without adding any test material were used as negative control.

**Assessment of cytotoxicity**

Cytotoxicity was determined by a MTT reduction assay, which measures the metabolic reduction of MTT (3-4,5-dimethylethiazol-2-yl)-2,5-diphenyl tetrazolium bromide; Sigma, USA) to colored formazan by mitochondrial dehydrogenase.[18] Primary human gingival fibroblasts were seeded into each well of 24-well culture plate at a concentration of $5 \times 10^4$ cells/ml with 0.4 ml/well, and incubated for at 37 °C with a 5% $CO_2$ atmosphere. After 24 h of incubation, cells were exposed to test materials or their extracts (0.2 ml/well). After another 24-h exposure, the medium was replaced with the same volume of fresh medium containing MTT. Following a 4-h incubation the formazan produced was dissolved in solubilizing reagent, and the optical density of the medium was determined using an ELISA reader at a wave length of 595 nm. Results are expressed as a percentage of the control.

**Morphological Study**

Human gingival fibroblasts at a concentration of $2 \times 10^4$ cell/ml were cultured in the presence of HA, DFDBA, NCHA, or β-TCP, respectively, in each well of an 8-well cell culture chamber for different periods. Then the morphology of the cells was observed under phase-contrast and scanning electron microscopy, respectively.

**Phase-contrast microscopic observation**

On days 1, 3, and 7, the cultured fibroblasts were

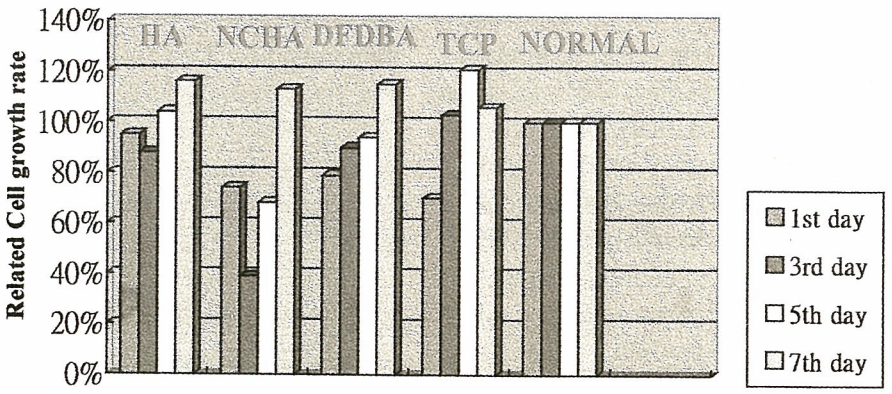


Fig. 1A. Effect of bone grafting material extracts on the viability of primary human gingival fibroblasts.

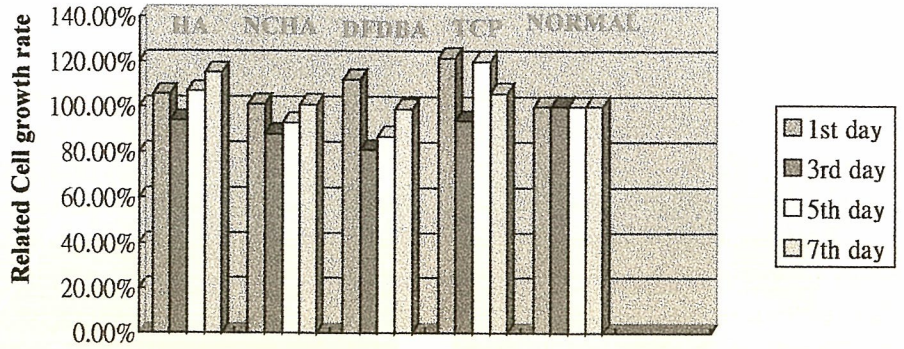


Fig. 1BEffect of bone grafting material extracts on the viability of primary human gingival fibroblasts. Cytotoxicity was measured by an MTT reduction assay, and the results are expressed as a percentage of the control. Each point represents the mean of 4 separate tests.