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Effect of PMMA polymer on the dynamic viscoelasticity and plasticizer leachability of PEMA-based tissue conditioners

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The purpose of this study was to determine the effect of PMMA polymer on dynamic viscoelasticity and plasticizer leachability of PEMA-based tissue conditioners. One PEMA polymer and one PMMA polymer were used in powder form with four formulations. The combination of 80 wt% ATBC, 15 wt% BPBG and 5 wt% ethyl alcohol was used as the liquid phase. The dynamic viscoelasticity and plasticizer leaching of each specimen were measured after 0, 1, 3, 7, and 14 days of immersion (37°C distilled water) using DMA and HPLC. A significant difference was found among the materials in the dynamic viscoelasticity and leaching of plasticizer. The materials containing 10 wt% PMMA showed the most stable dynamic viscoelasticity, and showed the lowest leaching of plasticizer. The results suggest that the addition of the PMMA polymer to the powder of a tissue conditioner can improve the durability of the PEMA-based tissue conditioner.

Keywords: Dynamic viscoelasticity, Tissue conditioner, HPLC

INTRODUCTION

Tissue conditioners are often used in edentulous patients to treat alveolar mucosa lesions due to ill-fitting dentures or following surgery, to record dynamic functional impressions, for temporary relining during the healing phase after implant placement as well as for other clinical applications that can utilize their specific viscoelastic properties[1-7]. The clinical use of tissue conditioners as temporary lining materials for dentures was first reported in 1961[8]. These materials are supplied as powder and liquid. The powder typically consists of polyethyl methacrylate polymer or a related copolymer[9]. The conventional liquid contains a mixture of an ester plasticizer and ethyl alcohol[9].

The dynamic viscoelastic properties as well as dimensional stability and surface properties of tissue conditioners are important factors to consider when these materials are used for tissue conditioning and temporary relining in clinical situations[3,10,11]. The main problem associated with this type of material is a rapid loss of viscoelasticity in clinical use[12] due to the leaching of ethyl alcohol and plasticizer into the oral environment[13-15]. This viscoelastic change can irritate denture-bearing areas and cause damage to edentulous soft tissues[16], and it can also accelerate the deterioration of the tissue conditioner[16]. Furthermore, the leached plasticizer can plasticize the heat-polymerized acrylic denture base resin and accelerate the deterioration of the denture base materials[17].

To solve these problems, it is necessary to improve the life span of tissue conditioners. Although a coating material is sometimes used on the tissue conditioner[18], it is also necessary to improve the tissue conditioner itself. Murata *et al.*[15] have reported that alcohol-free tissue conditioners containing n-butyl methacrylate/i-butyl methacrylate copolymer-based powder were the most stable over relatively long periods of time compared to tissue conditioners containing ethyl alcohol. To date, the mechanical properties, surface properties, and dimensional stability of tissue conditioners have been widely investigated[19-26]. However, few studies have examined the effect of addition polymethyl methacrylate (PMMA) polymer on the viscoelastic properties and plasticizer leaching. Information about these factors may be important for the efforts to improve the longevity of tissue conditioners. The PMMA is an unsuitable polymer to use as the main powder component of the tissue conditioner, because its solubility parameter for the hydrogen-bonded solvent is 0, and it only swells (not dissolves) very slowly in ethyl alcohol[9,15,26]. Therefore, the penetration of plasticizers into the PMMA polymer particle is a very slow process. Utilizing this characteristic of PMMA polymer, we suggest that it can act as a retarder if it constitutes a small portion of the powder in a tissue conditioner, and we think that long-term retention of the initial viscoelasticity is possible.

The purpose of this study was to determine the effect of addition of PMMA polymer on dynamic viscoelasticity and plasticizer leachability of PEMA-based tissue conditioners. We hypothesized that the PMMA polymer can reduce plasticizer leaching and improve the durability of tissue conditioners.

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MATERIALS AND METHODS

Sample preparation
The polymer powders and plasticizers used are listed in Table 1. One polyethyl methacrylate polymer (PEMA) with an average molecular weight (M_w) of 2.48×10^5 and average particle size (d_{50}) of 20.6 µm; and one polymethyl methacrylate polymer (PMMA) with M_w of 2.51×10^5 and d_{50} of 28.0 µm were used in powder form. Acetylcitric acid tributyl ester (ATBC), butyl phthalyl butyl glycolate (BPBG) and ethyl alcohol (EtOH) were used in liquid form.

Table 2 lists the four formulations used in this study and indicates the powder/liquid (P/L) ratio. The powder and liquid were mixed for 2 minutes at 23±2°C and 70% humidity, and then poured into a polypropylene container. Glass plates were placed on top of the containers and the containers were placed in a 37°C incubator for 15 minutes. After the incubation, the specimens were removed from the polypropylene containers and immersed in 100 ml distilled water in dark-brown bottles at 37°C.

Dynamic viscoelasticity measurement
Five pairs of specimens of each formulation were prepared in layers that were 10 (long) ×10 (wide) ×2 (thickness) mm. The dynamic viscoelasticity was determined using an automatic dynamic mechanical analyzer (DMA Q800, TA Instruments Co., New Castle, USA) based on the principle of non-resonance-forced vibration. The dynamic viscoelasticity test was conducted at 37°C on pairs of specimens with the use of shear sandwich jig (Figure 1) after 0 (no immersion in distilled water), 1, 3, 7, and 14 days of immersion. The shear storage modulus (G'), shear loss modulus (G'') and loss tangent (tan δ) were determined over a frequency range of 0.05-100 Hz with a 0.08% strain.

Plasticizer leaching measurement
Five specimens of each formulation were prepared as disks 18 (diameter)×2 (thickness) mm. One day after immersion, the specimens were removed from the water and blotted dry with filter paper. The specimens were then immersed in fresh volumes of water and returned to the 37°C storage. This procedure was repeated each day for 14 days. The water samples and corresponding filter-paper blotters were extracted into hexane. The plasticizer leaching was determined using a high performance liquid chromatograph (HPLC, LC-10ATvp, Shimadzu Co., Kyoto, Japan) with a degasser (DGU-12A, Shimadzu Co., Kyoto, Japan), auto sampler

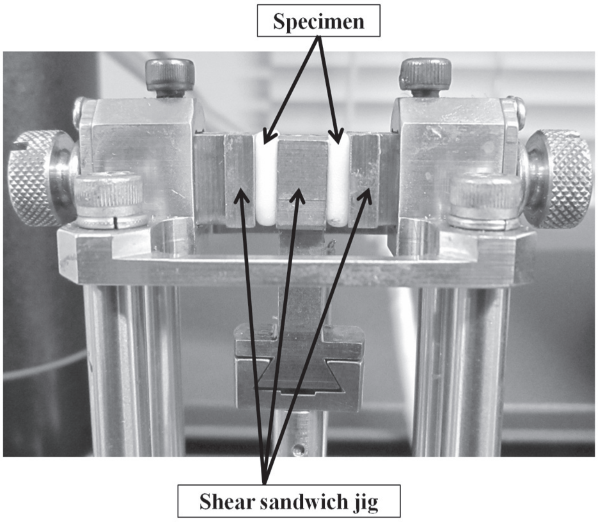

Fig. 1 Shear sandwich jig

Table 1 Polymer powders and plasticizers used

	Manufacturer	Lot. No.
Polymer		
Polyethyl methacrylate	Negami Chemical Industrial Co., LTD., Ishikawa, Japan	704051
Polymethyl methacrylate	Negami Chemical Industrial Co., LTD., Ishikawa, Japan	810021
Plasticizer		
Acetylcitric acid tributyl ester	Wako Pure Chemical Industries Ltd., Osaka, Japan	TSF0564
Butyl phthalyl butyl glycolate	Tokyo Chemical Industry Co., LTD., Tokyo, Japan	AGN01
Ethyl alcohol	Wako Pure Chemical Industries Ltd., Osaka, Japan	KWP4183

Table 2 Formulations of components

Code	Powders	Liquids
100 wt% PEMA	PEMA 100 wt%	ATBC 80 wt%, BPBG 15 wt%, EtOH 5 wt%
95 wt% PEMA+5 wt% PMMA	PEMA 95 wt%, PMMA 5 wt%	ATBC 80 wt%, BPBG 15 wt%, EtOH 5 wt%
90 wt% PEMA+10 wt% PMMA	PEMA 90 wt%, PMMA 10 wt%	ATBC 80 wt%, BPBG 15 wt%, EtOH 5 wt%
85 wt% PEMA+15 wt% PMMA	PEMA 85 wt%, PMMA 15 wt%	ATBC 80 wt%, BPBG 15 wt%, EtOH 5 wt%

(P/L by weight: 1.35)

(SIL-20AC, Shimadzu Co., Kyoto, Japan), column oven (CTO-10ASvp, Shimadzu Co., Kyoto, Japan; temperature: 40°C), UV-VIS detector (SPD-10Avp, Shimadzu Co., Kyoto, Japan) and a system controller (SCL-10Avp, Shimadzu Co., Kyoto, Japan) after 1, 3, 7, and 14 days of immersion. The column used was reversed phase HPLC packed column (Shim-pack VP-ODS, 150×4.6 mm, 5 μm particles, SN: 9062249; Shimadzu Co., Kyoto, Japan). The mobile phase was a 7:3 mixture of acetonitrile (HPLC grade) and water (HPLC grade). The flow rate was 1 ml/min and the injector volume was 20 μm. UV detection was performed at 235 nm. Based on the readings from a standard series of ATBC and BPBG in hexane, the results were calculated in milligram.

Statistical analysis

All the dynamic viscoelasticity data (G', G'', and tan δ) and plasticizer leaching data were analyzed independently by one-way analysis of variance (ANOVA) combined with an Student-Newman-Keuls (SNK) multiple comparison test at a 5% level of significance. Two-way ANOVA was used to determine the effect of the PMMA polymer content and immersion time on the dynamic viscoelasticity and plasticizer leachability of the tissue conditioner. One frequency of 1 Hz was selected for statistical analyses. The differences among materials and among immersion times were tested with an SNK test at a 5% level of significance. All analyses were computed with SPSS for the Windows operating system (SPSS 12, SPSS Japan Inc., Tokyo, Japan).

RESULTS

Figures 2 and 3 show the mean and standard deviations (S.D.) of the storage modulus (G'), loss modulus (G'') and loss tangent (tan δ) of tissue conditioners at 1 Hz on day 0 after immersion. Significant differences were found between the different materials ($p<0.05$, one-way ANOVA). Samples 100 wt% PEMA had significantly higher values of G' and G'' ($p<0.05$, SNK test) than the other three materials. No significant difference was found between the G' of samples 95 wt% PEMA+5 wt% PMMA and 90 wt% PEMA+10 wt% PMMA, which had significantly higher values than sample 85 wt% PEMA+15 wt% PMMA. No significant differences were found among the G'' of samples 95 wt% PEMA+5 wt% PMMA, 90 wt% PEMA+10 wt% PMMA, and 85 wt% PEMA+15 wt% PMMA ($p>0.05$, SNK test). Sample 85 wt% PEMA+15 wt% PMMA had a significantly higher value of tan δ ($p<0.05$, SNK test) than samples 100 wt% PEMA and 90 wt% PEMA+10 wt% PMMA.

Variations of G', G'', and tan δ at 1 Hz according to time of immersion of the four materials are shown in Figures 4, 5, and 6. The ANOVA results indicate significant differences among the materials for these rheological parameters ($p<0.05$). G' of sample 100 wt% PEMA decreased significantly after 1 day of immersion ($p<0.05$, one-way ANOVA), while that of sample 85 wt% PEMA+15 wt% PMMA increased significantly after 1 day of immersion ($p<0.05$). In addition, the tan δ of sample 100 wt% PEMA increased significantly after 1 day of immersion ($p<0.05$, one-way ANOVA), while that of sample 85 wt% PEMA+15 wt% PMMA decreased significantly after 1 day of immersion ($p<0.05$). The G'' of samples 100 wt% PEMA and 85 wt% PEMA+15 wt% PMMA increased with increases in immersion time. However, no significant difference was found between immersion time ($p>0.05$). The G' and G'' of sample 95 wt% PEMA+5 wt% PMMA increased until day 1 of water immersion, and decreased from day 1 to day 14. Sample 90 wt% PEMA+10 wt% PMMA showed most stability coupled with low change rates of G', G'', and tan δ ($p<0.05$). A

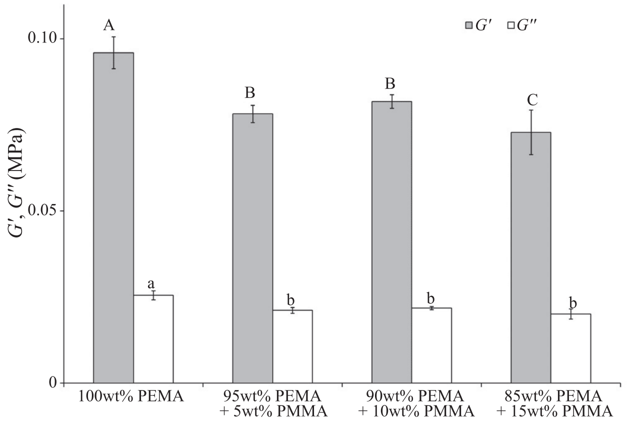

Fig. 2 Storage modulus (G') and loss modulus (G'') at 1 Hz, on day 0 after immersion. Identical letters indicate no significant differences ($p>0.05$).

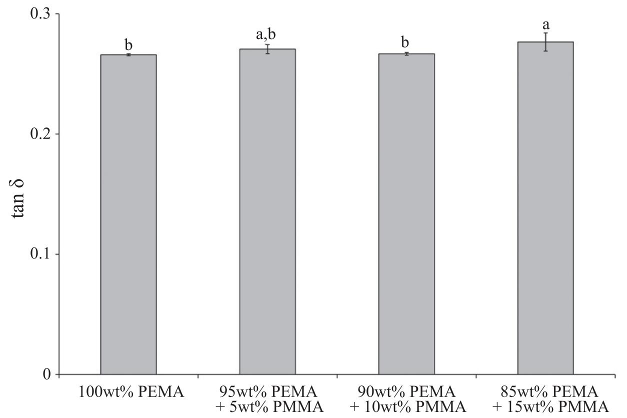

Fig. 3 Loss tangent (tan δ) at 1 Hz, day 0 after immersion. Identical letters indicate no significant differences ($p>0.05$).

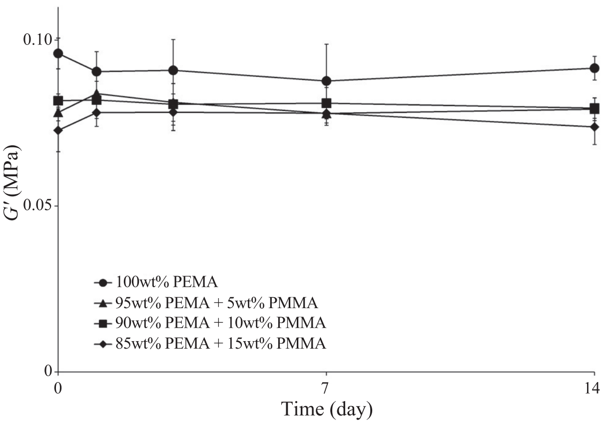

Fig. 4 Variations in storage modulus (G') with immersion time at 1 Hz.

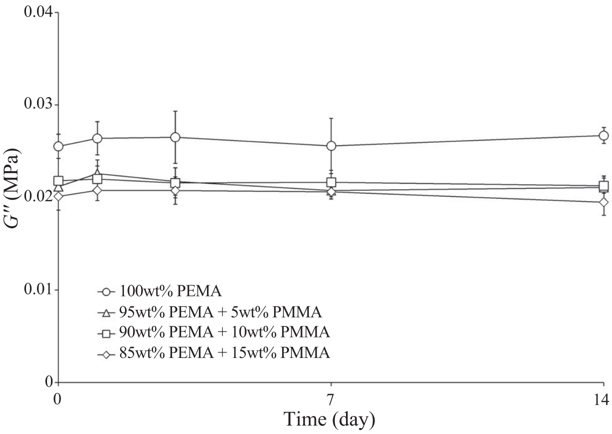

Fig. 5 Variations in loss modulus (G") with immersion time at 1 Hz.

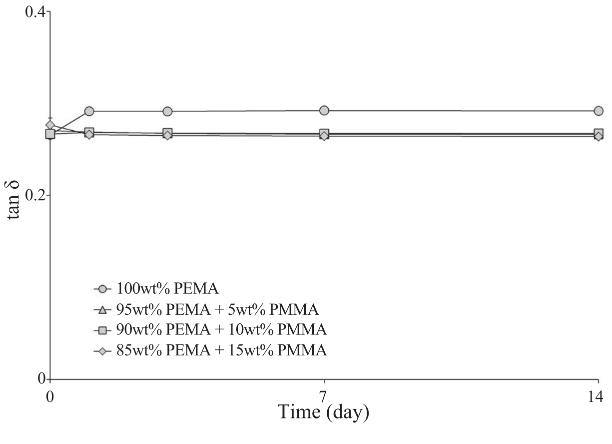

Fig. 6 Variations in loss tangent (tan δ) with immersion time at 1 Hz.

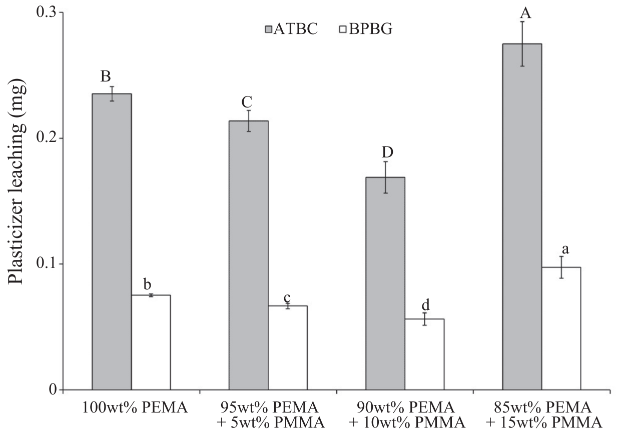

Fig. 7 Leaching of the plasticizer from specimens after day 1. Identical letters indicate no significant differences (p>0.05).

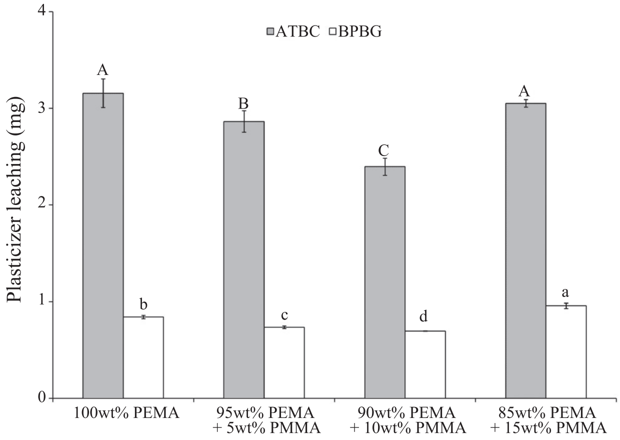

Fig. 8 Total leaching of the plasticizer from specimens during 14 days. Identical letters indicate no significant differences (p>0.05).

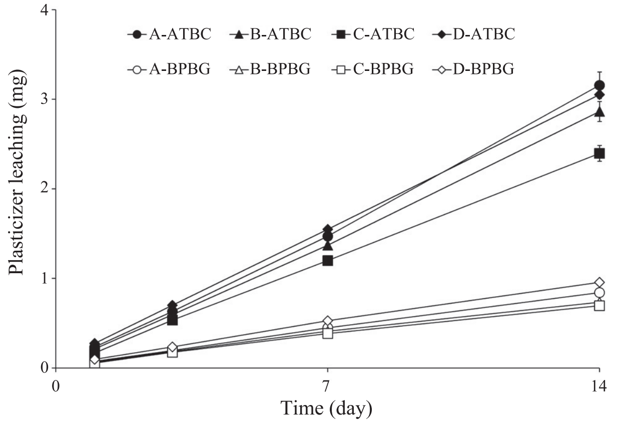

Fig. 9 Variations of plasticizer leaching with immersion time.

rapid change in viscoelasticity was recorded for samples 100 wt% PEMA and 85 wt% PEMA+15 wt% PMMA until day 1 of water immersion.

The leached amounts of plasticizer from specimens on day 1 after immersion are Figure 7. Significant differences were found between the different materials (p<0.05, one-way ANOVA). Samples 90 wt% PEMA+10 wt% PMMA leached significantly lower amounts of ATBC and BPBG (p<0.05, SNK test) than did the other three materials. Sample 85 wt% PEMA+15 wt% PMMA leached a significantly higher amount than did the other materials (p<0.05). Sample 90 wt% PEMA+10 wt% PMMA also leached significantly lower total amounts of ATBC and BPBG (p<0.05, SNK test) than did the samples 100 wt% PEMA, 95 wt% PEMA+5 wt% PMMA, and 85 wt% PEMA+15 wt% PMMA even 14 days after immersion (Figure 8).

Figure 9 shows the means and standard deviations (S.D.) of the integration leached amounts of plasticizer from tissue conditioners and their changes with immersion period for all materials. The leaching of plasticizer from all materials significantly increased with increases in immersion time (p<0.05, one-way ANOVA).

DISCUSSION

The PMMA polymer is unsuitable for main component of tissue conditioner, because the penetration of plasticizers into the PMMA polymer particle is a very slowly[9)]. Utilizing this retarder characteristic of PMMA polymer, we think that the addition of PMMA polymer into the powder of tissue conditioner can improve the durability of tissue conditioner. It is presumed that the leaching out of plasticizers from the tissue conditioner into the water is prevented because the plasticizer continue to penetrate into the PMMA polymer particle for a while. Moreover, the best conditions for gel formation obtained by the suitable combination of the components of the tissue conditioner may reduce plasticizer leaching. From our study, the hypothesis that the PMMA polymer can reduce plasticizer leaching and improve the durability of tissue conditioners was proved to be true and accepted. However, a suitable amount of PMMA polymer is necessary. The longevity of tissue conditioners is a significant problem in prosthodontics[12)]. The viscoelasticity of a tissue conditioner is one of the most important factors for determining a clinician's acceptance of it[10,11)].

In clinical situations, tissue conditioners are often replaced every three to four days[27)]. Their usage is generally not extended beyond two weeks. Therefore, in this study, we tested the samples for two weeks. In this study, ATBC was used as a plasticizer and was the main liquid component in the tissue conditioners. The estrogenic effects of certain phthalates *in vivo* and *in vitro* have been reported previously[28)]. Hashimoto *et al.*[29)] reported that phthalate esters, which were included in various dental materials as plasticizers, showed estrogenic activity *in vitro*. However, ATBC is a citric acid ester, and it is the preferred plasticizer in food packing films[30)]. Moreover, ATBC is odor-less and safe according to its pharmacological properties. And it is one of the most economical plasticizers accepted by the American Food and Drug Administration (FDA). In this study, ethyl alcohol (5 wt%) was used as the liquid component. Several investigators have reported that the alcohol is completely lost from polymer gel materials within the first 24 hours[13,14)]. The influence of the leaching of ethyl alcohol on the viscoelasticity loss of the tissue conditioner within the first 24 hours is bigger than the effect of leaching of the plasticizer. However, PEMA-based tissue conditioners, like those used in this study, contain no ethyl alcohol and would not produce a clinically acceptable gelation time, because polymer particles are dissolved only very slowly by large plasticizer molecules[9)]. Therefore, ethyl alcohol is necessary for the PEMA-based materials because ethyl alcohol rapidly swells the polymer particles and facilitates the dissolution of the polymer into the plasticizer. Murata *et al.*[15)] reported that the viscoelastic characteristics at 1 Hz would simulate behavior under typical masticatory conditions. Therefore, the dynamic viscoelastic parameters at 1 Hz were selected for evaluation in this study.

Differences in dynamic viscoelastic behaviors were found among the materials. The PMMA polymer addition samples (95 wt% PEMA+5 wt% PMMA, 90 wt% PEMA+10 wt% PMMA and 85 wt% PEMA+15 wt% PMMA) were showed lower G' and G'', and higher tan δ than sample 100 wt% PEMA (without PMMA polymer addition). This means that the PMMA polymer addition samples have a large flow, which would allow the abused denture-bearing mucosa to recover to a healthy state and record the shape of the mucosa under functional stress more effectively. Sample 90 wt% PEMA+10 wt% PMMA showed the most stable dynamic viscoelastic characteristic with the passage of time of all the materials. The change rate of the dynamic viscoelastic parameters of sample 90 wt% PEMA+10 wt% PMMA was less than 1.5%. The tissue conditioner is often used for three main clinical purposes: tissue conditioning; dynamic impressions; and temporary relining. Murata *et al.*[31)] reported that materials that have a large early flow, and a lower rate of change of flow with the passage of time are suitable for tissue conditioning. Therefore, sample 90 wt% PEMA+10 wt% PMMA is probably suitable for tissue conditioning.

Sample 90 wt% PEMA+10 wt% PMMA showed the most stability coupled with a low change rate of all rheological parameters. Several investigators have reported on the leachability of plasticizers from soft dental polymers[9,13,32)]. The loss of viscoelasticity of tissue conditioners in clinical use may be due to the leaching out of the plasticizer from the soft polymer gel material. Sample 90 wt% PEMA+10 wt% PMMA showed the lowest leaching of plasticizer of all four materials. This could be the reason why sample 90 wt% PEMA+10 wt% PMMA also showed the most stable viscoelasticity. Samples 100 wt% PEMA and 85 wt% PEMA+15 wt% PMMA showed rapid changes in *G'*, *G"*, and tan δ during one day of water immersion. Jones *et al.*[13] have reported that the alcohol is completely lost within 24 hours from polymer gel materials stored in water. Also, Wilson[14] reported that most alcohol was lost within the first 12 hours. Based on these reports, we suggest that the alcohol loss might be the reason for the rapid changes in rheological parameters recorded during the first day. In addition, samples 100 wt% PEMA and 85 wt% PEMA+15 wt% PMMA showed more leaching of plasticizer than did the other materials until day 1, and this may have influenced the rapid change in viscoelasticity during the first day. However, *G'* of sample 100 wt% PEMA decreased during one day of water immersion, while that of sample 85 wt% PEMA+15 wt% PMMA increased. This may be due to water sorption of the sample 100 wt% PEMA exceeded the leaching of plasticizer and alcohol, and material became soft.

In this study, the total leached amount of plasticizer from materials was 3.10–4.01 mg in 14 days. Graham *et al.*[32] reported that the total leached amount of plasticizer from a commercial tissue conditioner was 8-13 mg when the tissue conditioner was immersed in water at 37°C for 14 days. The leached amount of plasticizer from tissue conditioners examined in this study was significantly lower. The molecular weights (M_w) of plasticizers used in this study are 402.48 (ATBC) and 336.38 (BPBG), which are higher than those of other plasticizers usually used in commercial tissue conditioners, such as dibutyl phthalate (M_w: 278.35), benzyl benzoate (M_w: 212.25), benzyl salicylate (M_w: 228.24) and dibutyl sebacate (M_w: 314.46). Jones *et al.*[13] have reported that plasticizers with higher molecular weights showed lower leaching from soft polymer gel materials than did lower molecular weight plasticizers. Based on this information, we believe that the durability of the materials manufactured in this study was improved over that of conventional commercial products.

When the powder and liquid of tissue conditioners are mixed, the polymer dissolves in the plasticizer, polymer chain entanglement takes place, and a gel is formed. The gel has pseudo cross-links consisting of polymer chain entanglements[21]. We surmise that when the best conditions for formation of the pseudo cross-links have been obtained by the suitable combination of the components of the tissue conditioner, plasticizer leaching will decrease, and durability of the tissue conditioner improve. In this study, when the concentration of the PMMA polymer was less than 10 wt%, the leaching of plasticizer decreased in step with increases in the concentration of the PMMA polymer. The sample containing 10 wt% of PMMA polymer showed the lowest leaching of plasticizer. This may be due to formation of the pseudo cross-links consisting of polymer chain entanglements, which becomes optimal when PMMA polymer is present at a low concentration. However, when the concentration of the PMMA polymer was increased to 15 wt%, the leaching of plasticizer tended to increase correspondingly. The leaching of plasticizer from this sample was higher than from any of the other materials. We suggest that the balance between the polymer and plasticizer collapses when a larger amount of PMMA polymer is added to the powder, resulting in an increase in the non-reacted plasticizer concentration because the plasticizer penetrates very slowly into the PMMA polymer[9], thus accounting for the increased leaching of plasticizer.

The results of this study indicated that the dynamic viscoelasticity and plasticizer leachability of tissue conditioners were greatly influenced by addition of the PMMA polymer to the powder. Furthermore, the flow of tissue conditioner tended to increase with higher PMMA polymer content. However, an improvement in durability of tissue conditioners was not seen if the concentration of the PMMA polymer in the powder was lower or higher than 10 wt%. The present study did not completely simulate clinical behavior because viscoelasticity specimens were tested in the dry state, and all specimens were immersed in distilled water. Several investigators have reported that the plasticizer loss was higher *in vivo* than *in vitro*[13,32]. To overcome the limitations of the *in vitro* tests, the viscoelasticity of tissue conditioners actually used by patients should be evaluated; and artificial saliva should be used as an immersion solution. Furthermore, in order to understand the influence of PMMA polymer on the mechanism of plasticizer leachability it is necessary to study how addition of PMMA polymer to the powder of tissue conditioners influence other properties of these materials, such as working time, gelation time, surface properties, dimensional change, leachability of ethyl alcohol, water sorption and solubility.

CONCLUSIONS

The results of this study are summarized as follows:

(1) Sample 90 wt% PEMA+10 wt% PMMA showed the most stable dynamic viscoelasticity among the four materials.

(2) Sample 90 wt% PEMA+10 wt% PMMA showed the lowest leaching of plasticizer among the four materials.

From the standpoint of dynamic viscoelasticity and plasticizer leachability, the addition of PMMA polymer to the powder of PEMA-based tissue conditioners can improve the durability of these materials. However, it is necessary to use an optimal amount of PMMA polymer.

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