als may be obtained from the patient (autogenous graft) or from another person (allogenic). Although the application of autogenous grafting has great osteogenic potential, its source is limited. For this reason, demineralized freeze-dried bone allografts (DFDBA) have been in clinic since 1980s.[2] Mellonig et al.[3,4] reported that attachment and new bone formation occurred following DFDBA implantation based on histological investigations. Shallhorn et al.[5] also reported that pocket reduction and attachment gain were observed 5 years after DFDBA implantation. In addition to the natural graft materials, artificial bone grafting materials have also been designed and used clinically. Most of these materials are chemical compounds similar to the inorganic components of the bone matrix, for example, hydroxyapatite and tricalcium phosphate.[6] In 1920, it was found that tricalcium phosphate could enhance the process of osteogenesis. Froum et al.[7] and Yukna et al.[8-10] reported that following hydroxyapatite implantation bony filling and clinical probing depth reduction were observed. During the 1980s, Saffer et al.[11] and Bowers[12] reported clinical benefits in treating periodontal diseases following tricalcium phosphate implantation. In 1985, Kenneth[13] and Wagner et al.[14] reported achieving the clinical benefitst in bony defect following nonceramic implantation.

Amar,[15] and Pitaru et al.[16] reported that fibroblasts contributed to the wound healing process on the third day after surgery. Wang et al.[17] also reported that fibroblasts migrated to the bony defect on the first day after surgery. Generally, fibroblasts are considered to play an important role in wound healing after periodontal surgery.

With increasing clinical use, the safety and biocompatibility of dental materials are of significant concern. In vitro assays for cytotoxic effects are increasingly being used for initial screening of new dental materials intended for use in humans. It would be better to perform in vitro cytotoxicity tests using cells homologous to human tissue of ultimate concern.

Generally, in vivo exposure of tissues to dental materials occurs both directly and indirectly. Direct cell-material contact can be tested by placing the material with the cultured cells, while indirect contact has been achieved by using extracts of materials. In the present study, we attempted to evaluate the cytotoxicity of HA, NCHA, TCP, and DFDBA to primary human gingival fibroblast under direct and indirect contact conditions.

MATERIALS AND METHODS

The bone grafting materials used in this study included hydroxyapatite (Orthomatrix ® HA 500, Lifecore, USA), 250-420 μm in size; demineralized freeze-dried bone allograft (DEMBONE ®, Pacific Tissue Bank, USA) 250-500 μm in size; β-tricalcium phosphate (Synthograft ®, Johnson & Johnson, USA), 200-400 μm in size; and non-ceramic hydroxyapatite (Osteogen ® Impladent, USA) 300-400 μm in size.

Primary Human Gingival Fibroblasts Culture

Healthy gingival tissues were obtained during oral surgery on human donors. The tissue explants obtained were cut into small pieces. The tissue fragments were placed into a culture flask in DMEM containing 10% FCS, penicillin, and streptomycin and incubated at 37 °C in an atmosphere of 5% CO_2 with 100% humidity. After the cells grew to confluentluence, they were detached with 0.25% trypsin and subcultured. Fibroblasts from passages 3-8 were used in this experiment.

Cytotoxicity Assay

Indirect cytotoxicity ttest

The 4 (HA, DFDBA, β-TCP and NCHA) test materials were individually immersed in DMEM containing 10% FCS, penicillin, and streptomycin for 1, 3, 5,or 7 days (see below). Extracts were then tested for cytotoxicity. And equal volume of culture medium was incubated under the same conditions but without materials to serve as the negative control.

Direct cytotoxicity test

Human gingival fibroblasts were co-cultured together with each of the 4 test materials. Cells cultured