fixed with 3% glutaraldehyde in 0.1 M cacodylate buffer and observed under a phase-contrast microscope.

Scanning electron microscope observation

On days 1, 3, and 7, the cultured fibroblasts were fixed the same as for phase-contrast microscopy, followed by washing with 0.1 M cacodylate buffer, post-fixing with 0.1% OsO_4, dehydration with alcohol, drying, and gold ion coating. Specimens were then observed under a scanning electron microscope (Hitachi-2400, Japan).

RESULTS

Assessment of Cytotoxicity

As shown in Fig. 1, the cytotoxic behavior differed for different groups. Human gingival fibroblasts cultured without materials/extracts were defined as 100%. In general, the cytotoxic effect was mild; even the most-toxic effect caused by DFDBA on day 3 was only about 80% of the control cell viability (Fig. 1B). Under direct conditions, NCHA showed the greatest cytotoxicity with 40% cell viability on day 3 (Fig. 1A). The maximum reduction of cell viability for the remainder was about 20% on day 3. Immediate toxicity on day 1 was observed for NCHA, DFDBA, and β-TCP. None of the 4 test materials showed any additional cytotoxicity on day 7; even an increase in cell viability was observed for HA. With indirect contact conditions using extracts of materials, the highest level of toxicity was caused by DFDBA with only 20% inhibition on day 3 (Fig. 1B). In contrast, acute toxicity on day 1 was not seen, and a general increase in cell viability, even higher than that of the negative control, was seen for all test materials on day 7 under indirect contact conditions.

Morphological Observation

Phase-contrast microscope observation

Fibroblast accumulation and aggregation around β-TCP particles were initially observed on day 1 (Fig. 2), and phagocytosed particles were seen in the cytoplasm of fibroblasts. Cell accumulation and aggregation were seen for DFDBA and NCHA, on day 3 and For HA on day 7.

Scanning electron microscope observation

The accumulation and aggregation of human gingival fibroblasts around HA particles were initially observed on days 3 and 7. In the DFDBA group, this phenomenon was observed initially on day 3. On day

Fig. 2. Photomicrograph showing fibroblasts aggregated around β-TCP particles and some small particles found on the fibroblasts after 3 days of co-culturing. (100X).

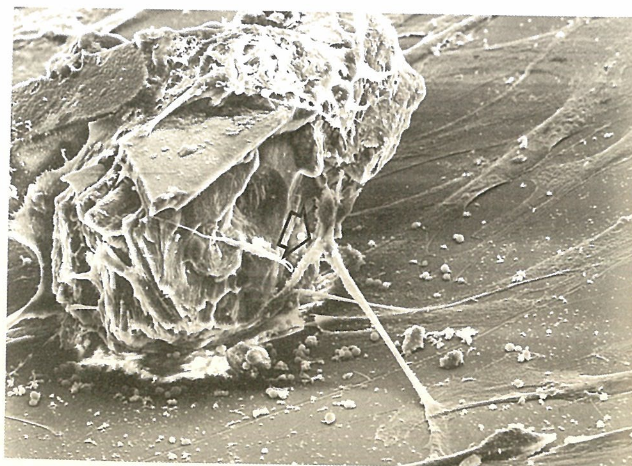

Fig. 3. Fibroblast processes contacting DFDBA particles. (⇨) (1500X).