MATERIALS AND METHODS

Cell Culture

Human oral fibroblast-like cells were obtained from the gingival tissue of the patients who had surgical removal of impacted teeth. After washing the tissue with an iodine-based antibacterial agent, the mucosal layer was stripped to remove keratinocyes using a scalpel blade and scissors. The tissue was then cut into small pieces following the primary explant technique[10] which utilized Dulbecco's Modified Eagle Medium (DMEM) (Gibco BRL, New York, USA) with 10% fetal calf serum (Seromed , Berlin , Germany), 100 units/ml Penicillin, 100 μg/ml Streptomycin (Gibco BRL, New York, USA), and 1% amphotericin B. The resulting material was cultured at 37 °C with 5% CO_2 , 95% air. After the outgrowth of cells from the explants covered nearly all the culture plate, subculture was done by 0.25% Trypsin (Gibco BRL, New York, USA). Cells used for the cytotoxicity test were taken from the subculture between the fourth to twentieth passages .

Materials

2-Cyanoacrylic acid esters (Sigma, MO, USA), which is approximately a 1:1 mixture of ethyl and methyl esters, were used as the standard cyanoacrylate (std CA).

Three different brands of commercial cyanoacrylate adhesives in liquid form were purchased from local markets in Thailand and were coded as:

CA1 - Yamayo (Super Glue), Japan

CA2 - Alteco (Super Glue) made from Alpha Techno group, Japan and Singapore

CA3 - KenJI brand made from Taoka chemical company, LTD, Japan, which is stated to be composed of α-Cyanoacrylate.

Number 1 Whatman filter papers (Sringfield, Kent, England) were cut in a half circular shape with a diameter of 6 mm (this diameter was smaller than the diameter of the well in 96 multiwell culture plate so that the media will be exposed to CO_2 and air). The paper was autoclaved, then saturated with cyanoacrylate adhesive by adding about 5 µl of the adhesive. It was then left for 24 hours to allow full polymerization. Fully polymerized cyanoacrylate will float in the media, hence two control systems were used, one with cells and normal media and the other with paper coated with wax to imitate the cells in the test material so that the paper floats over them.

Methods

The cytotoxicity of substances that may be released from polymerized commercial cyanoacrylate adhesive was compared to standard cyanoacrylate by observation of cell

morhology, which included the zone of inhibition, and cytotoxicity was measured by MTT method. Substances released from CA-coated paper were stained with crystal violet and allowed to stand for 24 hours and then compared to controls, which included the wax-coated paper. Crystal violet staining was also used to investigate cytotoxicity of releasing substance from CA-coated paper that was incubated in the culture media at 37 °C with 5% CO_2 , 95% air for 1 day, 3 days, one week and two weeks, with the media changed daily.

Cell morphology and migration

Oral fibroblast cells were subcultured and approximately 1×10^5 cells were plated out on 35 mm culture dishes that already had one circular shape with diameter 6 mm. filter paper saturated with cyanoacrylate attached at the middle of the dish. These were left for 24 hours to allow full polymerization. The cells were also plated to the culture dish which had wax coating applied to the middle of the dish as one control.

Also the same quanity of cells was added to the culture plate without any test material applied to act as a further control. Culture medium was changed every 2 days and the morphology of cells were observed with an inverted microscope.

Cytotoxicity

MTT test

This method was first described by Mosmann in 1983[11]. Cells were subcultured and seeded to 96-well microtitre plates at about 3500 cells/200 µl of media in each well, except the blanks. After 24 hours, filter paper with each type of CA and wax coating were placed (floated) into each well

except for the controls. After 3 days, the filter paper and media were removed and 200 µl of fresh media was added to each well including the blanks. Then 50 µl of 5 mg/ml of MTT (Sigma, M-5655) in sterile Phosphate buffer system (PBS) was added. The plate were then wrapped in aluminium foil and incubated at 37.5 C for 4 hours. The media was then removed and MTT and 200µl of DMSO were added in each well, followed with 25 µl of Sorensen's buffer solution. After mixing, the optical density was read against a blank at 590 nm using a microplate spectrophotometer (Titertek, Multiscan Plus MKII, Switzerland).

Crystal violet staining

This technique followed the method modified by Ikarashi, et al. (1993)[12]. After seeding the cells at the rate of about 3500 cells/200µl per well and leaving for 24 hours, the CA-coated and wax-coated filter papers were added in each well, except for the controls. After incubating for 3 days, the cells were fixed with 25% gluteraldehyde applied for 20 min. The cells were stained with 0.4% crystal violet in methanol for 30 min, rinsed with running water and then air-dried. Absorbances were read at 590 nm using the same microplate reader. The absorbance of the control wells which contained no test material was regarded as 100% and the percentage absorbance for each well was calculated.

This method also used to investigate cytotoxicity of substances that may be released from CA-coated paper after incubation with the media at 1 day, 3 days, one week and two weeks respectively.

Statistical Analysis

One way analysis of variance and Bonferroni multiple comparison were used to test the differences in optical density as the result of MTT and crystal violet staining methods for each of the tested materials. These tests were also employed for the analysis of the cytotoxicity test of each type of CA after incubation in culture media for various periods of time.