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Effect of rapidly resorbable bone substitute materials and various dental implant surfaces on the temporal expression of the osteoblastic phenotype *in vitro*

Habilitationsschrift
zur Erlangung der Venia legendi
für das Fach Zahn-, Mund- und Kieferheilkunde
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vorgelegt von
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To:

Paul

and

my parents, Gisela & Helmut Knabe

Words are inadequate to express my gratitude to you all.

Glossary of Abbreviations

Akt	Protein Kinase B
ALP	Alkaline phosphatase
AP-1	Activator protein 1
Asc-2-P	L-Ascorbic acid 2-phosphate
BALP	biotinylated alkaline phosphatase
BG	Bioactive glass
Bioc	Biocement D
BSA	Bovine serum albumin
BSP	Bone sialoprotein
cAMP	Cyclic adenosine monophosphate
CDHA	Calcium deficient hydroxyapatite
cDNA	Complimentary deoxyribonucleic acid
Co	Control
Col I	Type I collagen
Col III	Type III collagen
CTP	Calcium titanium phosphate
CTZP	Calcium titanium zirconium phosphate
CZP	Calcium zirconium phosphate
DNase	Deoxyribonuclease
ECM	Extracellular matrix
EDTA	Ethylendiaminetetra-acetic acid (disodium edetate)
EDX	Energy dispersive X-Ray analysis
Erk	Extracellular signal-regulated kinase

FAK	Focal adhesion kinase
Fn	Fibronectin
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GBR	Guided Bone Regeneration
Grb2	Growth factor receptor-bound protein 2
GTP	Guanosine triphosphate
HA	Hydroxyapatite
HBDC	Human bone-derived cells
HBSS	Hanks' Balanced Salt Solution
ICP	Ion-Coupled Plasma (ICP) analysis
ISH	*in situ* hybridization
mAb	Monoclonal antibody
MAPK	Mitogen-activated protein kinase
MAS-NMR measurements	^{31}P magic angle spinning-nuclear magnetic resonance measurements
α-MEM	Minimum Essential Medium
mRNA	Messenger ribonucleic acid
NASICON	Sodium superionic conductor
p-NP	p-nitrophenyl
p-NPP	p-nitrophenyl phosphate
NZP	Sodium zirconium phosphate
OC	Osteocalcin
ON	Osteonectin
OP	Osteopontin
pAb	Polyclonal antibody

PMMA	Polymethylmethacrylate
PI3K/Akt survival pathway	Phosphatidylinositol-3-kinase/Protein Kinase B survival pathway
QISH	Quantitative *in situ* hybridization
RGD	Arginine-glycine-aspartic acid
RNase	Ribonuclease
SD	Standard deviation
SEM	Scanning electron microscopy
Shc	Src-homology collagen
SOS	Son of sevenless
SSC	Sodium citrate solution
TCP	Tricalcium phosphate ceramic
Ti	Titanium
cp Ti	Commercially pure titanium
Ti-6Al-4V	Titanium-6-alumina-4-vanadium
TPS	Titanium plasma-sprayed
XPS	X-ray photoelectron spectroscopy
XRD	Quantitative X-ray diffraction analysis

General Introduction

Over the last three decades implant dentistry has established itself as a key discipline within the science and clinical practice of dental medicine. The utilisation of endosseous implants for the rehabilitation of completely or partially edentulous patients has become a standard treatment modality in dentistry. In this context, there has been an ongoing effort to enhance and accelerate osseointegration of dental implants by optimizing their surface design. To design implant surfaces which elicit excellent cell and tissue responses, furthering our knowledge base regarding cell and tissue responses to specific materials characteristics is necessary.

Resorption of the alveolar ridge after tooth extraction frequently mandates site development by augmentation before implants can be placed. The use of biodegradable bone substitutes is advantageous for alveolar ridge augmentation, since it avoids second-site surgery for autograft harvesting. As a result, over the past decade there has been great demand and an ongoing search for synthetic, biodegradable bone substitute materials which degrade rapidly, but still stimulate osteogenesis at the same time.

The work presented here is part of ongoing research of which the overall goal is to obtain a fundamental understanding of the processes involved in tissue integration of endosseous implant materials at a molecular level. Developing this understanding has been hampered by the inadequacy of the experimental techniques that could be used. In recent years, though, methods have become available that make it possible to extend the boundaries of knowledge, and these studies have used some of these novel methods. Once these processes involved in tissue integration are understood, it should be possible to create a novel generation of implant materials in which the surface properties can be engineered so as to elicit

specific biological responses resulting in the enhancement of osteogenesis and thus enhanced bone formation. This way a totally new concept would be introduced to biomaterials research and development in implant dentistry. Rather than following an empirical approach by implanting new materials and then characterizing the tissue response, the knowledge about the molecular mechanisms of tissue integration can then be used to strategically design biomaterials with the goal to elicit the desired tissue responses.

In vitro osteogenic cell cultures have been proven to be valuable for initial biological testing of endosseous implant materials. The quantitative evaluation of the gene and protein expression of osteogenic markers by putative osteoblasts grown on different biomaterials can generate valuable information concerning the osteogenic capacity of an implant material. Methodologies employing *in situ* hybridization and immunocytochemical techniques permit study of the expression of markers of the osteoblast phenotype. Techniques to quantitatively relate the expression of bone-related mRNAs (messenger ribonucleic acids) to their respective proteins as a measure of phenotypic differentiation have recently been developed. In the present thesis, this methodology was used to determine how osteoblastic cell differentiation is influenced by novel endosseous implant materials. Thereby, the osteogenic potential of a range of novel bone substitute materials and various dental implant surfaces was assessed. The investigations of novel, rapidly resorbable bioactive bone substitute materials comprise part I of the thesis and the studies regarding various dental implant surfaces part II.