science and Metallurgical Engineering, University of Pretoria for the different microscopic utilities especially Prof Von Moltke, Robert Ehlers and Prem Premachandra.

Gerrit Myburg for doing the Radio Frequency Glow Discharge Treatment of samples.

Wynand Louw - from the Council for Scientific & Industrial Research for the Depth profile analysis of samples.

Department of Electron Microscopy, University of Pretoria and especially Ian Hall and Chris van de Merwe for samples preparation, training and assistance in using the Scanning Electron Microscope.

Dr PJ Becker from the Medical Research Council, for guidance assistance and processing of the statistics.

Gerald Grossberg - L N administrator, School of Dentistry for help with the network, digital camera and computer technicalities.

Library staff (Susan Marsh, Maria Skosana, Antionette Kemp, Patrick Maibelo and Marinda Maritz) of the Pre Clinical Library of the University of Pretoria for their tireless effort in retrieving of publications.

Colleagues from the Assistant Dental Officer's Training School/Faculty of Dentistry, Muhimbili University College of Health Sciences, Tanzania.

Primo, my husband, for his love, support, prompting, advice and encouragement to persevere.

My children, Ryan, Elton and Michael who besides many sacrifices were deprived of my presence.

My parents for their continued love and support.

Ivan, my brother for his spiritual support besides the many other times he has been there for me.

Riantes Residence - The Rossi's -you provided not only boarding and meals during the many times I was busy working but love, care and consideration.

My friends Monica, Hulda, Marna, Carol, Cecilia and lice -both near and far, for their prayers, friendship and words of encouragement.

Lastly not forgetting the families of Dr Hennie and Annelie van Jaarsveld, Monica and Guillermo Hamity, Fanie and Francien Botha, Rina and Hennie de Wet, Ina and Jan de Wet and Hulda and Fidelis Swai - you all provided me a family to be part of. Thank-you.

## SUMMARY

Properties making Ti and its alloys popular implant materials are determined by manufacturing conditions. With introduction of cast Ti into the dental fraternity, alternative methods of implant fabrication are possible. This study determined and compared differences in bio-acceptability between surface characteristics of machined and cast Ti and Ti-alloy in relation to materials used, fabrication procedure employed, surface enhancements and Radio Frequency Glow Discharge Treatment (RFGDT). Discs of 6.35mm diameter, 2mm thick, were prepared using cpTi and Ti6Al4V by machining and casting, and specific topographies were introduced. The first group of surfaces was from machining and casting procedures (controls). The second group was surfaces enhanced according to proprietary specifications of Southern Implants (SI). The third group was experimentally enhanced surfaces (ES). Enhancement included grit blasting and acid etching. From each group 21 of 24 samples were RFGDT. Electron Spectroscopy and Profilometric analysis of the Ti surfaces determined chemical composition, oxide thickness and surface roughness. Growth of human gingival fibroblasts and osteoblast-like cells, and scanning electron microscopy (SEM) determined *in vitro* bio-acceptability of different samples. Surface chemical composition was the same for cpTi and Ti6Al4V samples. Cast and enhanced samples were different from machined samples with higher % concentration of Sodium and Aluminium ( $p < 0.05$ ). RFGDT reduced Carbon and other surface contaminants and enhanced the Oxygen and Titanium atomic % concentration ( $p < 0.05$ ). The Sodium and Aluminium atomic % concentration was not affected. The major surface peak was  $TiO_2$  for Ti and oxygen peaks varied considerably between machined and cast samples. Surface topography of cast samples had higher surface analysis values compared to machined samples ( $p < 0.05$ ). RFGDT increased surface area and  $R_p$  values ( $p < 0.05$ ). No significant differences in

oxide thickness were observed between materials employed, but it was significantly higher for cast and enhanced samples. RFGDT significantly increased oxide thickness of samples. Fibroblasts showed significant increases in % attachment efficiency and proliferation (%AEP) with time while osteoblast-like cells showed a significant decrease with time. The %AEP of fibroblasts and osteoblast-like cells on different samples was not significantly different. Cast Ti6Al4V control and machined Ti6Al4V SI samples had relatively higher %AEP for osteoblast-like cells than the control or other samples. SEM revealed that fibroblasts and osteoblast-like cells displayed similar attachment behaviour. On machined surfaces cells spread, displaying the underlying topography while on cast and enhanced surfaces cells attached to the available peaks and used these attachments to suspend themselves over the surface. Filopodia were responsible for the attachment of cells. Significant differences in chemical composition were introduced by casting and surface enhancement procedures. RFGDT significantly reduced the concentration of Carbon and other contaminants on the surface exposing the surface Titanium oxide. Cast samples had rougher surface topography than machined samples. RFGDT significantly increased surface area and peak height. Casting, surface enhancement and RFGDT significantly increased oxide thickness. With time fibroblasts showed significant increases in %AEP while osteoblast-like cells significant decreases. Fibroblasts tended to proliferate on relatively smooth surfaces whereas osteoblast-like cells favored rougher surface topography produced by casting and surface enhancement.

# CONTENTS

	<b>PAGE</b>
Declaration	ii
Dedication	iii
Words of Wisdom	iv
Acknowledgements	v
Summary	vii
List of Figures	xvi
List of Tables	xxiv
<b>Chapter 1 Introduction</b>	<b>1</b>
<b>Chapter 2 Review of Literature</b>	<b>4</b>
<b>2.1 Overview on Titanium</b>	<b>4</b>
2.1.1 Titanium Development	4
2.1.2 Pure/Unalloyed Titanium	4
2.1.3 Titanium Alloy	5
2.1.4 Properties of Titanium	6
2.1.5 Titanium Oxides	8
2.1.6 Corrosion of Titanium	10
<b>2.2 Fabrication of Titanium</b>	<b>12</b>
2.2.1 Machining of Titanium	12
2.2.2 Casting of Titanium	12
2.2.2.1 Casting Machines	13

2.2.2.2	Cyclarc Titanium Casting Machine	13
2.2.2.3	Investment Material	14
<b>2.3</b>	<b>Osseointegration</b>	15
<b>2.4</b>	<b>Titanium and its Alloys as Dental Implants</b>	18
<b>2.5</b>	<b>Classification of Dental Implants</b>	19
2.5.1	The Implant Surface	20
2.5.1.1	Machined Surfaces	20
2.5.1.2	Osseotite Surface	21
2.5.1.3	Grit Blasted Surfaces	22
2.5.1.4	Bioactive Coated Surfaces	23
<b>2.6</b>	<b>Surface Characterization</b>	24
2.6.1	Compositional-Considerations	26
2.6.1.1	X-ray Photoelectron Spectroscopy (XPS)	27
2.6.2	Surface Topography	29
2.6.2.1	Atomic Force Microscope (AFM)	31
2.6.3	Depth Profiling	34
2.6.4	Cleaning and Sterilization of Implants	37
2.6.4.1	Radio Frequency Glow Discharge Treatment (RFGDT)	39
<b>2.7</b>	<b>Cell Culturing</b>	41
2.7.1	Types of Cells used in Culture	42

2.7.2	Cell Attachment	46
2.7.3	Cell Detachment	47
2.7.4	Inoculation/Seeding of Cells	48
2.7.5	Estimation of Cell Number	48
2.7.6	Cell Proliferation	49
<b>2.8</b>	<b>Bio-acceptibility</b>	<b>50</b>
2.8.1	Scanning Electron Microscopy (SEM)	51
<b>Chapter 3</b>	<b>Aim of the Study</b>	<b>52</b>
<b>Chapter 4</b>	<b>Materials and Methods</b>	<b>54</b>
<b>4.1</b>	<b>Fabrication of Specimen Discs</b>	<b>54</b>
4.1.1	Preparation of Machined Discs	54
4.1.2	Fabrication of Cast Discs	55
4.1.2.1	Preparation of Resin Disc Patterns	55
4.1.2.2	Casting Procedures	57
<b>4.2</b>	<b>Preparation of Different Surfaces</b>	<b>59</b>
4.2.1	Non-enhanced Surfaces	60
4.2.2	Enhanced Surfaces	60
4.2.2.1	Surface Enhancement of Samples according to Southern Implants (SI)	60
4.2.2.2	Experimental Enhancement of Samples (ES)	61

<b>4.3</b>	<b>Sterilization by Radio Frequency Glow Discharge Treatment</b>	61
<b>4.4</b>	<b>Analysis of Surface Characterization</b>	62
4.4.1	X-ray Photoelectron Spectroscopy (XPS)	62
4.4.2	Atomic Force Microscope (AFM)	63
4.4.3	The Quantum 2000 Scanning ESCA Microprobe	65
<b>4.5</b>	<b>Cell Culturing</b>	66
4.5.1	Cell Cultures	66
4.5.1.1	Fibroblasts	66
4.5.1.2	Osteoblast-like cells	66
4.5.2	Cultivation of Cell cultures	66
4.5.3	Cell Concentration	67
4.5.4	Inoculation of Cells onto Sample Discs	67
4.5.5	Incubation	68
4.5.6	Counting Procedures	68
4.5.6.1	Preparation of Trypan Blue	68
4.5.6.2	Cell Detachment for Osteoblast-like cells	69
4.5.6.3	Cell Detachment for Fibroblasts	69
4.5.6.4	Neubauer Haemocytometer	70

4.5.6.5	Estimation of Cell Number and Proliferation	71
4.5.7	Fixation Procedures	71
4.6	<b>Scanning Electron Microscopy (SEM)</b>	72
4.7	<b>Statistical Analysis</b>	72
<b>Chapter 5</b>	<b>Results</b>	74
5.1	<b>Surface Characterization</b>	74
5.1.1	Chemical Composition	74
5.1.1.1	Atomic Percent Concentration	74
	XPS Survey Spetra	76
	Carbon	78
	Oxygen	79
	Titanium	80
	Aluminium	81
	Sodium	82
5.1.1.2	Curve Fitting	83
	Carbon sub peaks	83
	Oxygen sub peaks	85
	Titanium sub peaks	86
	Aluminium sub peaks	88
	Sodium sub peaks	88
5.1.2	Surface Roughness	89
5.1.2.1	Area Analysis	89

	Area Ra and RMS	92
	Average height and Maximum range	94
	Surface Area	96
5.1.2.2	Line Analysis	99
	Ra	104
	Rt	105
	Rtm	105
	Rp	105
	Rpm	105
5.1.3	Depth Profile	106
<b>5.2</b>	<b>Cell Culturing</b>	109
5.2.1	Cell Attachment	109
5.2.1.1	Fibroblasts	109
5.2.1.2	Osteoblast-like Cells	116
5.2.2	Scanning Electron Microscope Analysis	123
5.2.2.1	Fibroblasts	123
	Two days	123
	Twenty-eight days	126
5.2.2.2	Osteoblast-like Cells	129
	Two days	129
	Twenty-eight days	133

<b>Chapter 6</b>	<b>Discussion</b>	137
6.1	Chemical Composition	139
6.2	Surface Roughness	143
6.2.1	Area Analysis	144
6.2.2	Line Analysis	146
6.3	Depth Profile	147
6.4	Cell Culturing	150
6.5	Scanning Electron Microscopy	154
6.6	Bio-acceptability	157
<b>Chapter 7</b>	<b>Conclusion and Recommendations</b>	159
7.1	Conclusions	159
7.2	Recommendations	162
<b>Chapter 8</b>	<b>References</b>	164
<b>Addendum A</b>		178
<b>Addendum B</b>		179
<b>Addendum C</b>		180
<b>Addendum D</b>		183
<b>Addendum E</b>		188
<b>Addendum F</b>		193
<b>Addendum G</b>		196
<b>Addendum H</b>		200

## LIST OF FIGURES

	<b>PAGE</b>
Fig 2-1:	Three separate phenomena of osseointegration taken from The Colgate Oral Care Report (2000) 17
Fig 2-2:	a) Cast surface (An isotropic surface) and b) Machined surface (An anisotropic surface) taken from Mummery (1992) 30
Fig 4-1:	Sample discs of cpTi (Grade 3) and Ti6Al4V (Grade 5) as received from Southern Implants 55
Fig 4-2:	DuraLay resin rods as fabricated from the duplicating mould 56
Fig 4-3:	Slow speed cutting saw sectioning resin rods into discs 57
Fig 4-4:	The Morita Cyclarc Casting Machine taken from (J.Morita, Europe, GMBA) 58
Fig 4-5:	Coded sample discs 60
Fig 4-6:	Placing of sample discs into tissue culture wells 68
Fig 4-7:	Neubauer haemocytometer being filled by capillary action prior to cell counting 70
Fig 5-1:	XPS survey spectra for cpTi cast control sample surfaces before RFGDT 76

Fig 5-2:	XPS survey spectra for cpTi cast control sample surface after RFGDT	77
Fig 5-3:	XPS survey spectra of cpTi machined RFGDT sample (AG=green) and cpTi cast RFGDT sample (FG=blue) of control surfaces	77
Fig 5-4:	XPS survey spectra of Ti6Al4V machined RFGDT sample (CG=green) and Ti6Al4V cast RFGDT sample (HG=blue) of control surfaces	78
Fig 5-5:	Atomic percent concentration of Carbon before and after RFGDT of sample surfaces	78
Fig 5-6:	Atomic percent concentration of Oxygen before and after RFGDT of sample surfaces	80
Fig 5-7:	Atomic percent concentration of Titanium before and after RFGDT of sample surfaces	81
Fig 5-8:	Atomic percent concentration of Aluminium before and after RFGDT of sample surfaces	82
Fig 5-9:	Atomic percent concentration of Sodium before and after RFGDT of sample surfaces	83
Fig 5-10:	Deconvolution of XPS Carbon envelope of Ti6Al4V cast control RFGDT sample surfaces	84

Fig 5-11:	Deconvolution of XPS Oxygen envelope of Ti6Al4V cast control RFGDT sample surface	86
Fig 5-12:	Deconvolution of XPS Titanium envelope of Ti6Al4V cast control RFGDT sample surface	87
Fig 5-13:	Deconvolution of Aluminium envelope for Ti6Al4V cast control sample surface	88
Fig 5-14:	AFM 3D images of 20µm scans of cpTi machined control sample surface	89
Fig 5-15:	AFM 3D images of 20µm scans of Ti6Al4V machined control sample surface	89
Fig 5-16:	AFM 3D images of 20µm scans of cpTi cast control sample surface	90
Fig 5-17:	AFM 3D images of 20µm scans of Ti6Al4V cast control sample	90
Fig 5-18:	Average roughness (Ra and RMS) of the different sample surfaces by material employed and RFGDT for 20µm scans	93
Fig 5-19:	Average roughness (Ra and RMS) of the different samples by material employed and RFGDT for 5µm scans	94
Fig 5-20:	Average height and Maximum range for 20µm scans of sample surfaces	95
Fig 5-21:	Average height and Maximum range for 5µm scans of sample surfaces	95

Fig 5-22:	Average Projected surface area of the 20µm scans for sample surfaces	96
Fig 5-23:	Average Projected surface area of the 5µm scans for sample surfaces	97
Fig 5-24:	Percent increase of Projected surface areas of samples for the 20µm scans	98
Fig 5-25:	Line analysis of the 20µm scans of cpTi machined control sample surfaces	99
Fig 5-26:	Line analysis of the 20µm scans of cpTi cast control sample surfaces	100
Fig 5-27:	Line analysis of the 20µm scans of Ti6Al4V machined control sample surfaces	100
Fig 5-28:	Line analysis of the 20µm scans of Ti6Al4V cast control sample surface	101
Fig 5-29:	Line analysis of the 5µm scans of cpTi machined control sample surfaces	102
Fig 5-30:	Line analysis of the 5µm scans of cpTi cast control sample surfaces	102
Fig 5-31:	Line analysis of the 5µm scans of Ti6Al4V machined control sample surfaces	103
Fig 5-32:	Line analysis of the 5µm scans of Ti6Al4V cast control sample surfaces	103
Fig 5-33:	Different values of line analysis of sample surfaces as related to fabrication procedures for the 20µm scan	106

Fig 5-34:	Tracing of the depth profile of Ti6Al4V cast control RFGDT sample	107
Fig 5-35:	Oxide thickness of samples	109
Fig 5-36:	Percent attachment efficiency and proliferation of fibroblasts on machined cpTi samples	110
Fig 5-37:	Percent attachment efficiency and proliferation of fibroblasts on machined Ti6Al4V samples	111
Fig 5-38:	Percent attachment efficiency and proliferation of fibroblasts exposed to cast cpTi samples	112
Fig 5-39:	Percent attachment efficiency and proliferation of fibroblasts exposed to cast Ti6Al4V samples	112
Fig 5-40:	Percent attachment efficiency and proliferation of fibroblasts exposed to machined samples	113
Fig 5-41:	Percent attachment efficiency and proliferation of fibroblasts exposed to cast samples	114
Fig 5-42:	Percent attachment efficiency and proliferation of fibroblasts exposed to different control samples	114
Fig 5-43:	Percent attachment efficiency and proliferation of fibroblasts exposed to SI enhanced samples	115
Fig 5-44:	Percent attachment efficiency and proliferation of fibroblasts exposed to ES samples	116

Fig 5-45:	Percent attachment efficiency and proliferation of osteoblast-like cells exposed to machined cpTi samples	117
Fig 5-46:	Percent attachment efficiency and proliferation of osteoblast-like cells exposed to machined Ti6Al4V samples	118
Fig 5-47:	Percent attachment efficiency and proliferation of osteoblast-like cells exposed to cast cpTi samples	118
Fig 5-48:	Percent attachment efficiency and proliferation of osteoblast-like cells exposed to cast Ti6Al4V samples	119
Fig 5-49:	Percent attachment efficiency and proliferation of osteoblast-like cells exposed to machined samples	120
Fig 5-50:	Percent attachment efficiency and proliferation of osteoblast-like cells exposed to cast samples	121
Fig 5-51:	Percent attachment efficiency and proliferation of osteoblast-like cells exposed to control surfaces	121
Fig 5-52:	Percent attachment efficiency and proliferation of osteoblast-like cells exposed to SI samples	122
Fig 5-53:	Percent attachment efficiency and proliferation of osteoblast-like cells exposed to ES samples	122
Fig 5-54:	Growth of fibroblasts on surfaces of machined cpTi samples after 2 days incubation	124

Fig 5-55:	Growth of fibroblasts on surfaces of machined Ti6Al4V samples after 2 days incubation	124
Fig 5-56:	Fibroblasts aligned according to the grooves created by machining on surfaces of machined Ti6Al4V control sample	125
Fig 5-57:	Growth of fibroblasts on surfaces of cast cpTi samples after 2 days incubation	125
Fig 5-58:	Growth of fibroblasts on cast Ti6Al4V samples after 2 days incubation	126
Fig 5-59:	Growth of fibroblasts on surfaces of control samples after 28 days incubation	127
Fig 5-60:	Growth of fibroblasts on surfaces of enhanced samples after 28 days incubation	128
Fig 5-61:	Growth of fibroblasts on surfaces of SI enhanced samples after 28 days incubation	129
Fig 5-62:	Osteoblast-like cells have completely covered the surface of the machined cpTi and Ti6Al4V control sample after 2 days incubation	130
Fig 5-63:	Growth of osteoblast-like cells on surfaces of machined enhanced samples after 2 days incubation	131

Fig 5-64:	Growth of osteoblast-like cells on surfaces of cast control samples after 2 days incubation	131
Fig 5-65:	Growth of osteoblast-like cells on cast enhanced samples after 2 days incubation	132
Fig 5-66:	Attached osteoblast-like cell seen on the different Titanium surfaces	133
Fig 5-67:	Growth of osteoblast-like cells on surfaces of machined samples after 28 days incubation	134
Fig 5-68:	Growth of osteoblasts on surfaces of cast samples after 28 days incubation	135
Fig 5-69:	Network of osteoblast-like cells formed on surfaces of cast Ti6Al4V control samples after 28 days incubation	136

## LIST OF TABLES

		<b>PAGE</b>
Table 2-1	Maximum impurity limits of pure Titanium taken from Donachie (1984)	5
Table 2-2	Normal values of Titanium, Aluminium and Vanadium in Humans taken from Vargas <i>et al</i> (1992)	11
Table 2-3	Surface analytic techniques used to study material interaction with host fluids and tissues taken from Baier & Meyer (1988)	25
Table 4-1	Description of materials used in this study for sample fabrication	54
Table 4-2	Summary of the designation of sample material, fabrication procedure adopted and introduced surface enhancement	59
Table 5-1	A summary of the elemental composition of sample surfaces (At %)	75
Table 5-2	Deconvoluted Carbon 1s envelope with respective sub peak positions (Bold figures indicate highest percent)	84
Table 5-3	Deconvoluted Oxygen 1s envelope with respective sub peak positions (Bold figures indicate highest percent)	85
Table 5-4	Deconvoluted Titanium 2p envelope with respective sub peak positions (Bold figures indicate highest percent)	87

Table 5-5	Average tabulated values of the surface topography of samples for 20 $\mu$ m scans (N=5)	91
Table 5-6	Average tabulated values of the surface topography of samples for 5 $\mu$ m scans (N=5)	92
Table 5-7	Projected surface area of samples with their relative percent increase	98
Table 5-8	Averages of the line analysis done for the different sample surfaces for the 20 $\mu$ m scan	101
Table 5-9	Averages of the line analysis done for the different sample surfaces for the 5 $\mu$ m scan	104
Table 5-10	Sputter time and Oxide thickness of samples	108