Analysis of Implant Biofunctionalized with Bioactive Glass in Response to Bone Marrow Stem Cells: An In Vitro Study

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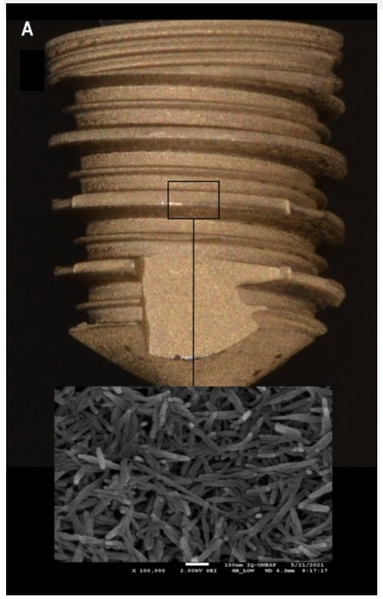
Research Funding: The Sao Paulo Research Foundation (FAPESP) n. 2013/07793-6; n. 2021/00632-3; n. 2021/14557-3

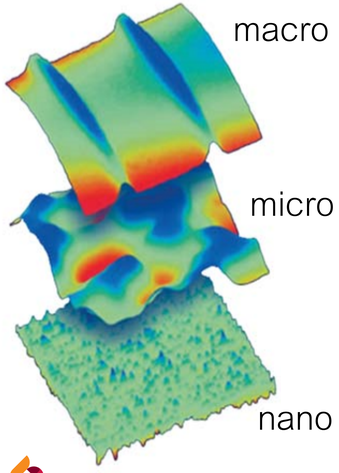
Introduction

OSSEOINTEGRATION OF DENTAL IMPLANTS:

- implant design
- **physicochemical properties of the surface**
- bone quality
- surgical techniques

Albrektsson 1983
Buser et al., 2017





S.I.N. Implant System

Bioactive glasses are highly active materials that can induce the upregulation of osteoblasts proliferation and differentiation resulting in a biologically active bone on their surface.

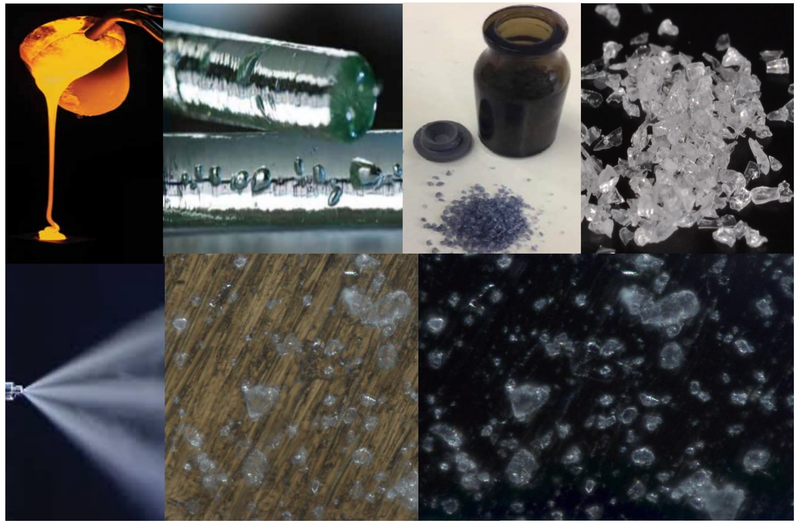
CeRTEV Center for Research, Technology and Education in Vitreous Materials

UFSCAr, Brazil
Zanotto Lab

A new bioglass (BSF18) protected by patent (2014; 003817-5, Brazil), was developed to improve the osseointegration response.

Biofuncionalization with bioactive glass F18 into surfaces

SiO_2-CaO-Na_2O-MgO-K_2O-P_2O_5 system







Preliminary studies (*in vitro*) with F18

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J Tissue Eng Regen Med (2015)
Published online in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/term.2017

RESEARCH ARTICLE

Characterization and biocompatibility of a fibrous glassy scaffold

P. R. Gabbai-Armelin[1,4], M. T. Souza[2], H. W. Kido[1,4], C. R. Tim[1,4], P. S. Bossini[3], K. R. Fernandes[3], A. M. P. Magri[3], N. A. Parizotto[5], K. P. S. Fernandes[5], R. A. Mesquita-Ferrari[5], D. A. Ribeiro[3], E. D. Zanotto[2], O. Peitl[2] and A. C. M. Renno[3]*

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Translational Materials Research

PAPER

New highly bioactive crystallization-resistant glass for tissue engineering applications

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J Mater Sci: Mater Med (2015) 26:177
DOI 10.1007/s10856-015-5516-1

BIOMATERIALS SYNTHESIS AND CHARACTERIZATION

Effect of a new bioactive fibrous glassy scaffold on bone repair

P. R. Gabbai-Armelin[1,5] · M. T. Souza[2] · H. W. Kido[1,5] · C. R. Tim[1,5] · P. S. Bossini[3] · A. M. P. Magri[3] · K. R. Fernandes[3] · F. A. C. Pastor[4] · E. D. Zanotto[2] · N. A. Parizotto[5] · O. Peitl[2] · A. C. M. Renno[3]

Aim

The aim of this study was to evaluate the osteoinductive potential of a nano-hydroxyapatite surface biofunctionalized by BSF18 in rabbit bone marrow stem cells

Methods

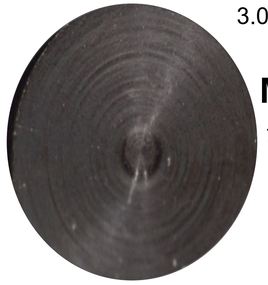
Animal Ethics Committee number
02/2021 – **UNESP/FOAr, Brazil**

Experimental design outline (*in vitro*)
All tests were performed 3x in triplicate for each sample/group

Discs/groups

S.I.N. Implant System

S.I.N. Implants, Brazil
6.0mm diameter
3.0 mm thickness

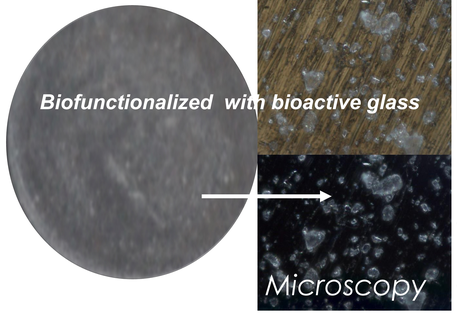


Machined
Titanium grade IV

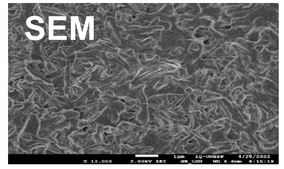


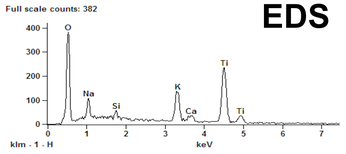
Nano-Ha
Nano-hydroxyapatite
Pomimic Hanano method

BSF18

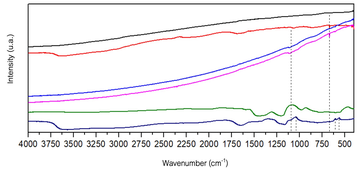


Titanium surface characterization

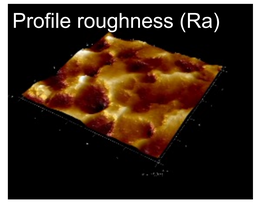




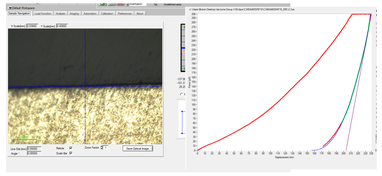
Bioactivity (0-24h)



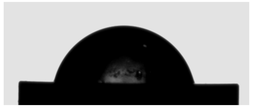
Atomic Force Microscopy



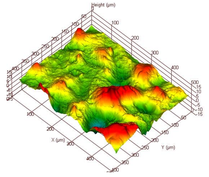
Nanoindentation
Elastic modulus (GPa)
Hardness (GPa)



Contact Angle



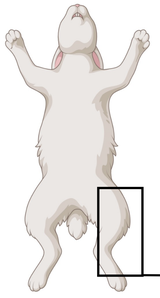
3D Surface Roughness
Sa, Sq, Sds and Sdr

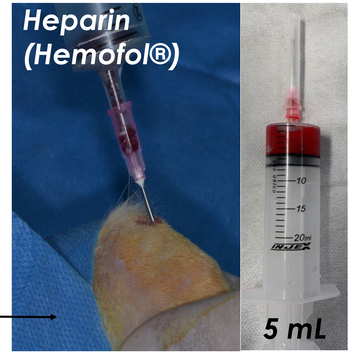


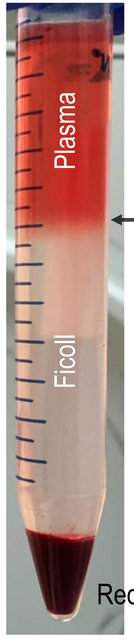
Biofunctionalization with bioglass particles (SiO_2-Na_2O-K_2O-MgO-CaO-P_2O_5 system) through the pneumatic atomization technique (50 µm) – **Patent 2014 003817 5 (Brazil)**
Vitreous Materials Laboratory, UFSCar

Bone marrow aspiration & Cell culture (BMSCs)

n=8 albino rabbits

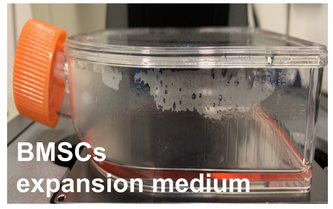




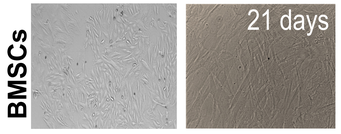


Bone Marrow Stem Cells isolation
Density-gradient centrifugation
FICOLL®

Mononuclear Phase



Differentiation in osteoblasts (osteogenic medium)



Brightfield Microscopy

Biological analyses

BMSCs ($2x10^4$) into surfaces:

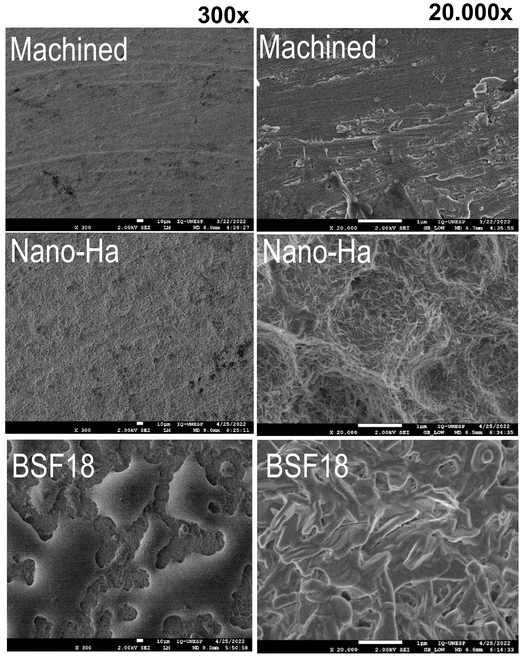
- **Cell adhesion and proliferation**
  Hemocytometer (count cells)
  24,48 and 72 hours
- **Direct Fluorescence Microscopy**
  Alexa Fluor phalloidin and DAPI
  Day 3
- **Cellular morphology and spreading**
  Scanning electron microscopy images
  (300x, 800x and 1000x magnifications)
  Day 3 and 7
- **MTT Assay for Cell Viability**
  Days 3 and 7
- **Reverse transcriptase PCR**
  RUNX-2 gene
  Runt-related transcription factor 2
  Day 7
- **Alkaline Phosphatase (ALP) formation**
  Day 10
- **Mineralized matrix formation**
  Alizarin Red Staining
  Days 14 and 21

Statistical analysis:
Kruskal-Wallis; ANOVA; t test
Pos-hoc Tukey's comparisons
Level of significance 5%
GraphPad Prism Software

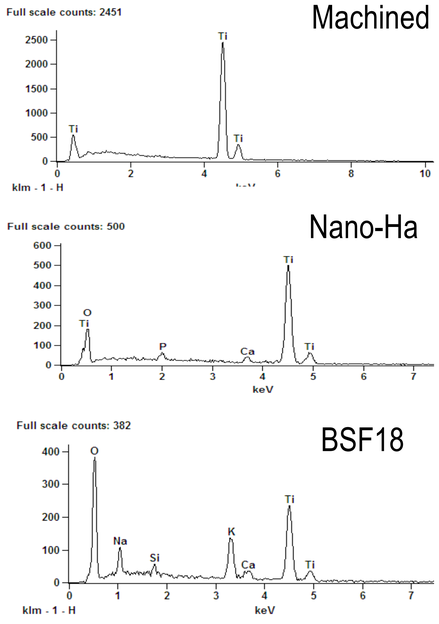
Results

SURFACE CHARACTERIZATION

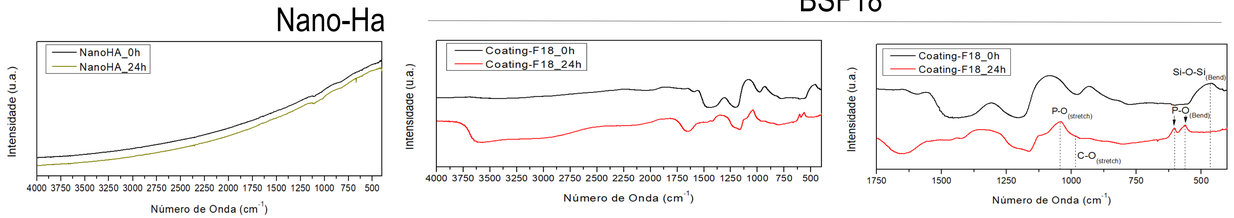
SEM



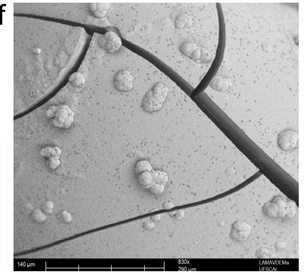
EDS



Bioactivity



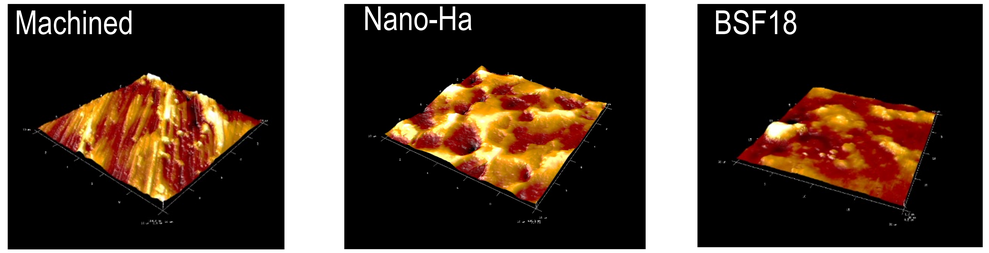
Formation of HCA layer



Atomic Force Microscopy

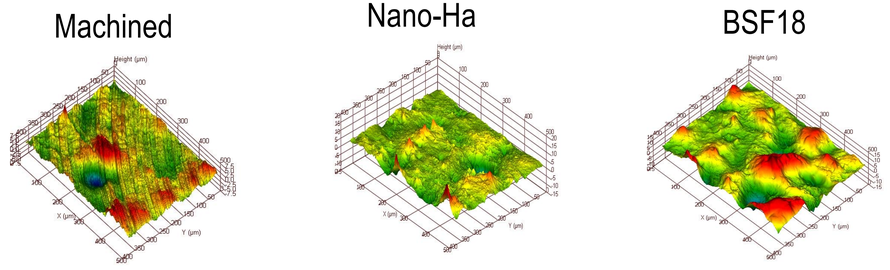
Groups	Ra (nm)
Machined	28.72±10.52*
Nano-HA	200.6±59.84*
BSF18	114.0±53.43

*Significant differences (p<0.05).
Kruskal-Wallis p<0.0003



3D Roughness

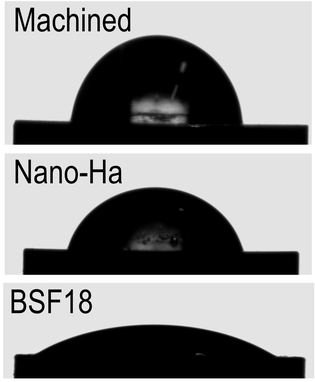
Groups	Sa (µm)	Sq (µm)	Sds (mm²)	Sdr (%)
Machined	1,39±0,54	1,70±0,64	21164,5±49506,8	4,596±7,02
Nano-HA	0,72±0,40	0,92±0,51	101613,3±6402,7	1,31±1,069
BSF18	0,90±0,51	1,13±0,65	4683,9±4002,4	1,50±1,573



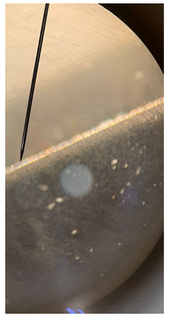
Contact Angle

Groups	CAº
Machined	81.75±4.33*
Nano-HA	49.54±3.74
BSF18	26.04±10.0*

*Significant differences (p<0.05).
Kruskal-Wallis p<0.0219



Nanoindentation



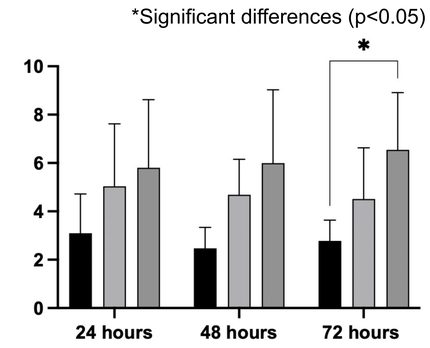
Load function: 4000 µN
9 indentation per sample and area

Groups	Em (GPa)	H (GPa)
Machined	113,51±47,53	3,18±1,50
Nano-HA	126,76±33,65	3,92±1,36
BSF18	56,12±20,40	4,79±1,16

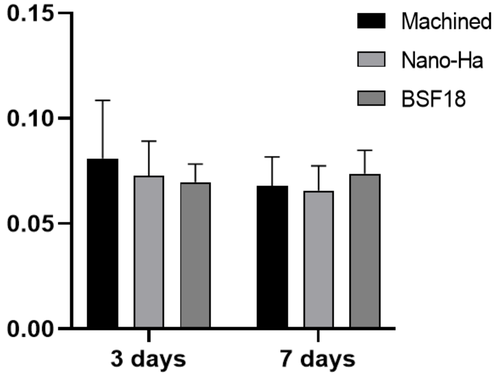
Results

BIOLOGICAL ANALYSES

Cell adhesion and proliferation

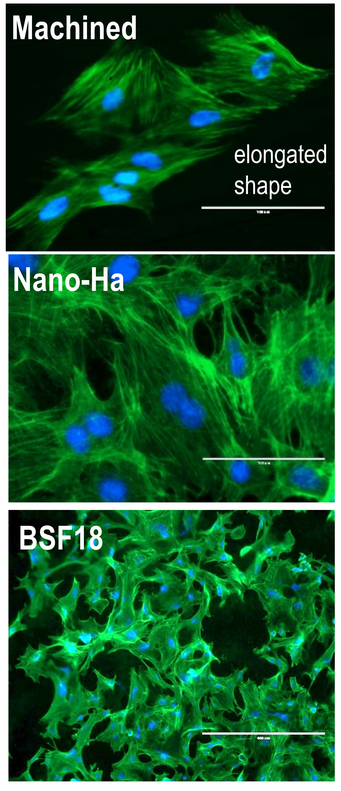


MTT Assay for Cell Viability

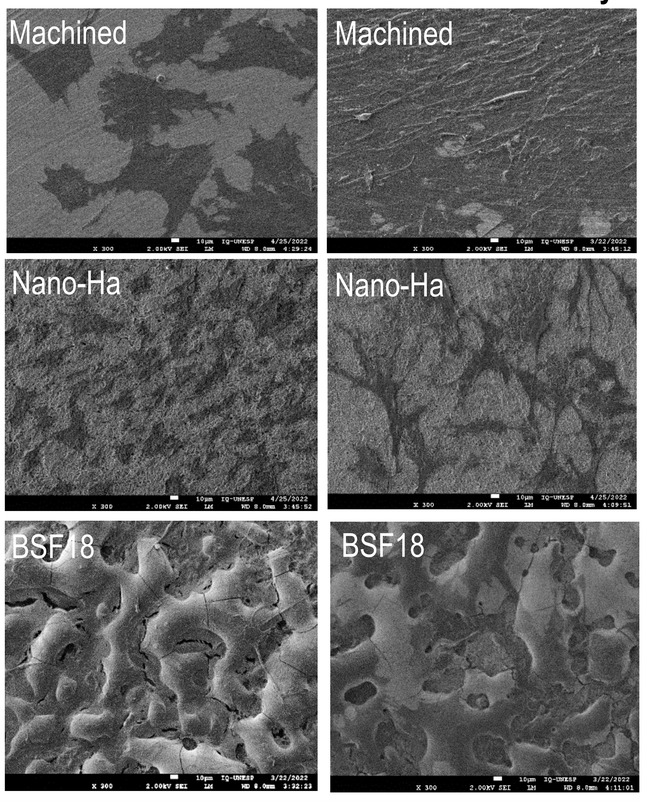


Direct Fluorescence Microscopy- 3 days

small prolongations
polygonal shape

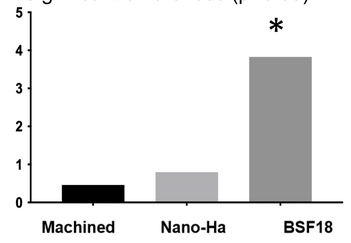


Cellular morphology and spreading- 3 and 7 days



Reverse transcriptase PCR

Runx2



Cell morphology

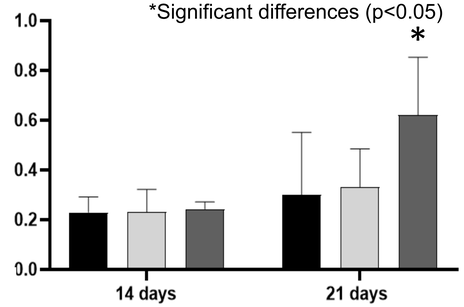
3 days:
elongated shape emitting their first extensions called filopodia

7 days:
more prolongations, forming agglomerates

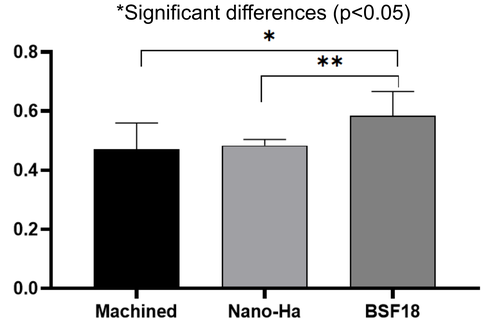
mineralized nodules



Mineralized matrix formation



Alkaline Phosphatase formation



Conclusion & Clinical implication

The surface biofunctionalized with a new bioactive glass system (BSF18) was able to stimulate the differentiation and activity of pre-osteoblastic cells, which indicates that it can be a promising alternative for dental implants.

Acknowledgements

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Center for Research Technology and Education in Vitreous Materials, Sao Carlos, Brazil

SIN Implants, Sao Paulo, Brazil