



Fig. 4. Fibroblasts laying over NCHA particles ( $\Rightarrow$ ) on the seventh day of culturing. There are some fibroblasts under the NCHA particle. (600X).

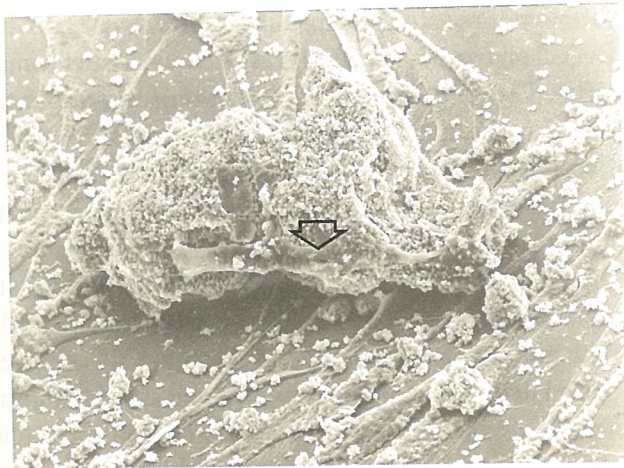


Fig. 5. Fibroblasts over the  $\beta$ -TCP surfaces ( $\Rightarrow$ ) and lots of small particles around the  $\beta$ -TCP particles after culturing. (1500X).

7, the process or the cell body of fibroblasts attached to DFDBA particles was noted (Fig. 3). In NCHA group, human gingival fibroblasts aggregating around the NCHA particle was observed initially on the first day. Attachment of human gingival fibroblasts to NCHA particles was observed on days 1, 3, and 7 (Fig. 4). Most observations showed that the human gingival fibroblasts lay on the NCHA particles.  $\beta$ -TCP particles are irregularly shaped, dense and microporous particles with rough surfaces. On the

first day, the aggregation and attachment of human gingival fibroblasts to  $\beta$ -TCP particles were initially observed. On the third and seventh days, similar phenomena were also observed. Observations also showed that human gingival fibroblasts lay on the  $\beta$ -TCP particles. In addition, there were many small particles on the surfaces of the human gingival fibroblasts or around the cells (Fig. 5). The particles were loosely formed and elliptical, and they differed from the  $\beta$ -TCP particles.

## DISCUSSION

When dental materials are used clinically, they come into contact, directly or indirectly, with living tissue. It is obviously required that they be evaluated for biocompatibility, especially cytotoxicity. Many systems are used for the determination of these complicated properties: *in vivo* systems and *in vitro* studies. The MTT test, which measures the metabolic reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to colored formazan by mitochondrial dehydrogenase in viable cells, is easy to perform and reproducible. Since it is known that gingival fibroblasts play an important role in the process of wound healing of gingival tissue after periodontal surgery,<sup>16,17</sup> the responses of gingival fibroblasts to dental materials could influence the healing process, the local fixation of grafting materials, and even subsequent following new bone formation.<sup>19</sup> In the present study, primary human gingival fibroblasts were used as target cells for MTT reduction assay to evaluate the cytotoxicity of DFDBA,  $\beta$ -TCP, HA, and NCHA, which are materials commonly used in periodontal therapy.

In most *in vitro* tests, cells are exposed to test materials for a short period, usually within 24 h. This assesses the initial acute cytotoxicity only. In the present study, exposure times of more than 24 h of contact were chosen. This allows for the prolonged exposure of fibroblasts to test materials, thus permitting investigation of delayed toxic effects or cell recovery.

The effects of the 4 test materials and their extracts