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Crystallization and hardening of poly(ethylene-co-vinyl acetate) mouthguards during routine use

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Mouthguards (MGs) made from poly(ethylene-co-vinyl acetate) (EVA) are widely used in contact sports to prevent injuries such as breaking teeth and lip lacerations and to reduce brain concussion. However, the changes in morphology and the molecular mobility of EVA, which can affect its physical properties during practical usage, have not been precisely examined. Therefore, we attempted to determine the main factors which lead to changes in MG performance after one season of practical use by high school rugby players. Solid-state nuclear magnetic resonance (NMR) and pulse NMR measurements showed the hardening of MGs, which was associated with an increased crystallinity of the EVA resulting from prolonged usage. Furthermore, our data indicated that the increase in the relative amount of the crystalline phase may be primarily attributed to temperature fluctuations and repeated changes in pressure, which could cause the hardening of EVA and eventually diminish the protective ability of MGs.

Mouthguards (MGs) can prevent sports-related oral injuries and reduce concussions, therefore, they are recently being employed for various sports^{1–12}. Materials employed for MGs are very limited, and only poly(ethylene-co-vinyl acetate) (EVA), olefin-based thermoplastic elastomers and styrene-based thermoplastic elastomers have been certified in Japan^{13–18}. EVA is primarily used because of its low cost and facile processability—the melting point of EVA (*ca.* 30–80 °C) is particularly low, for example¹⁹. Indeed, EVA sheets can easily be treated with commercially available small MG manufacturing machines; therefore, EVA MGs can be prepared not only at dental clinics but also at sporting grounds.

MGs are prepared by melting EVA sheets and subsequent molding with dental casts. MGs are detached from the molds after the temperature reaches room temperature. Following this, MGs undergo final occlusal checking by dentists before they are supplied to users. After MGs are used for long periods of time, some users report discomfort; in particular, they feel that their MGs are becoming hard. If hardening occurs, along with increased brittleness and reduced energy absorption capability, deterioration in the protective ability of the MG will occur concomitantly. To ensure that MGs are providing adequate safety levels to users, the guidelines for renewing MGs should preferably be based on scientific indicators.

Because EVA is a macromolecule with entangled polymer chains comprising crystalline and amorphous phases^{20,21}, we presumed that the discomfort relating to the fit of MGs would mainly be derived not from chemical degradation but from the state of those phases, which may be influenced by temperature fluctuations and/or repeated changes in pressure. In this study, we precisely analysed routinely used MGs and EVA films using differential scanning calorimetry (DSC), solid-state NMR and pulse NMR measurements^{22–27} to identify the factors that affect MG morphology and molecular mobility.

Results and Discussion

NMR spectroscopic analyses of MGs after one season of use. The solid-state cross-polarization magic-angle spinning (CP/MAS) ¹³C NMR spectra of a piece from MG ϕ 1 and another from MG1 that contact on tooth#16, which is one of the most compressed occlusion parts of the MG, are displayed in Fig. 1. The notation MG1 represents a MG that was routinely used by user 1 for one season (10 months), whereas MG ϕ 1 represents the excess portions of the MG material, which were obtained after the lamination and subsequent trimming of

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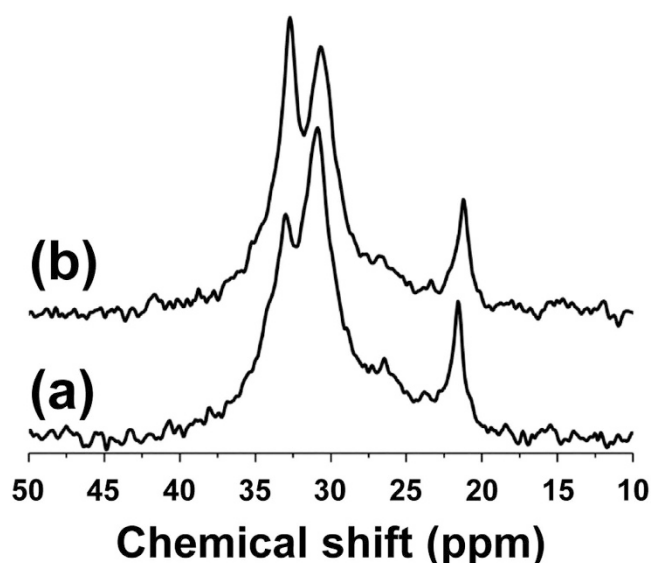


Figure 1. Solid-state CP/MAS ^{13}C NMR spectra of (a) a piece from MG ϕ 1 and (b) the piece of MG1 that contacts on tooth#16.

MG1	MG2	MG3	MG4	MG5	MG6	MG7	MG8
44.1	42.5	37.9	39.7	41.6	42.1	36.9	41.2
MG ϕ 1	MG ϕ 2	MG ϕ 3	MG ϕ 4	MG ϕ 5	MG ϕ 6	MG ϕ 7	MG ϕ 8
33.0	35.0	34.6	32.7	34.4	38.0	33.5	35.9

Table 1. Crystalline ratio (%) determined by solid-state CP/MAS ^{13}C NMR spectroscopy.

MG1	MG2	MG3	MG4	MG5	MG6	MG7	MG8
30.6	27.0	26.5	25.5	24.5	26.8	24.9	24.5
MG ϕ 1	MG ϕ 2	MG ϕ 3	MG ϕ 4	MG ϕ 5	MG ϕ 6	MG ϕ 7	MG ϕ 8
22.7	22.1	23.7	24.0	23.1	22.4	22.7	23.5

Table 2. Rigid component (%) of MG determined using pulse NMR spectroscopy.

MG1 and preserved at room temperature for one season. Peaks at approximately 33 and 31 ppm are known to result from CH_2 units in the ethylene groups of the crystalline and amorphous phases, respectively, whereas the peak corresponding to CH_3 in the methyl group of acetate appears at 22 ppm^{20,21}. As shown in Fig. 1a, the intensity of the peak corresponding to the amorphous phase was higher than that corresponding to the crystalline phase. Conversely, in Fig. 1b, the intensity of the peak corresponding to the crystalline phase was higher than that corresponding to the amorphous phase. Such phenomena were observed in all the MGs examined in this study (Table 1). These results indicate that the usage of MGs can increase the ratio of the crystalline phase present in EVA. Incidentally, the solution ^{13}C NMR spectra of used MGs remained unchanged compared with those of unused MGs (data not shown), indicating that no chemical decomposition of EVA (e.g., hydrolysis of the acetate group)²⁸ occurred during the usage period.

In solid-state CP/MAS ^{13}C NMR, the efficiency of the cross-polarization from ^1H to ^{13}C in the crystalline phase is known to be higher than that in the amorphous phase. Therefore, in Fig. 1a, the ratio of the amount of the crystalline phase to that of the amorphous phase is, in fact, not as large as that indicated by the ratio of the two corresponding peaks. However, the change in the ratio of the peaks between unused and used MGs can be compared, and accordingly, the difference between the spectra (Fig. 1a,b) could be attributed to the crystallization during routine use.

We then assumed that the increase in the crystalline ratio may increase the fraction of restricted components in MGs. Pulse NMR measurements were therefore performed using the above-mentioned pieces of MG ϕ 1–8 and MG1–8 to evaluate molecular mobility^{29,30}. The observed data were fit to a hybrid of exponential and Gaussian functions to obtain the fraction ratios for the rigid, intermediate, and mobile components (Figure S1). As shown in Table 2, which summarizes the fractions of the rigid component for MG ϕ 1–8 and MG1–8, the magnetization fractions demonstrate that MG usage increases the rigid component instead of decreasing the intermediate and

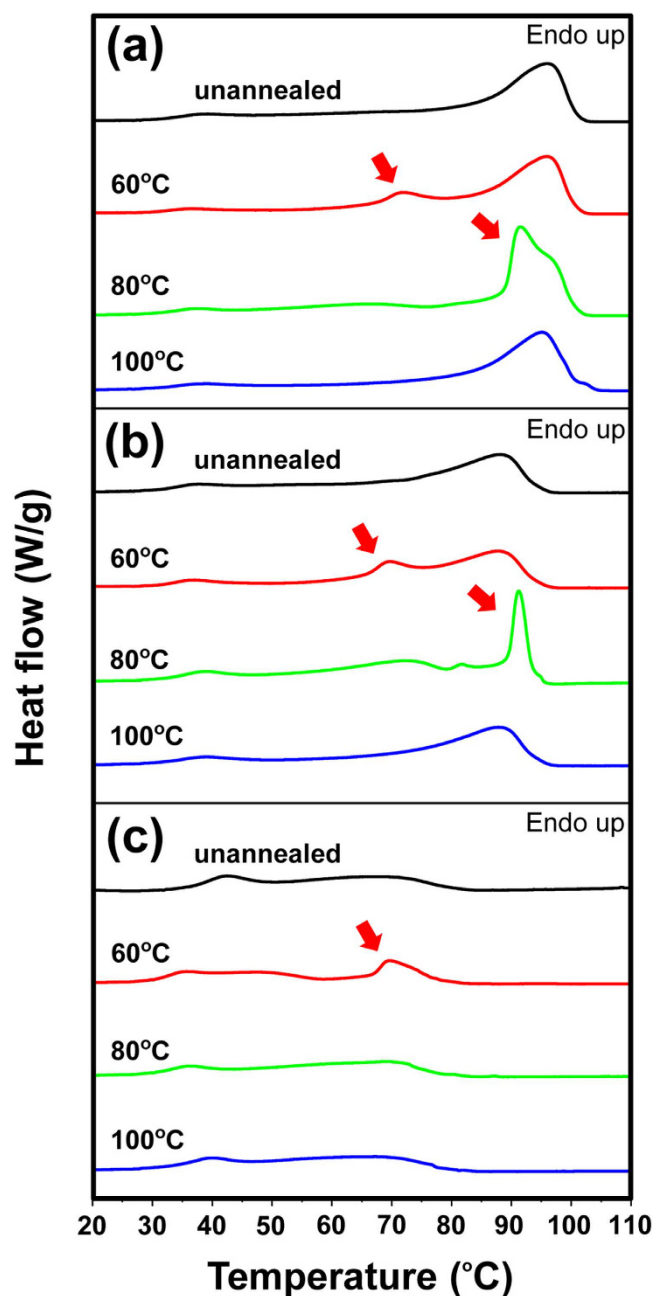


Figure 2. DSC curves of the heating process of (a) EVA9, (b) EVA14, and (c) EVA28. Samples: unannealed (black), annealed at 60 °C (red), annealed at 80 °C (green), and annealed at 100 °C (blue). New endothermic peaks were shown with red arrows.

In summary, the CP/MAS ^{13}C NMR and pulse NMR measurements show that the usage of EVA MG increases the crystalline fraction, which restricts molecular mobility and leads to the eventual hardening of the MG.

Verification of the effect of temperature fluctuations by DSC. We attempted to verify the effects of temperature fluctuations on the crystallization behaviour of EVA films (0.030 cm thickness) with different vinyl acetate (VA) contents of 9%, 14% and 28%, for EVA9, EVA14 and EVA28, respectively, using DSC analysis. According to the solution ^{13}C NMR spectra, the VA content of EVA28 was nearly equal to that of the EVA sheet (Drufosoft[®]) used to prepare the examined MGs.

Melting of unannealed EVA9 started at approximately 30 °C and ended after the stark endotherm at approximately 95 °C, as shown in the DSC curves of the heating process (Fig. 2a). In the cases of EVA14 and EVA28, melting started at approximately 30 °C; the former ended with a strong endotherm at approximately 90 °C, while the latter ended with a gently sloping approximately peak at approximately 70 °C (Fig. 2b,c). The decrease in the maximum intensity and temperature of the endothermic peaks depended on VA content, reflecting the thick-

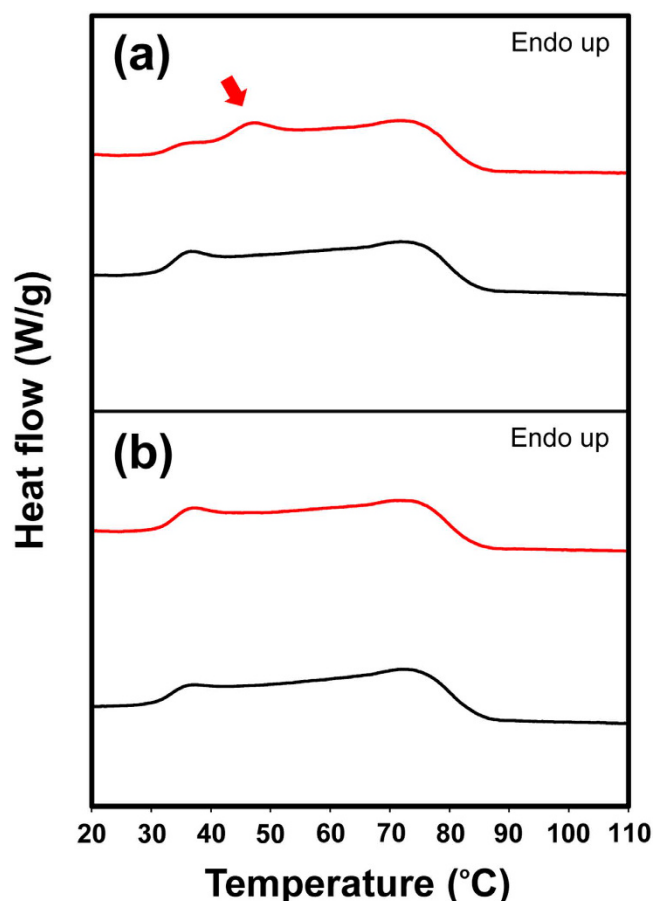


Figure 3. DSC curves of the heating process of Drufosoft® films with or without treatment by thermal cycles (100 times) executed using a protocol with repeated temperature fluctuations (a) between 25°C and 37°C or (b) between 6°C and 22°C. Samples: thermal-treated (red) and as-prepared (black). The new endothermic peak is shown with a red arrow.

provide thicker lamellae and enhance the crystallinity of EVA, although the broad melting ranges indicated that the lengths of the polyethylene strands between the VA units were widely distributed. Meanwhile, it can be stated that thin lamellae are produced regardless of the VA content because all films started melting at a low temperature (approximately 30°C). This suggested that the crystallization of MG could progress in the mouth, i.e. at body temperature, during routine use.

Annealing of EVA9, EVA14, and EVA 28 resulted in a new gentle endothermic peak at approximately 70°C when the films were annealed at 60°C (Fig. 2a–c), and annealing of EVA9 and EVA14 at 80°C resulted in a sharp endothermic peak at approximately 90°C (Fig. 2a,b). Although the new endothermic peaks are attributed to the melting of the crystalline phase by the annealing treatments, it was commonly observed that annealing of the EVA films at the crystallization temperature (T_c)¹⁹ or the closest temperature above T_c resulted in a relatively clear peak maximum for the melting temperature (T_m), i.e. $T_c = 80, 71$ and 48°C for EVA9, EVA14 and EVA28, respectively (Figs 2 and S2). Thus, these results indicate that the crystallinity of EVA MGs greatly depends on the status of the ethylene moieties and is significantly influenced by temperature fluctuations at ranges closer to T_c .

Furthermore, we analysed the effect of temperature fluctuations using Drufosoft® films (thickness = 0.30 cm, which is approximately equal to that of typical MGs) with or without treatment of repeated thermal cycles (100 times). Two different protocols of thermal cycling were employed; one was shuttling between 25°C and 37°C and the other was shuttling between 6°C and 22°C as a reference condition (Fig. 3). Intriguingly, in the former condition, a new endothermic peak appeared at approximately 45°C (Fig. 3a), but, in contrast, the latter condition caused little changes in the DSC thermogram (Fig. 3b). These results show that repeated temperature fluctuations even between close temperatures, i.e., ambient and body temperatures, can affect the crystallinity of EVA MGs.

Verification of the effect of temperature fluctuations using solid-state CP/MAS ^{13}C NMR. The tendency towards crystallinity discovered in the DSC measurements was also confirmed by solid-state CP/MAS ^{13}C NMR analyses. As shown in Fig. 4a, although the intensity of the peak corresponding to the crystalline phase (at 33 ppm) was higher than that corresponding to the amorphous phase (at 31 ppm), the highest difference was observed in the spectrum of EVA9 annealed at 80°C, which is equal to T_c . Similarly, the highest differences

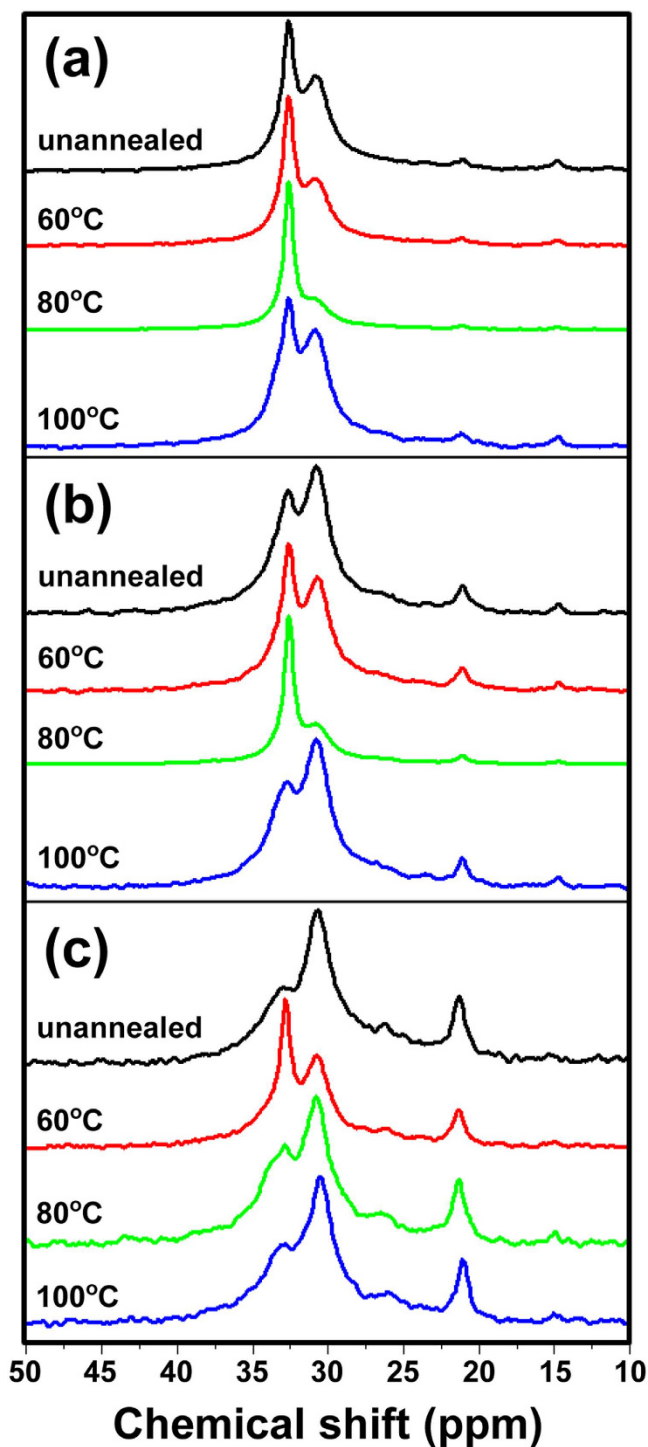


Figure 4. Solid-state CP/MAS ^{13}C NMR spectra of (a) EVA9, (b) EVA14, and (c) EVA28. Samples: unannealed (black), annealed at 60 °C (red), annealed at 80 °C (green), and annealed at 100 °C (blue).

(Fig. 4b,c). Furthermore, intriguingly, when the spectra of unannealed EVA films were compared, the ease of crystallization obviously reflected the ethylene content.

Verification of the effect of temperature fluctuations using pulse NMR. The molecular mobility in EVA films (0.030 cm thickness) was analysed using pulse NMR measurements. As compared with the unannealed EVA9 film, EVA9 films annealed at either 60 °C or 80 °C exhibited increased fraction ratios and decreased spin-spin relaxation times (T_2 values) for the rigid component (Table S1). EVA9 annealed at 100 °C provided similar values to unannealed EVA9. Among the fraction ratios and T_2 values obtained for the rigid component in

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