Evaluation of vancomycin impregnated bone grafts and vancomycin loaded poly(lactide-co-glycolide) (PLGA (75:25)) microspheres-bone grafts blend for the treatment of surgical bone infections

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Implantation of bone allografts has become an increasingly popular method of restoring bone defects and skeletal integrity following surgical removal of bone tumors, revision of total hip and knee arthroplasties and defects due to chronic osteomyelitis. Eradication of infections is critical for allograft survival. Local applications of antibiotics can provide high drug concentrations at the site of infection and can avoid systemic effects (1). For this reason, a study with vancomycin impregnated rabbit bone grafts and vancomycin loaded PLGA (75:25) microspheres is conducted. Characterization and in vitro release of these drug carriers were investigated in this study.

Vancomycin is a bactericidal glycopeptid antibiotic which is used because of its efficacy in the treatment of serious infections caused by meticillin-resistant Staphylococcus aureus (MRSA) (2). Antibiotic loaded microparticle systems are widely investigated to be used for the treatment of surgical bone infections (3-4). In this study, bone grafts which are commonly employed to augment skeletal defects and stimulate fracture healing, were impregnated with vancomycin and blended with vancomycin loaded microspheres. Microspheres were evaluated for particle size distribution, drug content and surface morphology. Besides, the in vitro release of the formulations were investigated and compared.

Vancomycin loaded PLGA (75:25) microspheres were prepared by emulsion/solvent evaporation process. Vancomycin was dissolved in dimethylsulfoxide and added to the polymer (PLGA 75:25) solution in methylene chloride. Then this dispersion was emulsified into the aqueous continuous phase containing polyvinyl alcohol : sodium oleate in a ratio of 4:1. The rabbit bone grafts were harvested from iliac crest and femur of the rabbits and morcellized into small particles by a bone mill. A series of shaking baths in ether, 70 %, 50 % and 30 % ethanol and 3 % hydrogen peroxide were used cleaning the bone grafts from remaining soft tissue and dried. Afterwards, the bone grafts were freeze-dried. Bone grafts (10 g) were impregnated with vancomycin in 10 ml of antibiotic solution and finally vancomycin impregnated bone grafts were freeze dried again (5). The second formulation was prepared by blending the vancomycin loaded microspheres with rabbit bone grafts in ratio of (5:1). The Laser Diffraction Particle Size Analysis was used for measuring particle size distribution of the microspheres. Drug content in microspheres was measured by UV-Visible Recording Spectrophotometer (Shimadzu-Japan) at 280 nm. Scanning electron microscopy (SEM) evaluation of the formulations was carried out to examine the surface morphology. For in vitro release studies; 600 mg vancomycin loaded vancomycin microspheres-rabbit bone graft blend and 1 g vancomycin impregnated rabbit bone grafts were placed in polypropylene tubes and 3 ml of phosphate buffer at pH 7,4 was added. Polypropylene tubes were shaken at 50 cpm and 37°C in a termostated bath. Then, samples were taken every 24 hours and after centrifugation at 5000 rpm, supernatant was removed, drug content was determined at 280 nm. Minimum Inhibitory Concentration (MIC) of the antibiotic was determined in every removed sample.

The particle size of the microspheres was measured as 64 ± 1,7 μm. The encapsulation efficiency of the microspheres was 3,99 %, while the yield value was 60%. SEM photographs revealed that microspheres were homogenous and had spherical surfaces. In vitro release of the vancomycin impregnated rabbit bone grafts was completed at the end of 35th day. In the other formulation, vancomycin loaded microspheres-rabbit bone graft blend; only 68,46 % of the antibiotic was released at the same period.

Our in vitro results suggest that, vancomycin impregnated rabbit bone grafts and vancomycin loaded microspheres-rabbit bone grafts blend could be suggested for application to treatment of surgical infection because of their efficacy of antibiotic release and lack of systemic toxicity, but vancomycin loaded microspheres-rabbit bone grafts blend seem to be a good choice for the protection of the antibiotic stability in PLGA matrix and controlled antibiotic release will be provided for up to 5 weeks with this formulation.

References

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