RESULTS

Cell Morphology and Inhibition Zone

After seeding cells in six 34 mm culture dishes, each type of polymerized CA-coated filter paper was attached to the middle of each of four of these culture dishes and one had the wax-coated filter paper attached. The remaining culture plate had only the cells set as the control.

After incubation for 24 hours, there was evidence of ruptured cells around all types of CA-coated paper, producing a zone of inhibition around each of the CA-coated filter paper, while the cells in the dish that had wax-coated paper maintained their fibroblast-like spindle shape and had begun to migrate and contact the wax-coated paper. The zones of inhibition around each of the CA-coated filter papers rangied from 200 μm to 1000μm. These inhibition zones persisted around each of the CA-coated filter papers for two weeks, even though media continued to replaced every 2 days (see Figures 1 to 3).

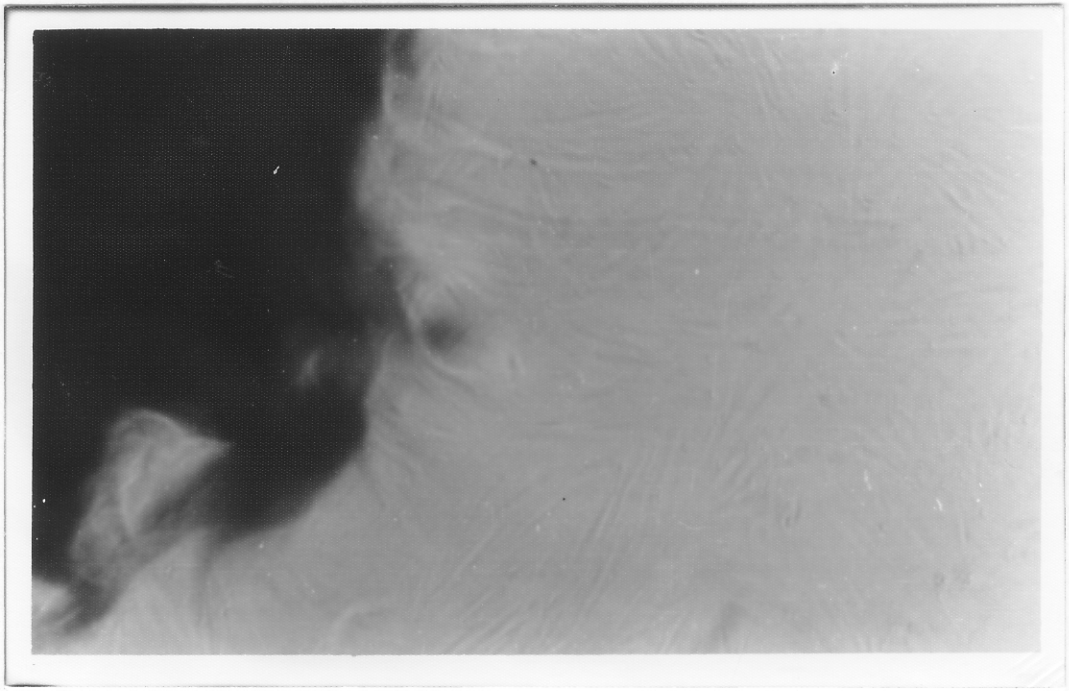

Figure 1. Oral fibroblast cells came close to wax coated paper after culture for 24 hours. (inverted microscope, original magnification × 10)

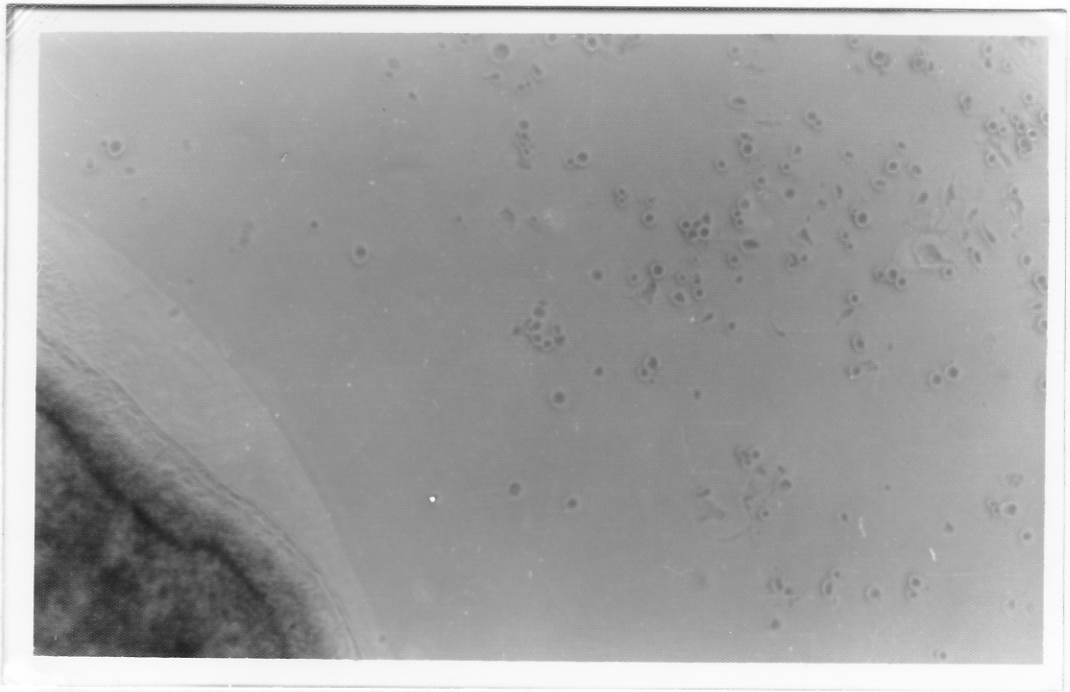

Figure 2. Cell apperance proximal to CA coated paper after 24 hours.

(inverted microscope original magnification × 4)

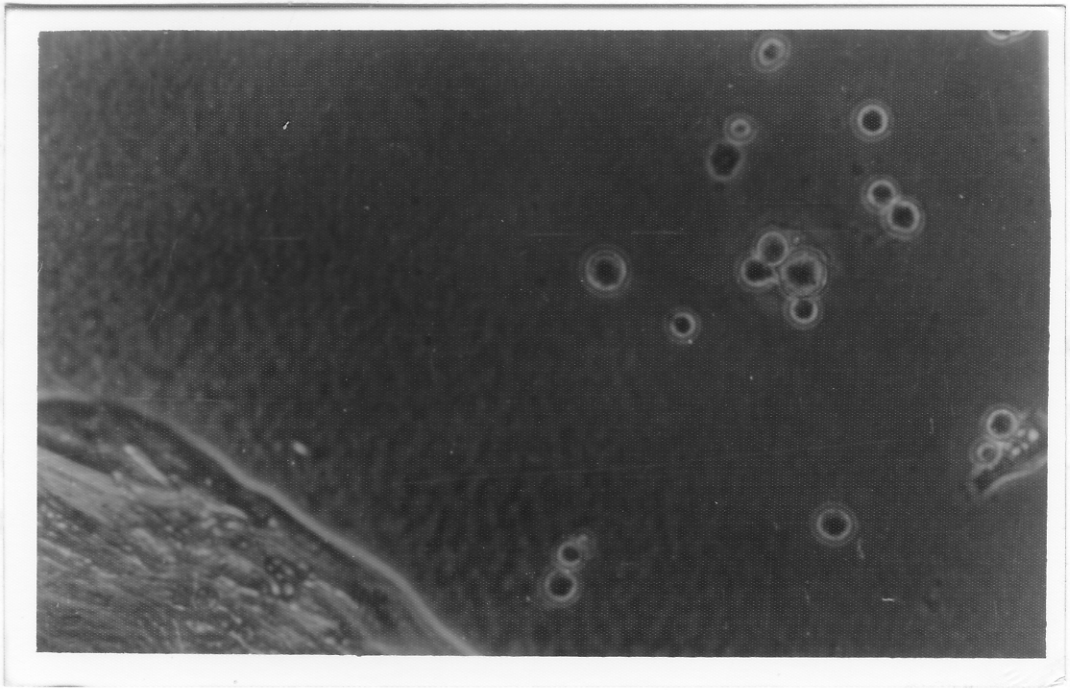

Figure 3. Cell appearance proximal to CA coated paper after 24 hours with higher magnification (inverted microscope original magnification × 10)

Cytotoxicity test

1. The polymerized CA adhesive compared with wax and control

MTT test

In the MTT test, the optical density (OD) of the cells treated with the three commercial CAs as well as the standard CA were significantly lower than the control and wax-coated filter paper ($p < 0.05$), but their OD were not significantly different from each other, when using the Bonforonni multiple comparison test. Wax-coated filter paper gave optical density nearly the same as the control (no significant difference).

(Table 1)

Crystal Violet Staining Method

The results from crystal violet staining method were similar to the MTT test, in that the cells which have commercial CA adhesive, including the std CA, gave absorbances of about half of the control, while the cells with the wax-coated filter paper gave an absorbance of about 96% of the control. Using the Bonforonni multiple comparison test, there was no significant difference between the percentage absorbance of all four CA, but they were significantly different from the wax-coated filter paper.

2. The releasing substances of CA after incubation in media for varying periods of time

There was no significant differences ($p > 0.05$) in the percentage absorbance comparing between the various time periods that the CA was allowed to float in the media in CA1, CA2 and CA3, except in the std CA where day 14 gave a significantly ($p < 0.05$) higher percentage absorbance

compared to day 1, 3 and 7. From Figure 3, the percentages of absorbance tended to be increased for std CA, CA2 and CA3 when compared to the cytotoxicity test of polymerized CA that were set for 24 hours and then tested without preincubation (Table 2) with the result of cytotoxicity test after incubation Cas for 1 day (Table 3), the optical density as perentage of control were increase about 73%, 37%,80% and 32% in CA1, CA2, CA3 and Std CA respectively.

Table 1. Cytotoxicity of polymerized cyanoacrylate adhesive compared with wax-coated filter paper and control using MTT test (n=10 in each group)

Test Material	Absorbance (against blank)
CA1	0.06±0.017
CA2	0.08±0.017
CA3	0.06±0.022
CA4	0.08±0.018
Std CA	0.09±0.015
Wax	0.43±0.058
Control	0.43±0.036
Wax	0.43±0.058
Control	0.43±0.036

Table 2. Cytotoxicity of polymerized cyanoacrylate adhesive coated filter paper compared with wax-coated filter paper using crystal violet staining method (n=30 in each group)

Test Material	Absorbance (% of control)
CA1	49.0±8
CA2	50.6±10
CA3	44.7±9
Std CA	46.8±7
Wax	96.0±13

Table 3. Mean ± SD (with 10 replications) of absorbance as a percentage of controls of incubated polymerized CA-coated filter paper in oral human fibroblast culture at day 1, 3, 7 and 14

Material	Time of incubation				P-value of
	1 day	3 days	7 days	14 days	ANOVA
CA1	84.8±23	76.8±25	81.7±13	76.0±12	0.7077
CA2	69.4±21	69.1±16	71.5±16	75.3±10	0.8190
CA3	80.8±23	95.5±18	81.7±20	87.3±13	0.2937
Std. CA	61.7±21	49.9±9	54.9±11	70.8±10	0.0104*

* Significant at $P<0.05$

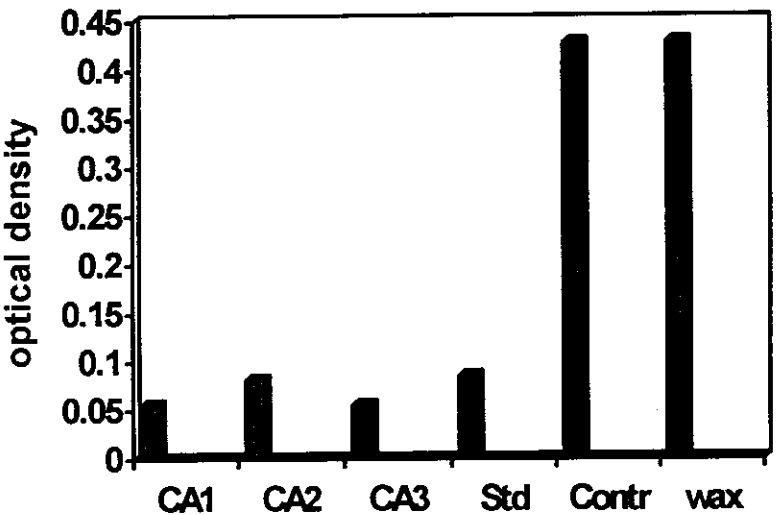

Figure4. MTT test on oral fibroblasts exposed to floating filter paper coated with various materials

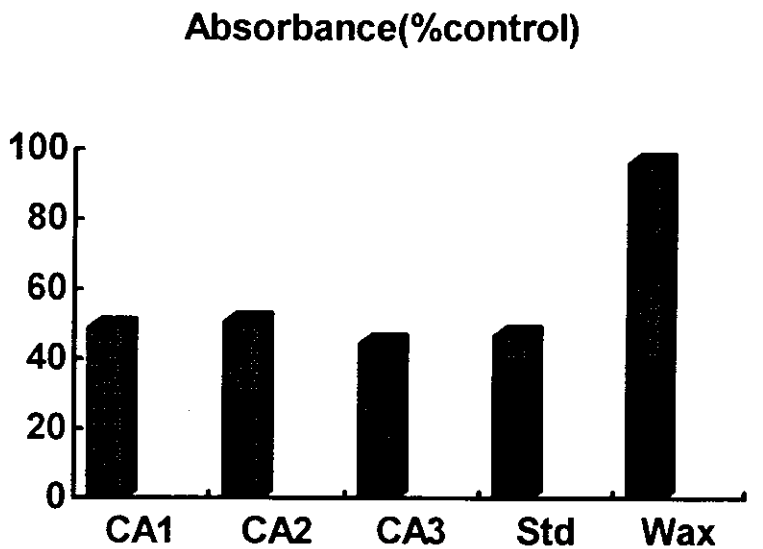

Figure 5. Absorbance of crystal violet staining of oral fibroblasts as percentage of controls

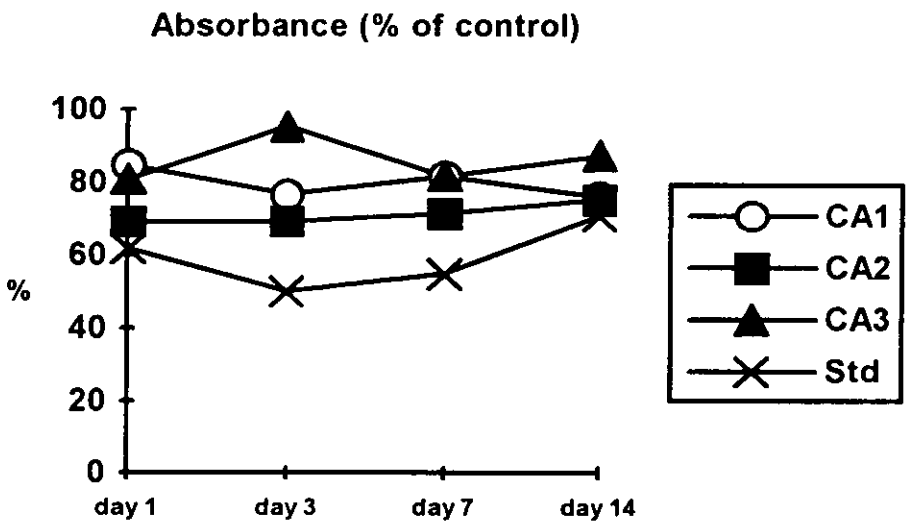

Figure 6. Absorbance of human oral fibroblast cells as a percentage of controls exposured to various types of cyanoacrylate-coated filter papers after incubation at day 1, 3, 7 and 14