Development of a 3D scaffold based model system for the study of bone infection

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Introduction

Staphylococcus aureus is a universal gram positive bacteria that lives harmlessly on the skin and mucous membranes. It can cause superficial skin lesions and wound infections as well as more serious debilitating invasive diseases such as osteomyelitis. Osteomyelitis is an acute or chronic infection of the bone and is characterized by suppurrative inflammation, abnormal bone remodelling, together with uncontrolled bone resorption. S. aureus is the causative agent in 80% of osteomyelitis cases and treatment of the disease is difficult due to the emergence of antibiotic resistance, in particular methicillin resistant S. aureus (MRSA). Thus further understanding of the disease is necessary in developing novel treatment strategies for osteomyelitis.

Aims

To facilitate and improve our understanding of the effects of S. aureus on osteoblasts, this project aims to develop a 3D scaffold based model system of osteomyelitis based on a collagen-glycosaminoglycan (CG) scaffold, which is designed to support bone formation.

Methods

S. aureus were grown in Brain Heart Infusion Broth overnight (18h) to stationary phase. The mouse clonal MC3T3-E1 pre-osteoblastic cell line were grown in flasks containing a-MEM supplemented with 10% FBS, 2% penicillin-streptomycin solution and 1% L-glutamine. The CG scaffolds are fabricated by a freeze-drying process in which chondroitin 6-sulfate is mixed with acetic acid and fibrillar collagen type I. The CG scaffold was seeded with 500, 000 and 1 million osteoblasts for 7 days then infected with S. aureus at an OD1 and OD1.6, respectively, for 48hrs. Analysis of the scaffold is conducted using gram, H&E and DAPI staining.

Results

S. aureus 1 were grown in Brain Heart Infusion Broth overnight (18h) to stationary phase. The mouse clonal MC3T3-E1 pre-osteoblastic cell line were grown in flasks containing a-MEM supplemented with 10% FBS, 2% penicillin-streptomycin solution and 1% L-glutamine. The CG scaffolds are fabricated by a freeze-drying process in which chondroitin 6-sulfate is mixed with acetic acid and fibrillar collagen type I. The CG scaffold was seeded with 500,000 and 1 million osteoblasts for 7 days then infected with S. aureus at an OD1 and OD1.6, respectively, for 48hrs. Analysis of the scaffold is conducted using gram, H&E and DAPI staining.

Conclusion

- Results for 500,000 MC3T3 cells with an OD1 S. aureus proves to be the most appropriate working model with greater migration of both cells and bacteria into the scaffold, with scaffold integrity also being maintained.
- Scaffold architecture is negatively affected when seeded with 1,000,000 cells and OD1.6 S. aureus.
- Due to the ability of Cowan to express a collagen binding adhesin (Cna), permeation of the scaffold by this S. aureus species is less successful than Newman, which has a truncated Cna.
- Results are promising with early indication of cellular and bacterial infiltration into the CG scaffold.
- However, there is a substantial degree of localisation of osteoblast cells around the scaffold periphery.
- To conclude, these results are promising demonstrating early migration and infiltration of osteoblasts into the CG scaffold in conjunction with S. aureus; further contributing to the development of a 3D scaffold based model system for osteomyelitis.

Future Work

- Staining techniques are purposeful however a single differential stain is required to demonstrate the spatial interactions between the osteoblasts and bacteria within a 3D environment.
- In order to achieve a higher degree of infiltration, longer seeding period for osteoblasts must be conducted with a shorter infection time.