Development of an antibacterial polymer/calcium phosphate-based cement for surgical prophylaxis

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DISCLOSURES: Lin (N); Caldwell (N); Bhaduri (N); Goel (1: Spinal Balance, OsteoNovus, Turning Point, Depuy, SI Bone, Apex/Spine/Medyssey, Spine Soft Fusion, Spinal Elements, AO Foundation/FORE, K2M, NIH, NSF, Third Frontier Program, ODSA.); Agarwal (1: Spinal Balance, OsteoNovus, Element Orthopedics, Endosphere Spine, Butterfly Spine, B.O.N.E., NSF, Third Frontier Program, ODSA.)

INTRODUCTION:

Surgical site infections (SSIs) occur in 2% of surgical procedures and account for increased morbidity and mortality of a patient, average hospital stay and heath expenses. Some surgeons have been applying vancomycin powder directly into the surgical site immediately before the wound closure to reduce SSIs and have achieved some success (1). However, the drug can easily diffuse to surrounding tissue or enter the circulatory system causing toxic side effects. This problem can potentially be solved by incorporating a drug delivery system to control the release of the antibiotic. Synthetic bone grafts serve as ideal carriers to deliver therapeutic agents to the site of surgery to maximize local drug concentration while minimizing systemic concentration and toxicity. Release of antibiotics from calcium phosphate-based cements (CPCs) has been extensively investigated since these materials are widely used in bone replacement in traumatology or dentistry. A desirable antibiotic-carrying CPC has to perform sustained release of the drug at its minimum inhibitory concentration during a short period of time (<7 days) without the effect of subinhibitory concentrations, which causes development of antibiotic resistance of bacteria. Most drug-loaded CPCs exhibit burst release in 8-24 hours. Incorporating polymers (e.g. poly(lactic-co-glycolic acid), alginate or chitosan) into CPCs is one of the promising approaches for control of the antibiotic release (2). Carboxymethyl cellulose (CMC) is a biocompatible and biodegradable material widely used in tissue engineering, and its chemical structure resembles sugar-based polymers. We assessed the *in vitro* antibiotic release of CMC-incorporated CPC and found that by adding CMC, the CPC's antibiotic delivery properties can be manipulated and optimized.

METHODS:

The monetite (DCPA, $CaHPO_4$)-based powder was prepared according to the method described elsewhere (3). This cement powder was mixed with sodium CMC, sodium alginate, or poly(vinyl alcohol) (PVA) (all polymers purchased from Sigma, MO, USA) at 10 or 20%w/w and 10%w/w vancomycin hydrochloride (Sigma, MO, USA), and double-distilled water was added to generate a paste which was then molded into 0.1 g pellets and air dried. Each pellet was immersed in 1 ml PBS at 37°C. PBS samples were collected at different time points and measured by UV/visible spectroscopy at 280 nm. The vancomycin concentration in each sample was obtained by using a standard curve of absorbance vs concentration. Since the functional properties of CMC depend on the chain length of the cellulose backbone structure as well as the degree of substitution (i.e. the number of hydroxyl groups involved in the substitution reaction), cement pellets with CMC polymers of various chain lengths, expressed in terms of the molecular weight (90,000, 250,000 or 700,000), or different degrees of substitution (0.7, 0.9 or 1.2) were also assessed for their antibiotic-carrying capabilities. To confirm if the released antibiotic remained bioactive, 100 µl of the PBS sample was added into 5 ml freshly inoculated methicillin-resistant *Staphylococcus aureus* (MRSA) culture, and bacterial density measured at 600 nm. The mean and SD were calculated based on at least three independent experiments.

RESULTS:

The burst release effect was observed on the vancomycin-loaded CPC since within 24 hours, significant amount (>70%) of the antibiotic was released from this composite. This effect was prevented by adding 10%w/w (Fig. 1A) or 20%w/w CMC or alginate in the CPC, whereas adding PVA did not improve the CPC's drug carrying property. The antibiotic in all CPC composites was released rapidly during the first 7 days (Fig. 1A), and it became undetectable in PBS samples of the 10%w/w CMC-containing CPC composite after 2 weeks (Fig. 1B) and of the 20%w/w CMC-containing CPC composite after 3 weeks. In addition, high molecular weight (700,000) CMC in the CPC retarded drug release whereas high degree of substitution (1.2) CMC accelerated it in the first 7 days (Fig. 1C and D). Antibacterial activity of the released antibiotic was demonstrated by inhibition of MRSA growth.

DISCUSSION:

Our data suggested that, by incorporating CMC or alginate, the vancomycin release from CPC composites can be optimized to prevent burst release, and the majority of the loaded antibiotic can be released in the first 7 days with a more sustained pattern. Since complete release can be achieved in 2 weeks by using 10%w/w CMC, development of antibiotic resistant bacteria due to subinhibitory concentrations is expected to be minimal. The chain length of the polymer and the degree of substitution are two critical factors that need to be considered for improvement of this vancomycin/polymer/CPC complex.

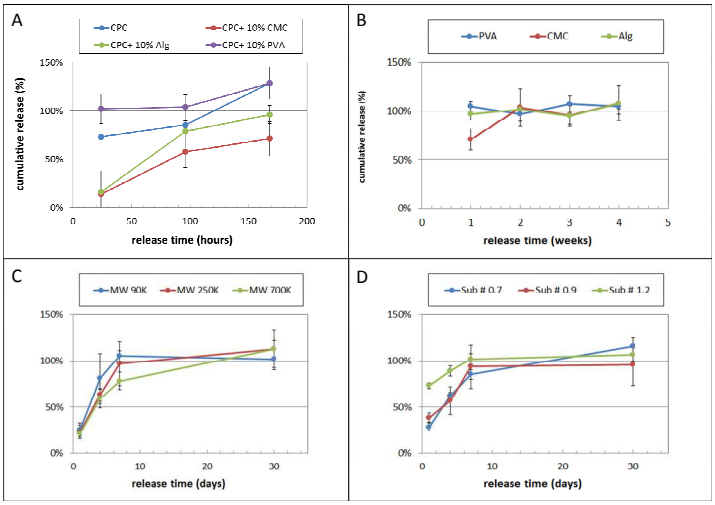

Fig 1 A) Vancomycin release from CPC and CPC with 10% polymers for 1, 4 and 7 days. B) Vancomycin release from CPC with 10% polymers for 1, 2, 3 and 4 weeks. C) Vancomycin release from CPC with CMC of various molecular weights for 1, 4, 7 and 30 weeks. D) Vancomycin release from CPC with CMC of various degrees of substitution for 1, 4, 7 and 30 weeks

SIGNIFICANCE:

A simple method is introduced in this study to prevent osteomyelitis by utilizing a bone filler material CPC as a carrier for antibiotics. To optimize the administration of antimicrobial agents, the drug release profile of this composite was made tunable by proper blending with sugar-based polymers such as CMC and alginate. The aims of better controlled drug release kinetics are to avert the adverse effects due to burst release and the antibiotic resistance of bacteria due to exposure to non-lethal concentrations of the drug.

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