Surface Modification by Gas Cluster Ion Beam (GCIB) as a Novel Polymer-free Drug Delivery Method

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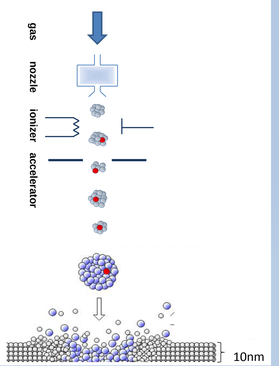
Abstract

Much discussion has focused on the possible inflammatory and thrombogenic influences of the polymer coating on drug-eluting stents (DES) and the potential for late stent thrombosis. Biodegradable polymers and / or microporous materials are being evaluated by others. Similarly, a means to deliver proteins such as bone morphogenic protein (BMP) or antibiotics on orthopedic or dental implants without a binding polymer would be ideal. GCIB, a process which employs accelerated ions comprised of a few hundred to several thousand atoms under high vacuum, is being developed into a new field of ion beam technology. The process is characterized by low energy surface interaction effects, lateral sputtering phenomena and high-rate chemical reaction effects. In this study, we have used Argon based GCIB to adhere a drug to the surface of a metal. GCIB bombardment of the surface of the deposited drug results in modification of the top 100Å of the surface, allowing a thin coating of drug or therapeutic to control the rate of elution without polymer.

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
  - Permits changing surface properties without changing the surface chemistry of a material
  - Very shallow penetration – at atomic levels
  - Extremely localized process that only impacts the surface



Materials and Methods

DES: To study the controlled elution of drugs by GCIB, CoCr bare metal stents were initially processed with GCIB at $5x10^{14}$ Ar clusters/cm^2. 50µg of rapamycin (RAP) was placed on the surface; one group received a GCIB drug surface processing while another group did not. Stents were placed in human plasma at 37˚C on a rotator to simulate *in vivo* drug elution for up to 7 days (n=3) and drug weights were recorded daily and compared to historical data of the commercially available Cypher® stent. Pre- and post- eluted stents were then placed in plates and 2,500 mouse endothelial cells were seeded, allowed to attach for 24 hours, and counts were measured by MTS assay.

BMP: GCIB processing has the ability to physically change the surface up to 100Å, this modification to a protein could be detrimental; therefore to study the effects of GCIB processing on potential protein degradation, 1µg of BMP-2 was applied on the surface of 1cm^2 titanium foils. BMP from untreated or GCIB-treated foils (n=3) was then extracted from the surface and concentrations of BMP-2 was evaluated by ELISA or was subjected to SDS-PAGE and silver-stained to visualize for degraded protein products.

Antibiotics: To study the effects of GCIB on temperature sensitive therapeutics such as antibiotics, 30µg of kanamycin was applied to 1cm titanium disks. Untreated and GCIB-treated disks were then placed on Nutrient agar dishes containing *S.aureus* bacteria and incubated for 7 days. Measurements of the zone of inhibition was recorded daily and compared to kanamycin impregnated filter disks and bare titanium disks.

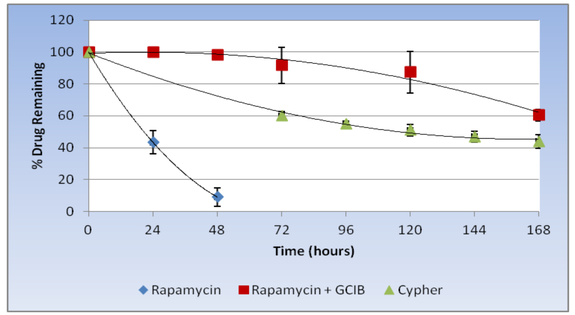
Results

DES: Rapamycin on stents not receiving the second modification by GCIB eluted off within 24 hours and increased cell attachment from 672±187 cells (pre-eluted) to 2,249±124 (24h post-eluted, $p<0.01$). Stents that received a second GCIB modification caused the rapamycin to be eluted off more slowly, and did not have any additional cell attachment on post-eluted stents over the 7 day study as compared to pre-eluted stents. Elution from GCIB-treated rapamycin stents eluted at a slower rate than Cypher stents over the initial 7 day period. However, unlike Cypher, where an initial burst of drug is released, and the polymer holds in the remainder of the drug for over 60 days, GCIB elution of the drug is progressive and expected to be gone in about 14 days, allowing endothelialization to occur.

BMP: ELISA results from BMP-2 elution revealed 0.8µg±0.03µg remaining after GCIB-treatment. The loss of nearly 0.2µg of the initial protein may be due to the high vacuum pressure which may remove weakly adhered material from the surface. However, the remaining protein displayed no degradation as assayed by silver stain.

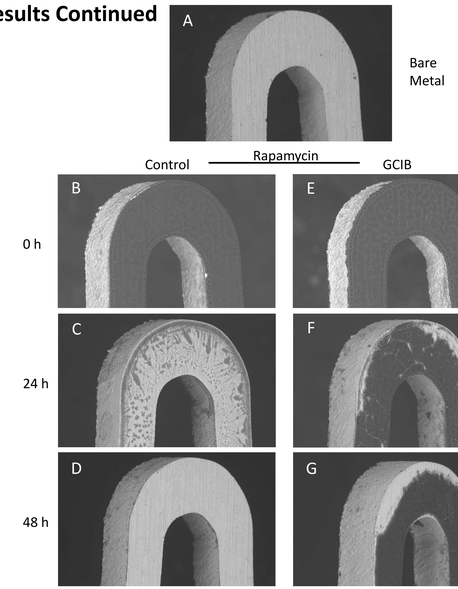
Antibiotics: Studies on antibiotics revealed an 8mm zone of inhibition (ZI) by kanamycin on filter paper at 48 hours which decreased to 6mm at 72h and 0mm at 96h post placement on *S.aureus*. Kanamycin placed on titanium disks revealed a 6mm ZI at 48h, 4mm at 72h, and 2mm at 96h. Disks that have been treated with GCIB revealed a 6mm ZI at 48h, 5mm at 72h, and 4mm at 96h. Bare Ti disks had no ZI. The decreased initial ZI of kanamycin on Ti may be related to the original spreading on the foil as compared to filter paper which absorbs the liquid evenly. GCIB-treated disks showed slower degradation of the antibiotic as compared to untreated or filter disks by means of ZI, hence a controlled elution.

DES:

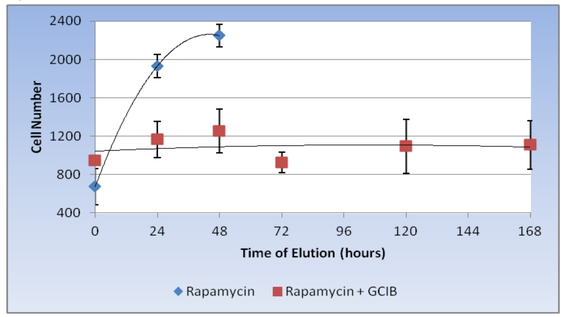


Drug elution profile reveals the use of GCIB allows for a more controlled release of rapamycin than without treatment and has a better release profile than Cypher for the first 7 days.

Results Continued



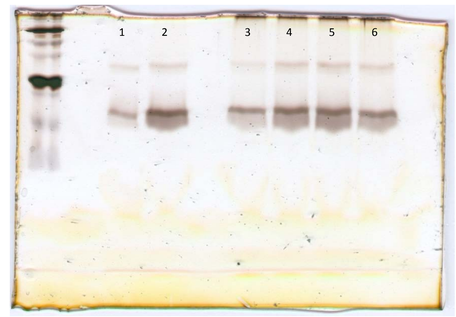
SEM images of stents following elution. Bare metal CoCr stents (A) were coated with approximately 50µg of rapamycin (B-G) and were treated with GCIB at $5x10^{14}$ Ar clusters/cm^2 (E-G). Stents were then either un-eluted (B,E) or eluted for 24h (C,F); 48h (D,G); or up to 168h (data not shown). Stents that were coated with rapamycin but not treated by GCIB resulted in drug being completely eluted off within 48h (D); however, stents that were treated with GCIB show a much slower and progressive decrease of drug over time of elution.



Following drug elution, 2500 EOMA cells were seeded per stent and allowed to attach for 24 hours then assayed by MTS. GCIB-treatment of rapamycin-coated stents (■) results in a continuous suppression of cell attachment to the surface over 7 days. Without GCIB treatment (◆), cells attach rapidly as the drug elutes off.

Results Continued

BMP:



BMP-2 was coated on Ti foils and then either left as untreated (2) or treated by GCIB processing (3-6, increased dose). Protein was then extracted from the surface and subjected to SDS-PAGE and silver stained. Native BMP-2 (1) was used as a control, no evidence of protein degradation is seen.

Antibiotics:

S. aureus	48 h	72 h	96 h
Kanamycin Filter	8 mm	6 mm	0 mm
Kanamycin on Ti	6 mm	4 mm	2 mm
Kanamycin on Ti + GCIB	6 mm	5 mm	4 mm

GCIB treatment of antibiotics on Ti surface result in a longer elution profile able of inhibiting bacteria as measured by the Zone of Inhibition assay on LB Agar plates.

Conclusions

GCIB processing results in a controlled release of drugs and therapeutics without the use of a binding polymer. GCIB offers the potential to create implants containing predetermined precise doses of drugs or therapeutics. Controlling the release rate of the therapeutic may be achieved by multi-layering drug and GCIB treatments. This process has been evaluated in this study on small molecules such as Rapamycin, on proteins such as BMP-2, and on fragile antibiotics such as Kanamycin. This controlled release without the use of binding polymers is crucial in development of next generation drug eluting stents as well as orthopedic and dental implants.

Questions or Further Information
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