Gas Cluster Ion Beam Surface Modification of Titanium Enhances Osteoblast Proliferation and Bone Formation *in vitro*

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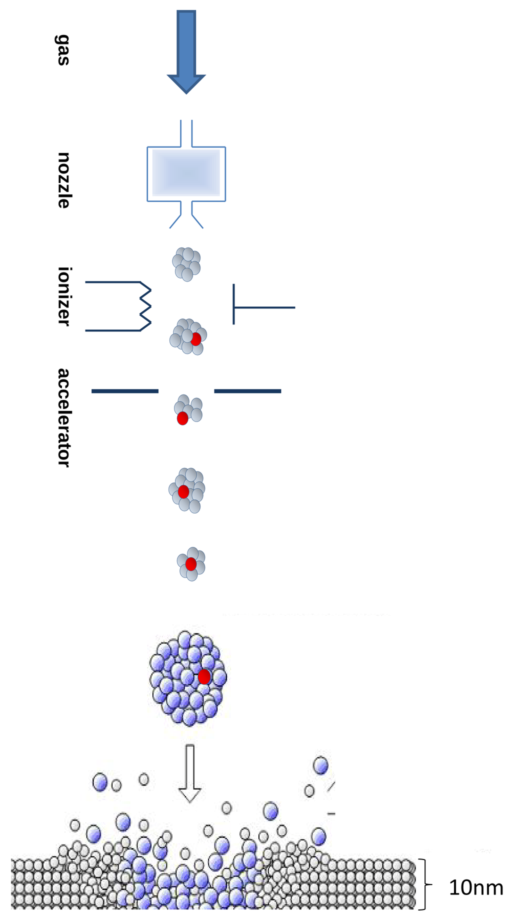
Abstract

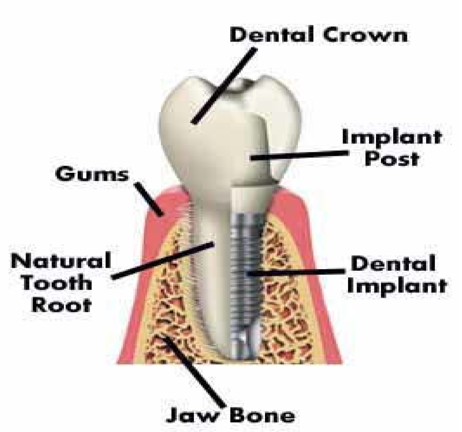
Titanium implants are considered to be bioactive, but osseointegration is often slow. Many have increased surface area of the implant to which osteoblasts bind by etching or sandblasting, and applied additive coatings such as hydroxyapatite. We have altered the atomic-level structure of the TiO_2 surface without adding material onto the surface. GCIB utilizes high energy ionized gas clusters of inert argon atoms. Bombardment of TiO_2 results in increased surface wettability and amorphization. Ti pieces were divided into 2 groups: untreated, or GCIB irradiated with 5×10^{14} Ar clusters/cm^2. Osteoblasts were seeded at 2000 cells/cm^2, allowed to attach and proliferate up to 10 days (n=3). Cell counts were measured by MTS assay. RNA was extracted over 10 days and amplified for alkaline phosphatase (ALPL). Protein was extracted at 4, 24, and 48h and probed for total and phosphorylated p42, as a marker for cell proliferation. GCIB-treated TiO_2 showed: increased proliferation by day 10 (12,213 ± 1,570 vs 6,880 ±700 cells $p<0.05$); ALPL upregulated by 3.4 ± 0.6 ($p<0.01$) fold by day 10, indicating bone formation; phosphorylation of p42 is seen at 4h and sustained at 24h on GCIB-treated, and only 4h on non-treated surfaces. Results suggest that osteoblasts adhere and proliferate better on GCIB-treated TiO_2. GCIB treatment of dental implants has potential to enhance bone formation and significantly decrease osseointegration time.

Introduction to GCIB

- Unique energetic ion bombardment technique.
- Clusters can be formed from a number of gases, Argon is typically used due to inert properties.
- Clusters that collide with surface have capability to modify material to about 10nm below the surface.
- Surface Treatment using Gas Cluster Ion Beams :
  - Changes the surface properties without changing the surface chemistry of a material
  - Very shallow penetration – at atomic levels
  - Extremely localized process that only impacts the surface



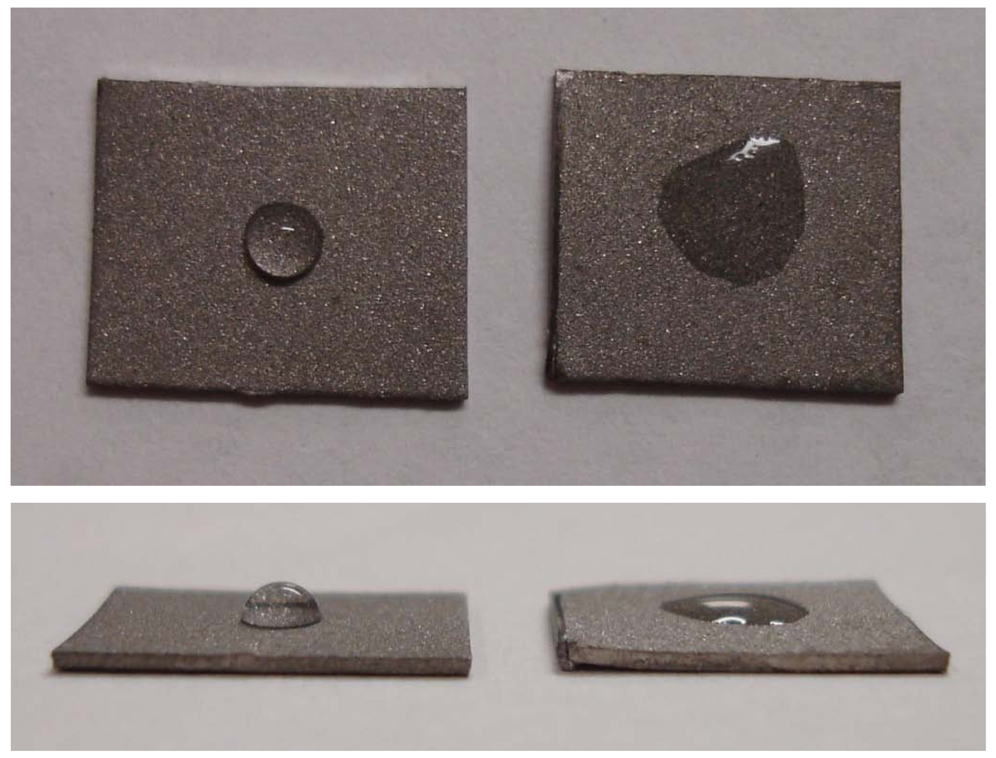
Dental Implant Integration



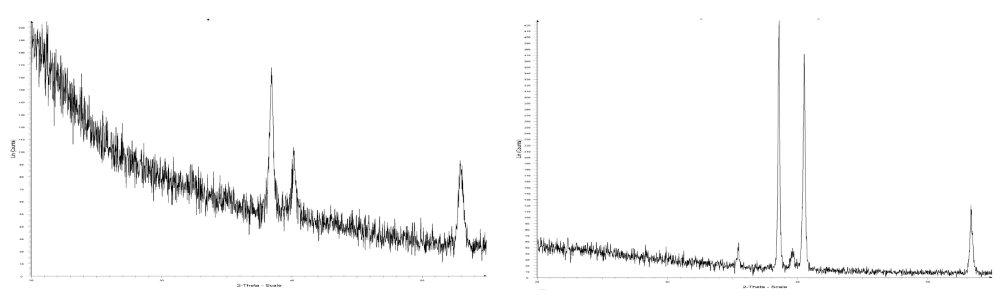
- Integration of dental implant into bone is often slow or incomplete
- Improvement found with increased surface area modifications such as DAE or SLA
- Could the integration time and strength be improved with GCIB?

Initial Findings

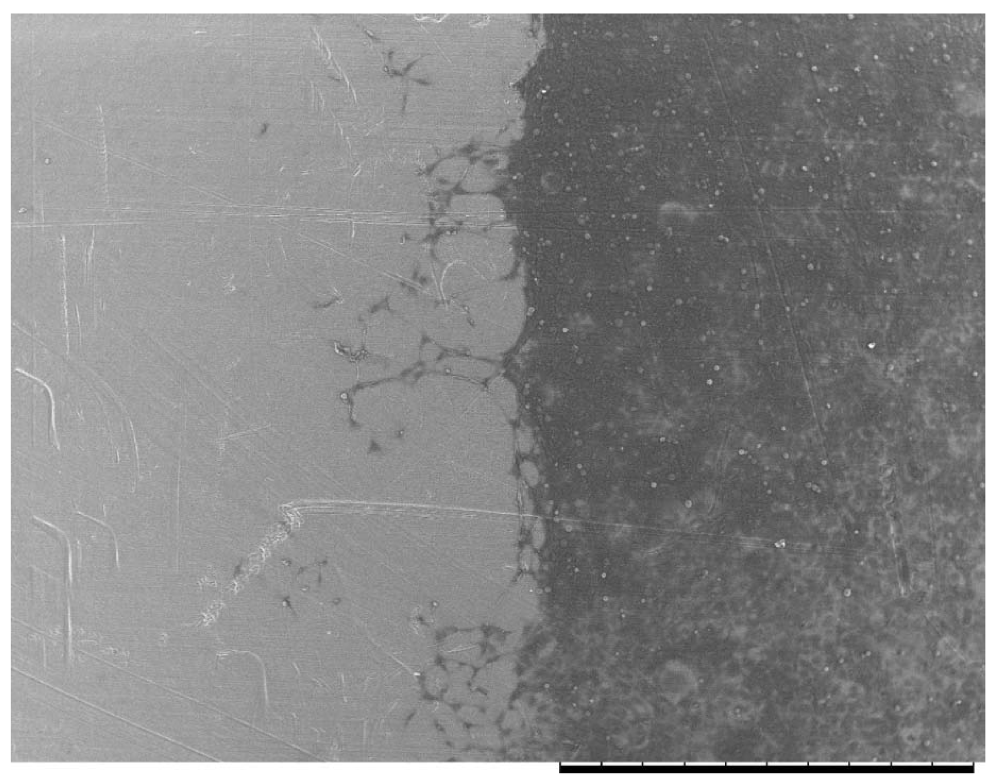
Control GCIB



As compared to the hydrophobic properties normally seen on Control Titanium (left), GCIB treatment (right) results in a hydrophilic surface.



GCIB-treatment of Titanium results in the surface crystallinity changing from an amorphous form to a rutile crystalline form.

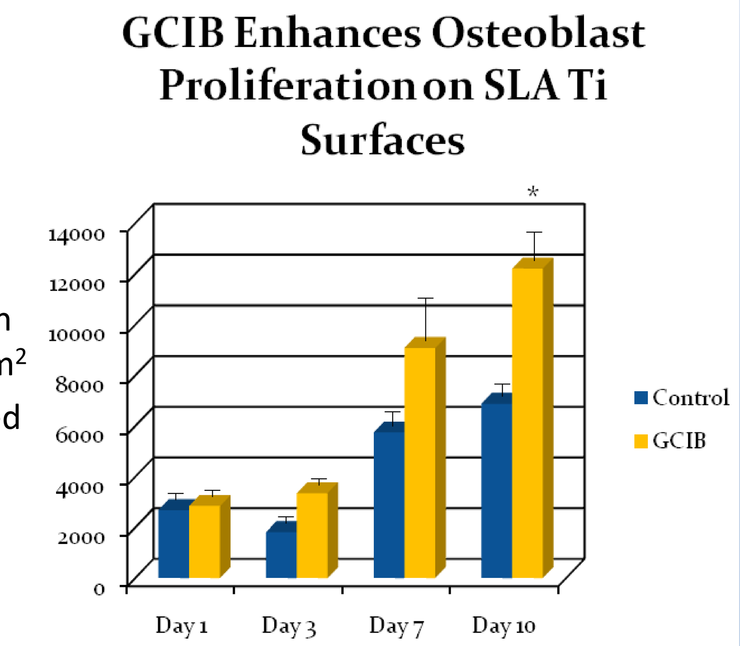


GCIB-treatment results in Teflon (PTFE) becoming cytocompatible and allowing cells to attach and proliferate only on the GCIB-treated side.

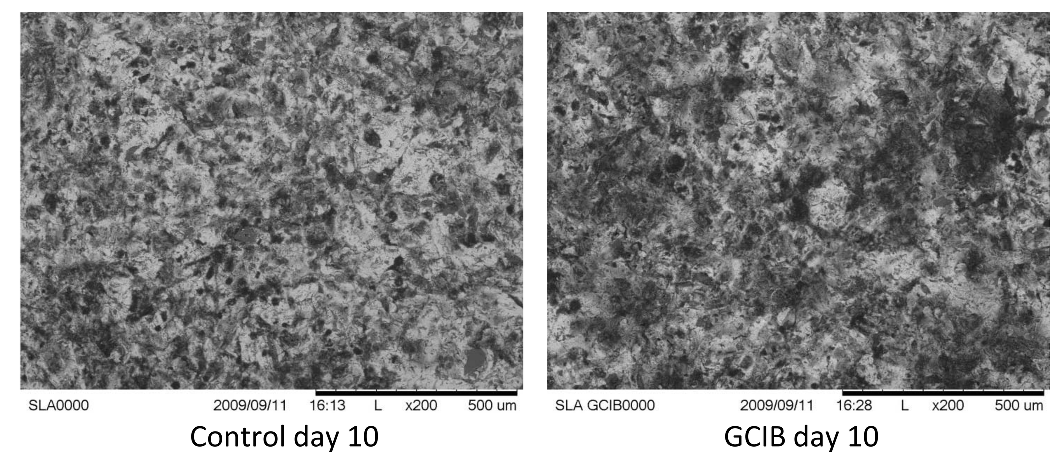
Hypothesis: GCIB will lead to enhanced cell attachment and proliferation

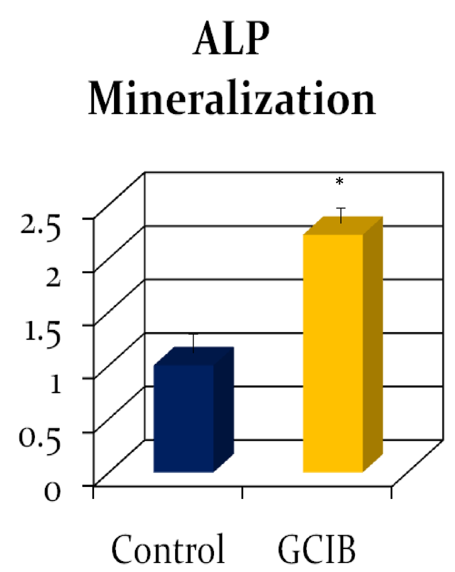
Results

- Titanium coupons ± GCIB irradiation
- Primary human osteoblasts seeded on the surface 2,000 / cm^2
- Proliferation measured by MTS assay



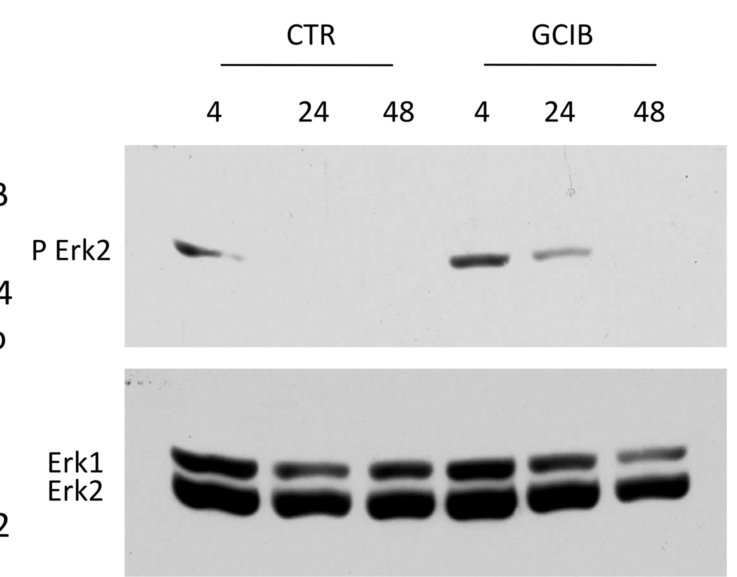
GCIB-treatment of titanium results in 77.5% enhanced cell proliferation by 10 days as compared to controls (* p<0.02).





SEM images of GCIB-treated Titanium (above) displays visible differences in cell proliferation as compared to controls at 10 days. Cells growing on GCIB-treated titanium result in increased alkaline phosphatase mineralization (left) as seen by the alizarin red stain (*p<0.05) indicating enhanced bone formation.

- Protein extracted from cells plated on Ti ± GCIB at 4h, 24h, 48h
- Western blot for p42/44 (Erk) pathway known to be involved in proliferation
- Determine phosphorylation of Erk2



GCIB-treatment results in prolonged phosphorylation of Erk2 (p42), part of the MAPK pathway and known to be involved in proliferation. Phosphorylation of p42 is seen at 24 hours on GCIB-treated surfaces but only at 4 hours on controls.

Results Continued

- RNA collected at days 1, 7, 10 post seeding onto SLA treated Ti ± GCIB
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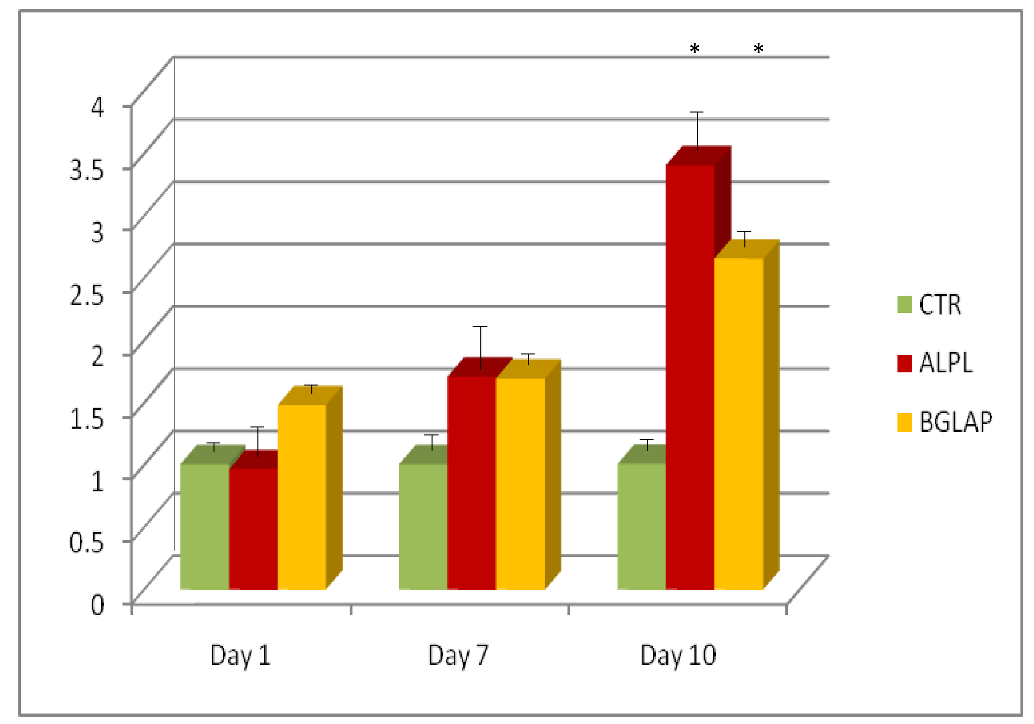

GCIB-treatment of titanium results in enhanced osteogenic gene expression as seen by increased ALPL and BGLAP by 10 days as compared to controls.

Conclusions

- GCIB alters the surface structure to a depth of ~10nm (100Å)
- GCIB modification enhances surface hydrophilicity and modifies the crystallinity of the surface
- GCIB leads to 77.5% increased osteoblast proliferation on SLA Ti by 10 days
- GCIB allows longer enhanced cell adhesion properties
- GCIB leads to earlier bone formation as seen by gene expression analysis leading to cell differentiation pathway

Benefits of GCIB Processing

- Non-additive technology
- GCIB uses the inert gas argon which allows modification of the surface physically without changing the surface chemistry
- GCIB treatment of dental implants has the potential to enhance bone formation and significantly decrease osseointegration time

Questions or Further Information
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