on fibroblast somewhat differed. Acute cytotoxicity, which occurs within 24 after exposure, was not noted under indirect contact conditions, but it was obvious when fibroblasts were directly exposed to the materials. After 24 h of exposure of fibroblasts to 1-day extracts, no responses were observed. In contrast, a decrease in cell viability was observed after 24-h direct contact with the test materials. The reasons for this discrepancy are unclear, but 1of the possible explanations is that direct contact of fibroblasts with test materials may impede cells due to the higher concentrations of released substances in the vicinity of target cells as a foreign body reaction. It is also possible that substances capable of promoting cell growth are released later. This possibility is supported by the result that the 7-day extract caused no reduction in cell viability. In general, all of the test materials exerted a mild cytotoxicity on fibroblasts on day 3, followed by recovery on day 7, and the cytotoxicity become attenuated under indirect contact conditions.

The data obtained in the direct contact assay may be more significant because it more closely simulates to the situation with living tissue. Substances are being released and metabolized, and substances surrounding cells are being changed both qualitatively and quantitatively.[20,21] After 3 days of exposure to the materials, there was a reduction in fibroblast viability, especially with NCHA. Upon further incubation, of up to 5 or 7 days, there was an increase in cell viability as compared with the control. Based on these data, it is proposed that the wound healing process is somewhat delayed due to a reduction of fibroblast viability on the first 3 days after NCHA implantation.

The phenomena of fibroblasts accumulating and aggregating around particles were found for all 4 test materials. These phenomena occurred more rapidly, belong seen on the first day, after culturing of fibroblasts with β-TCP, and were delayed for HA. These results indicate that all of the test materials have the potential to attract fibroblasts to migrate towards them and to interact with the particles. The initial interaction of cells and subsequent adherence to an implanted surface is of considerable significance since this process will influence subsequent tissue responses to the material, such as fixation of the material and wound healing. It has been proposed that cell aggregation stimulates the initial cell-cell interaction and promotes production of pericellular matrix.[22,23] In turn, cell attachment is highly dependent on the protein film that adsorbs onto the surface of the material.

In the present study, small particles on or around the surfaces of the fibroblasts could be seen by SEM, which revealed that fibroblasts acted as nonprofessional phagocytes. Alliot-Licht[24] reported that ingested β-TCP is further metabolized into calcium ions, which can enhance calcification. Wu et al.[21] also reported that the enzymes released upon hydrolyzation of β-TCP particles enhance the bone-coupling process.

Fibroblast accumulation around the material was observed for all 4 test materials, but it took place at different times, for example, it occurred on day 1 with β-TCP and on day 7 with HA after exposure to the material. The time that cell accumulation occurs is determined by both the potential of the material to attract fibroblast and its cytotoxicity. It seems that fibroblast accumulation can be used as an index of biocompatibility.

Frank et al.[25] reported new bone formation and attachment of fibroblasts for bone grafting materials with small particle size. Alliot-Licht et al[25] also reported that the HA particles with small size (< 20 μm) were more easily phagocytosed by fibroblasts. In this study, the size of β-TCP was shown to be smaller than NCHA, DFDBA, and HA, and the SEM photographs also revealed the microporous structure and rough surface of β-TCP. There were a lot of small, loosely formed, elliptical particles observed on the fibroblasts. The small particles may be caused by structural "breakdown" of β-TCP particles. These characteristics of the aggregation of fibroblasts around particles and attachment of fibroblasts were more significant than for the other materials, and the phagocytosis effect was only observed in the β-TCP group.

The purpose of bone grafting surgery is to enhance bone repair or regeneration. In this study, fibroblasts showed a better response to β-TCP than to other bone grafting materials: mild cytotoxicity to fibroblasts,