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Acceleration of new bone formation by an electrically polarized hydroxyapatite microgranule/platelet-rich plasma composite

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ABSTRACT

We have developed a technology for the electrical polarization and electrical characterization of hydroxyapatite (HA) microgranules. In order to improve handling of ceramic powders to fulfill complex geometrical demands, platelet-rich plasma (PRP) containing many growth factors was chosen as a carrier of HA microgranules. In this study, the effects of this electrically polarized HA microgranule/PRP composite on new bone formation were examined. To compare osteoconductivity, HA microgranules with or without electrical polarization/PRP composite gel, HA microgranules alone with or without electrical polarization, or PRP gel were implanted into holes in the medial femoral condyles of rabbits. As a control, some of the bone holes were left empty ($n = 6$ in each group). Histological examination was performed 3 and 6 weeks after the surgical operation. It was suggested that PRP alone could not induce new bone formation until 6 weeks after implantation. HA microgranules with or without electrical polarization/PRP composite, especially the former, activated osteogenic cells, resulting in enhanced bone formation. It was confirmed that electrical polarization treatment of HA microgranules can accelerate new bone formation, and that this effect is enhanced by forming a complex within the PRP.

1. Introduction

Calcium phosphate compounds, especially hydroxyapatite (HA), have been widely used as a bone substitute due to their biocompatibility and bone-resembling mineral composition. HA is used as a bulk body, as a porous body as well as in granule form. HA granules are available in different sizes corresponding to various configurations of bone defect. A disadvantage with granules is the difficulty of handling them when they are implanted, and keeping them in the desired location. Therefore, attempts to create composites by combining granules with different carriers (fibrin glue, collagen, sodium hyaluronan and lipids) have been made [1–4]. These studies suggest that the nonimmunogenic, biodegradable, biocompatible, osteoconductive and handling properties of the composite have to be optimized for different clinical applications and implantation sites. Platelet-rich plasma (PRP) contains a number of growth factors: platelet-derived growth factor (PDGF), transforming growth factor (TGF)-β1, TGF-β2, insulin-like growth factors (IGFs), epidermal growth factor (EGF) and epithelial cell growth factor (ECGF) [5]. Eppley et al. reported that the PRP was produced from 10 healthy volunteers, following thrombin/$CaCl_2$-induced activation, and determined the following levels of growth factors: PDGF, 17 ± 8 ng ml^{-1}; TGF-β1, 120 ± 42 ng ml^{-1}; VEGF, 955 ± 1030 ng ml^{-1}; EGF, 129 ± 61 ng ml^{-1}; and IGF-1, 72 ± 25 ng ml^{-1} (average ± SD) [6]. Furthermore, PRP can be obtained on the day of surgery from autogenous whole blood. There are some reports suggesting that PRP may support bone and soft tissue healing [7–9]. For this reason, PRP may be a suitable carrier of HA microgranules used for bone substitute to accelerate new bone formation.

Proton transport polarization induces large surface charges on HA blocks [10,11] that can enhance the osteogenic cell activity, resulting in increased new bone formation in the regions bordering the charged surface of HA [12]. Our previous study anticipated that electrical polarization would improve the wettability of the HA surface because of polar interaction energy with water, and the improved surface wettability would affect osteoblastic adhesion [13]. Bovine serum albumin (BSA) was absorbed on the electrically polarized biphasic calcium phosphate surface [14]. This can be explained in terms of strong electrostatic interactions between the positive entities (H^+, Ca^{2+}) present at the surface and the negative terminals ($-COO^-$) of the BSA. Kumar et al. seeded osteoblastic MC3T3-E1 cells on electrically polarized HA discs [15]. An alamar blue assay at 7 days demonstrated significant cell metabolic

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