# Preclinical Summary

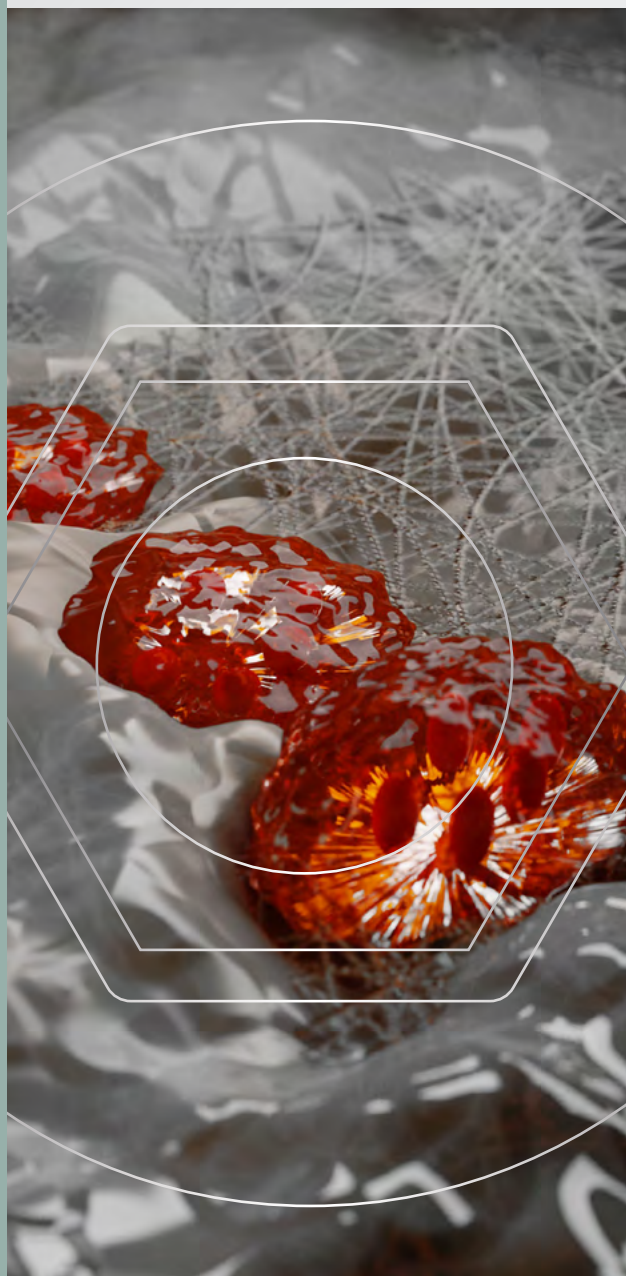
## Scientific Overview

NiNA™ Nano Hydrophilic Surface

AlphaBio TEC Simplantology

Nina™

# In vitro studies



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# 01 PART Characterization of Alpha-Bio Tec. implants with and without nano-topography surface

## Test Report

### Aim

The aim of this study was to investigate and characterize the evolution of nanostructures on the Alpha-Bio Tec. (ABT) implant surface with visual and quantitative comparison of standard ABT implants versus nanostructure surface implants.

### Methods

Analysis made by mechanical profilometry, XPS and SEM:

- **Group A - ABT NanoTec™ implants (control group)**

  **Group B - Nanostructure implants**

For AFM and SEM analysis:

- **Group C - Discs:** These discs were intended only for AFM testing and represent the surface treatment of group A implants in the nanometric scale.
- **Group D - Discs:** These discs were intended only for AFM testing and, with nanometric aspect, represent the surface treatment of group B implants in the nanometric scale.

### Characterization

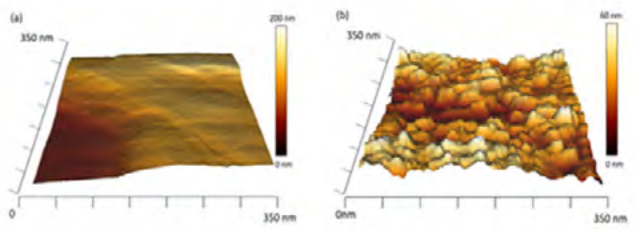
☑ SEM ☑ AFM ☑ XPS

☑ **Ra - roughness average** is arithmetic mean of the absolute values of the height of the surface profile.

☑ **Rq - Root mean square roughness (RMS)** is a function that takes the square of the measures.

☑ **Rt** – peak to valley roughness defines the vertical distance between the deepest valley and highest peak.

### Results




| Group | Ra [nm] | | Rq [nm] | | Rt [nm] | |
|---|---|---|---|---|---|---|
| NanoTec™ | 1.80 | (±1.02) | 2.29 | (±1.27) | 10.03 | (±5.08) |
| Nina™ | 7.41 | (±0.18) | 9.01 | (±0.25) | 40.86 | (±3.44) |

**Fig 1**

AFM analysis for 2 types of surfaces: Left is SLA the right one is a nanoscale surface.

### Conclusion

AFM showed a major increase in average roughness (Ra) of the surfaces.

More than 400% increase for the nano-structured surface at group D. The other parameters also show a similar increase in surface roughness.

# 02 PART Microscopic characterization of bioactivate implant surfaces: increasing wettability using salts and dry technology

Francesco Gianfreda, Donato Antonacci, Carlo Raffone Maurizio Muzzi, Valeria Pistilli, Patrizio Bollero

## Aim

The aim of the present study is to describe from a micrometric and nanometric point of view, the characteristics of a new bioactivated surface obtained by the use of salts dried on the surface.

## Methods

Topographic analysis, energy-dispersive X-ray spectroscopy, and contact angle characterization were performed on the samples of a sandblasted and dual acid-etched surface (ABT), a nano-surface (Nano) derived from the former but with the addition of salts air dried, and a nano-surface with salts dissolved with distilled water (Nano $H_2O$).

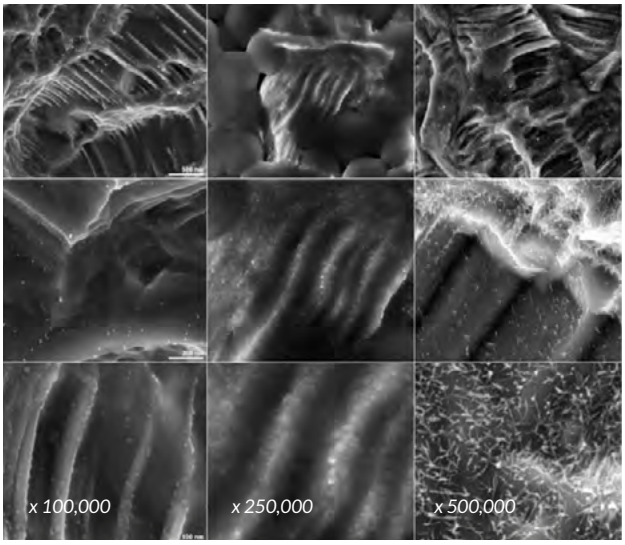
## Results

The analysis revealed promising results for nanostructured surfaces with increased wettability and a more articulated surface nano-topography than the traditional ABT surface.

a b c

**Fig 1**

*High-magnification SEM micrographs showing the different appearance and density of the nanostructures.*




a b c

**Fig 2**

AFM analysis for 2 types of surfaces: Left is SLA the right one is a nanoscale surface.

*High-magnification SEM micrographs showing the different appearance and density of the nanostructures.*

## Conclusion

This study validates surface topography and hydrophilicity of a new promising ultra-hydrophilic nano surface obtained by sandblasting, double acid etching and surface salt deposition using dry technology.

# 03 PART

# Early Biological Response of an Ultra-Hydrophilic Implant Surface Activated by Salts and Dry Technology: an In-Vitro Study

Francesco Gianfreda, Carlo Raffone, Donato Antonacci, Federico Mussano, Tullio Genova, Giorgia Chinigò, Luigi Canullo and Patrizio Bollero

## Aim

The aim of this study is to evaluate the osteogenic response of pre-osteoblast cell lines to dry bioactivated surface.

## Methods

MC3T3-E1 osteogenic cell lines were cultured on SM (sandblasted and dual acid-etched surface) and HNS (SM surface with dry salts bioactive technology). Cell adhesion assay, proliferation assay and cell morphology were performed. Osteogenic activity was performed using Alizarin Red S and alkaline phosphatase.

## Results

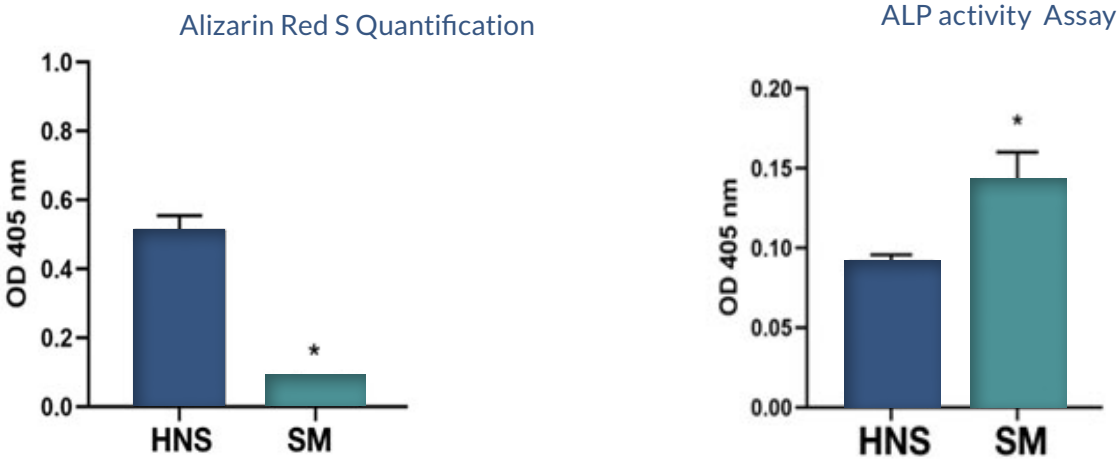



**Fig 1** Cell culture after 3 weeks: Alizarin red stains calcified material.

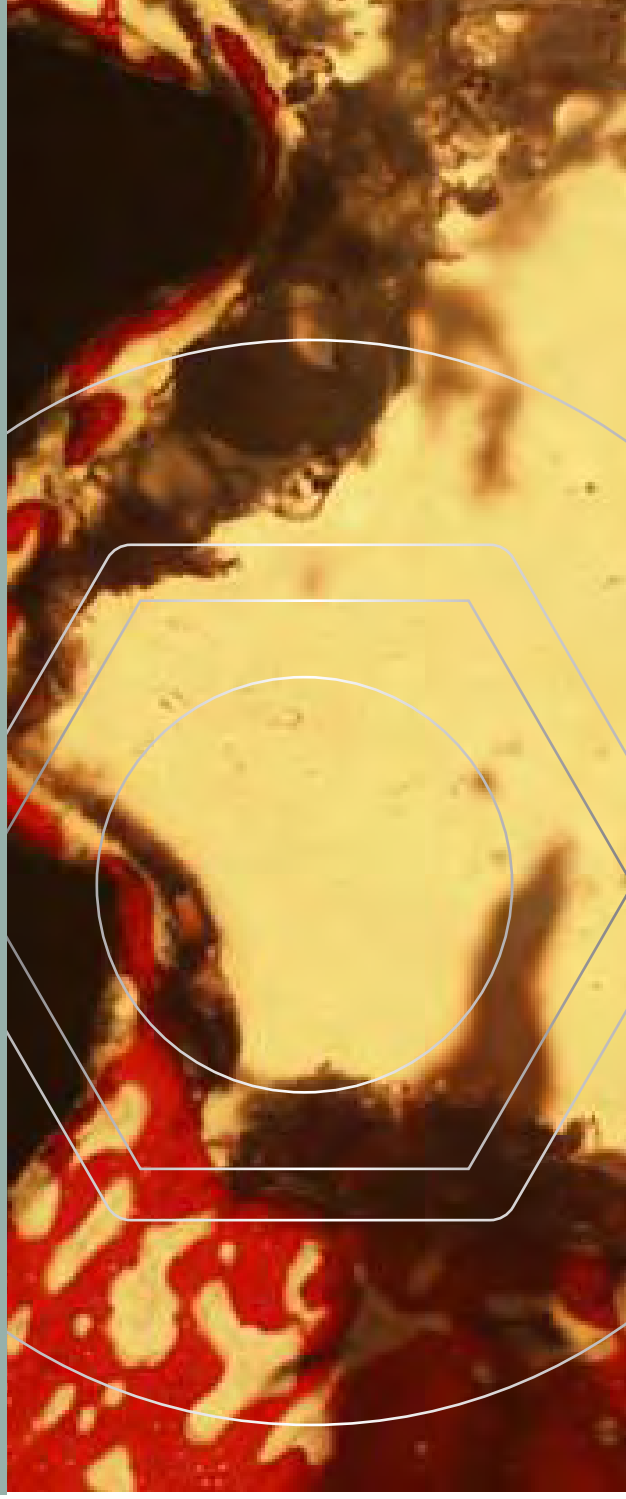
Notice that bone was more produced on Nano-H than on SLA while activity of ALK. Phosphatase is more pronounced on SLA. Bone apposition is earlier in Nano-H while ALK phosphatase peak appears late in SLA.

## Main conclusion

Nano-hydrophilic surface allows early mineralized deposition excreted by osteogenic cell line in-Vitro compared with SLA surface

Nina™

# In vivo studies



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# 01 PART

# Bioactivated implant surfaces installed in healed sites or into extraction sockets. An experimental study in dogs.

Luigi Canullo, Franco Bengazi, Mauro Ferri, Francesco Gianfreda, Tomaso Mainetti, Daniele Botticelli, Ofer Moses.

## Aim

Aim of the present pilot study on canine model is to investigate the early bone reaction to a conventional sandblasted and dual acid etched implant surface (ABT), a nanostructured-hydrophilic surface (Nano), a dry salts bioactivated super-hydrophilic surface (Hydro) and a bioactivated nano-surface obtained from the addition of dry salts to Nano surface (Nano-Active).

## Methods

Implantation of ABT, Nano, Hydro and Nano-Active implants were performed on twelve dogs. A split mouth design was adopted. One implant for each type was implanted in the mandible 3 months after tooth extraction in healed sites, at the first molar regions, bilaterally. In the same session, the third and fourth premolars were extracted bilaterally and one implant for each type was immediately implanted into the extraction socket. Euthanasia was performed at 14 and 28 days from surgery and peri-implant bone reaction was assessed using Stevenel's blue and alizarin red.

## Results

The postoperative healing was uneventful for dogs. Indeed, no complications, no allergic reactions, no abscesses or infections were present. The 14-day histological studies reported non-significant results in terms of difference between the groups, while significant results were found 28 days after surgery.

In fact, a significantly higher rate of new bone around the implant was reported between the "Nano-Active" and "Nano" groups (51.0 ± 10.2% vs 36.0 ± 10.2%) and "Hydro" and "Nano" groups (47.3 ± 10.7 vs 36.0 ± 10.2%).

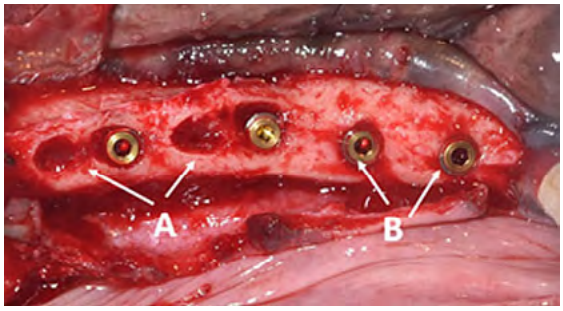


**Fig 1** **28 days postop**.

**Type A** defects: immediate implantation
**Type B** defects: delayed implantation

b c

**Fig 2**

**b** Bone reaction to SLA

**c** New bone attracted by Nina surface

# 01 PART Bioactivated implant surfaces installed in healed sites or into extraction sockets. An experimental study in dogs.

Table 3. Merged histometric measurements at implants installed in both healed sites and extraction sockets. Mean values and standard deviations (SD).

| | | 2 weeks | | | | | 4 weeks | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | NEW | OLD | TOTAL B | SOFT | | NEW | OLD | TOTAL B | SOFT |
| FINAL | Mean | 24,6 | 17,4 | 42,0 | 58,0 | Mean | 51,0[a] | 2,6 | 53,6 | 46,4 |
| | SD | 9,5 | 15,5 | 25,0 | 13,4 | SD | 10,2 | 2,0 | 12,3 | 11,7 |
| HYDRO | Mean | 24,2 | 10,5 | 34,7 | 65,3 | Mean | 47,3[b] | 0,9 | 48,2 | 51,8 |
| | SD | 6,4 | 8,0 | 14,3 | 7,9 | SD | 10,7 | 2,1 | 12,8 | 10,3 |
| SLA | Mean | 22,0 | 17,6 | 39,5 | 56,9 | Mean | 46,9 | 3,9 | 50,8 | 49,2 |
| | SD | 12,4 | 12,1 | 24,5 | 11,7 | SD | 10,8 | 4,6 | 15,3 | 11,9 |
| NANO | Mean | 26,7 | 13,8 | 40,5 | 56,7 | Mean | 36,0[a,b] | 3,6 | 39,6 | 60,4 |
| | SD | 10,5 | 6,4 | 16,9 | 15,6 | SD | 10,2 | 2,2 | 12,4 | 10,3 |

Friedman test 2 weeks p=0.682; 4 weeks p=0.037. [a] p=0.018; [b] p=0.018.

**Fig 1**
Summary table

## Conclusion

The results obtained in terms of new bone formed after 4 weeks demonstrate a tendency of dry salt treated bioactivated surfaces to improve histological outcomes in the early stages of healing. This study is the first to validate in vivo safety and the promising osseointegration capacity of dry salts bioactive implant surfaces.

# 02 PART

# BIC comparison of SLA surface implants vs. hyper hydrophilic nano-surface implants. A study in the rabbit tibia

Raz P, Slutzki G, Beitletum I, Shem-Tov K, Seaboun A, Moses O, Cohen O.

## Study design

2 types of implant surfaces involving one type of macroscopic design were implanted in both sides of the rabbit tibia.

One side was operated 3 weeks and the other one 6 weeks before euthanasia. In each tibia, randomly assigned, one of each implant was inserted underneath the knee. Histological slides were processed for non-decalcified section, stained with alizarin red.

## Results

3 weeks postop BIC to the Nina surface was 20% higher compared to the SLA surface implant.
6 weeks later this advantage was maintained.

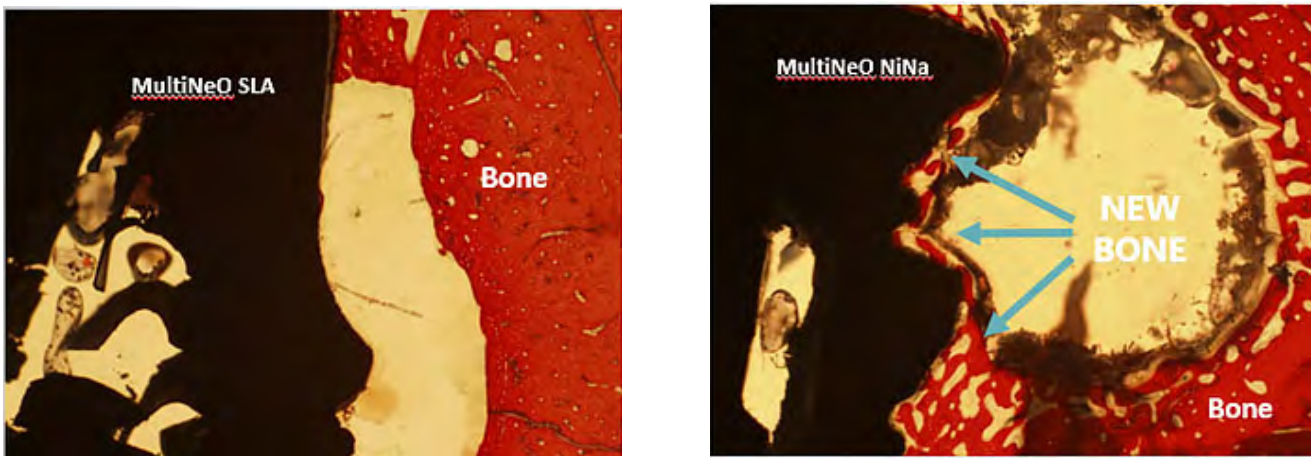


**Fig 1**

## Conclusion

Nina surface implant holds clinically a promising technique for immediate implantation. Further studies on Humans should be performed to assure these findings.

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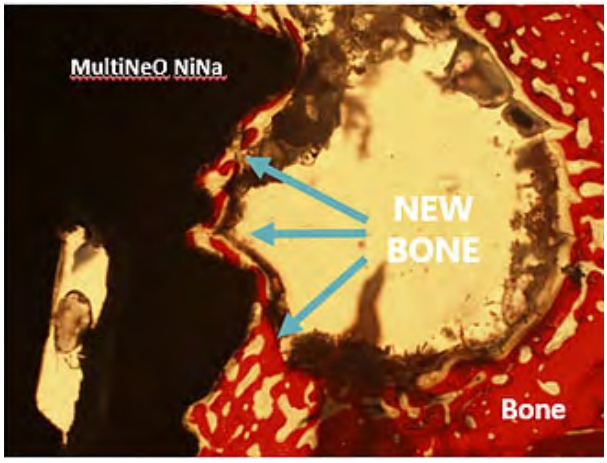


**Fig 1**

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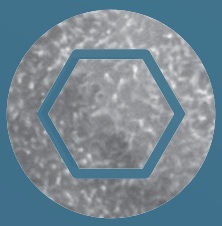

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