Antimicrobial and Bioactive Composite Scaffolds for Bone Tissue Engineering

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Statement of Purpose: Bioactive scaffolds which have antimicrobial properties are important in bone tissue engineering, since infection is one of the biggest problems in operations and especially in implanting bone grafts or bone prosthesis. On the other hand, vascularization of the operation site and regeneration of bone is a very slow event. Therefore, polymer matrices incorporated with inorganic ceramics and having antimicrobial properties gained importance in orthopedic and dental applications since they demonstrate osteo-conductive and osteo-inductive properties and good mechanical strength besides high biocompatibility. In this study, poly(ε-caprolactone) (PCL) composites which have an antimicrobial drug (gentamicin) and containing beta-tricalcium phosphate (β-TCP) crystals were prepared. The properties as well as cell and tissue responses were examined in situ, in vitro and in vivo conditions. The effects of the ratio of the β-TCP containing microspheres on the morphological, mechanical and stability properties of composites, as well as in vitro antibiotic release and antibacterial activities against E.Coli and S.Aureus were investigated. The results showed antibacterial activities for both bacteria. Culture studies with Saos-2 cells demonstrated high bioactivity, and in vivo applications on rabbits shoed enhanced incorporation with bone tissue.

Methods: Gentamicin loaded microspheres with composition of β-TCP/Gelatin were prepared with water-in-oil emulsion method and were mixed with PCL solution. The mixtures were molded in cylindrical tubes and lyophilized. The prepared scaffolds were examined by Scanning Electron Microscope (SEM), mechanical testing machine (Lloyd Instrument, Ltd., equipped with a 100 N load cell), and Mercury Intrusion Porosimeter (MIP). Gentamicin release studies as well as hydrolytic and enzymatic degradation analysis were carried out in PBS at 37°C. In vitro cell viabilities of the cells were determined by MTT assay. The ALP activity of Saos-2 cells was measured at the end of days 1st and 21st days of incubation period. In vivo applications were done on bilateral cylindrical bone defects (D:5 mm, H:4 mm) on the iliac crests of rabbits. The surgical protocols of this study were approved by Cukurova University Animal Research Ethical Committee (Adana, Turkey). After 8 weeks of implantation, rabbits were sacrificed and push-out tests were performed with Universal Testing Machine (Testometric) in the compression mode (Rate:2 mm.min^{-1}, Load Cell: 2500 kgf). For histological analysis, three sections were taken from each sample and stained with haematoxylin and eosin (H&E). These sections were evaluated via light microscopy (BX41, Olympus, Japan).

Results: The composite microspheres containing β-TCP were in spherical shape and distributed homogeneously in the matrices prepared by using PCL (Figure 1 A and B). In cell culture experiments high amount of Saos-2 cells adhered to the surfaces of the matrices prepared as porous 3D forms, and proliferated more than control groups which have no β-TCP (Figure 1 C and D). Diffusion tests demonstrated very effective antimicrobial activity against E.Coli and S.Aureus (Figure 2). Histological tests also demonstrated enhanced tissue regeneration around the implantation site.

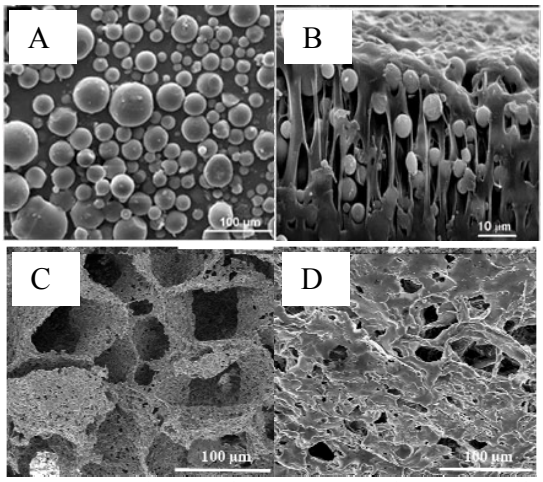

Figure 1. SEM micrographs. (A) Microspheres, (B) Films; (C) Saos-2 Cells on 3D scaffolds, Day 1, (D) Saos-2 Cells on 3D scaffolds, Day 21.

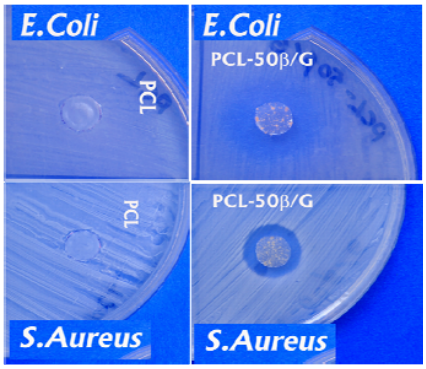

Figure 2. Antimicrobial efficacy for E.Coli and S.Aureus.

Conclusions: The matrices prepared as films and 3D structures showed high antimicrobial and bioactivity and have great potential for medical applications.

References: Sezer UA, Aksoy EA, Hasirci V, Hasirci N, J Appl Polym Sci, 2013;127:2132–2139.