

**EVALUATION OF IN VITRO ANTIBIOTIC RELEASE FROM VANCOMYCIN IMPREGNATED HUMAN BONE GRAFTS AND VANCOMYCIN LOADED POLY(LACTIDE-CO-GLYCOLIDE) PLGA (75:25) MICROSPHERES-HUMAN BONE GRAFTS BLEND**

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**ABSTRACT SUMMARY:**

In orthopaedic applications, allografts are used for restoration of bone defects for different reasons usually associated with chronic bone infections. For this reason, a glycopeptide antibiotic, vancomycin used to prepare vancomycin impregnated human bone grafts and vancomycin loaded PLGA (75:25) microspheres, they were characterized and their in vitro release were compared.

**INTRODUCTION:**

The bone grafts were used for; to promote osteogenesis between adjacent bones (for nonunion or arthrodesis), to fill cavities in bone (as after curettage of cysts) and to restore bone defects (in tumour resection and revision arthroplasty).

Vancomycin HCl, is a glycopeptide antibiotic often used to treat gram-positive infections and is the agent of choice for common causative agents of bone infections(1). In the last decades; antibiotic loaded microparticulate systems which are prepared with biodegradable poly(lactide-co-glycolide) (PLGA) polymers and natural polymers used for the treatment of bone infections for sustained release (2). In this study, combination of vancomycin and vancomycin loaded PLGA microspheres blended with human grafts were evaluated in vitro for the intention of using for bone repair and to prevent infections. For this purpose, particle size distribution, drug content in the microspheres, surface morphology, in vitro release of the formulations were investigated.

**EXPERIMENTAL METHODS:**

**1.Preparation of the formulations:**

**1.1.Preparation of PLGA (75:25) (MW 136000) Microspheres:**

Vancomycin loaded PLGA microspheres were prepared by emulsion/solvent evaporation process. For the formation of o/w type emulsion, 30 mg vancomycin was dissolved in dimethyl sulfoxide and added to the polymer solution (300 mg PLGA 75:25) in methylene chloride. Then, this dispersion was emulsified into the aqueous continuous phase containing polyvinyl alcohol (PVA): sodium oleate (SO). This medium was stirred continuously (750 rpm) at room temperature for 2 hours until the evaporation of methylene chloride was completed. Finally, the resulting microspheres were collected by centrifugation, washed with water and dried at room temperature (3). Blank microspheres were

also prepared in a similar way in order to be used as control in release studies.

**1.2.Preparation of human bone grafts:**

Human bone which was composed of cancellous and cortical parts was harvested and morcellized into small particles by a bone mill under sterile conditions. Fatty bone marrow, cells and small amounts of remaining soft tissue were removed by means of a series of shaking baths in ether, %70, %50 and %30 ethanol and %3 hydrogen peroxide, dried and cut into pieces with the bone mill. Finally, the bone pieces were freeze-dried (4).

**1.3.Preparation of vancomycin-impregnated human bone grafts:**

Vancomycin was dissolved in distilled water (50mg/ml concentration). 10g of bone was impregnated with antibiotic in 10ml of antibiotic solution after shaking 3 hours at room temperature. Finally vancomycin impregnated human bone sample was freeze dried again. (5)

**1.4.Preparation of vancomycin loaded microspheres human bone grafts blend:**

Human bone graft was blended with vancomycin loaded microspheres in ratio of (5:1) in a glass flask before in vitro release studies to obtain appropriate scales for implantation.

**2.Characterization of the formulations**

**2.1.Particle Size Distribution:**

The particle size distribution was measured using HELOS Laser Diffraction Particle Size Analysis (Sympa, Germany).

**2.2.Drug Content:**

5ml of methylene chloride was added on 50mg of vancomycin loaded PLGA microspheres and the polymer was dissolved. 10 ml of pH 7,4 phosphate buffer was added in which vancomycin was extracted for 2 hours. After the evaporation of methylene chloride, the polymer was removed and vancomycin amount in microspheres was measured by UV Visible Recording Spectrophotometer (Shimadzu-Japan) at 280 nm.

**2.3.Microscopic Evaluation:**

Scanning electron microscopy (SEM) was used to examine the surface characteristics of the formulations. They were mounted on metal stubs with conductive silver paint and then sputtered with a 150 Å layer of gold in a BIORAD (England) sputter apparatus. Microspheres and microspheres-bone graft blend were investigated in Jeol

Scanning Electron Microscope at 80 KV (SEM ASID-10 Device) (Japan).

#### 2.4. In vitro release studies:

##### 2.4.1. Vancomycin impregnated human bone grafts

1g of vancomycin impregnated human bone grafts were added into the polypropylene tubes and were placed in a thermostated bath shaken continuously at 50 cpm at 37°C. Samples were taken every 24 hours and replaced with fresh medium. The samples were filtered and assayed for vancomycin at 280 nm.

##### 2.4.2. Vancomycin loaded microspheres -human bone grafts blend:

600mg microsphere-human bone graft combination were added into the polypropylene tubes and were placed in a thermostated bath shaken continuously at 50 cpm at 37°C. Samples were taken every 24 hours and after centrifugation at 5000rpm, supernatant was removed and drug content was determined at 280 nm. Minimum Inhibitory Concentration of the antibiotic was determined in every removed sample.

#### RESULTS AND DISCUSSION:

Table 1. Characterization of PLGA (75:25) microspheres

Encapsulation efficiency (%)	3,99
Particle size(µm)	64±1,7
Yield value (%)	60

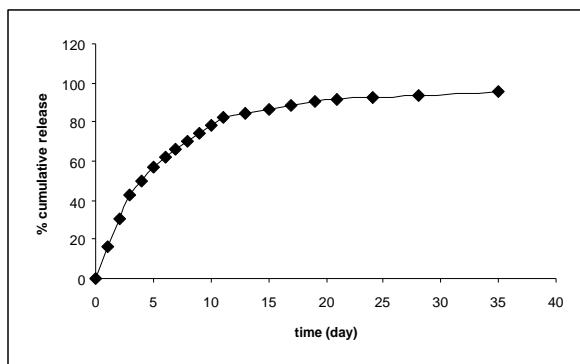


Figure 1. In vitro drug release from vancomycin impregnated human bone grafts

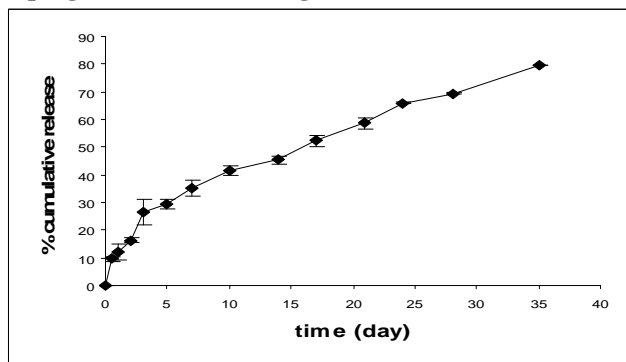
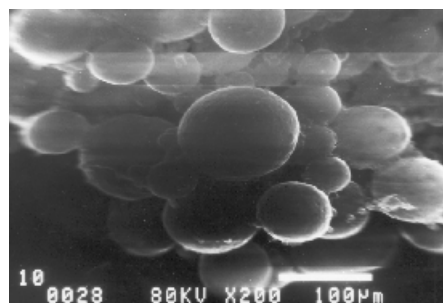
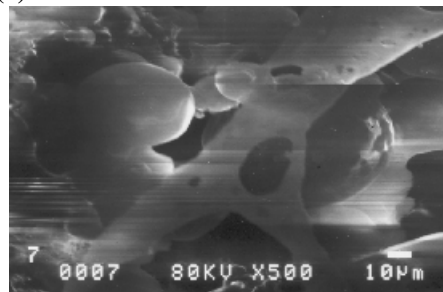


Figure 2. In vitro drug release from vancomycin loaded microspheres-human bone graft blend.



(a)



(b)

Figure 3. SEM photographs of (a) PLGA microspheres (b) vancomycin loaded microspheres - human bone grafts blend before in vitro release

#### CONCLUSION:

In vitro results obtained suggest that vancomycin impregnated human bone graft carriers could be used for the local treatment of bone infection. In the other hand; when release from vancomycin impregnated human bone grafts compared to vancomycin loaded biodegradable microspheres-bone graft blend, blend appeared to be a promising alternative for the local treatment of bone infection as the release determined to continue still at the end of 5<sup>th</sup> week (almost 80% released) and in which the stability of antibiotic could be protected in a polymer matrix. In vivo studies on animals are in progress to support in vitro findings.

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#### ACKNOWLEDGEMENTS:

Authors wish to acknowledge Dupont Merck (USA) for the generous supply of PLGA and Eli Lilly (Turkey) for vancomycin. This project was supported by Hacettepe University Research Fund (Project No: 0201301003)