PART IV

Results and Discussions

4.1 Preparation of high ammonium concentrated natural latex

In the process of high ammonium concentrated latex preparation, we added the water soluble chitosan as antifungal agent [58] and ammonium hydroxide as antibacterial agent. This was different from the previous studies [59, 60] that used only ammonium hydroxide. With this protocol, the latex could be easily centrifuged without coagulation and the concentrated latex was free from tetramethyl thiuramdisulphide (TMTD) which was usually used as a preservative reagent. It is known that TMTD causes allergic contact dermatitis and when this reagent degraded it formed the carcinogenic nitrosamines [61]. Several countries seriously concerned the contamination of TMTD in the rubber products. Therefore, the concentrated latex prepared by this protocol should be safe enough to be used as the raw material for production of soft lining material.

4.2 Determination the doses of gamma ray producing the high tensile strength

Since the gamma ray was used in vulcanization and grafting of the natural latex, the total dose and the appropriate dose for each use had to be determined. Up to now, there is no specification of the tensile strength for the soft lining materials. In this study, the total irradiation dose was defined as the dose that gave the final tensile strength of the product 6-7 MPa which was the average tensile strength of the rubber gloves and the dental dam sheets. To determine the total irradiation dose, the natural latex was vulcanized by different doses of gamma ray and the tensile strength of

rubber sheets casted from different irradiated latex was measured. The data of their tensile strengths were shown in Figure 4.1 and Table 4.1.
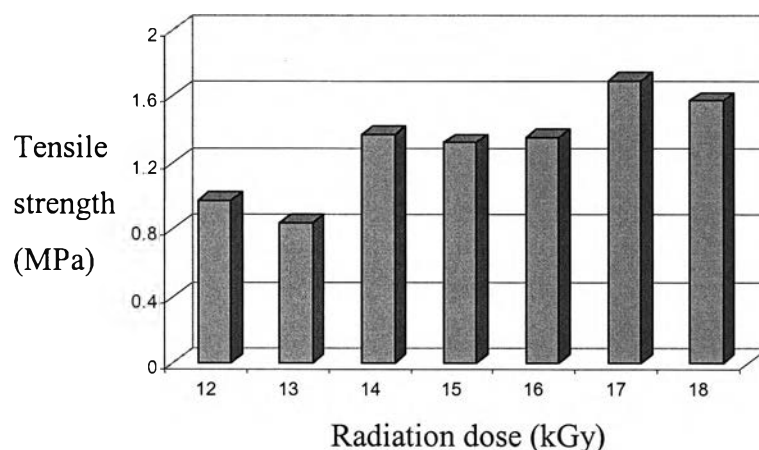

Figure 4.1. The bar graphs show the mean tensile strength of vulcanized rubber at different doses of gamma irradiation.
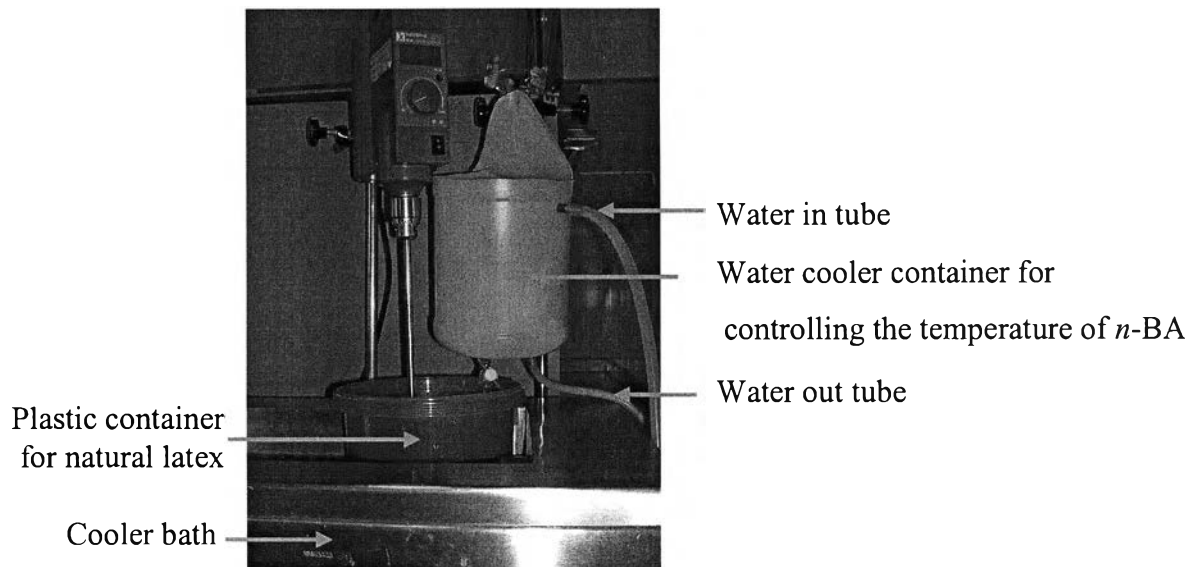
Table 4.1. The mean and standard deviation of tensile strength of vulcanized rubber at different doses of gamma irradiation

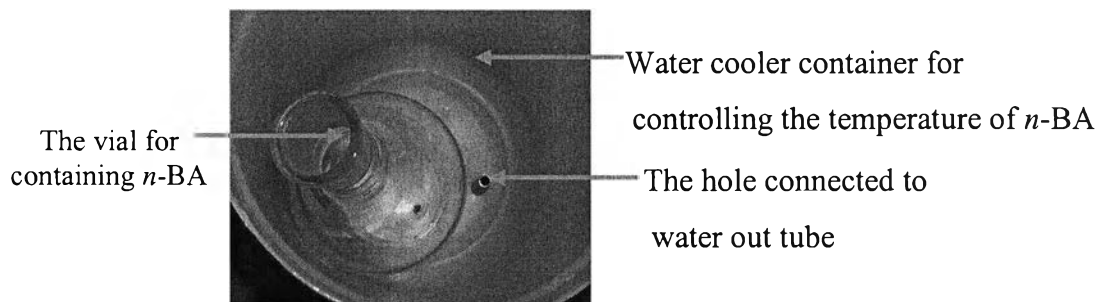| Tensile strength	Doses of gamma ray (kGy)						
(MPa)	12	13	14	15	16	17	18
Mean	0.97	0.84	1.37	1.32	1.35	1.69	1.57
SD.	0.16	0.11	0.21	0.18	0.14	0.24	0.19

The data showed that the tensile strengths of specimen performed from the vulcanized latex were lower than 2 MPa in all radiation doses. The data were statically analyzed with One-Sample T-test: test value = 6 at 95% confidence level. It was found that the mean tensile strength of vulcanized rubber by gamma radiation

doses from 12 to 18 kGy was significantly lower than the tensile strength of rubber dam sheet or the rubber examination grove ($p<0.05$).

From these results the experiment was reset with the new criteria that controlled the temperature of the mixing n-BA process. The hypothesis for this step was the lower environmental temperature during n-BA mixing process would enhance the tensile strength of vulcanized rubber. This idea came from the high vapor pressure of n-BA. Which very low dropping rate of n-BA to the latex, n-BA might be lost that caused the reduction of cross-linking accelerator. The experiment was set to prove this hypothesis. The cooler bath was used to control the temperature of both latex and n-BA (Figure 4.2). The temperature was controlled at $10°$, $15°$, $20°$, and $25°C$. At each temperature condition, the experiment was repeated as described previously. The specimens were prepared and tested again. The tensile strength from the repeated experiment was shown in Figure 4.3. It was found that the tensile strength of vulcanized rubber was still lower than 2 MPa. This result may be from the coagulation of rubber particles during storage in the refrigerator before mixing with n-BA. Therefore, the above hypothesis that the tensile strength increased if the temperature of environment during n-BA mixing decreased was rejected.

Water in tube

Water cooler container for

controlling the temperature of n-BA

Water out tube

Plastic container
for natural latex

Cooler bath

a

Water cooler container for

controlling the temperature of n-BA

The vial for
containing n-BA

The hole connected to

water out tube

b

Figure 4.2 a) The water bath was modified for controlling the temperature of latex

and n-BA.

b) The inner site of plastic container consists of glass vial for containing
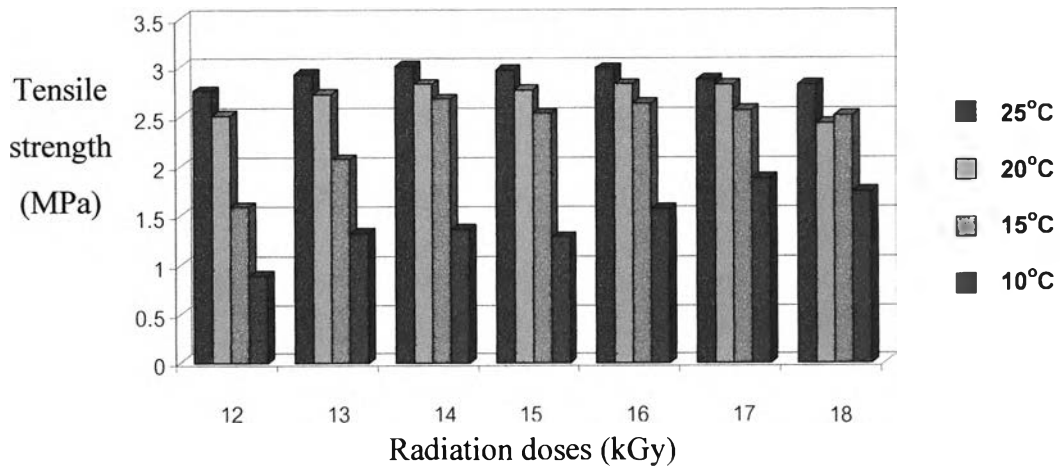
n-BA.

Figure 4.3. The tensile strength (MPa) of vulcanized rubber at different radiation

doses and temperature condition

From the above results, the low temperature might alter the physical

properties of concentrated latex. Therefore, the experiment was repeated for the third

time at room temperature. The concentrated latex kept in the room temperature was

used to evaluate the tensile strength after mixing with n-BA at room temperature and

irradiated by gamma ray at different doses:10,11,12,13,14,15,16,17,18,19, and 20

kGy. The results from the third trial revealed that the tensile strength of the

vulcanized rubber was higher than 2 MPa (Figure 4.4). After the data being

statically analyzed One-Sample T-test : test value = 6 at 95% confidence level, it

was found that the mean tensile strength of vulcanized rubber by gamma radiation

dose from 15 to 20 kGy was not significantly different from the tensile strength of

dental rubber dam sheet ($p>0.05$). Therefore fifteen kGy, the minimum dose

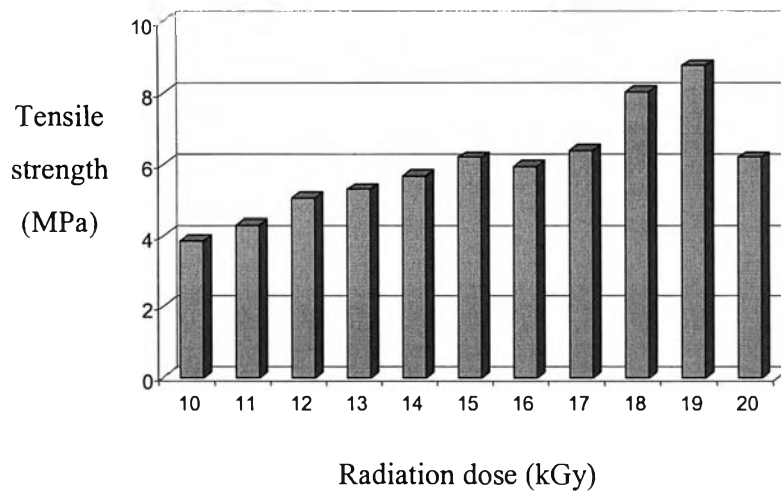producing the tensile strength equal to rubber dam sheet, was chosen.

Figure 4.4. The tensile strength (MPa) of vulcanized rubber irradiated with different

gamma radiation doses and prepared at room temperature.

These results suggested that the concentrated latex lost its tensile strength if

it was kept at low temperature and it should be stored at room temperature. It also

suggested that to get the high tensile strength as 6 MPa, the total irradiation dose

should be 15 kGy.

4.3 Grafting efficiency

In this study 100 phr of EMA was grafted to 50 % DRC vulcanized natural

latex. The concentration of EMA and % DRC of vulcanized natural latex used in this

study came from 2 reasons. First in the pilot study, the graft copolymer sheets made

from EMA lower than 100 phr was too soft and could not be ground with carbide

bur (Figure 4.5 a). In the dental laboratory process or in the dental clinic, the

permanent soft lining materials should be easily ground with carbide instrument for

boarder adjustment and smoothness (Figure 4.5 b and c). In the case of concentration

of EMA higher than 100 phr, it showed the mixture of latex and EMA did not blend

homogeneously. The solid granules of polyethyl methacrylate were formed (Figure 4.6). Secondly, the 30 % DRC of prevulcanized latex was grafted with 100 phr of EMA in another pilot study. The result showed that 30% DRC had high contraction because of its high water content and low viscosity that caused high shrinkage of dry graft copolymer sheet. This is not suitable for using as the soft ling material. The percentage of grafting efficiency from this method was 84 % which was lower than 88 % of the other study.[62] This result suggested that the high DRC (50%) of prevulcanized latex that had less water content might be necessary for producing free radical for the grafting process.[61]
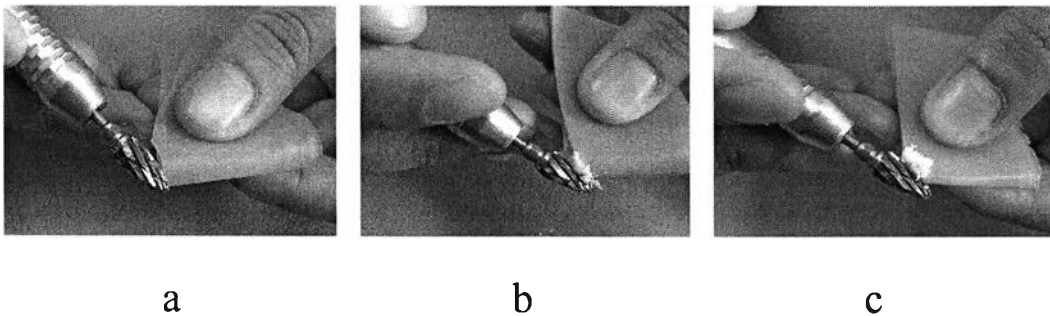

a b c

Figure 4.5 a) The graft copolymer sheet of natural rubber and EMA lower than

100 phr can not be ground with carbide bur.

b and c) The Coe Supersoft® sheet and the graft copolymer sheet with

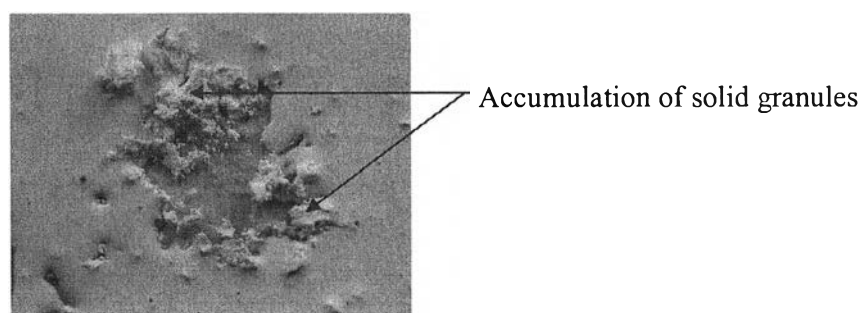100 phr EMA , respectively, can be ground easily with carbide bur.

Figure 4.6. The accumulation of solid granules of polyethyl methacrylate when

110 phr of EMA was used in the grafting process.

4.4 Ultrastructure of the graft copolymer

Ultrastructurally, the morphology of natural rubber particles is spherical in shape with different sizes (Figure 4.7) while the graft copolymer particles demonstrated the natural rubber core with the EMA polymer shells. The EMA polymer was not only coated the surfaces of rubber particles, but also linked these particles together to form polymeric networks (Figure 4.8). These linkages may lead the graft copolymers to have less porosity than natural rubber resulting in less water absorption of the graft copolymers. Less water absorption is advantageous to soft lining materials since it helps maintain the dimension of the materials as well as decrease water soluble food and microorganism absorption. In addition the linkages may enhance the tensile and tear strength of graft copolymer.
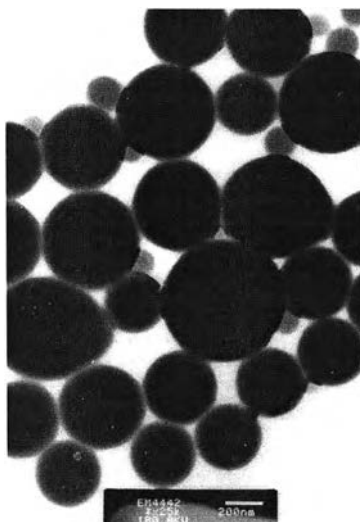
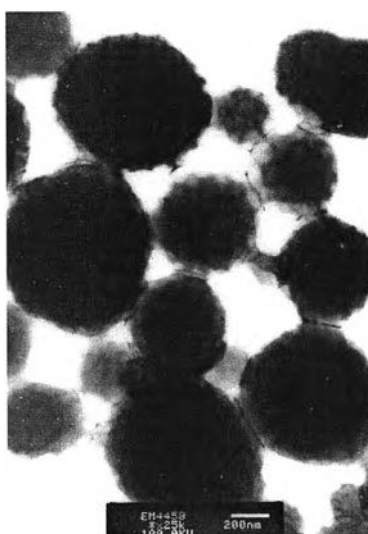Figure 4.7. Transmission electron micrograph of prevulcanized latex.

Figure 4.8. Transmission electron micrograph of graft copolymer by using

50% DRC of prevulcanized latex and 100 phr of EMA monomer.

4.5 Characteristics of graft copolymer

To investigate the functional group of EMA in the graft copolymer, the infrared spectroscopy was performed. The graphs of infrared spectrum (Figure 4.9 a, b) showed that the vulcanized rubber had the absorption peak at 1663 cm^{-1} (Figure 4.9 a) represented the -C=C-. On the other hand, it was referred that the unsaturated double bonds were still remained in the vulcanized rubber whereas graft copolymer, the prominent absorption peaks were found at 1732 cm^{-1} (-C=O stretch) and 1140 cm^{-1} (-C-O- stretch) (Figure 4.9 b). These results could be explained that the cross-linking site did not occur at the unsaturated double bond regions[61]. Therefore, in the grafting process the unsaturated double bonds were cleaved to form the radical and grafted with polyethyl methacrylate later. In the graft copolymer preparation, the latex was first irradiated for cross-linking and then was irradiated again for grafting. This protocol can enhance the tensile strength and tear strength of final product, graft copolymer, because of cross-linking and entanglement of polymer chains.
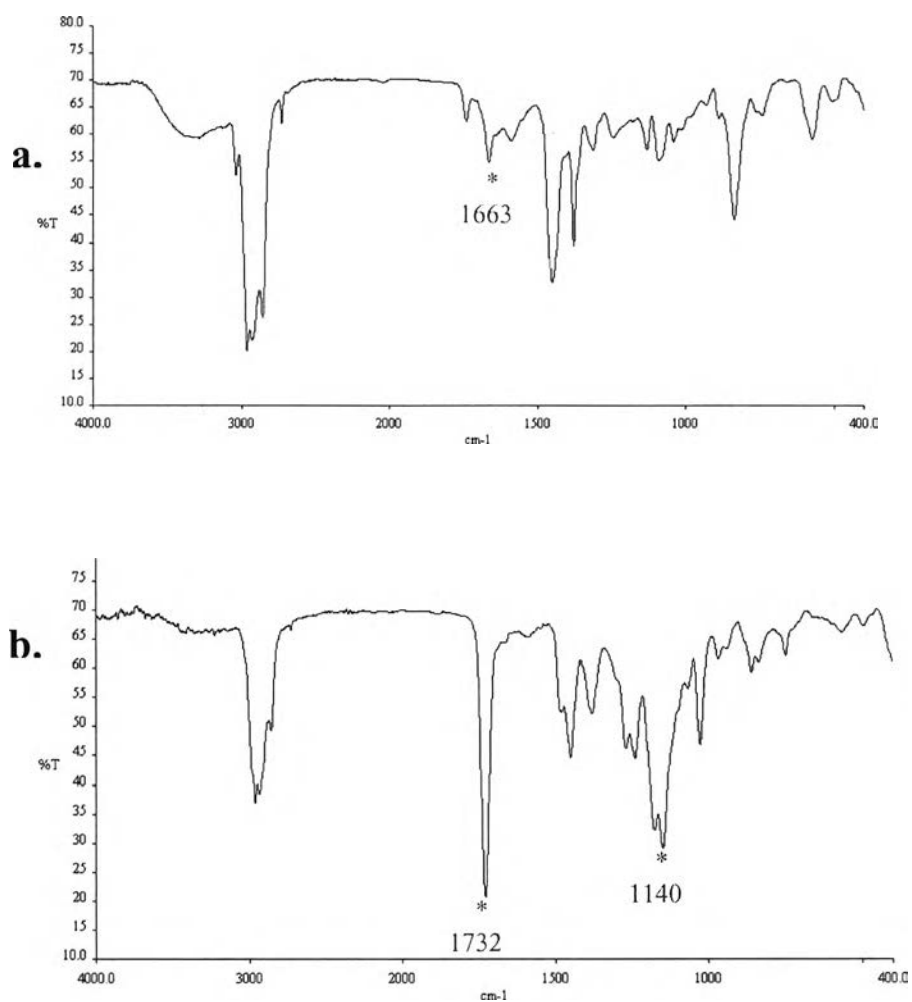
Figure 4.9   a) The infrared spectrum of latex vulcanized with 15 kGy irradiation.

b) The graft copolymer prepared by natural latex and 100 phr of EMA.

4.6 Glass transition temperature examination

The results from the Differential Scanning Calorimeter as of the T_g value (Figure 4.10) showed that the T_g of vulcanized rubber was found at -62.3 °C and graft copolymer was at -62.8 °C whereas, the T_g of the Coe Supersoft® could not found in the range of temperature from -80 to -50 °C. The T_g value of pure EMA is 66 °C that is much higher than the mouth temperature. Some manufacturers produced the soft acrylic resin by mechanical mixing of PEMA and plasticizer. This product has the lower T_g value. However, it will turn to the hard state after using it for a few

months because of the leaching out of plasticizers. Therefore, it is not suitable for this

product to be used as a soft lining. For the graft copolymer, PEMA was attached to

the polyisoprene chain by chemical bond and its T_g value is very low. The result

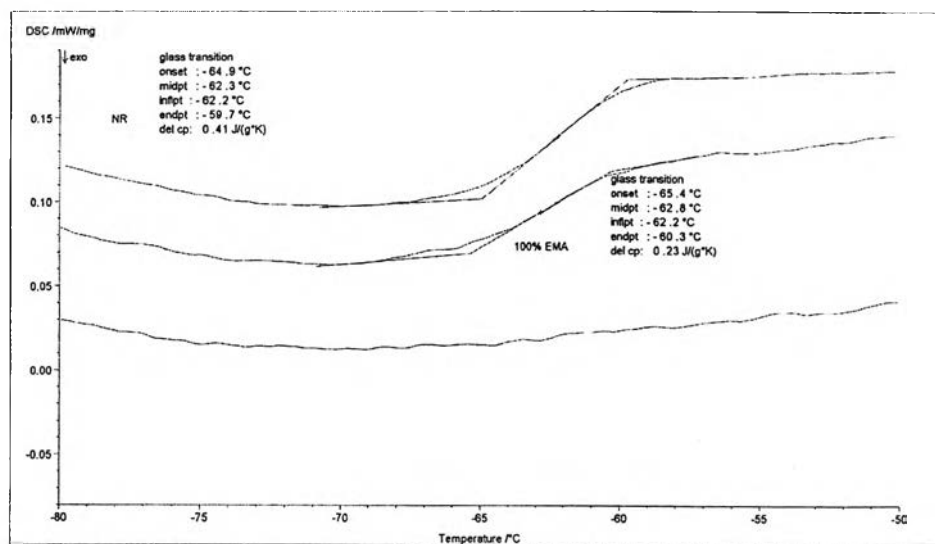suggested that the graft copolymer is able to keep the soft state for a long time.

Figure 4.10. T_g values of vulcanized rubber (top line), graft copolymer

(middle line), and Coe Supersoft ® (bottom line).

4.7 Mechanical properties

4.7.1 Surface hardness

The surface hardness of the graft copolymer was studied by immersion of

the graft copolymer as well as the Coe Supersoft® in distilled water. The result was

shown in Figure 4.11. From these results, we found that the median value of Shore A

of surface hardness was reduced after one day of immersion time in both materials. It

may be that water was absorbed into the specimens and acted as a plasticizer [63]. This

level of surface hardness stayed for three months and increased in the 4th month. However, over ten months of immersion in distilled water at 37 °C, the surface hardness of both graft copolymer and controlled group were not significant difference. The increasing of surface hardness in the 4th month can be explained as follow. For the Coe Supersoft®, soft acrylic resin material, is presented in powder and liquid format; comprising a higher methacrylate polymer powder (polyethyl methacrylate) with a liquid comprising a higher methacrylate monomer and a plasticizer (commonly a phthalate) [12]. The soft acrylic resin materials undergo two processes when immersed in water [47]. The low molecular weight plasticizer is leached out into the water and at the same time, water is absorbed into the polymer structure. The loss of plasticizer appears to be the most important process as far as the properties are concerned since ageing results not only in a dimensional change but also a loss of softness [64]. Whereas, the graft copolymer that natural particles were grafted with EMA polymer and act as the permanent plasticizing agents but the increasing of surface hardness may be from the degradation of free polyisoprene at the surface. This lead to the remaining PEMA which had hard consistency. If this idea is true, the high percentage of grafting efficiency or the surface coating on the graft copolymer might solve this problem.
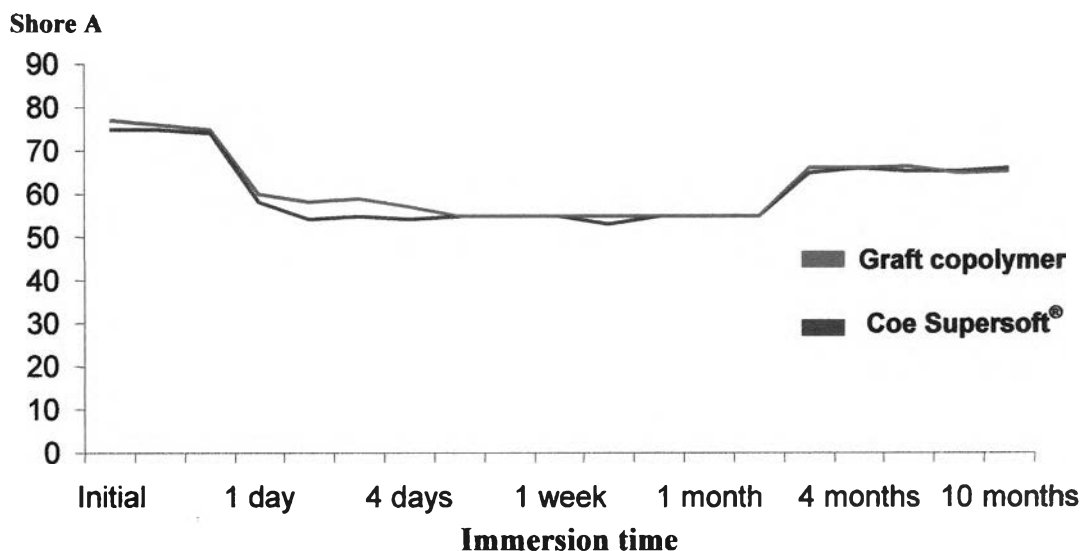
Shore A

Figure 4.11. The surface hardness of graft copolymer and Coe Supersoft® at

different period of immersion time

4.7.2 Tensile properties

The tensile properties of the graft copolymer and Coe Supersoft® was

shown in Table 4.2.The data were analyzed by using T-test at 95% confidence level.

It was found that the tensile strength, extension at break, and % elongation of graft

copolymer were significantly higher than Coe Supersoft® . On the contrary, the 100%

modulus of graft copolymer was lower than Coe Supersoft®. These results suggested

that the graft copolymer had higher elasticity than Coe Supersoft®. In the previous

study we have shown that the tensile strength of the vulcanized rubber with 15 kGy

gamma irradiation was 6 MPa. In this study, we found that the prevulcanized latex

grafted with 100 phr EMA. had twice higher tensile strength   than   that of the

vulcanized rubber. In this preparation can be explain that after vulcanization process,

the tensile strength increased by the cross-linking between the cis-1,4-polyisoprene

chains. And then the grafting process caused the entanglement between the polymer chains of polyethyl methacrylate themselves or between the polyethyl methacrylate and cis-1,4-polyisoprene. These possibly played a role in controlling the tensile strength (Figure 4.12).

Table 4.2. The mean and standard deviation of tensile properties of graft copolymer and Coe Supersoft®.

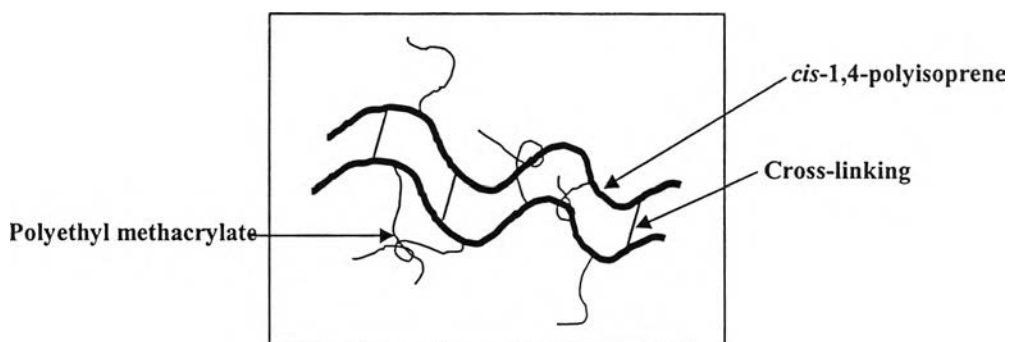| Materials	100 % modulus (MPa)	Tensile strength (MPa)	Extension at break (mm)	% Elongation
Graft copolymer	1.429 (0.18)	12.92 (2.07)	287.45 (22.35)	856.35 (77.53)
Coe Supersoft®	3.43 (0.62)	4.47 (0.77)	72.67 (2.59)	142.3 (8.87)

Figure 4.12. The illustration of cross-linking of cis-1,4-polyisoprene and entanglement of polyethyl methacrylate

4.7.3 Tear strength

From the pilot study it was found that the tear strength of the vulcanized rubber irradiated with 15 kGy produced the tear strength of 28.9 N/m. That was known that vulcanized rubber was high in tensile strength but low in tearing strength. This property is the disadvantage of all kind of vulcanization method: radiation, peroxide, and sulfur cured. In this study, the vulcanized latex grafted with ethyl methacrylate showed the significantly higher tear strength than Coe Supersoft[®] (p<0.05) (Figure 4.11). For this result, the high tear strength of the graft copolymer may be from the chain entanglement of polymer chain as described previously. Therefore, to solve the low tear strength of vulcanized rubber, the grafting method should be used. The high tear strength is one of the requirements of the soft lining materials because it provides the good edge of soft lining materials. From the clinical experience, most of the available soft lining materials in the market showed low tear strength that caused the irregular margin and irritated patients' oral tissues.
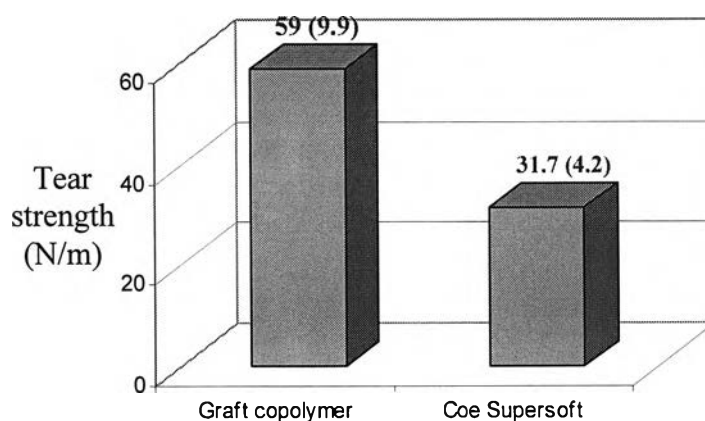

Figure 4.13. The mean and standard deviation of tear strength of graft copolymer and

Coe Supersoft[®]

4.8.4 Tensile bond strength

To study the nature of interface attachment between the tested materials and the PMMA (denture base material), investigation at the interface with SEM and the tensile bond strength measurement by Lloyd UTM were performed. It was found from the SEM examination that graft copolymer and Coe Supersoft® displayed differences in the nature of their interfaces with PMMA. Graft copolymer displayed a gap at the interface between itself and PMMA (Figure 4.14 a) suggested the incomplete bonding between these two materials. The Coe Supersoft® showed a complete junction with PMMA (Figure 4.14 b). When the specimens were measured the tensile bond strength by UTM, it was found the tensile bond strength between the graft copolymer and PMMA was not significantly difference from the tensile bond strength between Coe Supersoft® and PMMA ($p > 0.05$). The mean and standard deviation values of tensile bond strength of specimens are shown in Figure 4.15. The result demonstrated that graft copolymer and Coe Supersoft® had the different mode of failure of their bond with PMMA to external damaging loads. Coe Supersoft® showed the 8.26 MPa of bond strength and exhibited cohesive failure whereas graft copolymer provided 7.38 MPa of tensile bond strength and demonstrated the adhesive failure or interfacial failure. From the nature of their interface with PMMA, it can explain that PMMA and Coe Supersoft® have the hydrophobic behavior therefore they are compatible and exhibit the complete junction. In the case of graft copolymer when the latex which was in aqueous form was packed next to the dough stage of PMMA, the PMMA surface was contaminated with water from latex resulting in reduction of the complete contact between PMMA and graft copolymer. The incompatible between latex form of graft copolymer and PMMA caused the gap at the junction and enhanced the adhesive failure mode. To solve this problem,

elimination of water content in the graft copolymer before packing with the PMMA
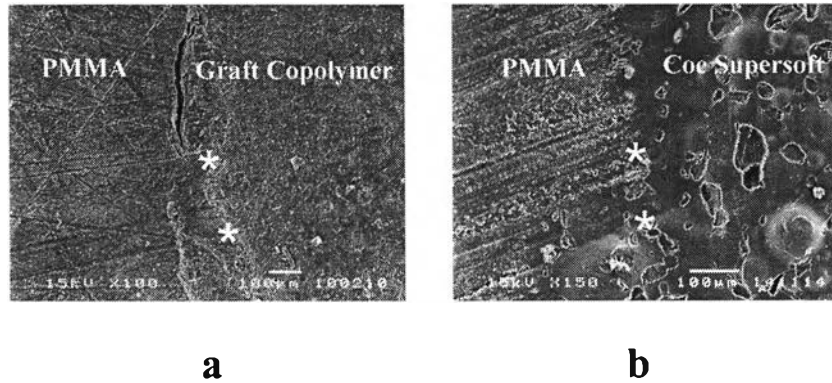
should be seriously considered.

a   b

Figure 4.14. Transmission electron micrographs

    (a) the interface (*) between PMMA and graft copolymer

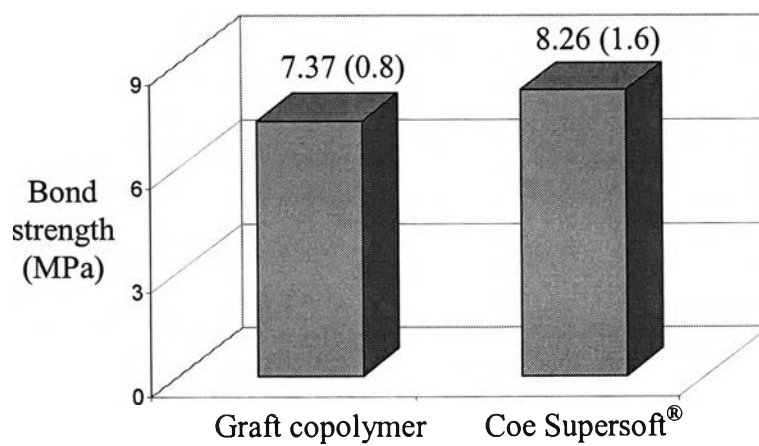   (b) the interface (*) between PMMA and Coe Supersoft®

Figure 4.15. The mean and standard deviation of tensile bond strength of graft

copolymer and Coe Supersoft®

3.7.5 Water absorption

In this study, the tested materials were immersed in distilled water up to 10 months. At each immersion time period, the tested materials were weighed and the data was collected as % of water absorption. The mean % of water absorption of graft copolymer, Coe Supersoft® and vulcanized rubber at each time interval was shown in Figure 4.16. The sorption values (standard deviation) for the materials tested ranged from a low to high at 10 months: 4.93 %(1.06) for graft copolymer, 6.75 (0.40)% for Coe Supersoft®, and 42.51 (4.50)% for vulcanized rubber, respectively. The water sorption values of all tested materials increased steadily over the 10 month period of this study. The graft copolymer and Coe Supersoft® had much less water sorption than that of natural rubber. One of the ideal properties of soft lining materials is less water absorption. In the past natural rubber had been used for denture base lining but it absorbed a lot of water. It is of interest that the vulcanized rubber had high water absorption even though the rubber is composed of hydrophobic hydrocarbon molecules. It should have had less water absorption. The increasing water absorption in the vulcanized rubber might be affected by the hydrophilic impurity materials contaminated in the latex [65] or the degraded of insoluble protein into soluble protein by irradiation [60]. In this study the vulcanized rubber was modified by grafting with EMA monomer which helped reduce water absorption from 42.51% to 4.9 %. For the Coe Supersoft®, water absorption increased steadily that may be caused by leaching out of the plasticizer from the materials and water replacement.
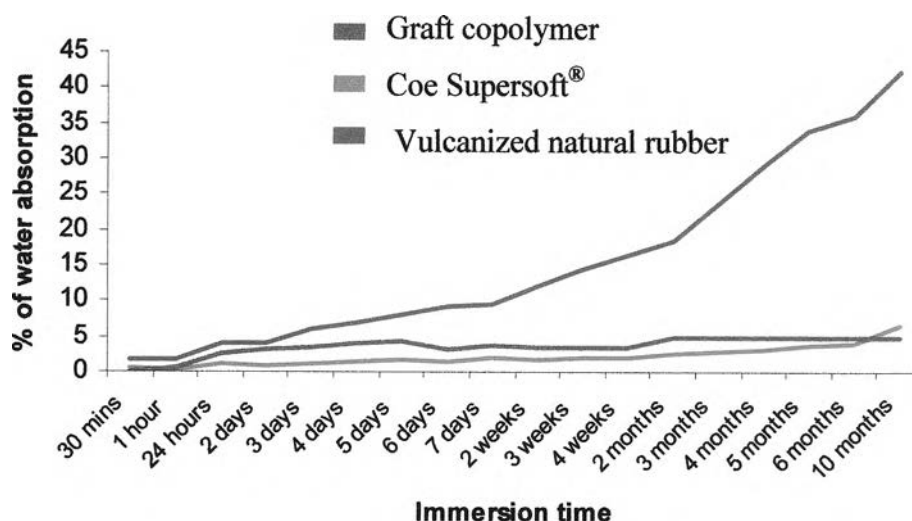
Figure 4.16. The % of water absorption of three materials: graft copolymer,

Coe Supersoft®, and vulcanized natural rubber.

3.7.6 Contact angle

The mean and standard deviation of contact angles of graft copolymer and Coe Supersoft® were 71 degree and 90 degree, respectively. The explanation for the difference of the contact angle of these 2 materials probably is that the graft copolymer had the surface energy higher than Coe Supersoft® did or, on the other hand, the surface of graft copolymer material had more hydrophilic property than Coe Supersoft® did. The hydrophilic surface of soft lining material will produce the more wettable than the hydrophobic surface. The wettable surface of the soft lining denture base materials is leading to the high capillary reaction and comfortable felling of the patients.

3.7.7 Staining test

Since the soft lining material has to be exposed to a lot of colored food and drink all the time, discoloration of this material is an unwanted effect. In this study, the discoloration of the graft copolymer and Coe Supersoft [®] were study by immersion of materials in coffee, tea, and capsaicin in oil. The discoloration of tested materials after immersion in three kinds of solutions was measured by spectrophotometer and the data was shown in Figure 4.17 a-c. For eight weeks of immersion period, the coffee solution produced the highest discoloration value in Coe Supersoft [®] ($\triangle$E = 17.02), followed by the graft copolymer ($\triangle$E = 14.13). It was known that if the color change value over than 3.3 the human eye can observe [(66)]. In this study, the coffee solution produced the discoloration value more than 3.3 in graft copolymer and Coe Supersoft [®] after 5 weeks and 3 weeks of immersion time, respectively. For the tea solution it was shown that discoloration values of both materials were lower than 3.3 over the 8 weeks of immersion time. For the immersion in capsaicin in oil, the discoloration value of graft copolymer was 90.07 whereas the Coe Supersoft[®] was 2.42 for over 8 weeks of immersion time. In this study the coffee and tea solution were used because to compare with the previous study [(67)]. The capsaicin in oil was chosen because Thai people like spicy hot food. The results in this study were similar to the previous study by Buyukyilmaz and Ruyter [(67)] who demonstrated that coffee produced greater color changes than tea on denture base resin. However, Polyzois et al. [(68)] showed that tea had higher staining effects than coffee on soft lining denture base materials. The disparity between these studies may be partly attributable to different polar properties of the tested materials that affect both the affinity of a material to extrinsic staining and the diffusion of

water molecules. In the previous study, Um and Ruyter [69] demonstrated that the yellow colorants of coffee were more hydrophobic than the yellow colorants of tea. This finding also agree to a certain extent with the statement that hydrophobic materials are stained by hydrophobic solutions, and hydrophilic materials are stained by hydrophilic solutions [70]. Also considering from the value of contact angle which showed that the Coe Supersoft® had more hydrophobicity than the graft copolymer, it confirmed the results of darker coffee staining in Coe Supersoft® than in graft copolymer. In the case of capsaicin in oil, it caused the severe discoloration and deformation in the graft copolymer. This result may be from the hydrocarbon component of rubber that is compatible with oil. Therefore, the orange color from capsaicin diffused into the graft copolymer and the oil diffusion caused the deformity of the specimens (Figure 4.18). The deformity of this material in oil is a major disadvantage of the graft copolymer. To solve these problems, natural latex might be grafted or blended with other polymer that can protect or resist the oil diffusion.
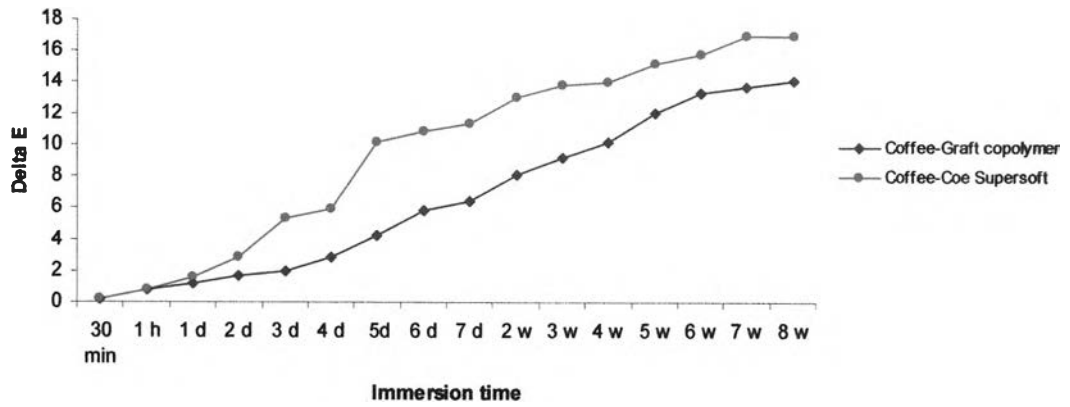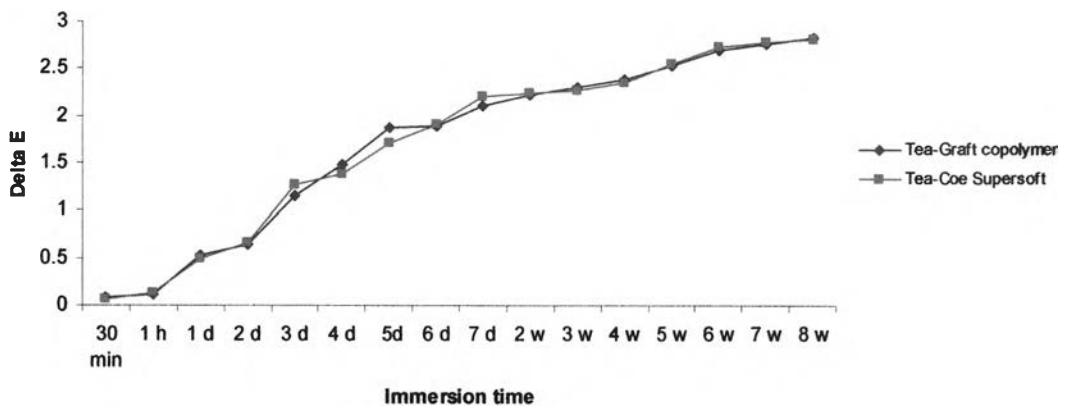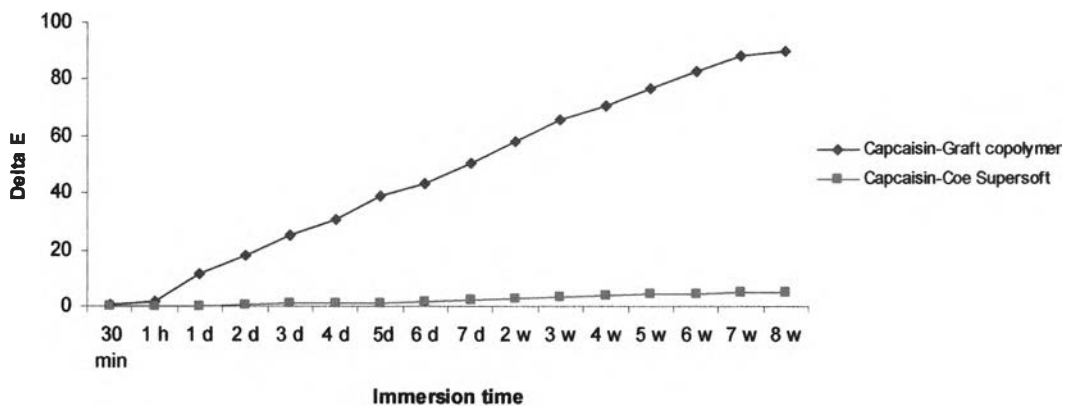
Figure 4.17 a) The discoloration ($\triangle$E) for tested materials in coffee solution

b) The discoloration ($\triangle$E) for tested materials in tea solution

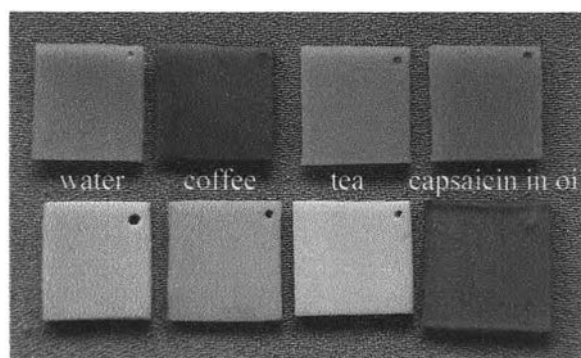c) The discoloration ($\triangle$E) for tested materials in capsaicin-oil solution

Figure 4.18. The stained specimens after immersion in different solutions over 8

weeks, Coe Supersoft® specimens are in the upper row and graft

copolymer specimens are in the lower row.

4.7.8. Cytotoxic effects of the graft copolymer and Coe Supersoft®

In the co-culture system, the graft copolymer demonstrated a lot of cells around the materials (Figure 4.19 a). These cells attached well on the surface of the graft copolymer (Figure 4.19 b) In contrast, the culture dish containing Coe Supersoft® showed a clear band with some cell debris around the specimen (Figure 4.20 a). At the surface of Coe Supersoft®, there were a few cells found poorly attaching to the material (Figure 4.20 b). These results suggested that the graft copolymer was not only noncytotoxic to the cells, but also offered a hospitable surface for the cells to attach while the Coe Supersoft® lack of these properties.
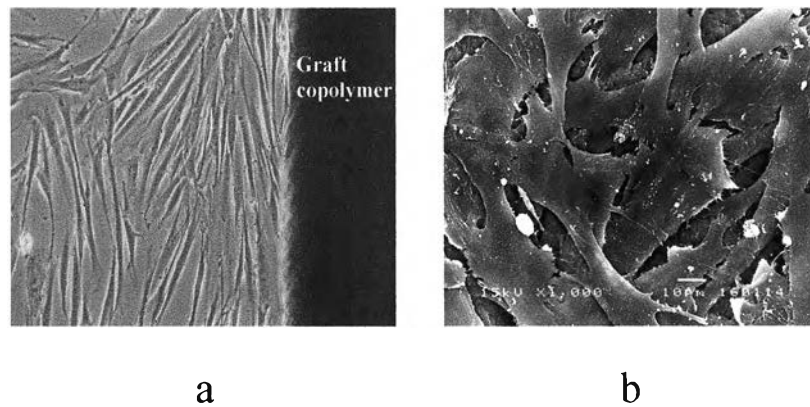
a   b

Fig.4.19. The inverted phase contrast micrograph (a) and scanning electron

micrograph (b) of the human gingival fibroblasts co-cultured with the graft

copolymer. Note the well proliferated and well attached human gingival

fibroblasts around and on the surface of the graft copolymer.
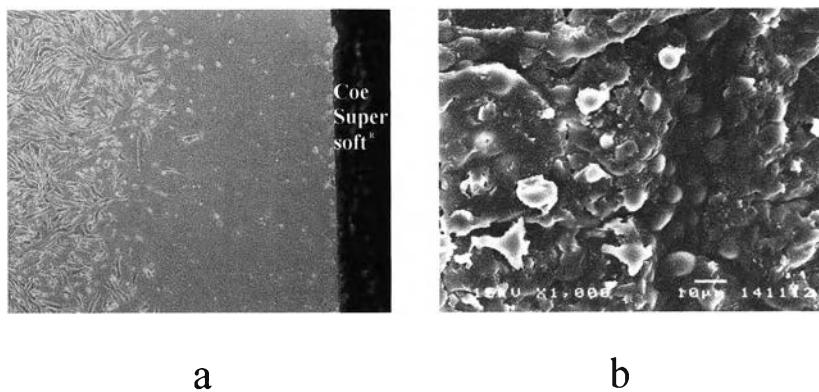

a   b

Figure 4.20. The inverted phase contrast micrograph (a) and scanning electron

micrograph (b) of the human gingival fibroblasts co-cultured with the

Coe Supersoft®. Note the clear band next to the material and a few

poorly attached cells on the material surface.

4.7.8. MTT assay

To test the cytotoxity of the graft copolymer and Coe Supersoft®, the MTT assay was performed to investigate the cell vitality in the co-culture system. In this study, the mitochondrial activity assessed with the MTT test was inhibited by the reagents leaching from the Coe Supersoft® specimen. The density of cell was reduced to zero in the presence of the Coe Supersoft® specimens for 48 hours. Whereas, the graft copolymer specimens did not affect the mitochondrial activity. Therefore, the cell density after incubation with graft copolymer for 48 hours was not different from the cells in non-exposed culture. The results from MTT test also confirmed that the graft copolymer did not have any cytotoxic effect to the human gingival fibroblasts.

In the presence of Coe Supersoft®, all the cultured cells died after 48 hours of the incubation evaluated by MTT test, suggested that there might be some leachable materials with cytotoxic effect from the Coe Supersoft®.