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 TiO2 surface without adding material onto the surface. GCIB utilizes high energy ionized gas clusters of inert argon atoms. Bombardment of TiO2 results in increased surface wettability and amorphization. Ti pieces were divided into 2 groups: untreated, or GCIB irradiated with 5x1014 Ar clusters/cm2. Osteoblasts were seeded at 2000 cells/cm2, 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 TiO2 showed increased proliferation by day 10 (12213 ± 1570 vs 6880 ± 700 cells/cm2; 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 TiO2. 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 1nm 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?

Hypothesis: GCIB will lead to enhanced cell attachment and proliferation

Results

- GCIB Enhances Osteoblast Proliferation on SLA Ti Surfaces
- ALP Mineralization 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.

Results Continued

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

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