Assessing the bioactive profile of anti-fungal loaded Calcium Sulphate against fungal biofilms

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Background

Calcium Sulphate (CS) has been used as a biomaterial for clinical applications for many years and has stood the test of time due to its well-tolerated nature by the human immune system, relative lack of expense and resorbable characteristics [1]. For these reasons, it has found a dedicated niche in the applications of bone cementing, dental-filling, and void-filling in a clinical setting. Perhaps more importantly than this, it has developed as a tool for the loading of antibiotics and pharmacological agents [2].

Currently, empirical fungal therapy favours fluconazole (FLZ), a drug distributed through oral dosing and subsequent absorption through the gastrointestinal system. Amphotericin B (AMB), a member of the polyene family, is also a systemically distributed antifungal agent. Echinocandins, such as caspofungin (CSP), are the most recent class of antifungals, having been first approved in 2001 as a direct response to increasing resistance to azole drugs [3]. Here, we have loaded CS beads with concentrations of FLZ (0.79mg), AMB (0.56mg) and CSP (0.38mg).

The capacity for CS to be used as a release mechanism for local antibiotic loads holds particular relevance within the management of such diseases as diabetic foot ulcers and peri-prosthetic joint infection, two such diseases that are heavily linked with bacterial and fungal colonisation in the form of biofilms [4].

Aims

The aim of this study was to show that antifungal loaded CS beads inhibit the in-vitro growth of fungal organisms planktonically; as a growing biofilm and finally as a pre-grown biofilm and that this pattern of inhibition remains visible over an extended period of time.

Methods

Biofilm formation and treatment samples were loaded with 5 mg of CS and beads loaded with antibiotics (0.79mg FLZ, 0.56mg AMB and 0.38mg CSP, respectively) or untreated control CS beads. Fluconazole, Amphotericin B, and Caspofungin were loaded onto the beads by using the Wako Pure Chemicals Carboxylated Methacrylate Copolymer (BioMat) for a period of 24 hours. This was followed by a wash out using dimethyl sulfoxide (DMSO) to remove any remaining antibiotic molecules that were not adsorbed to the beads. The CS beads were loaded with antibiotics and washed before being inoculated onto the 96-well plates for all biofilm experiments. The antibiotics were released over 7 days using a PBS elution cycle (200 µL at 37°C). The plates were then incubated at 37°C and 5% CO2 for 7 days.

Results

Figure 1. Planktonic MIC of antifungals released from CS beads over 7 days.

A) Control

B) FLZ CSP AMB

C) FLZ CSP AMB

Figure 2. Visualisation of In-vitro Biofilm inhibition from CS beads mixed with FLZ, AMB or CSP through SEM microscopy

A) Control

B) FLZ CSP AMB

C) FLZ CSP AMB

Discussion

Results from these studies show successful, sustained release of antifungal agents from STIMULAN® Rapid Cure CS beads which provide supra-MIC concentrations of drugs which inhibit growth of fungal biofilms

- Antifungal CS beads were observed to release concentrations far above planktonic MIC for a panel of 16 fungal species sustained over 7 days.

- SEM images display a clear inhibitory effect on established and growing in-vitro fungal biofilms for species C. albicans, C. auris, and A. brasilensis.

- Biofilm biomass and viability are significantly reduced across 5 key species when exposed to AMB and CSP loaded CS beads.

- Application of an in-vitro hydrogel model to better replicate a natural environment shows inhibition of fungal biofilms adhered to cellulose matrix when incubated with CS beads loaded with antifungal agents.

References