CHAPTER IV

RESULTS

4.1 Colorimetric (MTT) assay for cytotoxicity of extracted medium from materials (Elution)

When the extracted medium eluted from materials was cultured with human periodontal ligament cells, cytotoxicity test was performed by MTT assay. The survival rate of the cells shown as relative percent to the negative control (figure 3) in one-day-elution group revealed no significant difference in cytotoxicity among test materials ($p>0.05$). However, extracted medium eluted from Filtek™ Flow which revealed 85.5 percents survival rate appeared to be more cytotoxic than the others.

After materials were immersed for two days (two-day-elution), Filtek™ Flow which revealed 93.5 percents survival rate still presented some cytotoxicity but this was less than appeared in one-day-elution group. The cytotoxicity of all materials, however, was not significantly different from one another ($p>0.05$).

When extracted medium from three-day-elution and four-day-elution groups were tested, there was no significant difference in percent survival rate among materials. Filtek™ Flow was not cytotoxic at this period ($p>0.05$).

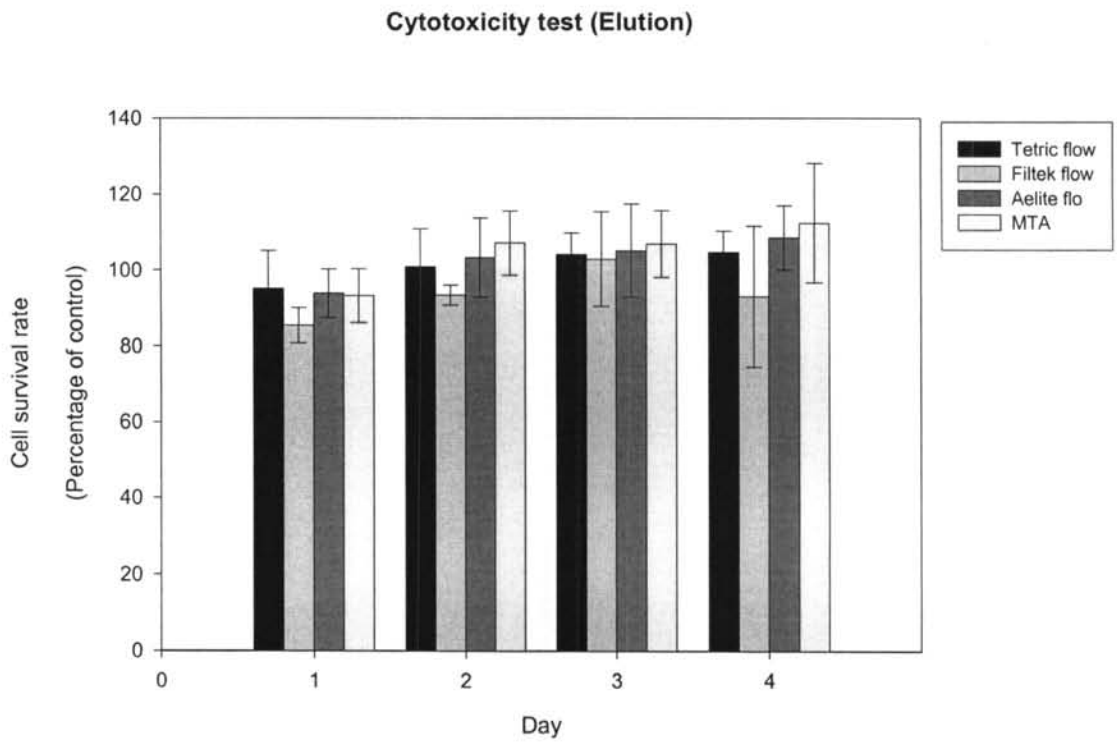

Figure 3 Cytotoxicity of extracted medium from materials (Elution) upon cultured human periodontal ligament cells at 1, 2, 3 and 4 days. The relative percent cell survival rate from four separate experiments was shown in mean percentage ± standard deviation

4.2 Colorimetric (MTT) assay for cytotoxicity of material (Direct contact)

When the materials were cultured with human periodontal ligament cells, cytotoxicity test was performed by MTT assay. The survival rate of the cells shown as relative percent to the control (figure 4) in freshly mixed group revealed MTA (35.2 %) and Aeliteflo™ (36 %) were significantly more cytotoxic than Tetric® Flow ($p<0.01$) but not different from Filtek™ Flow. All four materials express level of toxicity beyond control. However, Tetric® Flow at this point appeared to be the least cytotoxic among test materials.

After material was eluted for one day, cytotoxicity of MTA became less (72.8 % in cell survival rate) and was no longer different from Tetric® Flow and Filtek™ Flow. But Aeliteflo™ was still significantly more toxic than Tetric® Flow ($p<0.05$). Materials were still more toxic comparably to control.

Cytotoxicity of materials after eluted for two days revealed significant difference between Tetric® Flow and Aeliteflo™ ($p<0.05$). Filtek™ Flow also showed higher level of cell survival rate compared with Aeliteflo™ ($p<0.05$). At this period, Aeliteflo™ seemed to be the most toxic material compared to other materials.

After eluted for three days and four days, Aeliteflo™ which was toxic earlier, became less toxic and no longer different compared to other materials. There was no significant difference among materials as well. But Aeliteflo™ and MTA, however, showed significant difference in cell survival rate from control ($p<0.05$) (table 13).

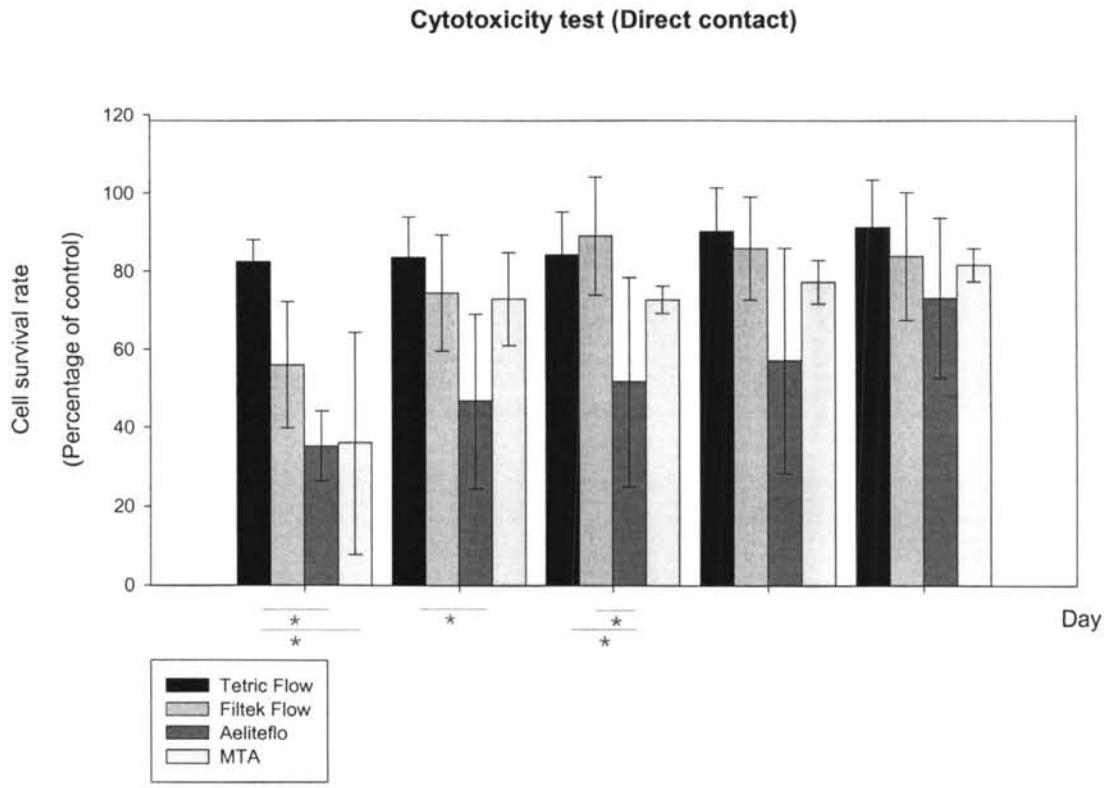

Figure 4 Cytotoxicity of material (Direct contact) upon cultured human periodontal ligament cells at freshly mixed state (0 day) and 1, 2, 3, 4 days after elution. The relative percent cell survival rate from four separate experiments was shown in mean percentage ± standard deviation

* = statistically significant difference at level 0.05 (Scheffe's test)

4.3 Cell morphology and attachment by scanning electron microscope

Morphology and attachment of human periodontal ligament cells after grown on materials was examined by using a scanning electron microscope at 15X, 200X and 1000X compared with the cells grown on bottom of culture plate. The result was similar to data obtained from cytotoxicity testing.

Human periodontal ligament cells grown on the bottom of culture plate demonstrated a large number of cells in similar shape and surface characteristics. They disperse normally on the culture plate (figure 5, 6). They appeared to be fully spread and well attached to the substrate by means of lamellipodia. The cell surface was relatively smooth (figure 7).

The morphology and dispersion of the human periodontal ligament cells observed on Tetric® Flow was different from that seen on the control (figure8). The cells were flat with spindle shape (figure 9) or fan shaped and had a smooth surface. They attached with lamellipodia and spread well on material. Parts of lamellipodia were inserted into material surface (figure 10).

Human periodontal ligament cells attached on Filtek™ Flow were less in density compared to control and Tetric® Flow (figure 11, 12). Cells appeared in spindle shape or fan shaped. They attached well on Filtek™ Flow with lamellipodia and filopodia (figure 13). Morphology was similar to that seen on the control but not well spread as on Tetric® Flow.

Human periodontal ligament cells on Aeliteflo™ were round to discoid in shape and had smooth surface. Spread cells attached to Aeliteflo™ with

filopodia were obviously seen (figure 16). Cells were few in number, were roughly round in shape, and had microvilli or filopodia (figure 14, 15).

The surface texture of the MTA was rough compared with Resin composites (figure 17). Well spread human periodontal ligament cells were illustrated with flat and smooth surface. Cells attached well on MTA with lamellipodia (figure 19). Cell morphology was similar to that seen in control but fewer cells were observed (figure 18).

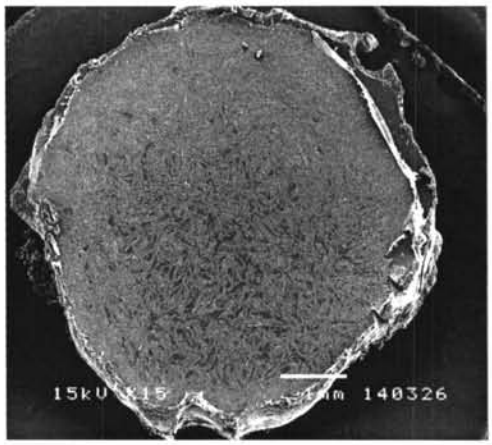

Figure 5 Scanning electron micrograph of HPDLs attached to cultured plate at 24-hour incubation. (original magnification x15).

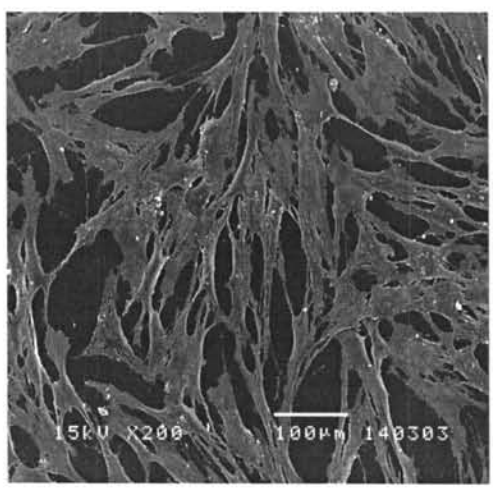

Figure 6 Scanning electron micrograph of HPDLs attached to cultured plateat 24-hour incubation. (central area)
(original magnification x200).

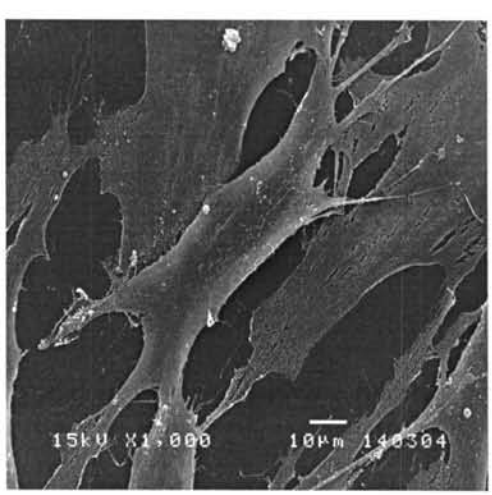

Figure 7 Scanning electron micrograph of HPDLs attached to cultured plate at 24-hour incubation. (central area)
(original magnification x1000).

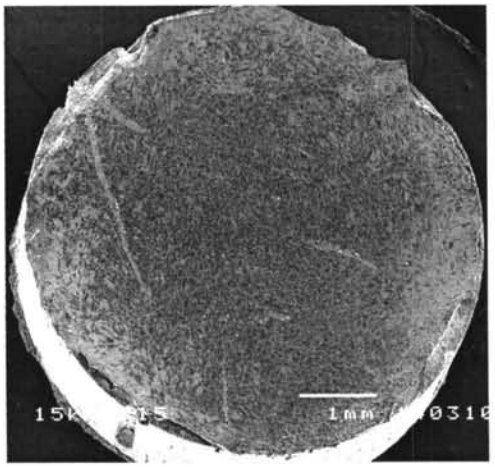

Figure 8 Scanning electron micrograph of HPDLs attached to Tetric® Flow at 24-hour incubation. (original magnification x15).

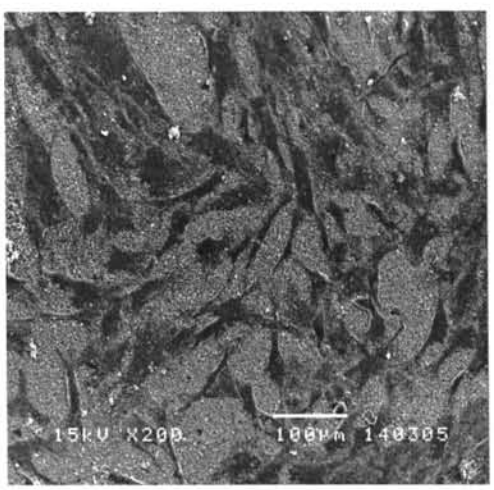

Figure 9 Scanning electron micrograph of HPDLs attached to Tetric® Flow at 24-hour incubation. (central area)
(original magnification x200).

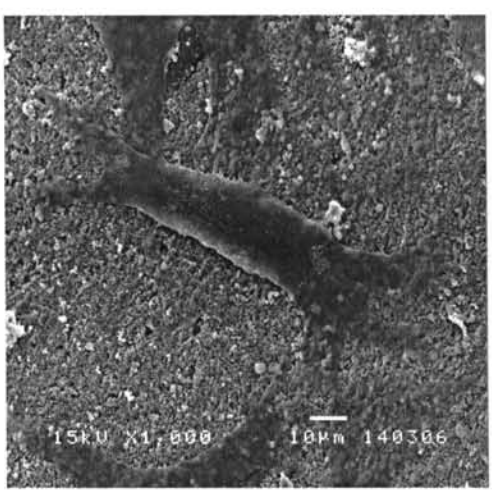

Figure 10 Scanning electron micrograph of HPDLs attached to Tetric® Flow at 24-hour incubation. (central area)
(original magnification x1000).

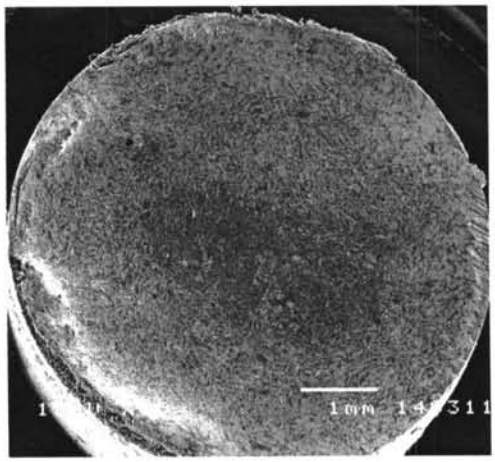

Figure 11 Scanning electron micrograph of HPDLs attached to Filtek™ Flow at 24-hour incubation. (original magnification x15).

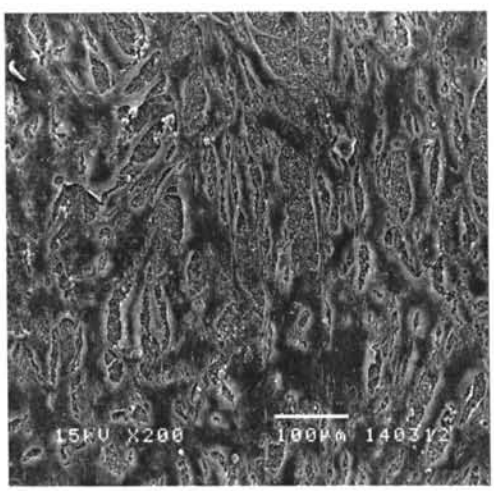

Figure 12 Scanning electron micrograph of HPDLs attached to Filtek™ Flow at 24-hour incubation. (central area)
(original magnification x200).

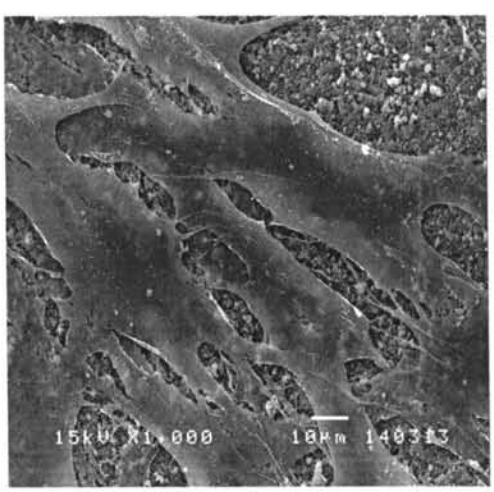

Figure 13 Scanning electron micrograph of HPDLs attached to Filtek™ Flow at 24-hour incubation. (central area)
(original magnification x1000).

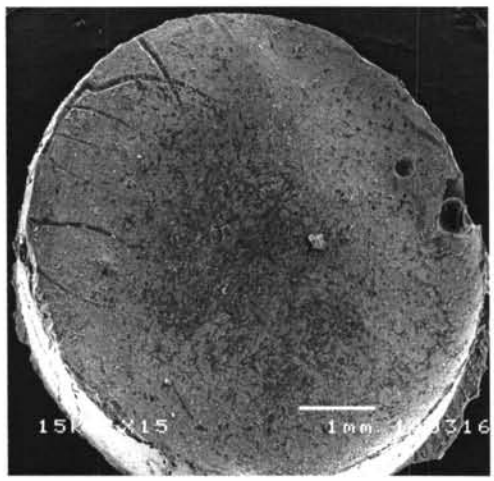

Figure14 Scanning electron micrograph of HPDLs attached to Aeliteflo™ at 24-hour incubation. (original magnification x15).

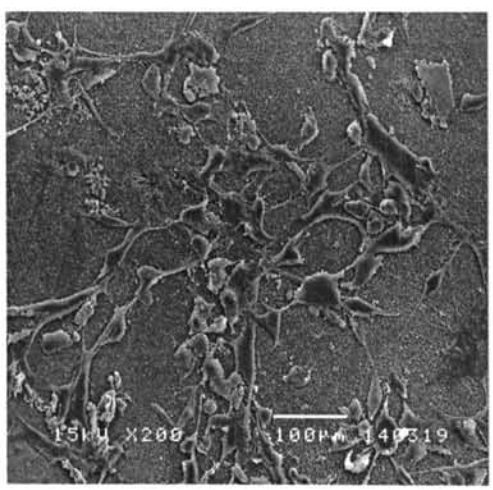

Figure 15 Scanning electron micrograph of HPDLs attached to Aeliteflo™ at 24-hour incubation. (central area) (original magnification x200).

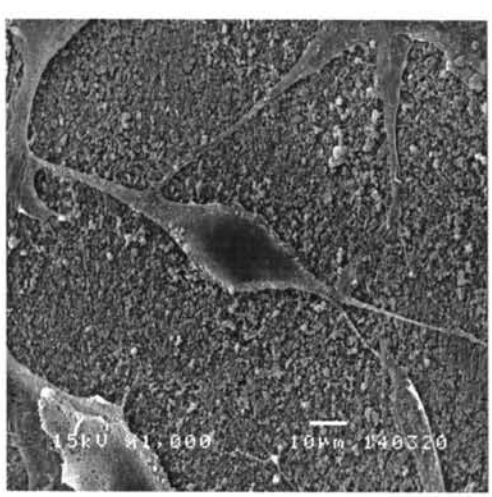

Figure 16 Scanning electron micrograph of HPDLs attached to Aeliteflo™ at 24-hour incubation. (central area) (original magnification x1000).

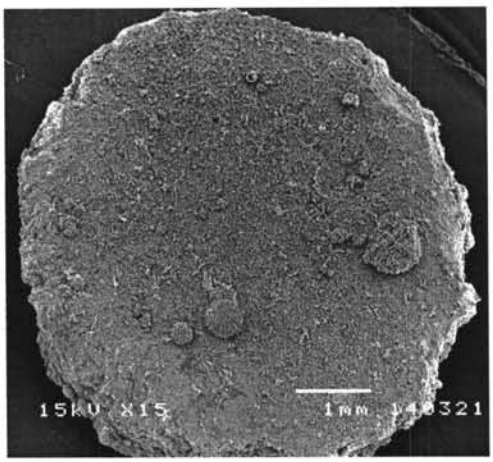

Figure 17 Scanning electron micrograph of HPDLs attached to MTA at 24-hour incubation. (original magnification x15).

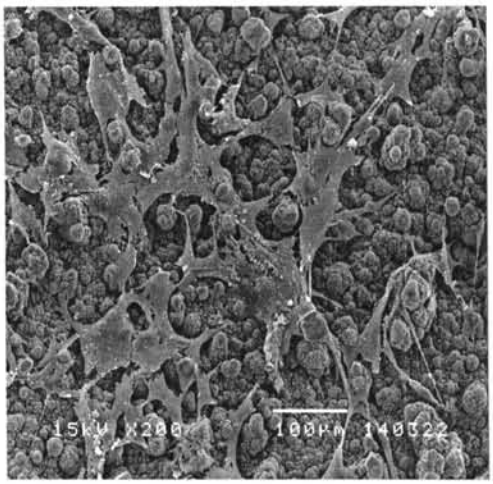

Figure 18 Scanning electron micrograph of HPDLs attached to MTA at 24-hour incubation. (central area) (original magnification x200).

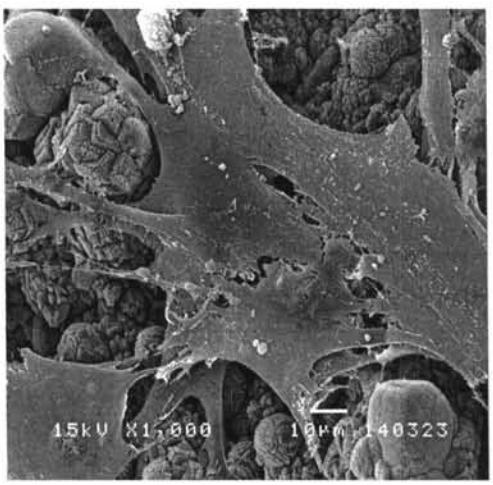

Figure 19 Scanning electron micrograph of HPDLs attached to MTA at 24-hour incubation. (central area) (original magnification x1000).