Characterization of In-vitro Release of Gentamicin from Biodegradable Polymer Thin Films Microstructure-Function Relationship by Confocal Raman Microscopy

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Statement of Purpose: Thin films of absorbable polymer matrix containing gentamicin sulfate particles were prepared by solvent casting process. The solvent cast films were fabricated into sleeves intended to fit over a metal plate for orthopedic fracture fixation using a custom heat welding process. These sleeves are designed to locally deliver gentamicin sulfate over a 2-3 week period when implanted along with a metal fixation plate during internal fixation of an orthopedic fracture to prevent implant related surgical site infection (SSI). The target antibiotic release profile is a high initial release to provide high concentrations close to the time of initial contamination, and a lower sustained release to provide local antibiotic coverage until soft tissue healing. Factors that affect the release of a drug from thin films of absorbable polymer include the solubility of drug, its particle size distribution, polymer molecular weight, film thickness, and drug content. The present study aims at understanding the mechanism of release of drug from implants and effect of its particle size distribution on release kinetics. An in-vitro dissolution test using USP dissolution apparatus I (basket configuration) has been developed to evaluate initial burst release of gentamicin sulfate that is followed by sustained release phase. The relationship of film microstructure to mechanism of drug release was further evaluated using Confocal Raman Microscopy (CRM).

Methods: Sleeves with different particles size distribution of micronized gentamicin sulfate were prepared. All sleeves contained an average of ~13% by weight of gentamicin sulfate. Release rates of gentamicin sulfate from the sleeves were monitored using USP dissolution apparatus I at 37°C in 0.01M PBS with basket rotating at 100 RPM maintaining sink conditions. The total amount of gentamicin sulfate released was determined by UPLC after derivatizing the eluted gentamicin sulfate using 9-fluorenylmethyloxycarbonyl chloride (FMOC) and separating the gentamicin components on a C18 column using a PDA detector. Sleeve samples were pulled from the dissolution media at different times, washed with deionized water and vacuum dried at room temperature. A 532 nm laser at 30mW power was used to obtain reference spectra of the gentamicin and polymer. Characteristic Raman shifts of the polymer and gentamicin were used for mapping of gentamicin within the polymer matrix using CRM. Depth scans were performed using a 100X objective and 50µm collection fiber.

Results: In-vitro release of gentamicin sulfate from the sleeves exhibited an initial rapid burst release followed by a sustained-release. The burst release of gentamicin sulfate was found to be highly sensitive to particle size distribution of micronized gentamicin sulfate. The observed burst release was approximately 20% with micronized gentamicin sulfate with a D_{90}value of 5.40µm and increased to about 45% of the total amount with micronized gentamicin sulfate with a D_{90}value of 12.0µm. On the contrary, changes in the drug loading (11%-15% by weight) did not have a significant effect on relative burst release of gentamicin sulfate. The observed increase in burst release with small changes in particle size is counter intuitive and the mechanism of release of API from these films was studied using CRM. Gentamicin mapping along the XY (lateral) and XZ direction (thickness) of the film revealed no agglomeration and uniform distribution in the polymer matrix. Differences in the initial particle size distribution were also confirmed by CRM images. The total amount of drug that is present at or near the surface of the implant that contributes towards the burst release was higher for implants with higher initial particle size distribution when compared to others.

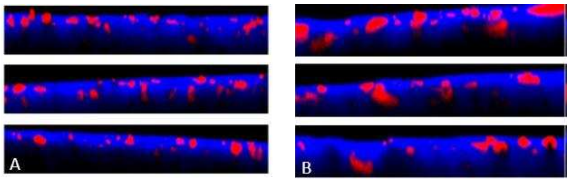

Figure 1: CRM depth image of polymer film (blue) with gentamicin sulfate (red). Image shows distribution of gentamicin sulfate in top 18µm thickness of the film containing gentamicin with different particle size.
A) D_{90}=5.4µm B) D_{90} = 12.0µm

Conclusions: The release kinetics of gentamicin sulfate from thin polymer films could be tailored to meet the desired release profile. Among other factors, gentamicin sulfate particle size seems to have the most pronounced effect on the initial burst release of drug. The mechanism of burst release was further confirmed using CRM. Tools like CRM that can detect and quantify spatial distribution of drug in similar formulation are critical in developing models that describe the kinetics of drug release, linking drug distribution on the microscopic scale to macroscopic performance of the product. This technique offers distinct advantages in comparison to other analytical techniques like imaging by light and electron microscopy that lack chemical information and require preparatory procedures that can introduce artifacts. With minimal sample preparation, both qualitative and quantitative analysis at ~0.3µm spatial resolution can be achieved. Future efforts will seek to improve the accuracy of the models to predict the API particle size distribution and efforts are underway to quantify spatial distribution by quantitative chemical imaging.

References:

1. Posyniak A. J of Chrom. A. 2001;914:59-66