LAMELIS

Lasers in Medicine and Life Sciences

Advanced summer school for under-graduate or postgraduate students of medicine and physics

July 2017

UNIVERSITAS SCIENTIARUM SZEGEDIENSIS · SZEGEDI TUDOMÁNYEGYETEM
VERITAS VIRTUS LIBERTAS

SZÉCHENYI 2020
HUNGARIAN GOVERNMENT
European Union
European Social Fund
INVESTING IN YOUR FUTURE

'ELITEAM'- ESTABLISHMENT OF THE ELI INSTITUTE AT THE UNIVERSITY OF SZEGED: FOUNDATION OF INTERDISCIPLINARY RESEARCH IN THE FIELD OF LASERS AND THEIR APPLICATIONS

LASERS FOR SURFACE MODIFICATION OF DENTAL IMPLANTS

Kinga TURZÓ, PhD

University of Szeged
Faculty of Dentistry

www.stoma.u-szeged.hu

SZÉCHENYI 2020

HUNGARIAN GOVERNMENT

European Union
European Social Fund

INVESTING IN YOUR FUTURE

Main topics

1. Introduction, Faculty of Dentistry, research program
   – Biomedical sciences and our research field
   – Main characteristics of dental implants
   – Surface aspects of biomaterials, biorecognition

2. Surface modifications enhancing biointegration/osseointegration
   – Physical-chemical surface modifications
   – Laser ablation of Ti surfaces, background/literature overview

3. Laser ablation of Ti discs
   – Bereznai et al., *Biomaterials,* 2003
   – A. Györgyey et al., *MSEC*, 2013
      - SEM, AFM and XPS studies
      - *In vitro* cell culture experiments - MG63 osteoblast cells

Main topics

1. Introduction, Faculty of Dentistry, research program

- Biomedical sciences and our research field
- Main characteristics of dental implants
- Surface aspects of biomaterials, biorecognition

2. Surface modifications enhancing biointegration/osseointegration

- Physical-chemical surface modifications
- Laser ablation of Ti surfaces, background/literature overview

3. Laser ablation of Ti discs

- Bereznai et al., *Biomaterials,* 2003
- A. Györgyey et al., *MSEC*, 2013:
  - SEM, AFM and XPS studies
  - *In vitro* cell culture experiments - MG63 osteoblast cells

Introduction

- 1989 – diploma in Physics, University of Bucharest (Romania)
- 1991 – 2001, JATE, Department of Biophysics, teaching and research
- 2000 - Ph.D. „Delayed fluorescence of bacterial reaction centers”, supervisor: Prof. Péter MARÓTI
- 2001 - Alfred Kastler grant: Université Louis Pasteur, Strasbourg (France)

RESEARCH IN DENTAL IMPLANTOLOGY

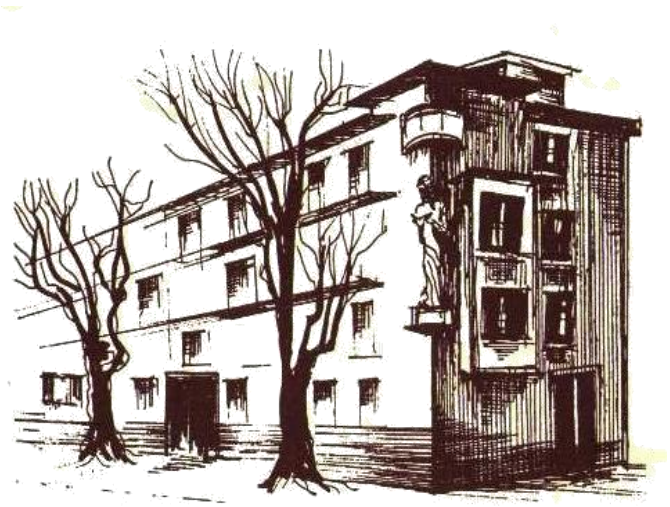
- 2001: research fellow at Department of Dentistry and Oral Surgery (University of Szeged, Medical Faculty): mainly research work, Prof. Dr. András FAZEKAS
- 2002 – teaching and developing dental subjects: Dental materials and technology, Oral biology, Dental inplantology, Physics for dental students, Dental biometry and evaluation of research results, Biomaterials and biocompatibility.
- 2004 – establishing the Research in Dental Medicine Program, Graduate School of Clinical Sciences (University of Szeged)
- Supervisor of „Biointegration of alloplastic materials” research topic.
- PhD school: 9 PhD students, 5 of them already defended their PhD

Scientific parameters:

- 25 publications, 4 book chapters
- IF: 43,377; citation index: 178; Hirsch index: 8
- Supervisor of 11 grants (EU5, GVOP, TÉT, ETT, FP7)
- 2003 – György BÉKÉSY postdoctoral fellowship
- 2011- 2014 - János BOLYAI fellowship, Hungarian Academy of Sciences
- 2014 - habilitation, School of Clinical Sciences (University of Szeged)
- 2015 –dean of the Faculty of Dentistry

Laboratory of the Faculty of Dentistry
Department of Oral Biology and Experimental Dental Research

- 1949 – year instruction started, Department of Dentistry and Oral Surgery, part of the Faculty of Medicine (WHO)
- 2007 - Faculty of Dentistry
  - 5 Departments, 46 academic staff, 23 medical/dental assistants, 180 dental students (Hungarian and from abroad).
  - All the academic staff takes part in research with different specialties (PhD program of the Faculty).



Patient care | **Education** | **Research**

- 2012 – New Department: Department of Oral Biology and Experimental Dental Research
  - To promote basic research in dental sciences
  - To develop and apply new materials and healing methods in the classic and modern dental practice
  - Assuring the teacher supply (dentists with academical carrier)
  - Laboratory of the Faculty of Dentistry is included in the structure of the Department of Oral Biology and Experimental Dental Research
- 2015 – 384 dental students, Hungarian and English program

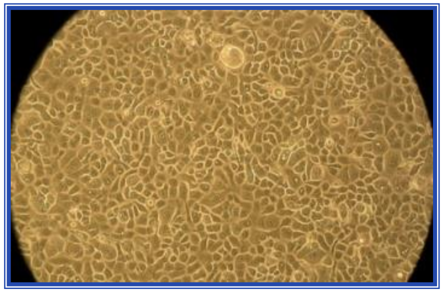
XE-100 PSIA AFM, Nikon Eclipse 80i optical microscope, Leica RM2255 rotary microtome, UV-visible spectrophotometer (ThermoSpectronic), Thermocycler (BioRad), Nikon TS100 invert microscope, and appliances for cell-culture experiments

Advanced SPM for Small Samples

Investigation methods

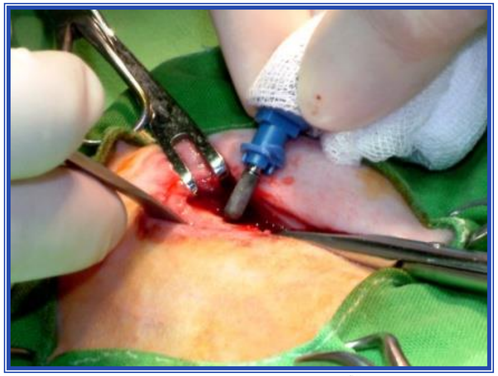
1. Surface science methods

- Atomic force microscopy (AFM): roughness (R_a)
- Scanning electron microscopy (SEM)
- X-ray photoelectron-spectroscopy (XPS): chemical composition of Ti surface
- X-ray diffraction (XRD): crystalline structure of the implant



2. *In vitro* cell culture studies

Determination of the attachment (24 h) and proliferation (72 and 168 h) of human oral epithelial (or fibroblast) cells via dimethylthiazol-diphenyl tetrazolium bromide (MTT) and alamarBlue (AB) assays, alkaline phosphatase quantification (ALP) and scanning electron microscopy (SEM)



3. *In vivo* animal experiments

Investigation of the osseointegration of Ti samples (discs or implants) in rabbit femur or Vietnamese pot bellied pigs calvarias.

Research group at the Faculty of Dentistry
Department of Oral Biology and Experimental Dental Research

Biointegration of alloplastic materials

- **Physical-chemical and biochemical surface modifications of titanium dental implants improving their biointegration**
  - Several publications and two PhD thesis (I. PELSŐCZI K, D. MATUSOVITS, Á. GYÖRGYEY, R. MASA)
- Effects of fluoride containing agents on titanium
  - Several publications and one PhD thesis (A. STÁJER, I. BARRAK)
- Effects of chemical agents used for decontaminating titanium implants (periimplantitis)
  - Several publications and one PhD thesis (K. UNGVÁRI, A. VENKEI)

University of Szeged, Graduate School of Clinical Sciences, Research in Dental Medicine Program

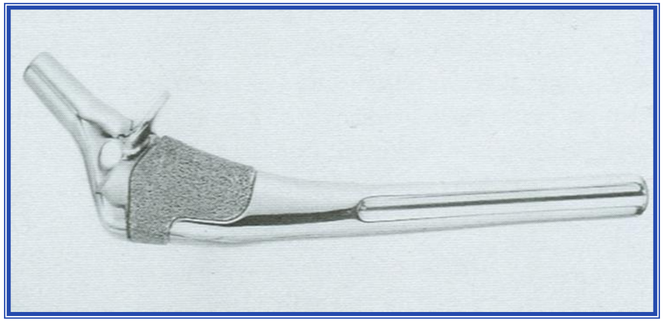
Head: : Prof. János MINÁROVITS, PhD., DSc.

SZEGED 2007 FOGORVOSTUDOMÁNYI KAR

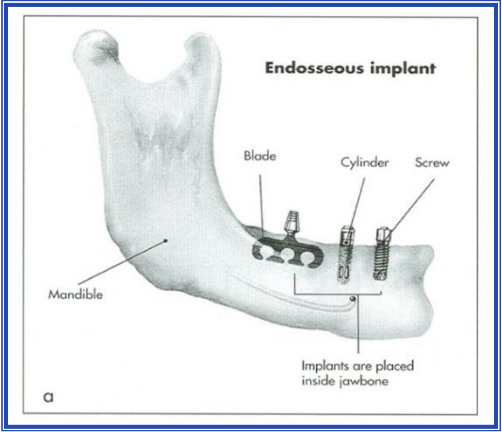
Research Program

Biointegration of alloplastic materials for replacement of missing parts of human body.

- Our studies relate to replacements of body structures in case of which the biological function requires significant load-bearing capability.



- Example for that are dental implants and artificial hip-joint replacements.



- They are generally made from titanium.
- Their biological integration depends on -among others- the surface structure of the metal.

❖ **General value of these studies: basic research highly applicable for industrial and biomedical use.**

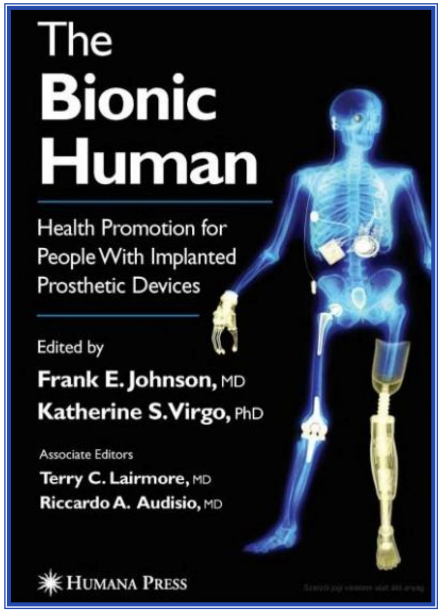
Biomedical Science

- Longer age, more people with missing or ill organs or tissues

⇨ we need more biomaterials replacing missing parts of human body!

Biomaterial Market (USA):

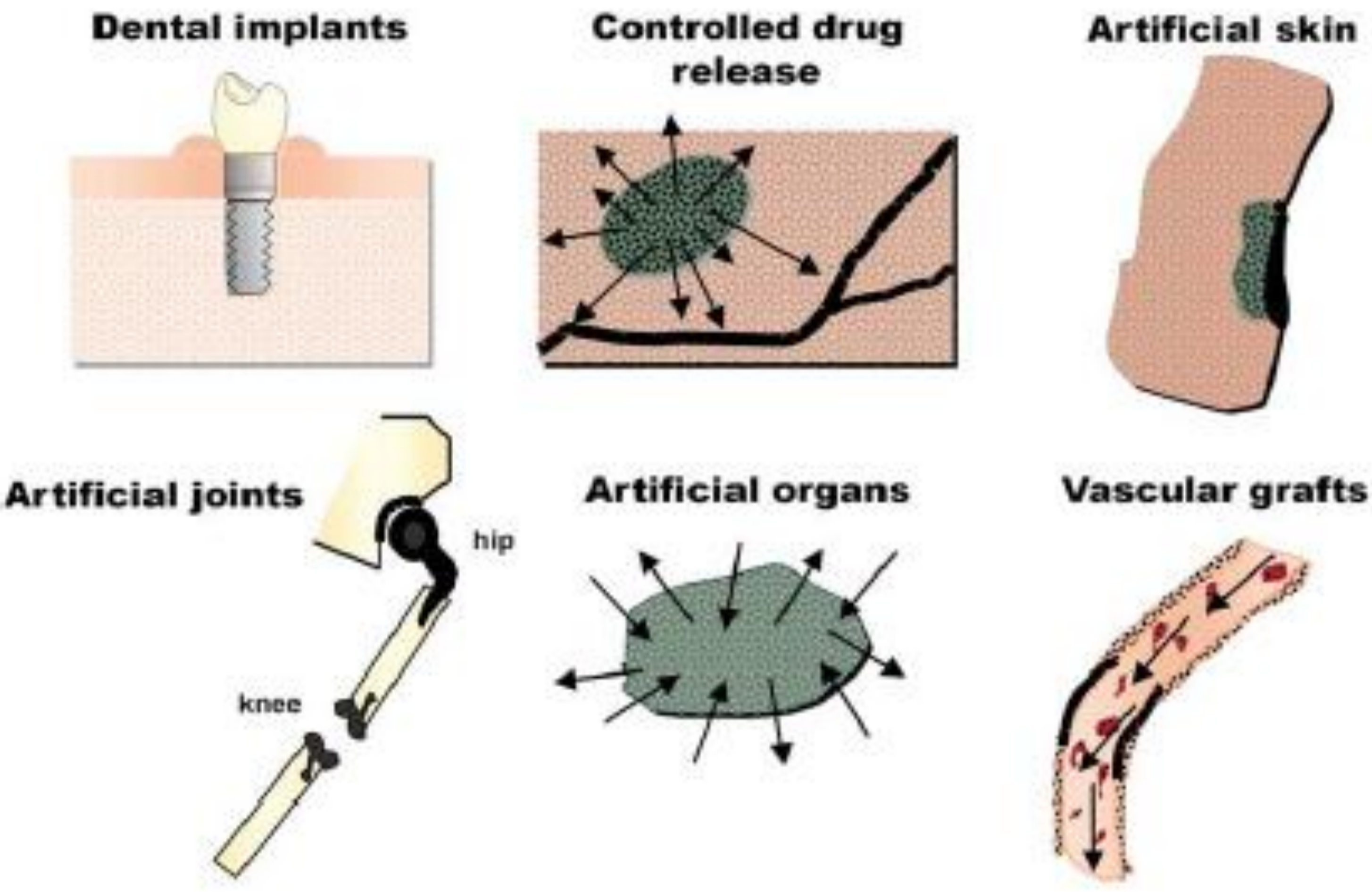
- 1988 - 11 million Americans (about 4.6% of the civilian population) had at least one implant
- 1991 - 2 billion $ for implants and instrumentation in orthopedics (1 billion $ metal joint prostheses)
- 1996 - 3.6 million orthopedic operations per year, four of ten involved metallic implants (reduction of a fracture and internal fixation)
- 2002 - 6 billion $ budget for biomaterials
- 2007 - 4.8 billion $ market for dental implants and bone grafting.



2006

- Study of the biointegration of alloplastic materials and development of biocompatible materials became one of the most important fields of research in biomedical science!

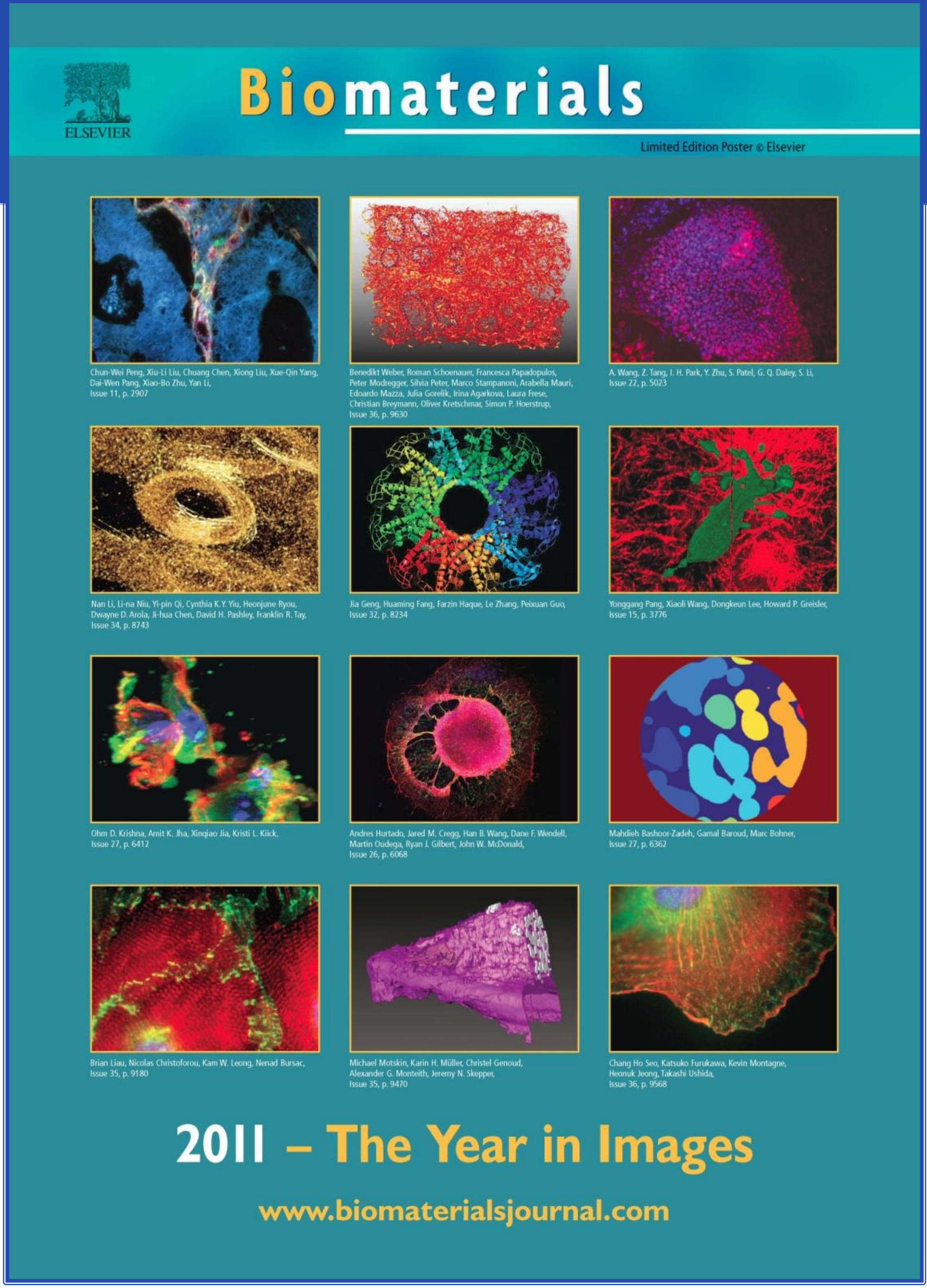
Biomaterial (alloplastic material)



Dental implants ----- most frequent!

2003

IF: 2.903



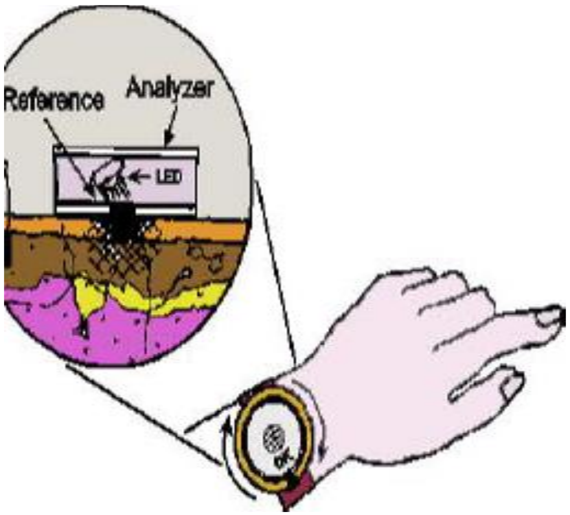
2014

IF: 8.557

Biological surface science -BioSS

2002- Bengt **KASEMO**, Department of Applied Physics, Göteborg University (*Surface Science 500:656-677).*

- Properties and processes at interfaces between synthetic materials and biological environments are investigated.
- Biofunctional surfaces are fabricated.



Research areas constituting a strong driving force for the current rapid development of BioSS

- Medical implants in the human body
- Biosensors and biochips for diagnostics
- Tissue engineering
- Bioelectronics
- Artificial photosynthesis
- Biomimetic materials

The successful biointegration of implants depends on many factors

- Bulk and surface characteristics
- Construction (design)
- Biocompatibility

} of the **material**

- Applied surgical technique
- General health condition and life-quality of the patient

Reliability of a biomaterial: $r = 1 - f$

f – probability of failure

Usually, there are multiple modes of failure, the total reliability:

$$r_t = r_1 \cdot r_2 \cdot ... \cdot r_n \quad \text{where} \quad r_i = 1 - f_i$$

Biointegration

The alloplastic material functions in intimate contact with the surrounding tissues for any period of time and does not have any adverse/damaging effect on the body as a whole.

Biocompatibility:

„Acceptance of an artificial implant by the surrounding tissues and by the body as a whole."

J.D. Bronzino, Ed.: *The Biomedical Engineering Handbook*, CRC and IEEE Press, 1999

„Biocompatibility is a dynamic, always changing phenomenon."

J.E. Lemons: *Journal of Prosthetic Dentistry*, 2001

Biocompatibility tests and standards

TABLE 5–1. Dentistry-Preclinical Evaluation of Medical Devices Used in Dentistry-Test Methods*

		Group I Primary (initial) Tests				Group II Secondary Tests							Group III Preclinical Usage Tests		
Nature of Contact	*Duration of Contact*	*Cytotoxicity Test ISO XXXX Clauses 6, 7*	*Cytotoxicity Test ISO 10993-5*	*Cytotoxicity Test ISO XXXX Annex A*	*Genotoxicity Test ISO 10993-3 Clause 4*	*Acute Systemic Toxicity—Oral Application ISO 10993-11 Clause 6.5.1*	*Acute Systemic Toxicity—Application by Inhalation ISO 10993-11 Clause 6.5.3*	*Subchronic Systemic Toxicity—Oral Application ISO 10993-11 Clause 6.7.1*	*Skin Irritation and Intracutaneous Reactivity ISO 10993-10 Clauses 5.2, 5.4*	*Sensitization ISO 10993-10 Clauses 6.2, 6.3*	*Subchronic Systemic Toxicity—Application by Inhalation ISO 10993-11 Clause 6.7.3*	*Local Effects After Implantation ISO 10993-6 Clauses 4, 5, 6*	*Pulp and Dentine Usage Test ISO XXXX Clause 8*	*Pulp Capping and Pulpotomy Test ISO XXXX Clause 9*	*Endodontic Usage Test ISO XXXX Clause 10*
Surface-Contacting Devices	≤24 h	X	X				X		X	X					
	24 h to 30 days	X	X				X	X	X	X	X				
	>30 days	X	X		X		X	X	X	X	X				
External Communicating Devices	≤24 h	X	X	X			X		X	X					
	24 h to 30 days	X	X	X	X		X	X	X	X	X	X	X	X	X
	>30 days	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Implant Devices	≤24 h	X	X						X	X				X	X
	24 h to 30 days	X	X		X			X	X	X		X		X	X
	>30 days	X	X		X			X	X	X		X		X	X

*X in the columns indicates test that shall be considered for use. ISO XXXX in the column heading indicates that the official number will be designated when the ISO grants approval.

This Draft International Standard was developed by a Technical Committee of the International Organization for Standardization (ISO). The American National Standards Institute (ANSI), the U.S. member of ISO, participates in its technical program and administers secretariats of various technical committees and subgroups. ANSI is also ISO's exclusive sale agent in the United States for all ISO standards, Draft International Standards, and Committee Drafts. The excerpt from this Draft International Standard is being distributed by W.B. Saunders Company through an arrangement with ANSI.

This is *not* an approved ISO International Standard. It is distributed for review and comment and may be modified during this process. It is subject to change without notice and may not be referred to as an International or ISO Standard until published as such.

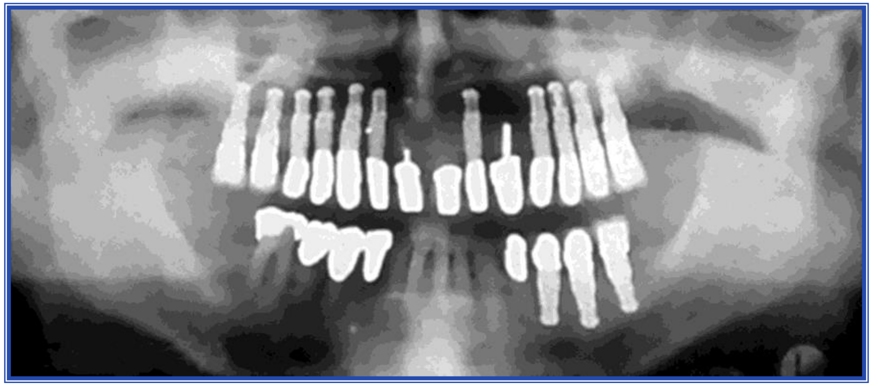
Anusavice, K.J. (Ed.), (1996) *Phillips' Science of Dental Materials*, 655-662, W.B. Saunders Company, 10th Edition, Philadelphia, Pennsylvania, USA

Importance of biocompatibility in dentistry/implantology

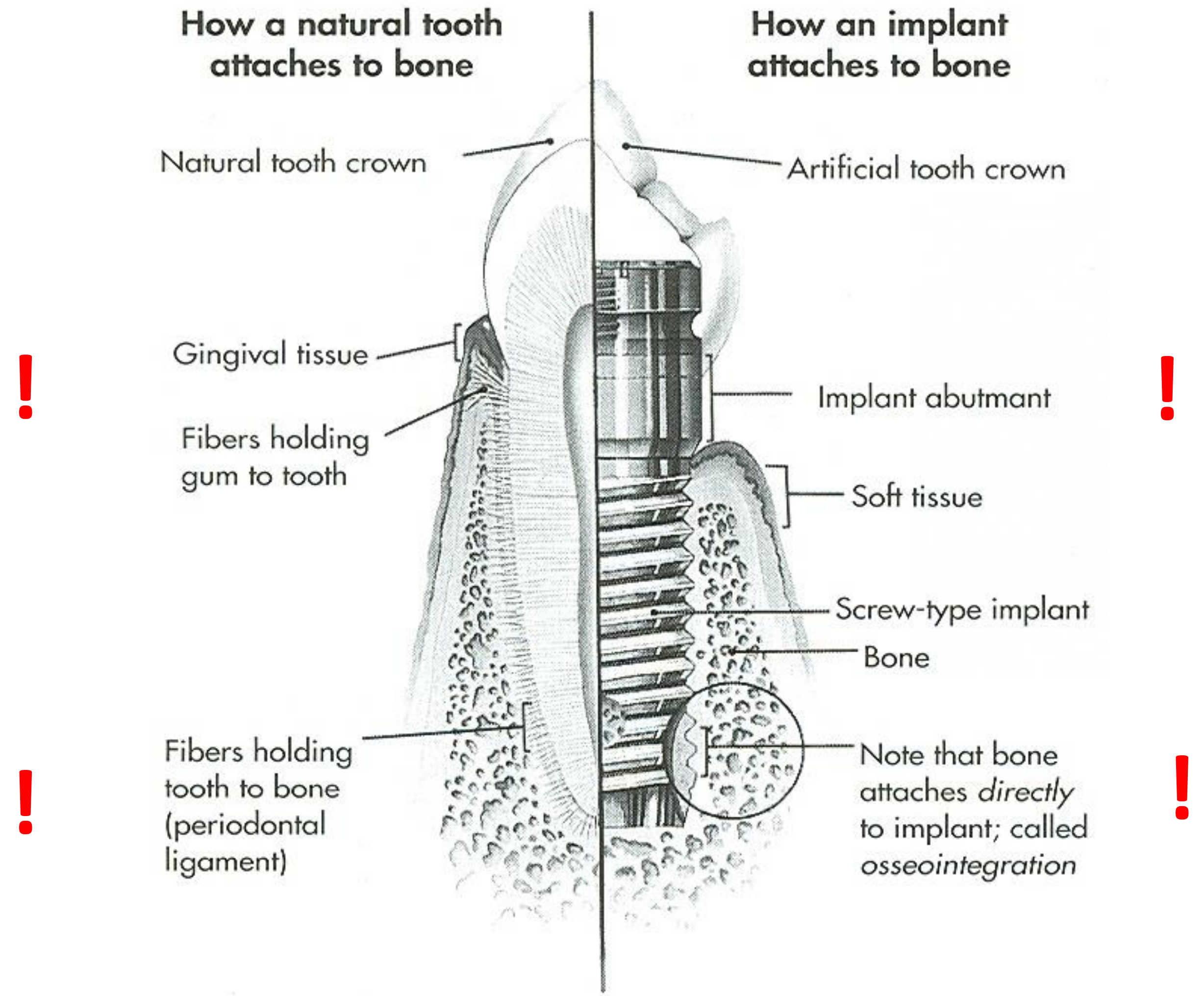
The main challenges in dentistry for centuries have been the development and selection of biocompatible prosthetic materials that can withstand the adverse conditions of the oral environment.

600 A.D.
Mayans used sea-shell segments for implant s

- Oral cavity represents a multivariant external environment with a wide range of circumstances:
  - Foods, abrasion, pH (3-9), temperatures from 5 to 55^{o}C, high magnitude masticatory forces, bacteria, etc.



Why do we need an optimized dental implant (surface)?

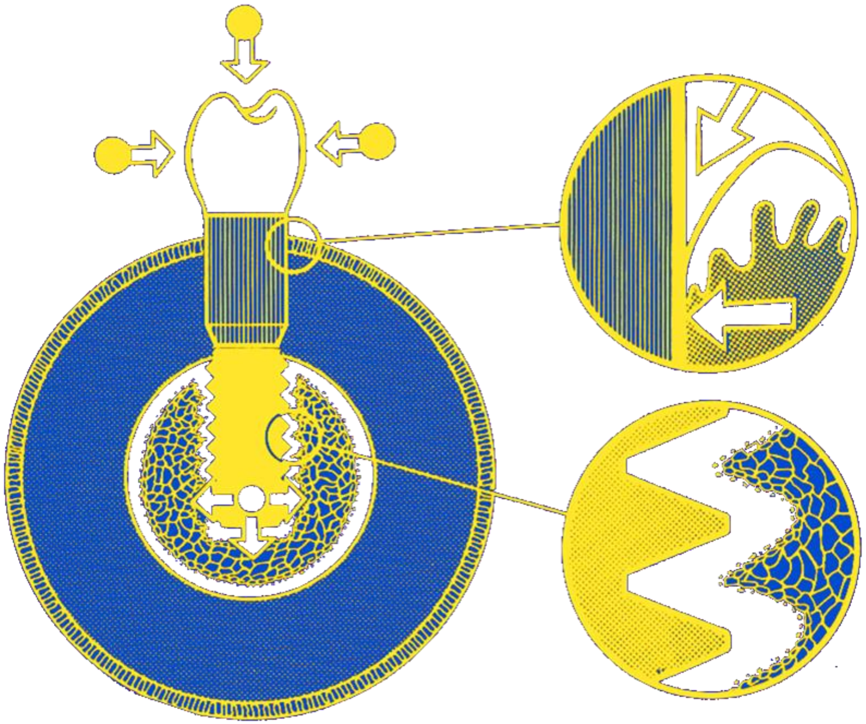


Schematic of natural tooth vs. implant attachment to bone.

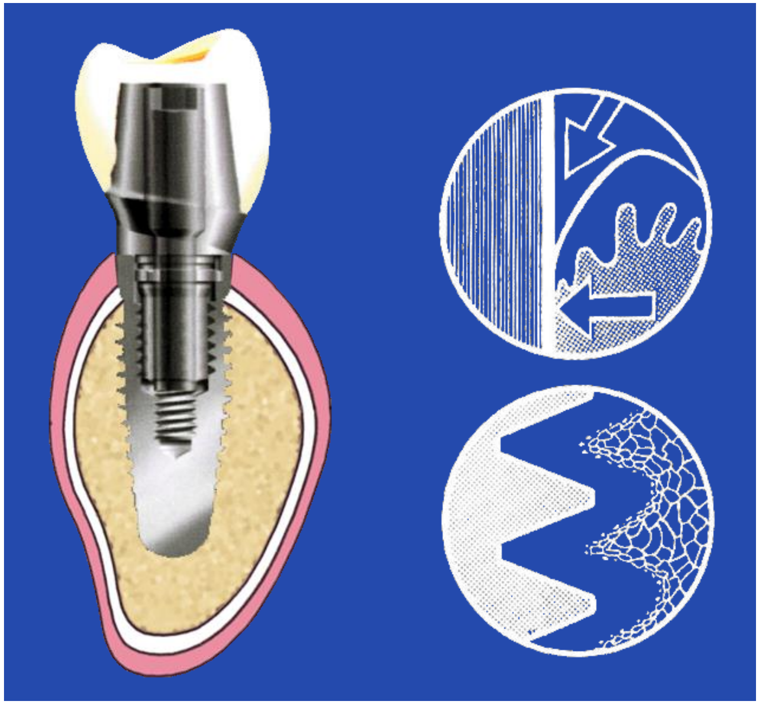
O'Brien, W.J. (2002). *Dental Materials and Their Selection*, Quintessence

The success and long-term prognosis of endosseous implants depend on...

- **Osseointegration**
  (anchorage in the host bone)
- **Gingival attachment**
  (periimplant mucosal seal)
- **Appropriate transmission of masticatory force**
  (load transfer capacity)



Functionally three different parts

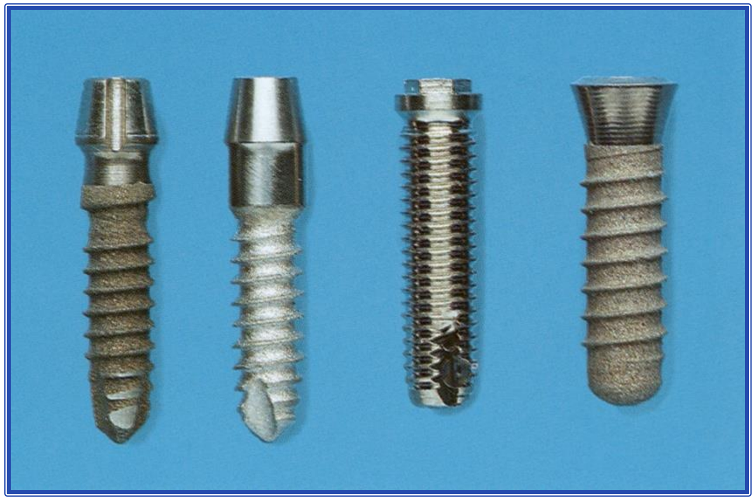


A - smooth surface for epithelial attachment and to prevent plaque formation

B - machined, oblique part for proper connective tissue attachment

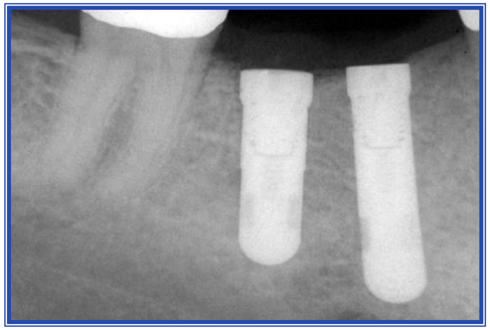
C - rough surface, developed for anchorage in the bone

Types of dental implants



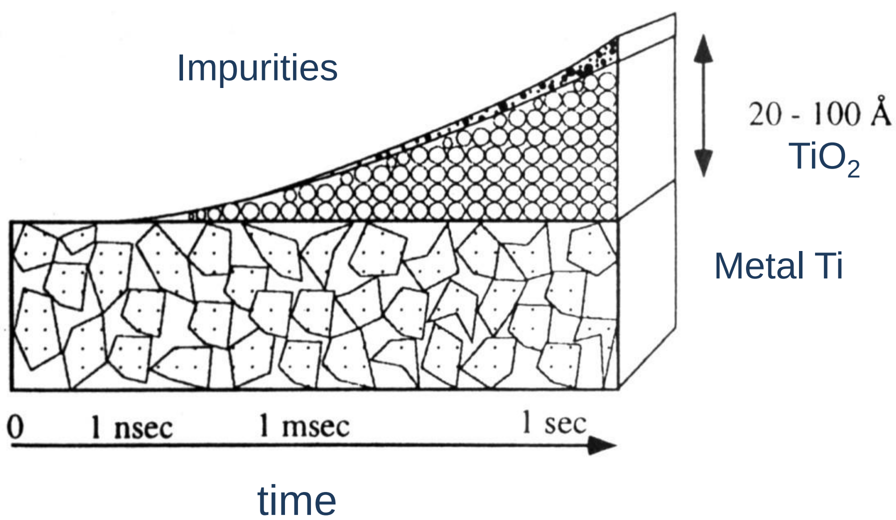
Favorable properties of titanium

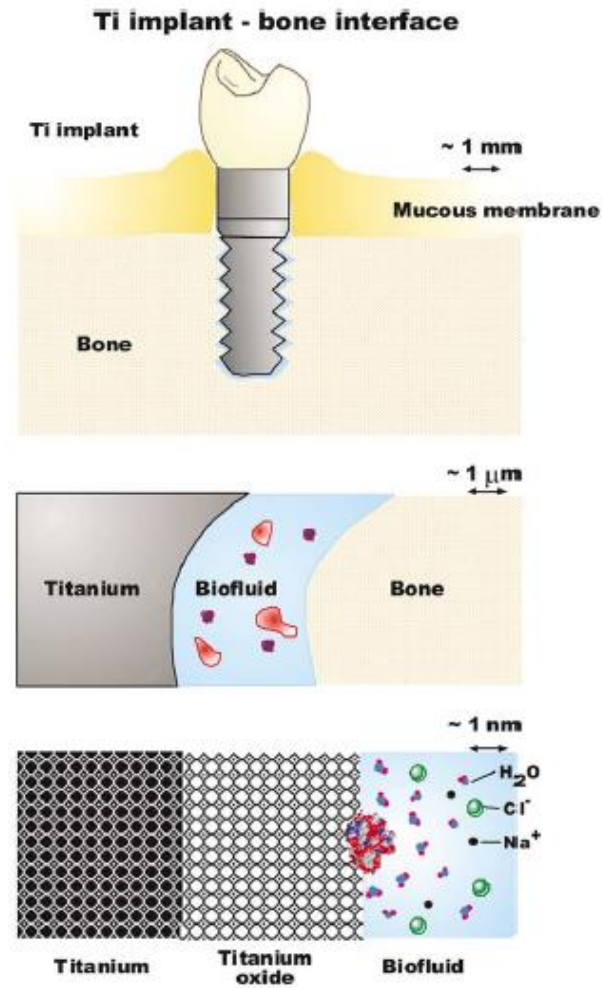
- Light material (atomic weight: 47.88)
- Low density (4.43 g/cm^3 ; almost as strong as steel, but 45% lighter)
- Low heat conductivity (21.9 J/(m·s·K))
- Easy to fabricate
- Good radiographic image
- Exceptional corrosion resistance
- High degree of biocompatibility



Widely used as a medical implant material (biomaterial) for fixating or replacing hard tissue in dentistry, implantology, oral and maxillofacial surgery.

Corrosion resistance and biocompatibility of titanium





TiO_2 has one of the highest heats of reaction:

$\Delta H = -912$ kJ/mol

Is very adherent to the parent titanium and impenetrable to oxygen.

Dielectric constant of TiO_2:

$\varepsilon = 50\text{-}170$

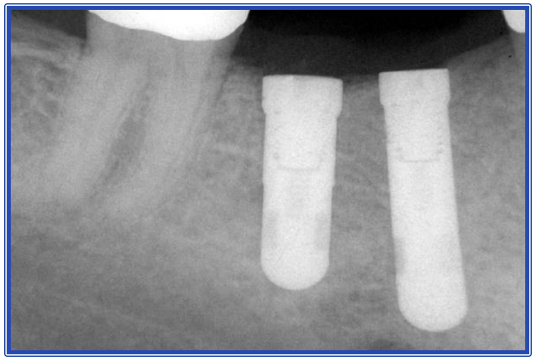
Osseointegrated implants replacing teeth

longer age, more people with missing teeth

- Hungary: ~ 300.000 yearly
- EC: ~ 2 million

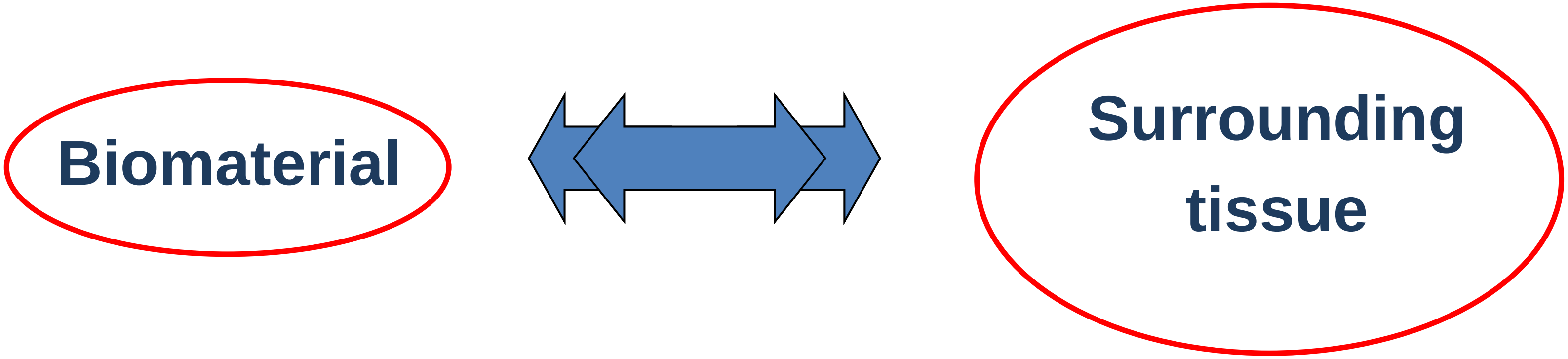
up-to-date demand: optimization of osseointegration

- reducing the 3-6 month healing period



Biointegration / Osseointegration

Interaction between the biomaterial and surrounding tissues



Place of interaction: **INTERFACE**

Surface properties of materials

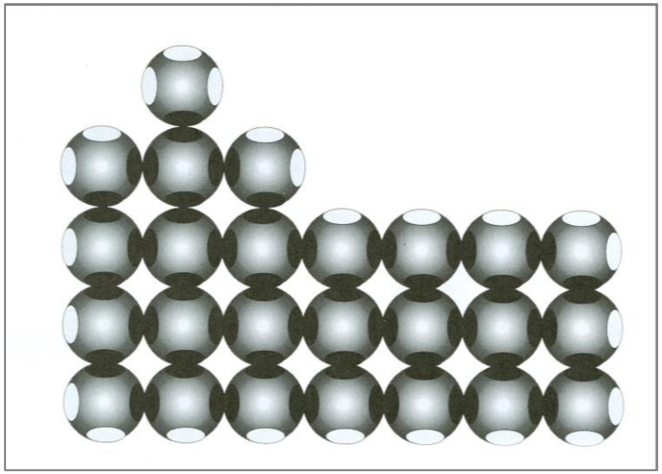
- The surface region has 4 important characteristics:
  - is uniquely reactive
  - different from the bulk
  - readily contaminate
  - variable or mobile.

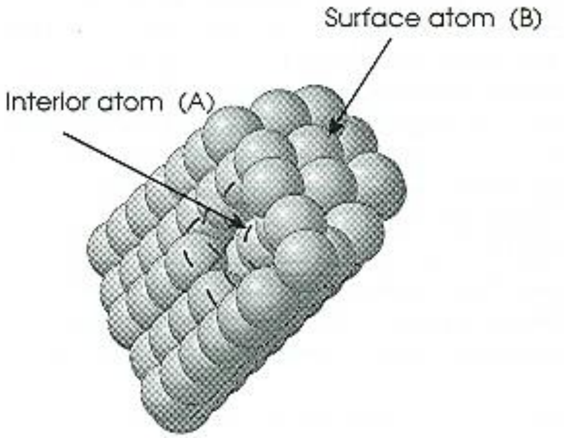
- Important question in biocompatibilty:

  - how the device or material „transduces” its structural makeup to direct or influence the response of proteins, cells and organisms?

- **Biorecognition:**

  This transduction occurs through the surface structure: the body „reads” the surface structure and responds.





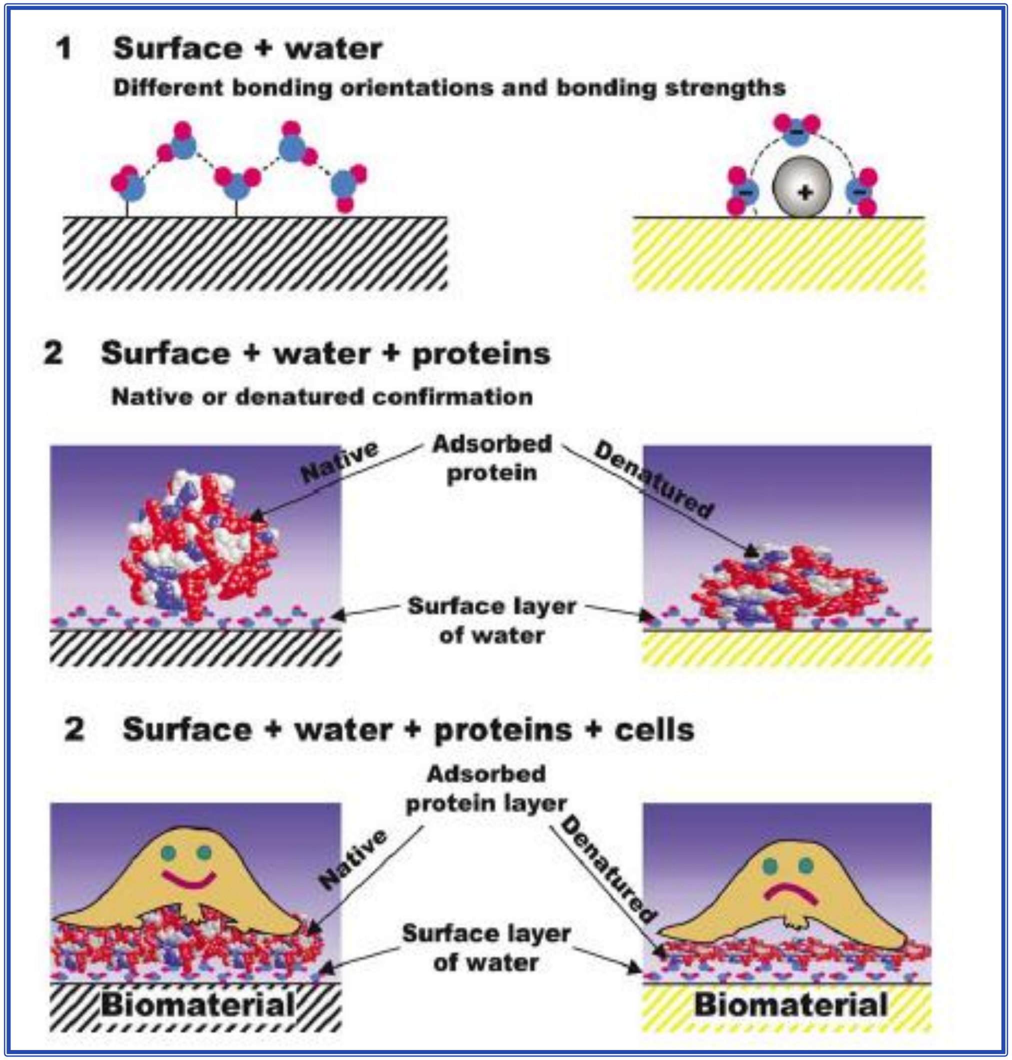
Biorecognition

Events following the implantation of a medical implant:

ns time scale: the first molecules to reach the surface are water molecules, water shell is formed.

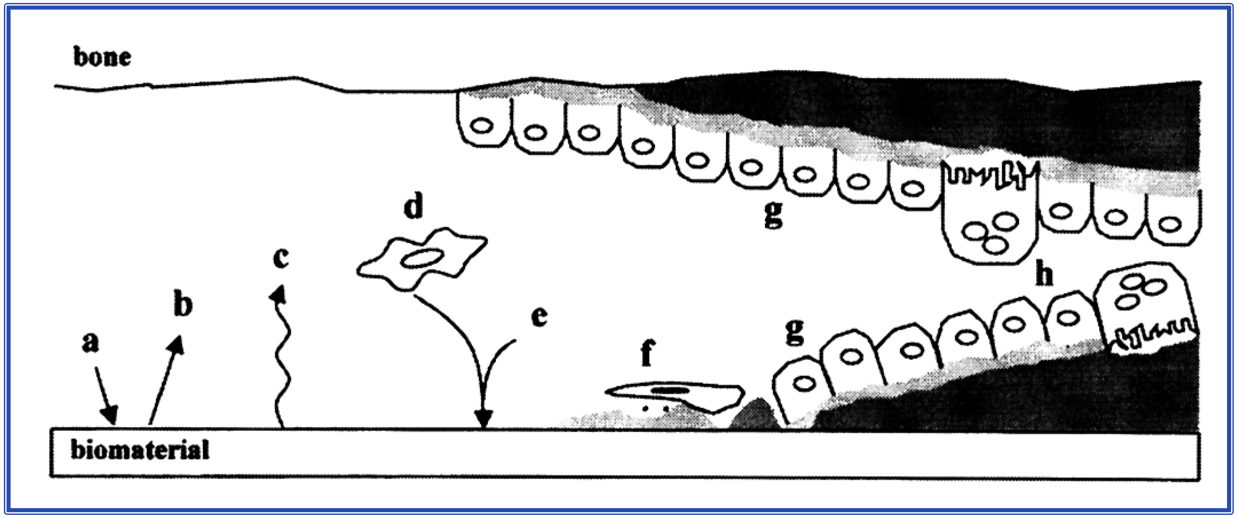
μs-ms time scale: the water shell that is formed affects the protein interactions, this continues for much longer times. Conditioning film is formed.

s time scale: cells reach the surface. Their surface interactions take place via the protein coating whose properties are determined by the surface and water adlayer properties.



B. Kasemo: *Surface Science*, 2002

Overview of the osseointegration process



~2 μm/day

Puleo & Nanci, Biomaterials,1999

a. Protein adsorption from blood and tissue-fluid

b. Conformational change and desorption of proteins, formation of a conditioning film.

c. Electrochemical modification of the implant surface and release of particles, ions (Ca, P).

d. Inflammatory and connective tissue cells arrive to the surface.

e. Release of matrix proteins, and selective adsorption of proteins.

f. Adhesion of osteogenic cells and formation of lamina limitans (afibrilar interfacial zone).

g, h. Formation of new bone.

Bio- and osseointegration

processes can be controlled at molecular and cellular level

↓

by modification of biomaterial (implant) surface

Possibilities:

- physical-chemical methods
- biochemical methods

Main topics

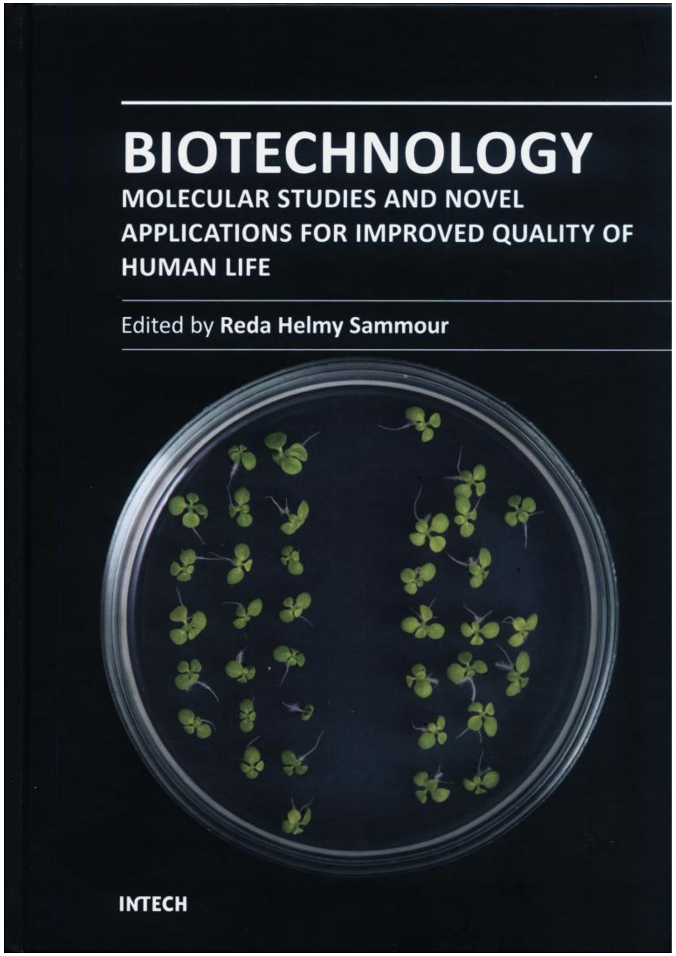
1. Introduction, Faculty of Dentistry, research program
   - Biomedical sciences and our research field
   - Main characteristics of dental implants
   - Surface aspects of biomaterials, biorecognition

2. Surface modifications enhancing biointegration/osseointegration
   - Physical-chemical surface modifications
   - Laser ablation of Ti surfaces, background/literature overview

3. Laser ablation of Ti discs
   - Bereznai et al., *Biomaterials,* 2003
   - A. Györgyey et al., *MSEC*, 2013:
     - SEM, AFM and XPS studies
     - *In vitro* cell culture experiments - MG63 osteoblast cells

K. Turzo: Surface aspects of titanium dental implants.

In: Biotechnology /Book 3, InTech Open Access Publisher, ISBN 978-953-51-0151-2, pp. 135-158, 2012



9

Surface Aspects of Titanium Dental Implants

Kinga Turzo
University of Szeged, Faculty of Dentistry, Hungary

1. Introduction

This book chapter presents a brief description of a new emerging field of science, the biological surface science and stresses its importance in the field of alloplastic materials and dental implants. It is not intended to present a comprehensive review of the field, but rather to indentify some important trends and directions in the surface modifications of titanium (Ti) dental implants targeting the improvement of their bio/osseointegration (second subchapter). The third subchapter outlines the impact of fluoride on surfaces of titanium implants or other dental devices. The fourth one will give an overview of the effects of some chemical cleaning agents on titanium implant surfaces. The interaction between Ti and fluoride containing prophylactic agents or chemical cleaning agents can result in a beneficial or/and destructive alteration of the surface of Ti dental appliances. The objective of our studies was to characterize these specific modifications and alterations of Ti surfaces. The fifth subchapter focuses on the relation between biological surface science and dental implants related research.

2. Improving osseointegration of titanium implants by surface modifications - latest trends

These days much effort goes into the design, synthesis, and fabrication of Ti dental implants to obtain a long term (lifelong) secure anchoring in the bone. First of all, implants must carry and sustain the dynamic and static loads they are subjected to. The bulk structure of the material governs this ability. Evidently, it is important to achieve a proper function with the shortest possible healing time, with a very low failure rate, and with minimal discomfort for the patient. These factors are important for cost reasons, too.

The success and the long-term prognosis of dental implants depend mainly on three factors: 1) on the anchorage of the artificial root in the host bone, i.e. on the osseointegration; 2) on the peri-implant mucosal seal; 3) finally on the adequate loading of the implant, transmitted by the abutment, i.e. the biomechanical factor (Figure 1.) (Adell et al., 1981; Brånemark, 1983a; Davies, 1998).

During osseointegration, which is the formation of a direct connection between the living bone and the surface of the load-carrying implant, strong links must be formed between the biomaterial and the surrounding bone tissue (Binon et al., 1992; Cochran, 1999; Morra & Cassinelli, 1997; Olefjord & Hansson, 1993).

Goal of physical-chemical methods

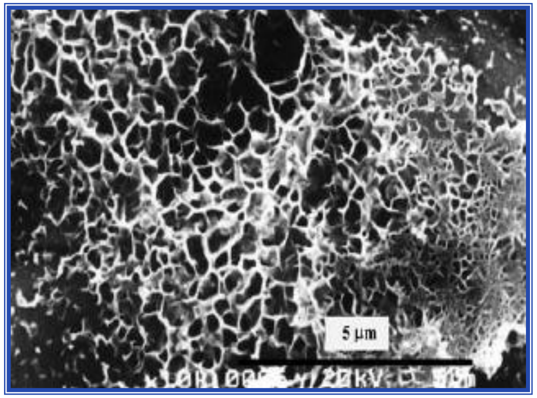
To modify the charge, chemical composition and morphology of the surface

↓

- to increase the adhesion of cells and the electrostatic interaction of the surface with biomolecules
- to insure the optimal surface for the biomolecules, proteins and CaP crystals in the process of osseointegration

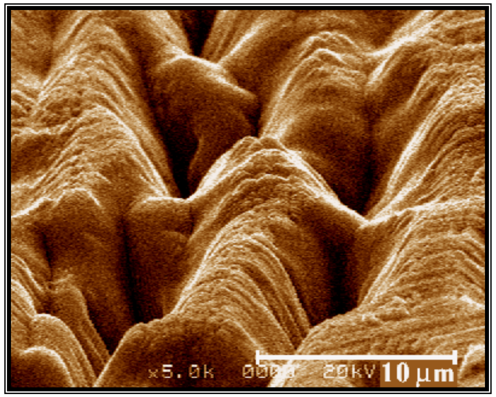
Physical-chemical methods

- **Chemical surface reactions** (e.g.: oxidation, acid-etching)
- **Sand blasting**
- **Ion implantation**
- **Pulsed laser deposition**
- **Coating the surface** (e.g. CaP)
- **Laser ablation**



M. Szekeres et al: *Colloid and Polymer Science* 283:587-592, 2005

Surfaces with modified roughness



M. Bereznai et al: *Biomaterials* 24, (23):4197-4203, 2003

Importance of surface roughness

- Osseointegration is enhanced on rough, micro structured surfaces, as the contact area will be bigger (Cochran et al., 1998; Joob-Fancsaly et al., 2004; Santis et al., 1996).
- Mechanical (morphological) fixation will help the bone ingrowth.

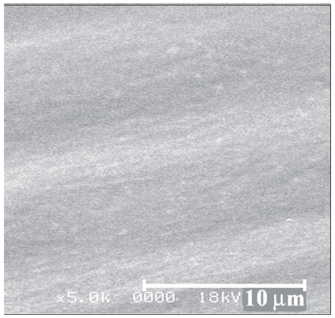
Influence of roughness on cells:

  - **Epithelial cells attach and proliferate better on smooth surfaces** (polished R_a < 0.5 mm) than on rough, acid-etched and sand-blasted surfaces (Klinge & Meyle, 2006, Baharloo et al., 2005).
  - **Fibroblast cells attach in the same way on both surface** (Klinge & Meyle, 2006)
  - **Osteoblast cells show an increased** metabolical activity (osteocalcin production and alkaline phosphatase activity) on rough surfaces (Boyan et al.,1998; Meyle, 1999).

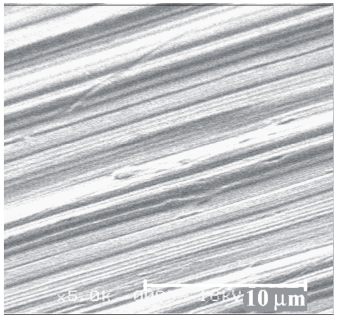
Meyle et al., 1999: the roughness of the surface control the cell activity and therefore determine the helaing process!

- Animal experiments (rabbit) demonstrated that the roughness and the thickness of TiO_2 layer influences the adhesion of bone in the early stage of implantation (1-7 weeks; Larsson et al., 1994; Larsson et al., 1996).
- Acid etched, sand blasted and Ti plasma prayed surfaces are typically rough surfaces, used at almost every implant. *In vitro and in* vivo studies prove their efficiency (Buser et al. 1991; Wennerberg et al., 1997; Wong et al., 1995).

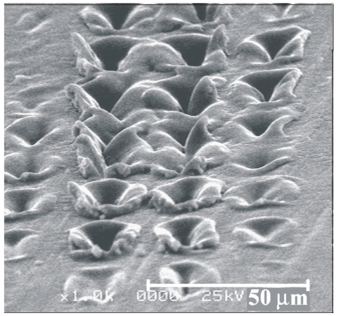
Polished surface



Machined surface



Rough surface, ablated with 18 ns ArF-laser



Application of lasers
Literature overview I.

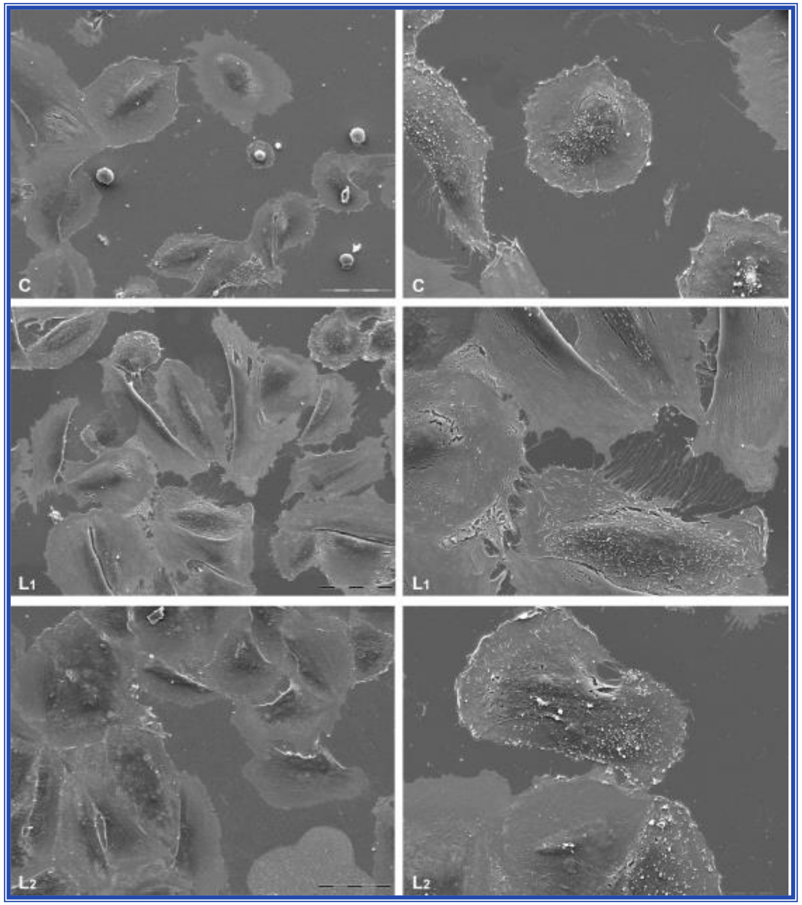
Laser type	Application	Surface	*In vitro* results	*In vivo* results	References
Er:YAG 2940 nm	• Irradiation, peri-implant treatment • shaping the place of the implant	Ti discs, different surfaces (SA, TPS, HA, machined), glass	Bacterial studies	Animal and human experiments	• M. Kreisler et al. *J Periodontol* 2002 • S. Sennhenn-Kirchner et al. *Lasers Med Sci* 2009 • G. Kesler et al. *Int J Oral Maxillofac Implants* 2006 • F. Schwarz et al. *Clin Oral Impl Res*, 2005
Er,Cr:YSGG 2780 nm	• Irradiation, peri-implant treatment	Ti discs, SLA surface	Cell culture studies (human osteoblast SaOs-2) MTT	Supragingival plaque biofilm, intraoral studies (3 patients)	• F. Schwarz et al. *J Periodontol*, 2006
Diode GaAlAs 830 nm LLLT: low-level laser therapy	• Irradiation, re-osseointegration • Direct irradiation of cells (fibroblast and osteoblast)	CP Ti implants	• SEM studies • cell culture • TGF-β_1 and osteokalcin production • Attachement and proliferation of osteoblasts increased	• Bone-to-implant contact - BIC increased • Re-osseointegration • rabbit tibia, dog studies •	• C.L. Pereira et al. *Int J Oral Maxillofac Implants*, 2009 • B.P. Campanha et al. *Photomedicine and Laser Surgery*, 2010 • J.A. Shibli et al. *Clin Oral Impl Res*, 2006 • C. B. Lopes et al. *Photomedicine and Laser Surgery*, 2005 • M. Khadra et al. *Clin Oral Impl. Res*, 2005 • M. Khadra et al. *Biomaterials*, 2005
CO_2 10600 nm	• **Irradiation, peri-implant treatment** • comparison with Nd:YAG laser	CP Ti implants TPS implants	• SEM studies • thermical studies	Beagle dogs Hystology, histomorphometry and X-ray	• C-Y Park et al. *J Oral Maxillofac Surg* 2005 • H. Deppe et al. *Int J Oral Maxillofac Implants* 2001

Diode laser treatment of cells

- Osteoblast and fibroblast cells irradiated with GaAlAs diode laser
- M. Khadra et al. *Biomaterials*, 2005; 26:3503-3509
- M. Khadra et al. *Clin Oral Impl Res*, 2005; 16:168-175
- Laser parameters:
  - 830 nm, 84 mW, 1.5-3 J/cm^2
  - 9 cm distance between laser and cell layer
  - 10 min, 3 consecutive days
  - HOB –human osteoblast: M. Khadra et al. *Biomaterials*, 2005; 26:3503-3509
  - HGF –human gingival fibroblast: M. Khadra et al. *Clin Oral Impl Res*, 2005; 16:168-175

- HOB: 3 J/cm^2 fluence was increasing significantly the production of osteokalcin and TGF-β_1 production.

- HGF: the irradiated cells were forming more effectively clones.



SEM images of fibroblast cells after 3 hours after attachment.
C: control
L1 : laser treatment , fluence: 1.5 J/cm^2
L2: laser treatment , fluence: 13 J/cm^2

Scale = 25 μm (left side)

Scale = 10 μm (right side)

Application of lasers
Literature overview II.

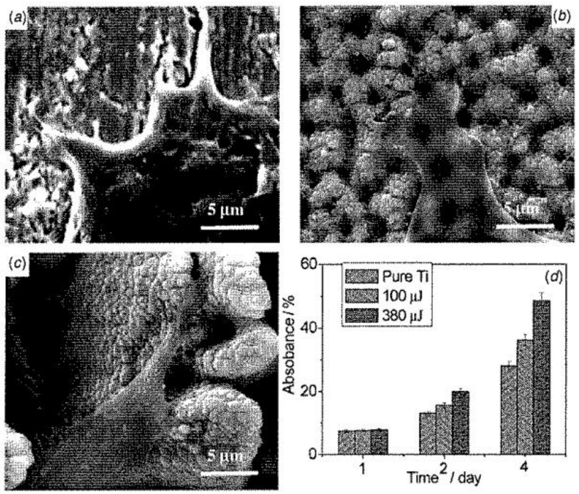
Laser type	Application	Surface	*In vitro* results	*In vivo* results	References
Nd:YAG 1064 nm 532 nm 355 nm	• **Irradiation** • **Ablation***	• Ti alloys, Ti6Al4V Sand blasted surface • CP Ti dental implants • Ti plates	• Microbial ablation • SEM, XPS • OM, AFM, XRD • 2T3 osteoblast ,MTT measurements	• Rabbit femur, tibia, torque tests • Histomorphometry	•R. Giannini et al. *Clin Oral Impl Res*, 2006 • R.S. Faeda et al. *Braz Oral Res.* 2009 • R.S. Faeda et al. *J Oral Maxillofac Surg*, 2009 • C. Hallgren et al. *Biomaterials* 2003 • M. Marticorena et al. *Journal of Physics: Conference Series* 2007 • M.W. Turner et al. *Applied Surface Science* 2005 • M Trtica et al. *Applied Surface Science*, 2006 • N. Mirhosseini et al. *Applied Surface Science* 2007
20 ns KrF excimer 248 nm	Pulsed laser deposition (PLD)	HA coatings on Ti6Al4V surface	SEM, AFM, XPS, XRD Nanoindentation	-	• G.P. Dinda et. al. *Acta Biomaterialia* 2009
50 fs laser 800 nm	• Irradiation in SBF • Ablation (1 atm) Irradiated area: 100-1200 μm	• Ti6Al4V • CP Ti plates	SEM, EDX Osteoblasts (OCT-1) MTT	• enhancement of Ca/P deposition • Osteoblast will grow better	• C. Symietz et al. *Acta Biomaterialia*, 2010 • H. Wang et al. *Biomed Mater*, 2010 • A.Y. Vorobyev & C. Guo, *Applied Surface Science* 2007; 253:7272–7280

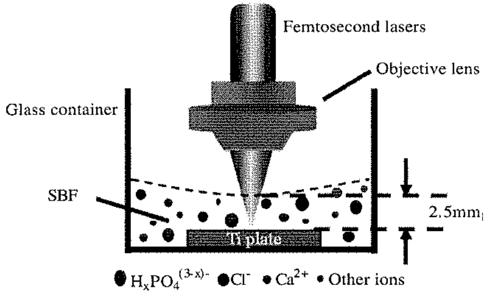
* Nd:YAG laser is used also for dentin ablation (formation of dentin craters): A. McDonald et al. *Biomaterials* 2002; 23:51-58

Femtosecond lasers

H.Wang et al. *Biomed Mater*, 2010;5:054115

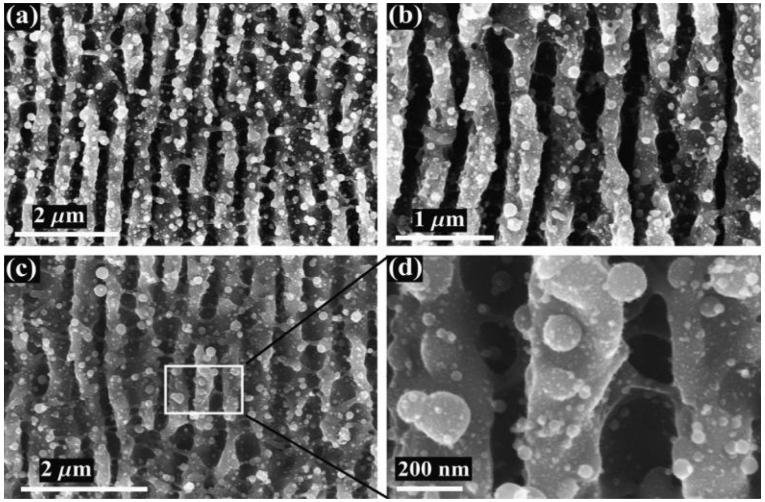
- 50 fs, 800 nm, 3.3-12.5 J/cm^2, 1000 Hz
- Irradiation of CP Ti plates in SBF (simulated body fluid)
- SEM, EDX studies: Ca/P deposition was enhanced.
- Osteoblast (OCT-1) MTT studies: cells were growing faster, more filopodia were observed.
- Double effect: increased roughness and the deposition of Ca/P salts improved cell attachment and proliferation.





A.Y. Vorobyev & C. Guo, Applied Surface Science 2007; 253:7272–7280

- 65-fs, Ti:sapphire laser, 800 nm, 1 mJ, 1 kHz
- Several micro- and nanostructures can be produced on Ti plates
- Parallel hollows will form
- 1–15 µm micro roughness
- Smooth surfaces and 10 nm diameter spherical nanostructures



F = 0.067 J/cm^2. (a) after 40 pulses (b) after 100 pulses
(c) After 400 pulses (d) magnifiication

Advantages of laser modifications

- Lasers are used more and more for surface modifications (Bauerle, 2000; Joob-Fancsaly et al., 2000).
- These techniques must be further improved, since medical applications require high accuracy in both mechanical and chemical characteristics.

- Advantages:
  - Precision in the wavelength of the light
  - Wide range of wavelength
  - High energy density ranges
  - Focusing or widening the light beam
  - Possibility of pulse mode and adjustment of FWHM
  - Advantage of excimer laser treatment compared to the others: the ultraviolet (UV) wavelength range has a sterilizing effect (*Bereznai et al*., 2003).

Main topics

1. Introduction, Faculty of Dentistry, research program
   - Biomedical sciences and our research field
   - Main characteristics of dental implants
   - Surface aspects of biomaterials, biorecognition

2. Surface modifications enhancing biointegration/osseointegration
   - Physical-chemical surface modifications
   - Laser ablation of Ti surfaces, background/literature overview

3. Laser ablation of Ti discs
   - Bereznai et al., *Biomaterials,* 2003
   - A. Györgyey et al., *MSEC*, 2013:
     - SEM, AFM and XPS studies
     - *In vitro* cell culture experiments - MG63 osteoblast cells

Biomaterials, 24; (23): 4197-4203, 2003

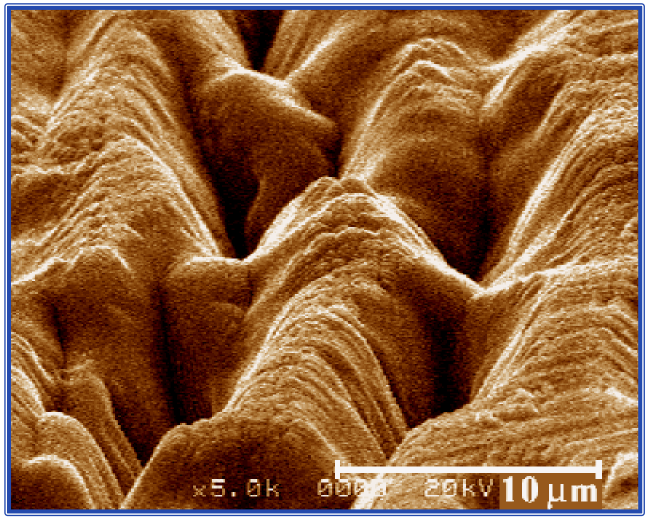
ELSEVIER
Available online at www.sciencedirect.com
SCIENCE DIRECT
Biomaterials 24 (2003) 4197–4203
Biomaterials
www.elsevier.com/locate/biomaterials

Surface modifications induced by ns and sub-ps excimer laser pulses on titanium implant material

M. Bereznai[a,*], I. Pelsöczi[b], Z. Tóth[c], K. Turzó[b], M. Radnai[b], Z. Bor[a], A. Fazekas[b]

[a] *Department of Optics and Quantum Electronics, University of Szeged, Dóm tér 9., Szeged, H-6720, Hungary*
[b] *Department of Dentistry and Oral Surgery, University of Szeged, Tisza Lajos krt. 64., Szeged, H-6720, Hungary*
[c] *Research Group on Laser Physics, Hungarian Academy of Sciences, Dóm tér 9., Szeged, H-6720, Hungary*

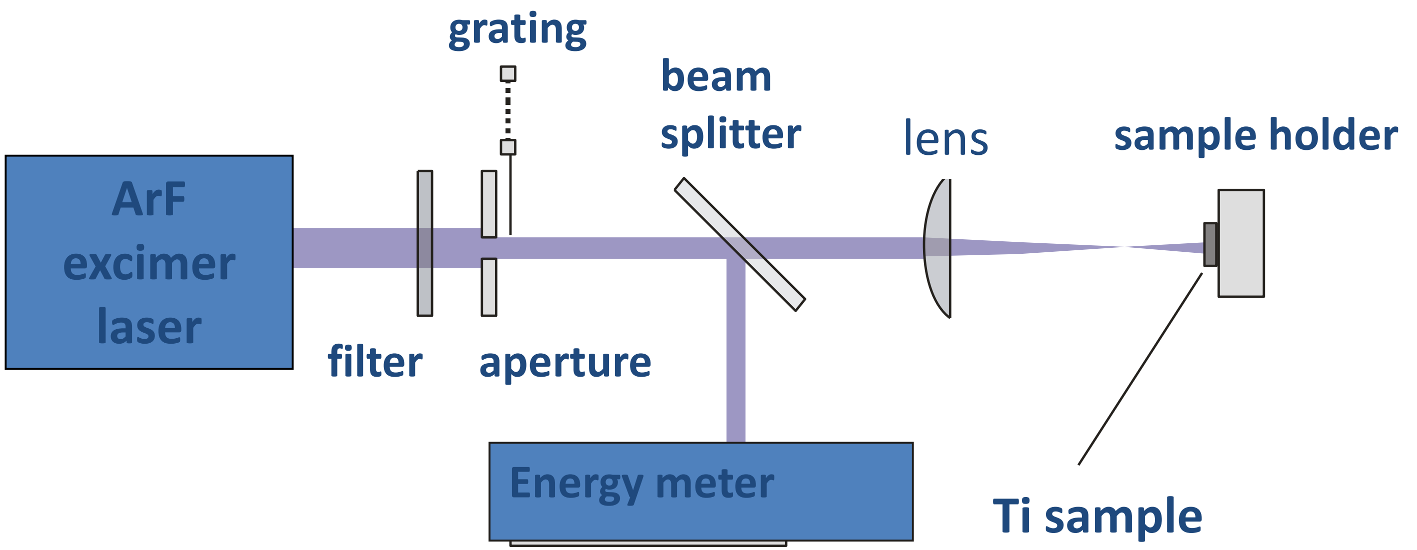
Received 21 December 2002; accepted 8 April 2003



Cooperation with Department of Optics and Quantum Electronics, Faculty of Science and Informatics

Excimer laser surface modification: polishing and ablation

Laser (Lambda Physics EMG 201):



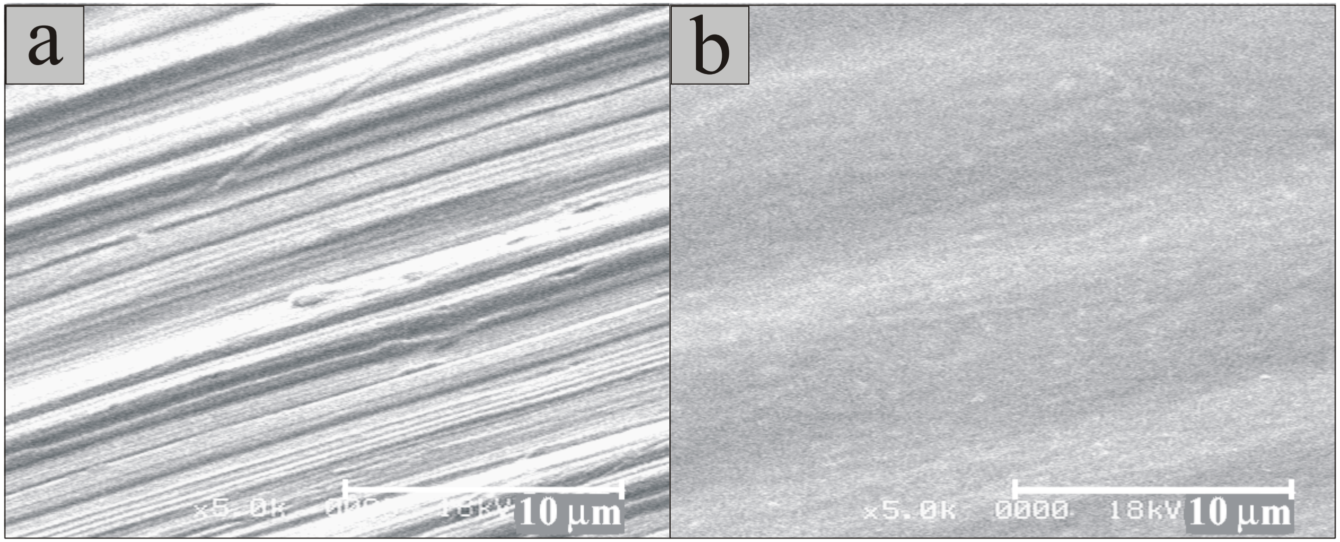
Laser:

wavelength 193 nm (UV)
pulse duration: 18 ns
pulse energy: 100 mJ

Sample: CP grade 1 Ti-disc

8 mm Ø, 1.25 mm thick

SEM image of laser polished Ti disc

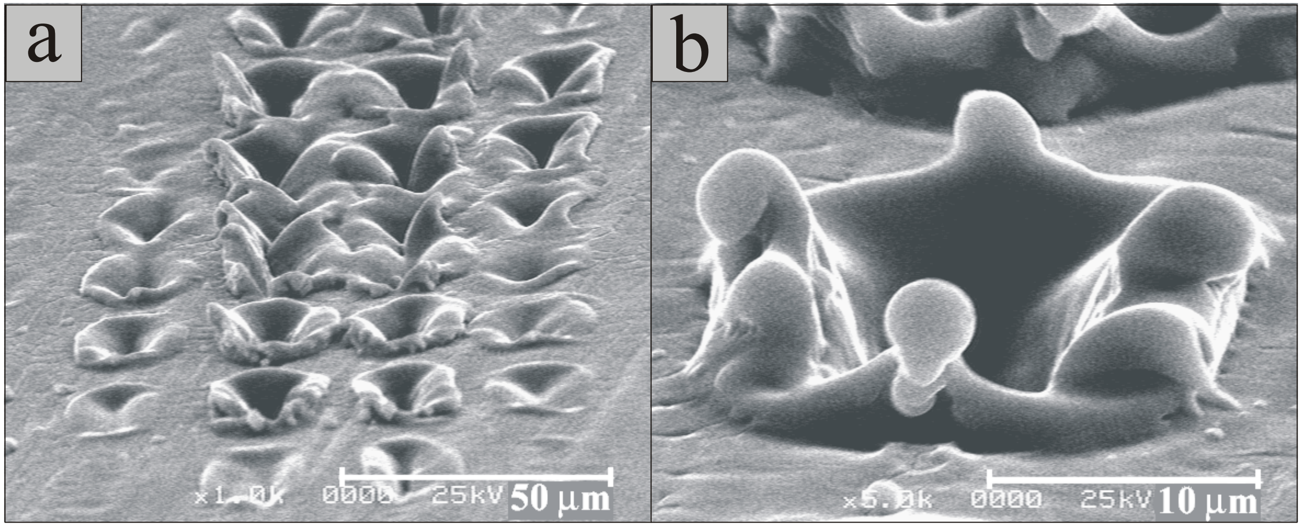


Non-irradiated **Laser-polished**

5000 x magnification, 10 laser pulses, 3.5 J/cm^2 incident fluence

Result: Ti sample with R_a < 1 mm can be effectively polished by homogenous 3-5 J/cm^2 fluence laser.

SEM images of 18 ns ArF- laser ablated Ti surface



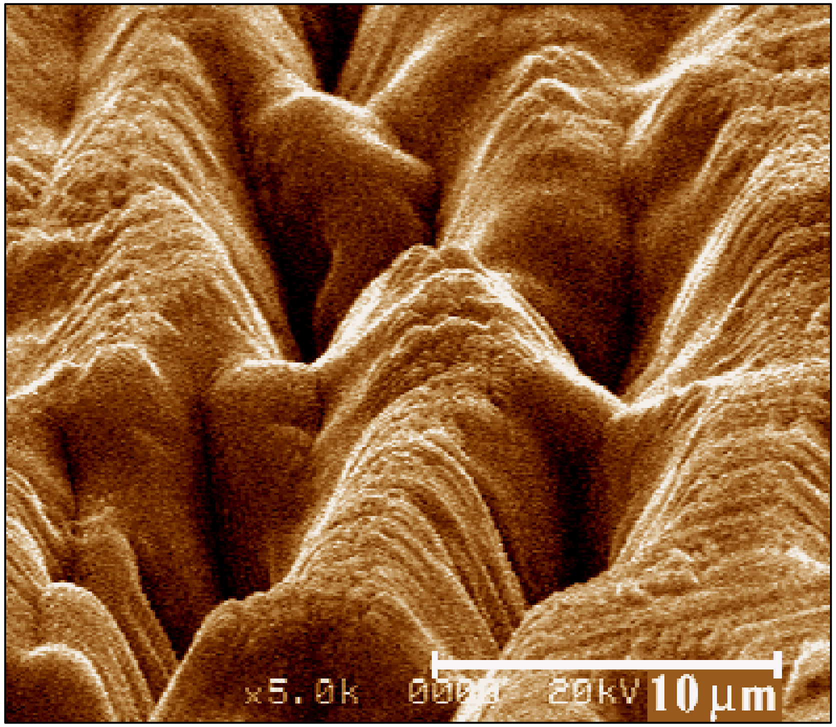
1000 x magnification

5000 x magnification

250 laser pulses, 8.5 J/cm^2 incident fluence

Result: Enlarged contact area, holes of about 20x10 µm, but fragile rims were formed around the holes (due to extensive evaporation and melting, plasma is formed). Inconvenient effect, as the rims may break away from the surface.

SEM image of the surface modifications induced by 0.5 ps KrF excimer laser pulses

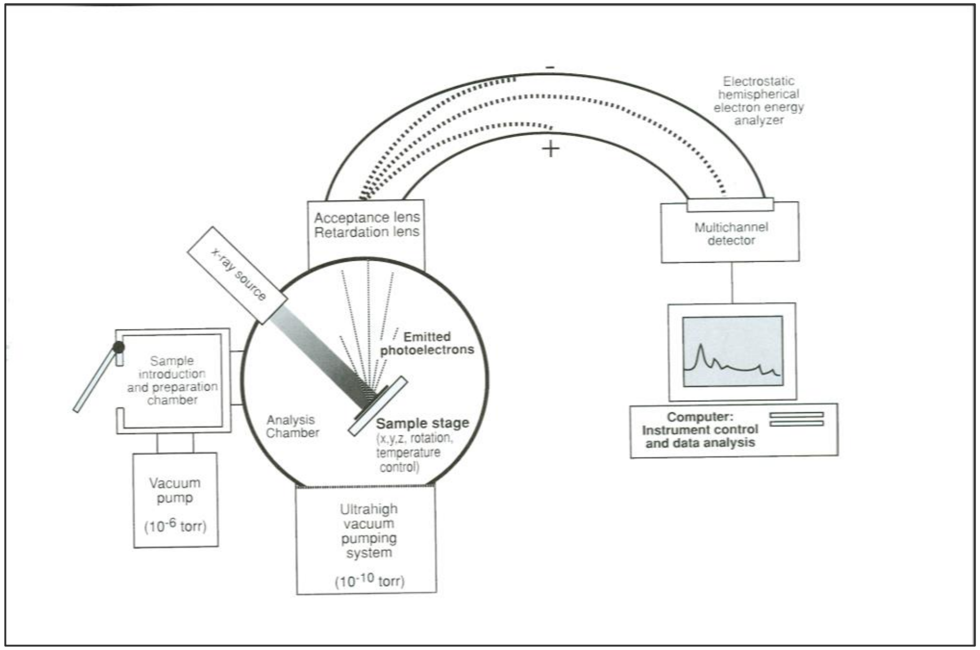


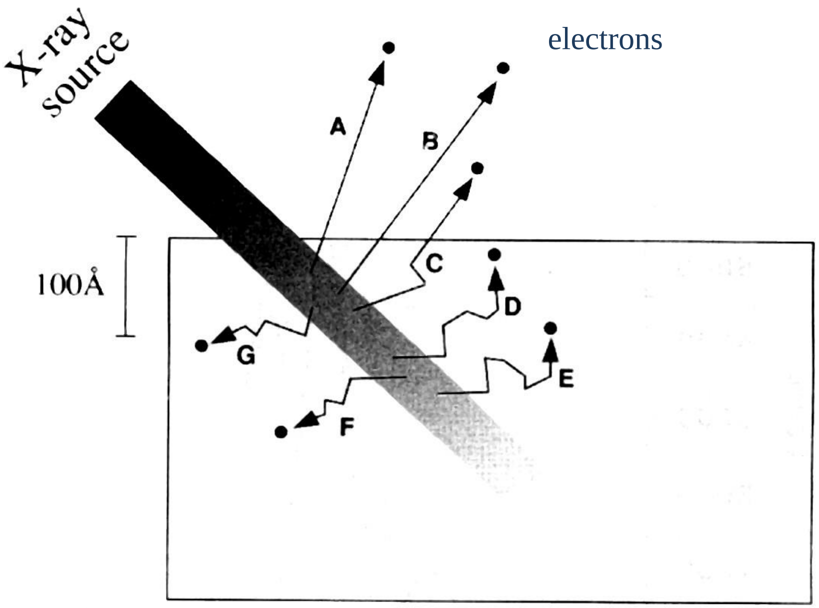
Results:

- Enlarged contact area
- Cleaner surface (XPS, XRD)
- Increased thickness of TiO_2

Laser ablation with KrF excimer laser (λ = 193 nm), pulse duration: 0.5 ps, 5000 x magnification, 1000 laser pulses, 2.4 J/cm^2 incident fluence.

X-ray photoelectron spectroscopy (XPS or ESCA)

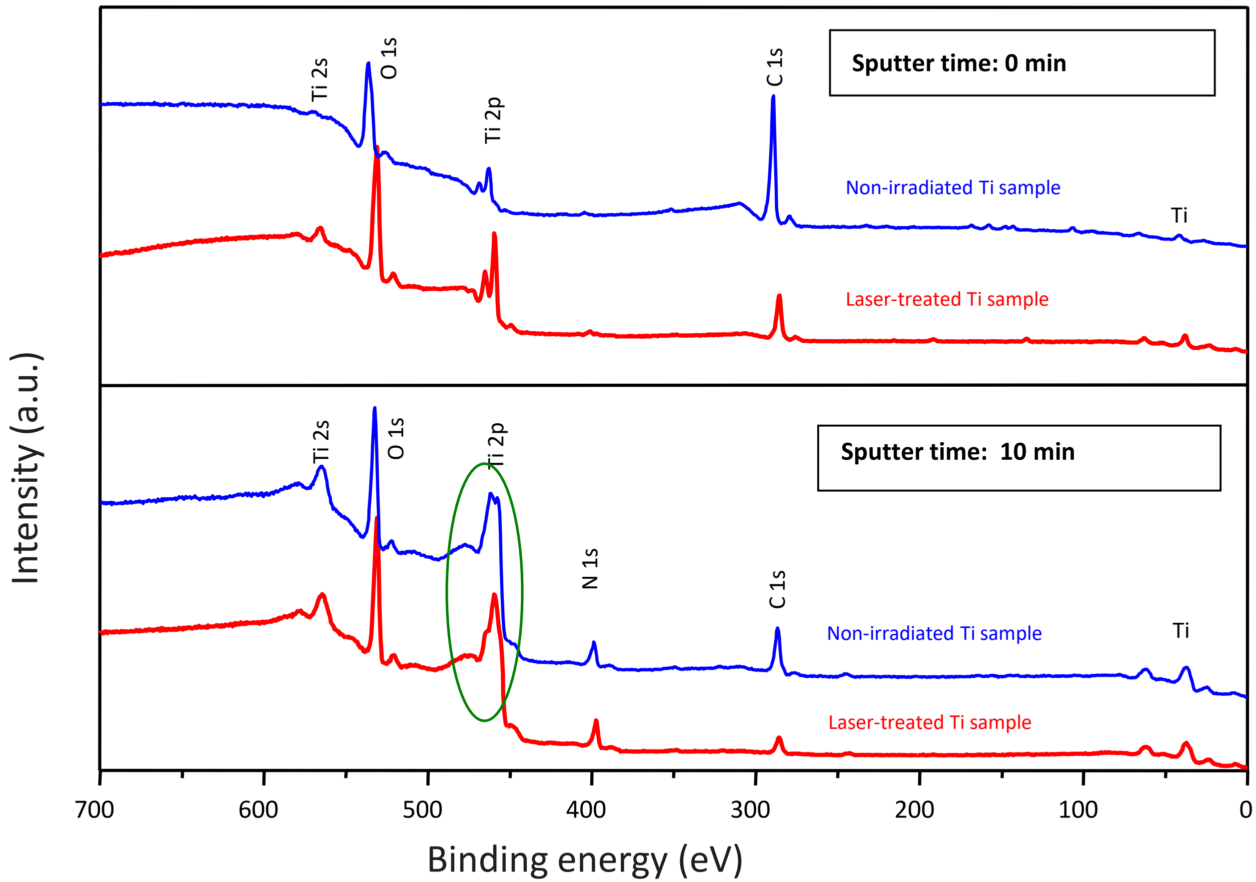




Ar⁺ bombardment:

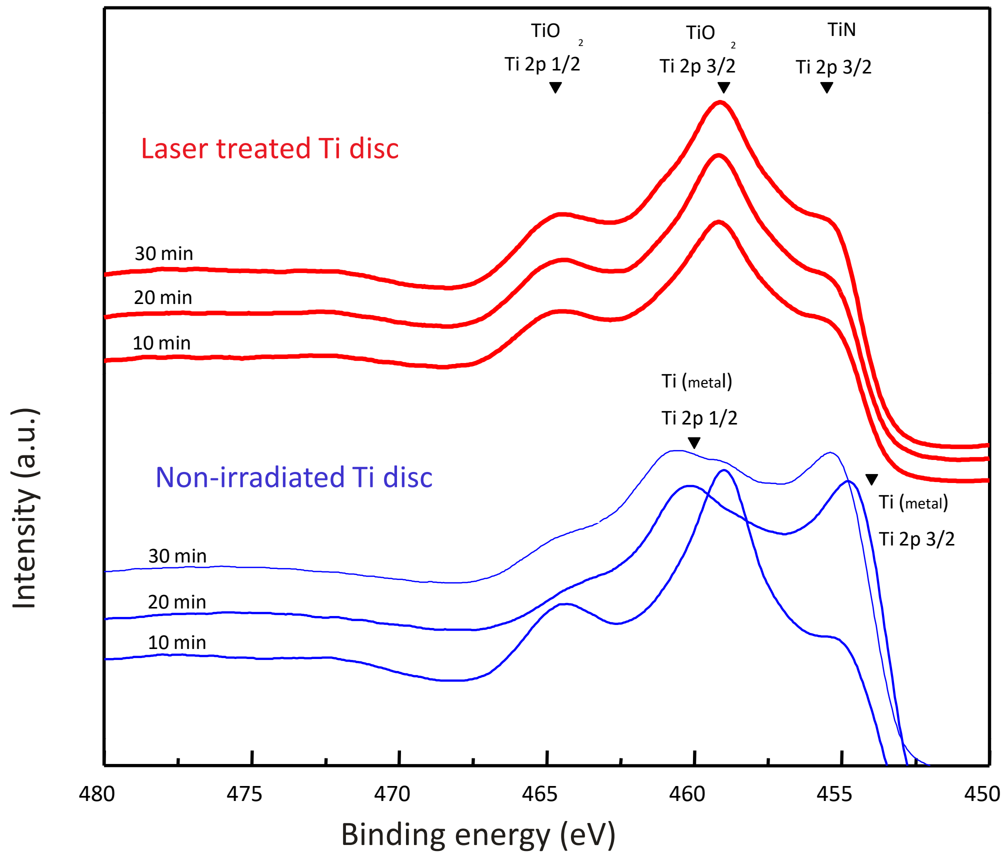
~ 10 nm of material was removed in 10 min.

X-ray photoelectron spectroscopy (ESCA) survey spectra of non-irradiated and ArF laser polished Ti discs



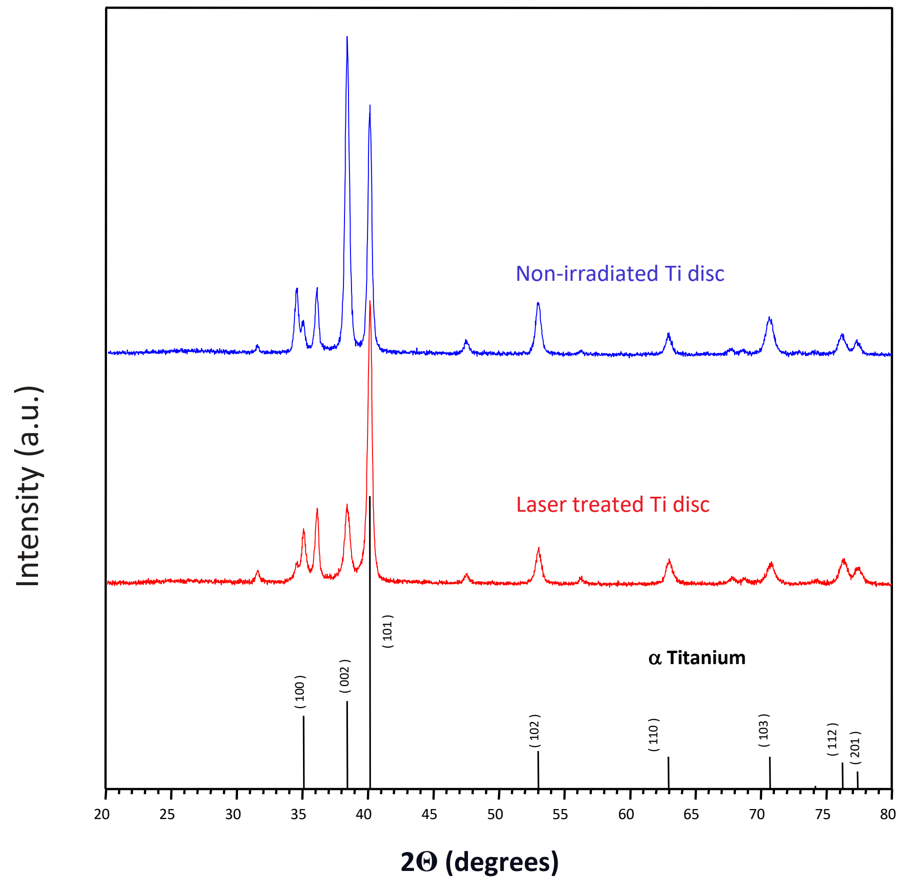
The laser cleans the surface and ...

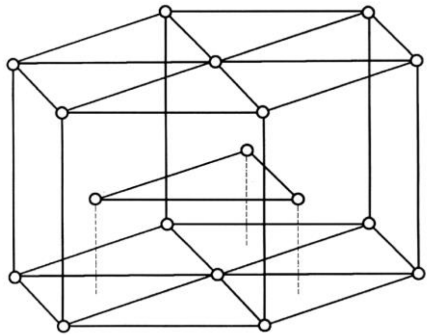
High-resolution XPS spectra of non-irradiated and ArF laser-treated titanium samples



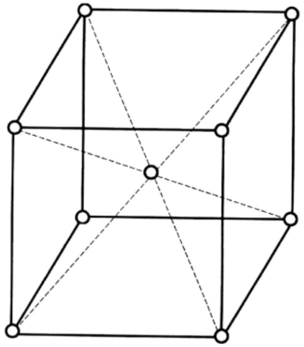
increased the thickness of the TiO_2 layer.

X-ray diffraction spectra of non-irradiated and irradiated Ti samples





α - Hexagonally Closed Packed



β (Body Centered Cubic), above 1158 K

The original crystalline structure is preserved.

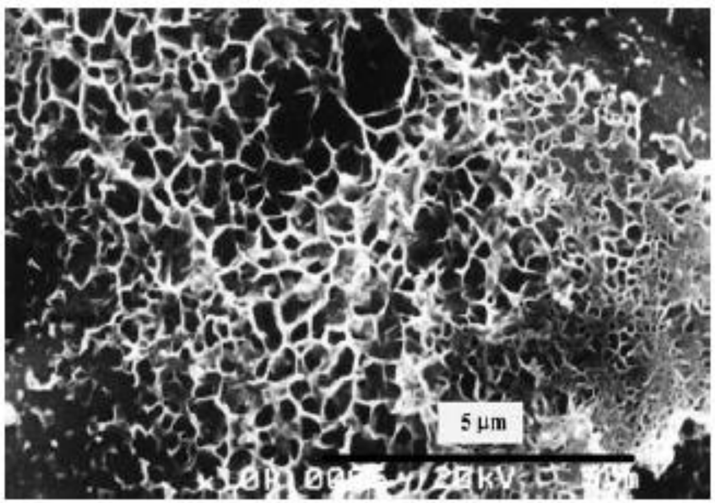
Ca-P coating of Ti surface

Colloid Polym Sci (2005) 283: 587–592
DOI 10.1007/s00396-004-1188-y

ORIGINAL CONTRIBUTION

Márta Szekeres
Gabriella Fodor
András Fazekas
Márta Radnai
Kinga Turzó
Imre Dékány

Formation of octacalcium phosphate by heterogeneous nucleation on a titania surface



SEM image of calcium phosphate nucleated on the surface of a titanium plate pretreated by laser ablation and heating at 450 °C for 3 days.

Cooperation with Dept. of Colloid Chemistry, Faculty of Science and Informatics, University of Szeged

Aims

- To investigate the effect of the Nd:YAG and excimer laser ablation on Ti implant surface and to compare with a typical dental implant surface (acid etched and sand blasted)

- Morphological (scanning electron microscopic-SEM and AFM images) and chemical composition studies (XPS)

- Testing the response of the biological environment:
  - *In vitro* cell culture studies
  - Investigation of the attachment and proliferation of human MG63 osteoblast-cells

Materials and methods

Samples:

- **Acid etched and sand blasted surfaces**, 9 mm Ø and 1.5 mm thickness
  Denti® System Ltd. (Hungary) CP grade 4 titanium discs.
- Samples were cleaned ultrasonically in acetone and then 70% ethanol, each for 15 min, and subsequently rinsed in ultrapure water.
- **Laser treatment - ablation** at Department of Optics and Quantum Electronics, Faculty of Science and Informatics
  - **Q-switched Nd:YAG laser:** λ= 532 nm, pulse energy 40 mJ, fluence 1.3 J/cm^2, FWHM: 10 ns, 200 pulses
  - **KrF excimer laser:** λ= 248 nm, fluence 0.4 J/cm^2, FWHM: 18 ns, 2000 pulses

Investigation methods:

Surface science methods

- Optical microscope, SEM, AFM, XPS

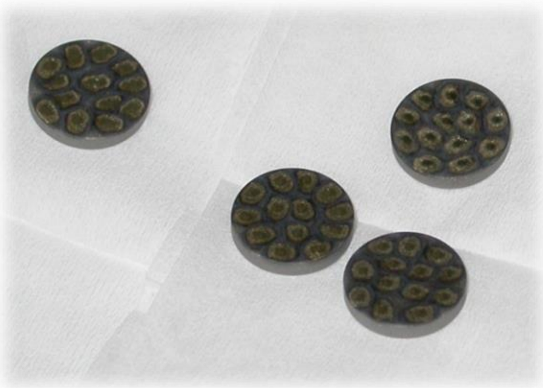
In vitro cell culture: attachment (24 hours) and proliferation (72 hours) of human MG63 osteoblast-cells by dimethylthiazol-diphenyl tetrazolium bromide (MTT) and Alamar Blue (AB) methods.

Laser treatment of Titanium surfaces

Laser type	Wavelength	Fluence	FWHM	Nr. of pulses
Nd:YAG	532 nm	1.3 J/cm^2	10 ns	200
KrF excimer	248 nm	0.4 J/cm^2	18 ns	2000

Denti -control

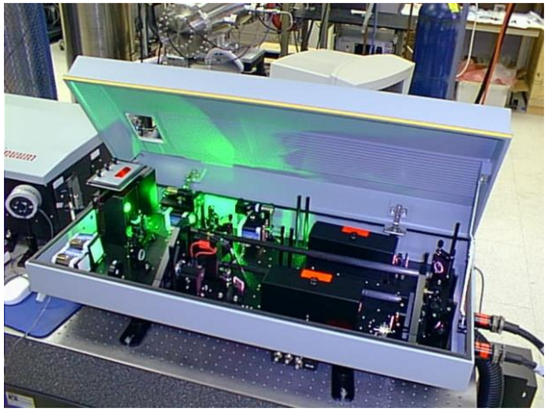
Nd:YAG laser



Excimer laser

Use of the applied lasers

Nd:YAG laser



- Solid state laser:
  - Nd-Neodimium; Y-Yttrium; A- Aluminum; G-Garnet crystal
- 1964, Bell Laboratory, J.E. Geusic
- Typically infrared range: 1064 nm, but frequency doubling: 532 nm (355 or 266 nm)
- Pulse and continuous mode
- FWHM: 10-25 ns
- Applications:
  - Industry: etching and engraving surfaces
  - Ophthalmology: glaucoma treatment, retina photocoagulation
  - Oncology: skin tumor removal
  - Cosmetics: treatment of cellulitis, removal of pelage
  - Oral medicine, dentistry: soft tissue operation, gingivectomy, periodontal applications

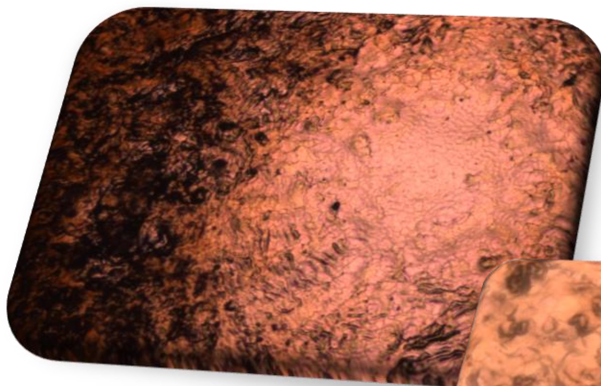
Excimer laser

- Pulse and gas laser family
- 1970, (Xe dimer) N. Basov, VA Danilychev, YM Popov, Lebedev Physical Institute, Moscow
- EXCIted diMER = excited molecule with two atoms
- Noble gas-halogen gas mixture (ArF, KrF, XeCl, etc.) electric discharge will form excited state noble gas-halid molecules.
- Ultraviolet range:

  λ = 126-351 nm; frequency: 100 Hz; UHWM: 10 ns
- Advantages:
  - Biological and organic materials absorb it well
  - Does not burn and cut the material, it disrupts the molecular binding of surface tissues, the material will be atomized, ablation will occur (material removal) without formation of heat
- Applications:
  - Micro fabrication of organic materials (polymers)
  - Photolithography, fabrication of chips
  - Precision eye surgery (LASIK), vein surgery
  - Dermatological applications: psoriasis, vitiligo, etc. treatment

Optical microscope images

Nikon Eclipse 80i, Japan
Faculty of Dentistry



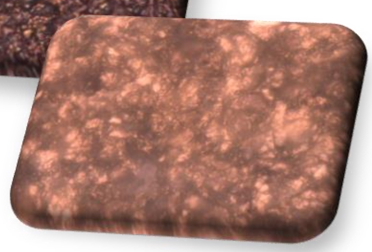
100x

400x

Nd:YAG modified

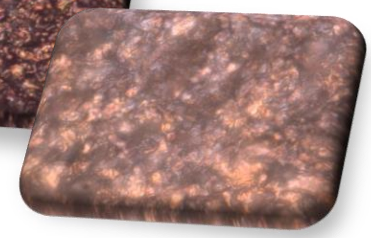
Control surface

200x



400x

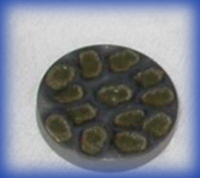
200x



400x

Excimer modified

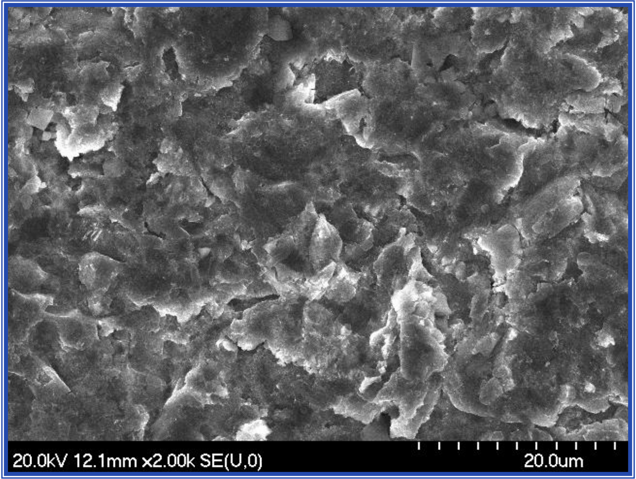
Scanning Electron Microscope images Nd:YAG laser ablation

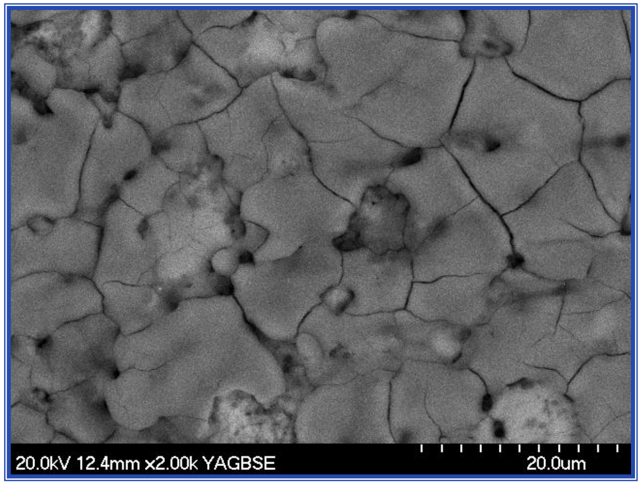


SEM: Hitachi S4700, Japan

Control:

Acid etched, sand blasted





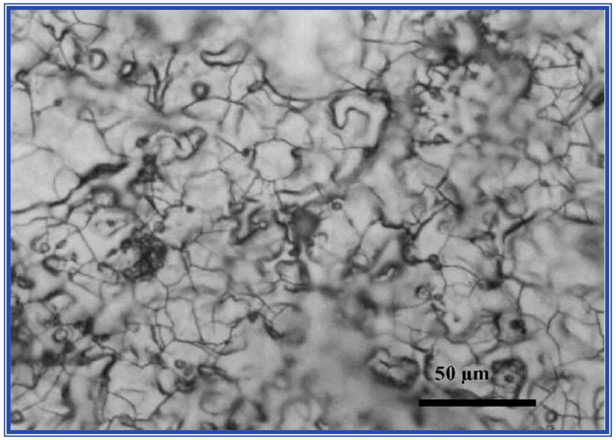
Nd:YAG:

lamellar structure, cracks, rims and holes

1300 mJ/cm^2, 200 pulses

Similar result:

- M.W. Turner et al. *Applied Surface Science* 2005;247:623-630
  - Micro cracks
  - 1385 mJ/ cm^2, 25 A, 25 kHz, 50 mm/s
  - 500x magnification

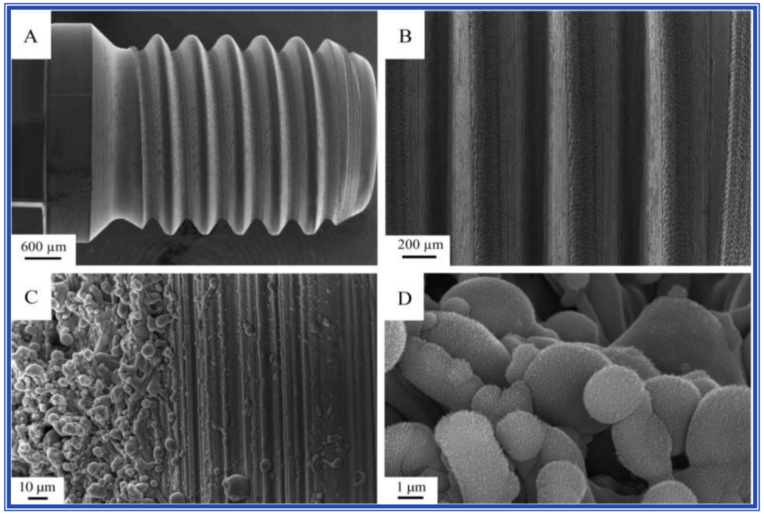


Surfaces modified with Nd:YAG lasern

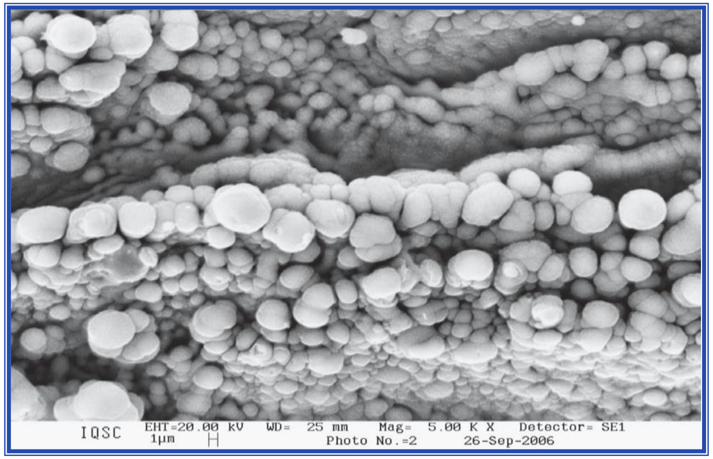
Most often Nd:YAG laser is applied for modification of CP Ti dental implants.

- SEM, AFM, XPS and animal experiments prove their effectiveness (torque tests and histomorphometry).

- R. Brånemark et al. *Nanomedicine: Nanotechnology, Biology and Medicine* 2011; 7:220-227
- R.S. Faeda et al. *Braz Oral Res (Implantology)*, 2009, 23(2):137-43
- R.S. Faeda et al. *J Oral Maxillofac Surg*, 2009, 67:1067-1715



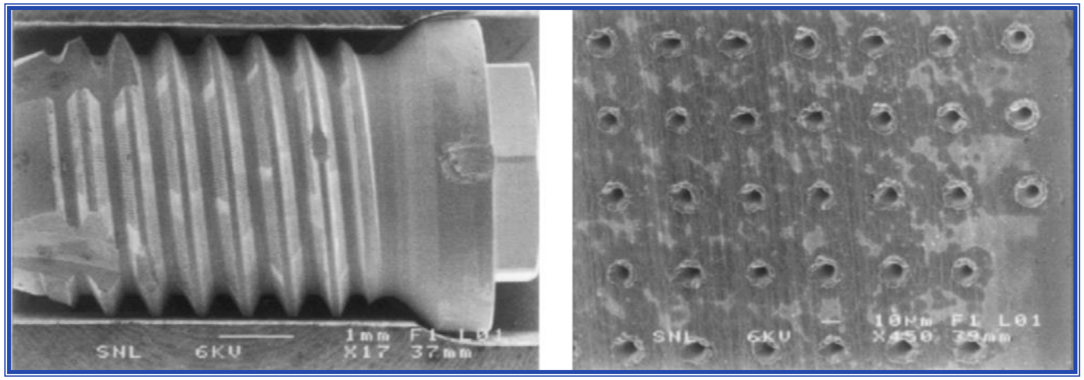
Brånemark et al., 2011



R.S. Faeda et al., 2009
5000x magnification

Surfaces modified with Nd:YAG lasern

- C. Hallgren et al. *Biomaterials* 2003; 24:701-710
- M. Marticorena et al. *Journal of Physics: Conference Series* 2007; 59:662-665

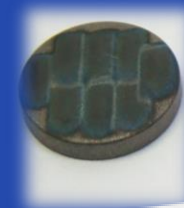


C. Hallgren et al., 2003

Disadvantages:
During Nd:YAG laser ablation due to the multiple illumination rims will form on the Titanium surface. Contamination will occur.

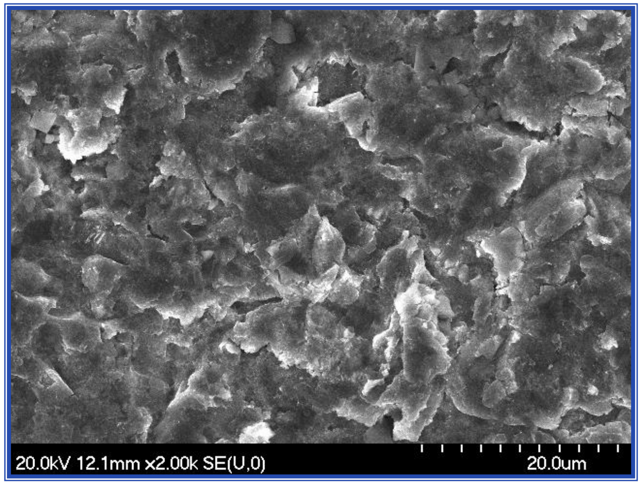
Gaggl et al., 2000; Pető et al., 2001, György et al., 2002

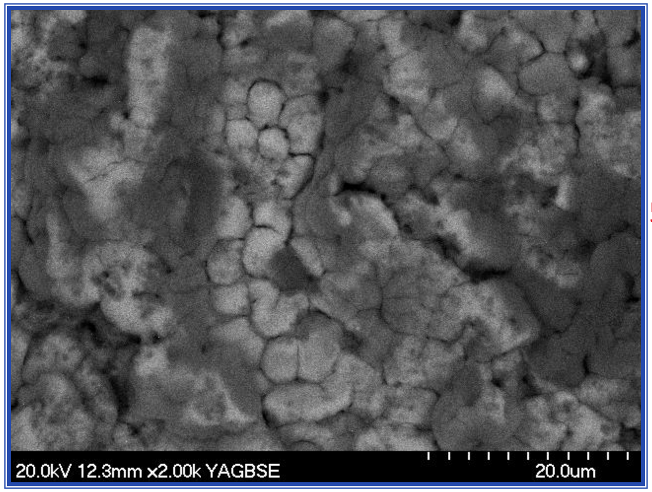
Scanning Electron Microscope images
Excimer laser ablation



SEM: Hitachi S4700, Japan

Control:
Acid etched,
sand blasted

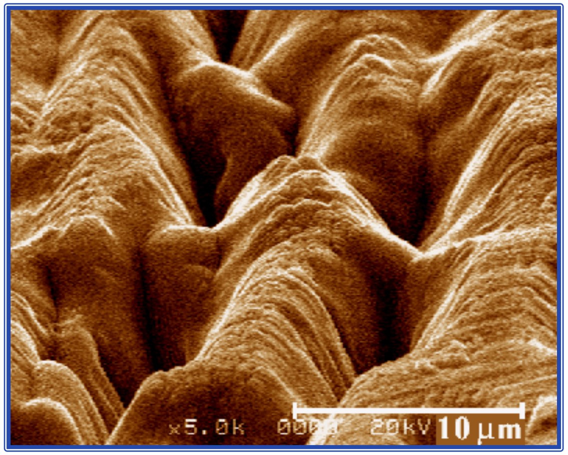




Excimer:
rounded, almost uniform structures ,
5–10 μm in diameter
248 nm
0,4 J/cm^2
18 ns, 2000 pulses

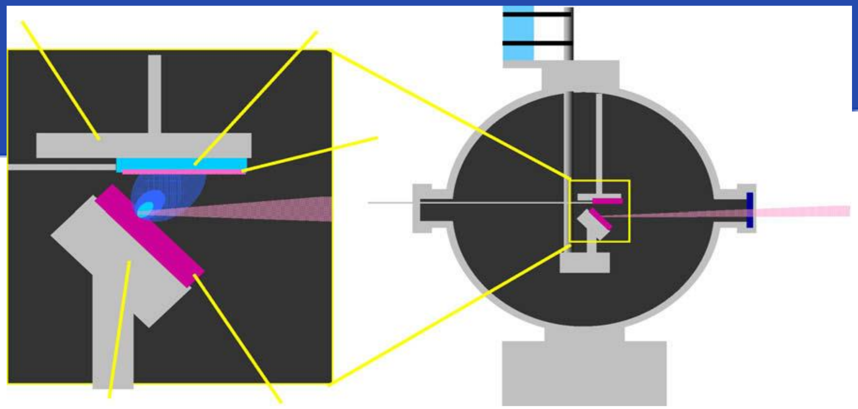
Comparison:

- *M. Bereznai et al. Biomaterials,* 2003, 24(23): 4197-4203



- The **KrF laser used in 2003** (248 nm) FWHM was much shorter**: 0,5 ps ,** pulse energy was 10 mJ, fluence was 2,4 J/cm^2 and 1000 pulses were applied.
- The samples were ablated under vacuum (10 Pa) , to avoid plasma formation in air (prevents light absorption).

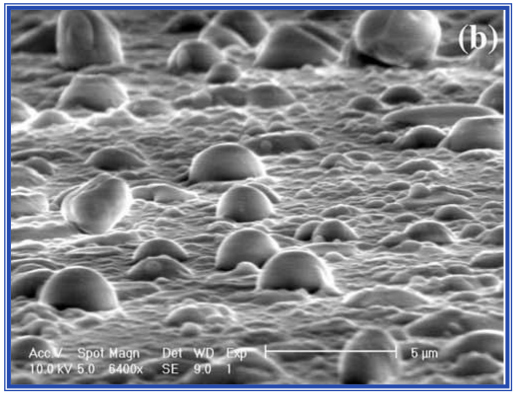
Pulsed Laser Deposition (PLD)with KrF excimer laser

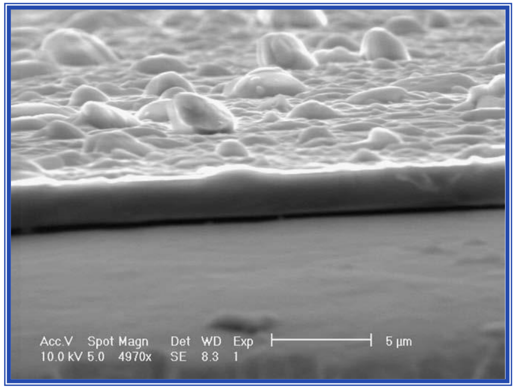


Excimer laser is rarely used for ablation of implants!

- T. Smausz et al. *Appl. Phys. A*, 2004; 79:1101-1103: ArF and KrF, PLD of tooth material

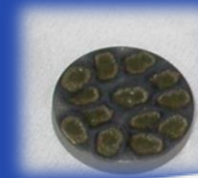
- Dinda et al. *Acta Biomaterialia 2009*; 5:1821–1830: Pulsed Laser Deposition of HA on Ti6Al4V surface
- KrF excimer laser (Lambda Physik EMG 201 MSC)
  - 248 nm, 20 ns, 10 Hz
  - 45° incident angle on HA sample
  - 3 x 1 mm size of spot
  - Fluence: 3 J/cm^2
- Results: the PLD and 300° C treatment gave a very clean , crystalline HA coating. It did not dissolve in SBF, very good attachment to the surface.





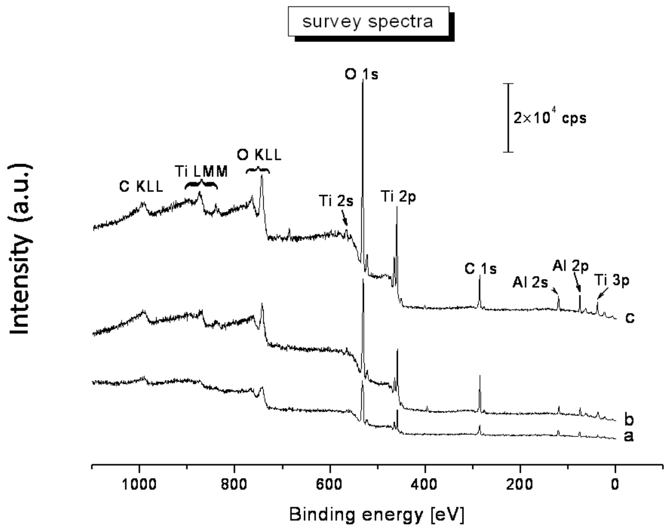
We did not find any publication investigating the response of osteoblasts on excimer-laser treated Ti implants.

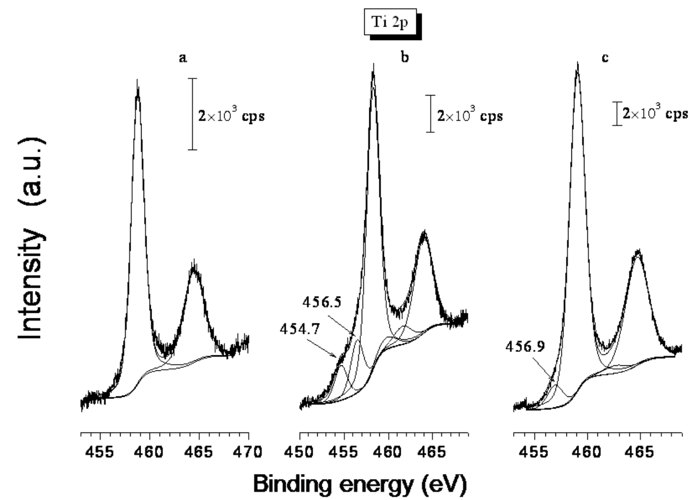
Results of XPS measurements



XPS: PHOIBOS 150 MCD 9, SPECS

Department of Physical Chemistry and Materials Science, Faculty of Science and Informatics





a) Control surface: acid etched and sand blasted Ti surface

b) Nd: YAG laser modified

c) KrF excimer laser modified survey **XPS spectra.**

High-resolution XPS spectra showing Ti 2p lines

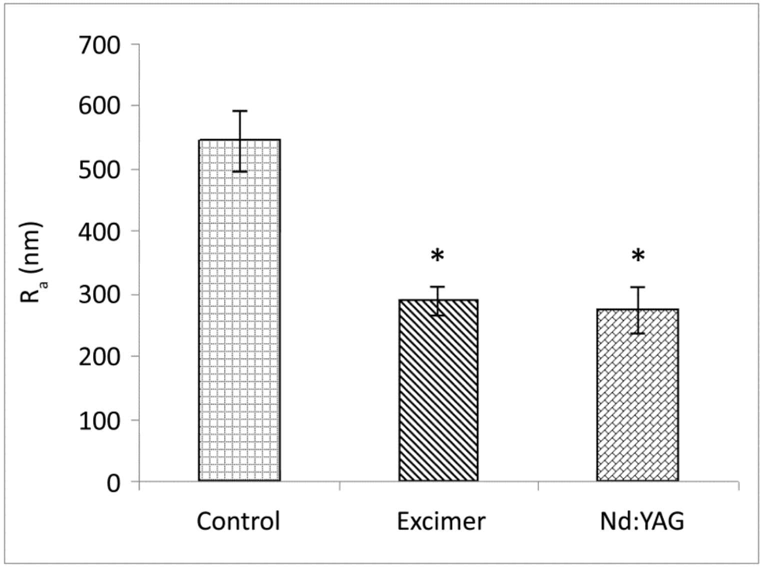
Deconvolution: at 454.7 and 456.5 eV new peaks!

An intact TiO_2 layer was present on all the samples.

Only the Nd:YAG laser treatment changed the surface composition: Ti^{3+} and also metallic forms of Ti were detected besides a thin TiO_2 layer.

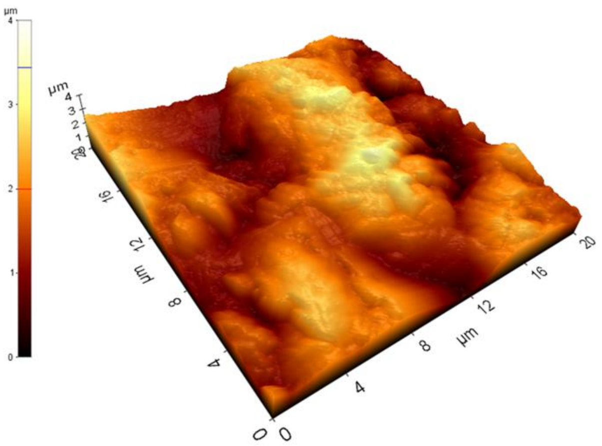
The excimer laser treatment did not provoke such a change, which favors this kind of ablation.

Results of AFM measurements (R_a –roughness)



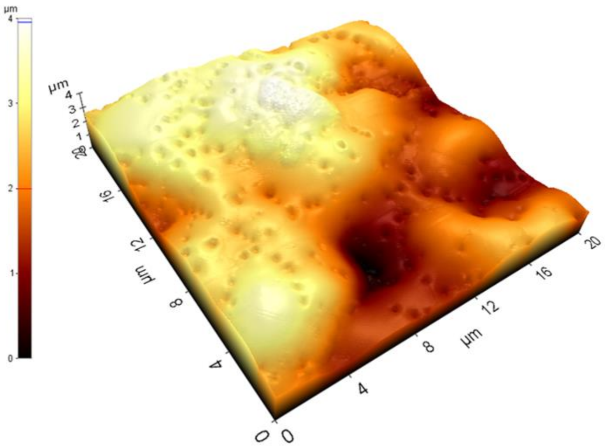
PSIA XE-100 (PSIA Inc., South Korea)

- Lasers significantly decreased surface roughness.



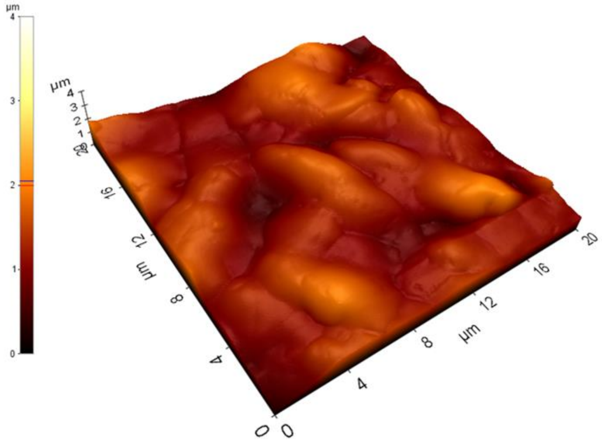
A $R_a = 544 \pm 47$ nm

Control: acid-etched and sand-blasted



B 200 laser shots
1.3 J/cm^2 fluence
FWHM = 10 ns
$R_a = 275 \pm 36$

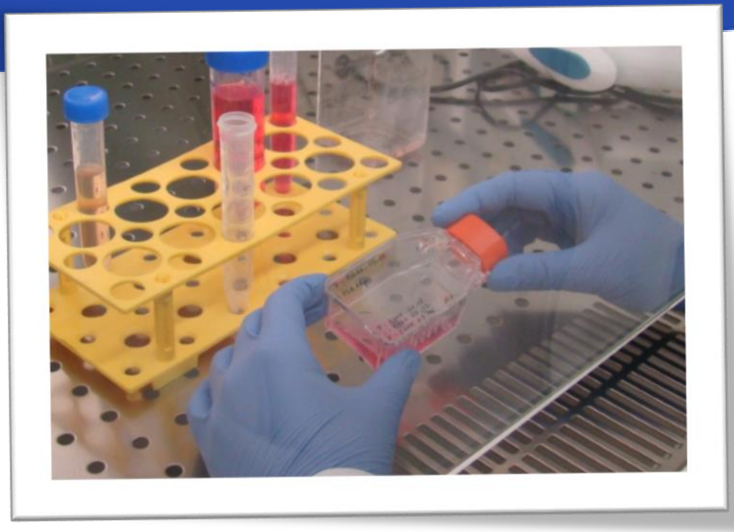
Nd:YAG laser treated



C 2000 laser shots
0.4 J/cm^2 fluence
FWHM = 18 ns
$R_a = 288 \pm 25$ nm

Excimer laser treated

In vitro cell culture studies



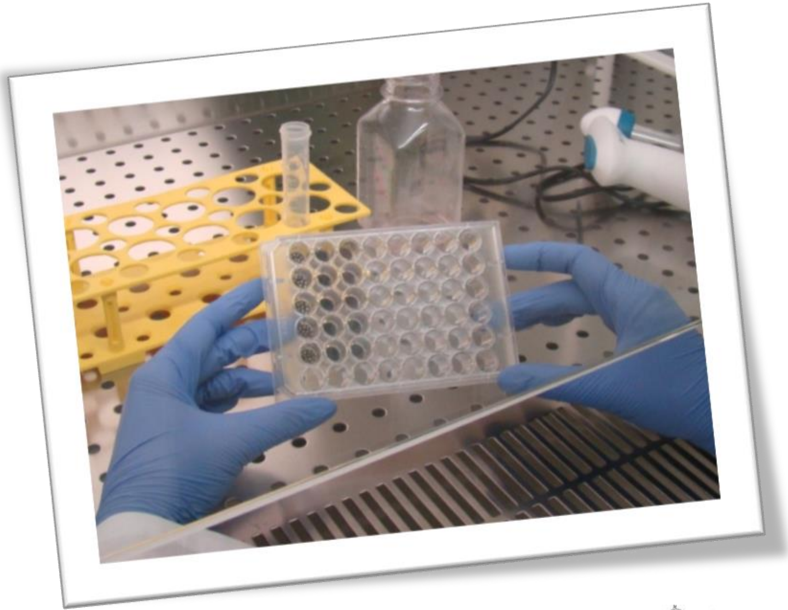
Testing the response of the biological environment

MG63 osteoblast cells (European Collection of Cell Cultures)

- Attachement (24 hours)
- Proliferation (72 hours) studies on the laser modified and non-treated (control) surfaces

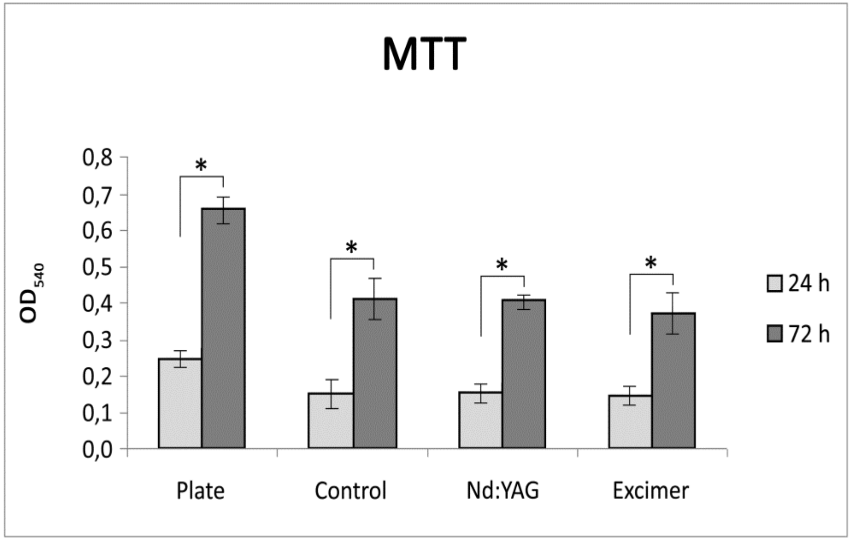
Methods/assays:

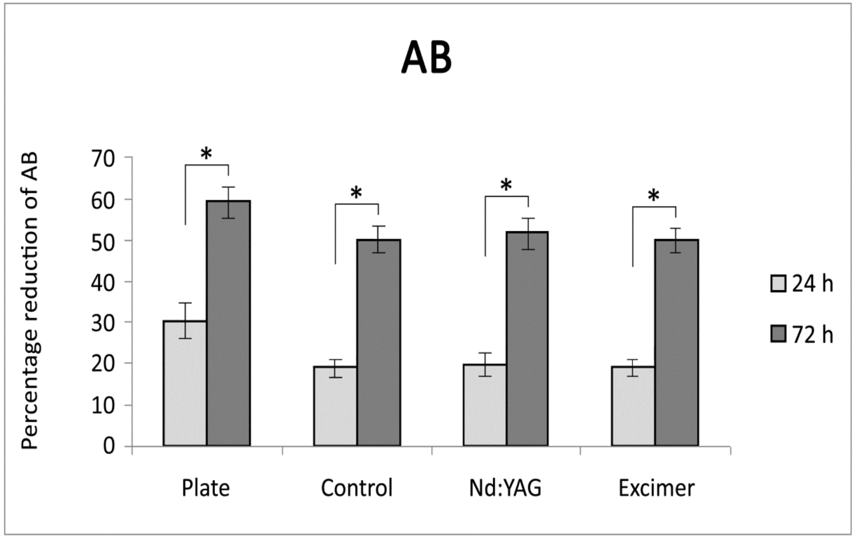
- MTT: dimethylthiazol-diphenyl tetrazolium,)
- AB: Alamar Blue
- ALP: alkaline phosphatase quantification
- SEM images



Cell culture results

- MG63 osteoblast cells attach and proliferate better on plate (positive control).
- MTT and AB measurements detected an increase in the number of cells between the 24- and 72 hour observations
- However, laser treatment did not affect cell attachment and proliferation significantly (ANOVA).





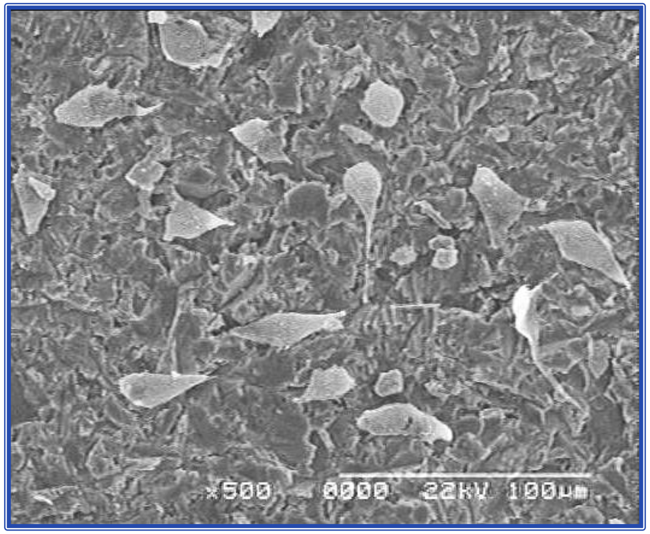
SZÉCHENYI 2020

HUNGARIAN GOVERNMENT

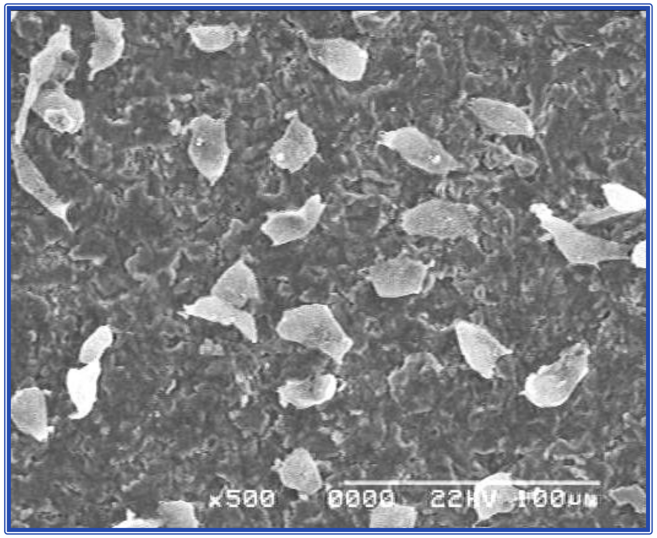
European Union European Social Fund

INVESTING IN YOUR FUTURE

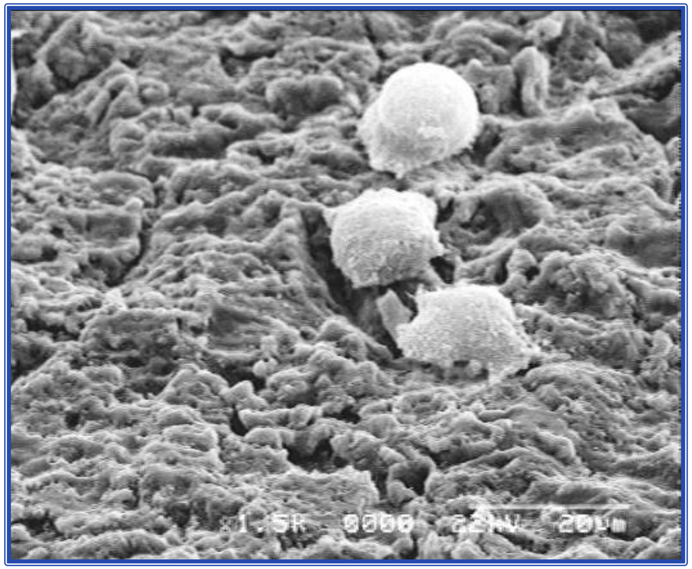
SEM images of MG63-cell attachment (24h)



Control: acid-etched and sand-blasted



Excimer laser treated



Nd:YAG laser treated

Results, conclusions

- SEM: significant differences in morphology of the original and laser-irradiated Ti samples were found; excimer laser: uniformly rounded forms, for Nd:YAG laser rims and holes appeared.
- XPS: intact TiO_2 layer on every surface, for Nd: YAG in the original TiO_2 layer appeared forms of reduced titanium (Ti^{3+}) and metallic form. Excimer laser ablation did not change the native TiO_2 layer, in contrary to the Nd:YAG laser!
- AFM: laser treatments significantly decreased the roughness of the surfaces (R_a).
- MTT and AB:
  - MTT, AB and ALP measurements detected an increase in the number of cells between the 24- and 72 hour observations;
  - however, laser treatment did not affect cell attachment and proliferation significantly

Roughness plays a significant role in determining the cell response and that the causes of implant failure are primarily **not changes in the oxide on the Ti surface**, but rather the appreciably more important biological and biomechanical factors !

Materials Science and Engineering C 33 (2013) 4251–4259

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

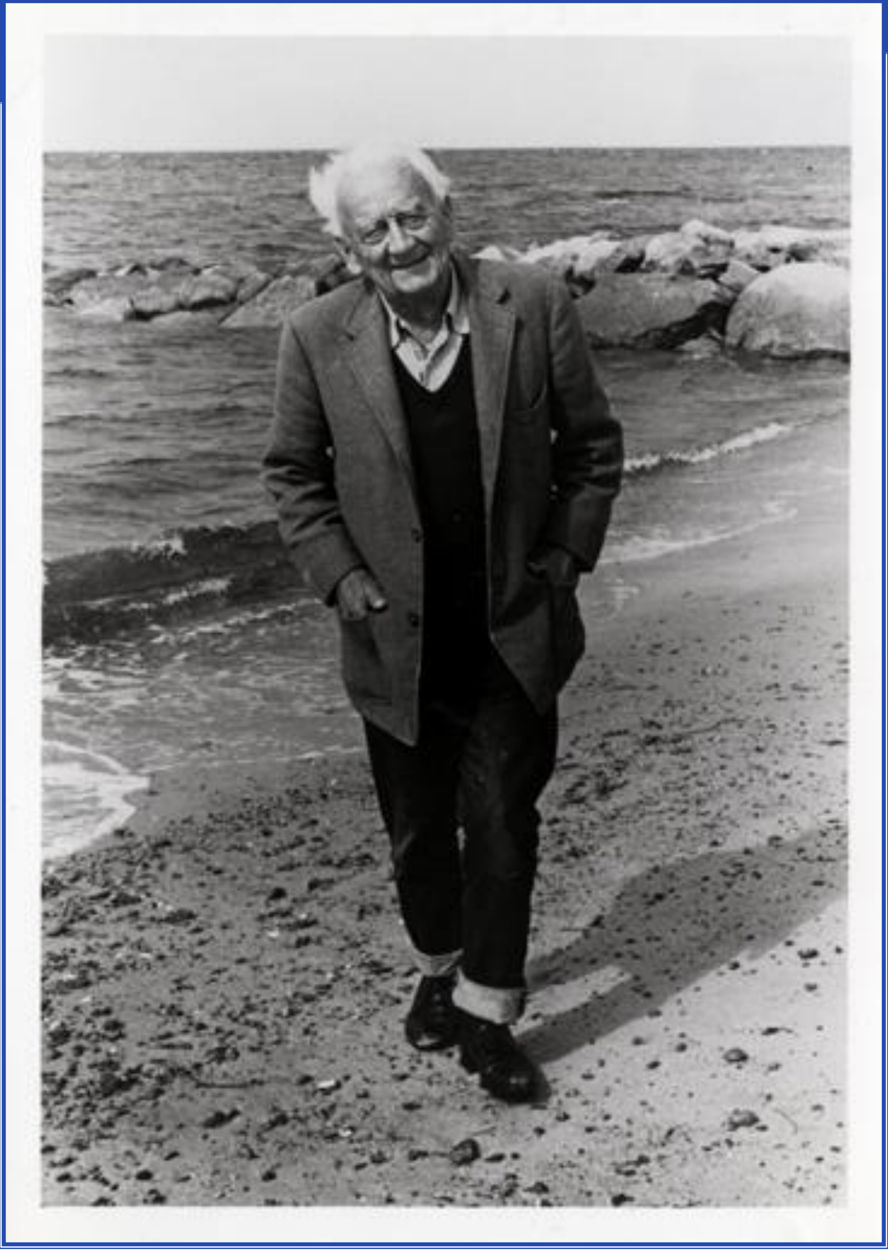
ELSEVIER

Attachment and proliferation of human osteoblast-like cells (MG-63) on laser-ablated titanium implant material

Ágnes Györgyey [a], Krisztina Ungvári [a], Gabriella Kecskeméti [b], Judit Kopniczky [b], Béla Hopp [c], Albert Oszkó [d], István Pelsöczi [a], Zoltán Rakonczay [a], Katalin Nagy [e], Kinga Turzó [a,*]

[a] Department of Oral Biology and Experimental Dental Research, Faculty of Dentistry, University of Szeged, H-6720 Szeged, Hungary
[b] Department of Optics and Quantum Electronics, Faculty of Science and Informatics, University of Szeged, H-6720 Szeged, Hungary
[c] Research Group on Laser Physics, Hungarian Academy of Sciences and University of Szeged, H-6720 Szeged, Hungary
[d] Department of Physical Chemistry and Materials Science, Faculty of Science and Informatics, University of Szeged, H-6720 Szeged, Hungary
[e] Department of Oral Surgery, Faculty of Dentistry, University of Szeged, H-6720 Szeged, Hungary

Thank you for your attention!



Albert Szent-Györgyi

„Research is to see what everybody else has seen, and to think what nobody else has thought."