Antibiotic Delivering Phosphatidylserine Coatings for Allograft Bone

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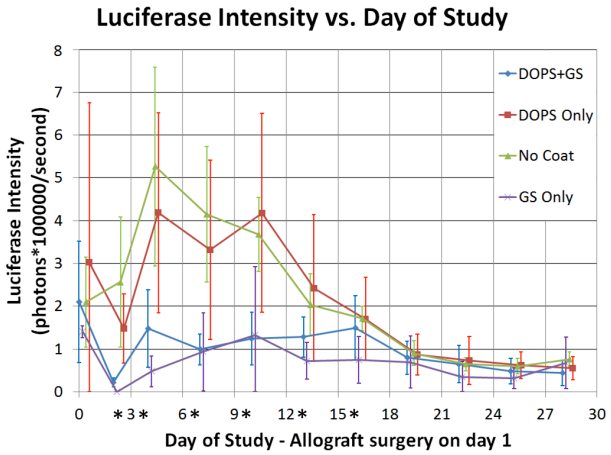
Statement of Purpose: This study was motivated by the need to improve outcomes for osteosarcoma patients receiving massive bone allografts. Osteomyelitis is a common bone infection often caused by the bacterium *Staphylococcus aureus*. It is particularly problematic for osteosarcoma patients with compromised immune systems. The purpose of this study was to investigate whether coatings of the phospholipid 1,2 dioleoyl-sn-glycero-3-phospho-l-serine (DOPS) and the antibiotic Gentamicin Sulfate (GS) are capable of reducing infection and enhancing osseointegration in *S. aureus* infected mice receiving massive femoral allografts. Previous research has demonstrated that DOPS is capable of enhancing the formation of calcium phosphate crystals which are suspected to act as precursors to new bone formation (1,2) and that GS is capable of reducing osteomyelitis (3).
Methods: Donor femurs obtained from BalbC mice were cleaned, decellularized and cut to 4 mm length. These were split into 4 groups and coated with DOPS+GS, DOPS only, GS only, or No Coating. The allografts were implanted into osteomyelitis infected and uninfected Black6 mice as follows:

Number of Mice Per Treatment Group

	DOPS+GS	DOPS	No Coat	GS
Infected	10	10	10	5
Uninfected	5	5	5	0

Seven days prior to allograft implantation mice in the infected treatment groups were infected with Luciferase expressing *S. aureus* by placing a bacterial suture laterally through the diaphysis of the femur. Treatment types were assigned randomly and allografts were fixed with an intramedullary pin. Infection was monitored over the course of the study by quantifying luciferase expression using an In Vivo Imaging System (IVIS) at 3-day intervals. At 4 weeks the mice were humanely euthanized and femurs were collected and analyzed with MicroCT as well as histologically.
Results: Infection was consistent between treatment groups prior to allograft implantation. As shown in the figure, IVIS showed a marked decrease in the level of infection in the osteomyelitis infected mice receiving GS coated allografts on the first imaging day following surgery, however luciferase expression rose and remained at a steady but low level on subsequent imaging days. Mice receiving allografts lacking GS showed an increase in infection that peaked between days 4 and 10 and then decreased. Analysis of Variance was performed on the data and significant difference between GS and non-GS treatment groups was observed between days 2 and 16. After day 16 there was no longer a significant difference between groups and this continued until the end of the study.



Asterisk indicates days with significant differences between GS and non-GS treatment groups ($p \leq 0.05$)

Histological analysis showed that all of the infected mice continued to show a high degree of inflammation and bacterial colonization at the time of euthanasia and osteogenesis was absent in all infected mice. The uninfected control mice did not show any significant inflammation or bacterial colonization and osteogenesis was observed in numerous mice from these treatment groups however statistical analysis using a Wilcoxon Rank Sum Test showed no significant difference between treatments. The MicroCT analysis yielded similar results, showing a high degree of bone deterioration and lack of osseointegration in the infected mice, while the uninfected mice showed varying degrees of osseointegration with the allograft.
Conclusions: While the GS coatings did demonstrate efficacy in reducing osteomyelitis they were unable to fully eliminate the infection. This may have been due to the dosage, infection method, elution kinetics or other factors. Further study is required and *in vitro* investigation of elution characteristics is currently underway. The study was unable to demonstrate whether DOPS coatings are capable of enhancing allograft osseointegration. Only the uninfected control mice showed any osseointegration and there were only 5 mice in each uninfected treatment group, which made statistical significance difficult to achieve from the histological data. Further research with larger sample sizes will be necessary to determine whether DOPS coatings are potentially useful for enhancing allograft osseointegration.
References:
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