Visualization of Strain in Elastic Silicone Polymers Using Fluorescence Energy Transfer

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There is an urgent need to develop new methods for assessing strain in polymers without damaging the material’s intrinsic properties. The disadvantages of current methods include: high costs; impregnation of the polymer with bulky mechanical devices; inability to measure in several dimensions; and interaction with electromagnetic radiation. In this paper, a method to detect strain in elastic polymers that is based on energy transfer between two fluorophores is presented. By incorporating donor and acceptor dyes in poly(dimethylsiloxane) (PDMS) and monitoring the change in emission spectral profile, evidence of strain is gathered. We first demonstrate the successful doping of the elastomer with the dyes fluorescein and rhodamine B by using different optical techniques. PDMS strips containing the two dyes are fabricated and their emission spectral profile upon stretching is analyzed using a spectrometer. As the strip is extended, the distance between donor and acceptor molecules increases, resulting in a decline in energy transfer efficiency. This is manifested by intensification of emission from fluorescein, accompanied by a decline in emission from rhodamine B. The effect of dye incorporation on the polymer’s elastic properties is explored with an extensometer. Next, strain in samples is visualized using a simple digital camera and basic image-processing methods. Finally, a gradual flexibility PDMS is manufactured and the nonuniform distribution of strain in it is charted.

Keywords: Strain, Polydimethylsiloxane, FRET, Polymer, Fluorescence

1. Introduction

The ability to analyze the forces exerted on polymers in an economical and non-disruptive fashion is of great importance. Classical methods to detect strain in elastic polymers (elastomers) include mechanical, electrical and optical ones [1]. For example, the integration of flexible fiber optic sensors (FOS) into polymers is used in biomedical applications where qualities of light travelling in the fiber, such as intensity, phase and wavelength, are modified in response to forces applied to the polymer [2]. The disadvantage of these techniques is that they all require integration of physical components into the polymer, which might alter the material’s physical properties. However, a new generation of strain-evaluation techniques that involve observing changes in the polymer’s intrinsic optical properties is currently under intensive investigation. These include changes in the polarization of light passing through a flexible elastomer or variation in the reflectance intensity of opaque (or coated) materials [3]. Another system related to this approach is the integration of fluorescent molecules into the material and observation of changes in intensity and wavelength of emission. For example, Weder et al. utilized the fact that cyano derivatives of oligo(p-phenylene vinylene) can form aggregates that emit light at greater wavelength compared to the monomer units [4]. They added these molecules to a polyethylene film and observed how stretching of the polymer separates the fluorophores from each other, resulting in decreased formation of aggregates and a hypsochromic shift in fluorescent emission.

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Further use of this concept was demonstrated by Nicholas et al. whereby pyrene molecules were covalently-bound to poly(hydromethylsiloxane) (PHMS) and integrated into poly(dimethylsiloxane) (PDMS) [5]. Upon stretching, a marked increase in the excimer/monomer intensity ratio was detected. Recently a biomimetic approach was devised by Zeng and coworkers, where stretching a fluorescein-coated PDMS reveals phosphorescent particles that change the color of emission from the polymer [6].

Another use of fluorescent phenomena that is gaining interest for the evaluation of strain is Förster resonance energy transfer (FRET). This physical process occurs when an excited fluorophore (“donor”) transfer its excess energy to an adjacent fluorophore (“acceptor”) which can then be emitted as a photon [7]. This energy transfer (ET) is non-radiative and occurs through dipole-dipole coupling, meaning its efficiency is proportional to the inverse of the sixth power of the distance between the two molecules. Another mechanism of the ET may apply, which is a radiative process due to the emission of the donor and reabsorption by the acceptor. In the radiative mechanism, the efficiency corresponds to the inverse of the second power of the dye distance.

For sufficient data to be analyzed, light in the wavelength of both the donor’s and the acceptor’s emission must be collected. By monitoring the decline in donor emission compared to increase in that of the acceptor, evidence of FRET can be gathered and spatial information be determined. FRET is extensively used for studying molecular interactions in biological research, for example observing protein structures and enzymatic activity [8]. Sachs and co-workers were the first to utilize this technique to measure changes in macroscopic objects, where they implanted 2 fluorophores bound by an amino acid linker into functionalized silicone rubber [9]. When the functionalized polymer was stretched up to 111%, an increase in the donor/acceptor emission ratio was observed. The researchers explained the rise by suggesting that in the relaxed state, the linker molecule is somewhat folded and the donor is close enough to the acceptor to allow energy transfer. However, when the polymer is stretched, the spacer is straightened and perhaps elongated, increasing the distance between fluorophores and decreasing FRET efficiency. Similarly, Karthikeyan and Sijbesma introduced donor and acceptor molecules into a poly(ether urea) thermoplastic elastomer and were successful in detecting elongation in the polymer using FRET ratiometric measurements [10]. The disadvantage of the above uses of FRET is that they require cumbersome polymer synthesis and are probably not suitable for commercial, large-scale production.

In this research we utilize energy transfer phenomena as a tool for assessing and visualizing the strain exerted on polymers. We chose as a model the widely-used, biocompatible PDMS elastomer which was doped with donor and acceptor dyes. When the polymer is stretched, the distance between the fluorophores increases, resulting in decreased emission of acceptor, accompanied by increased emission of the donor (Scheme 1). By monitoring the change in the emission ratios of the two fluorophores we detect evidence of strain. The advantage of our approach is that it utilizes widely used, non-toxic materials in a facile fabrication method that does not change the intrinsic properties of the polymer. The monitoring of stress can be carried out with a simple digital single-lens reflex (DSLR) camera and a set of filters.

Scheme 1. Strain-dependent ET efficiency. (A) When the polymer is relaxed, ET can occur between two adjacent dye molecules. (B) Upon stretching, ET decreases.

2. Materials and methods
2.1. Sample preparation
Fluorescein (Wako, Japan) and rhodamine B (Kanto chemical, Japan) were dissolved in alkaline (10 mM NaOH) ethanol and ethylene glycol (Wako) mixture (1:1). 25 µL of each fluorophore solution
was added into a 0.55 mL mixture (10:1) of the poly(dimethylsiloxane) (PDMS) reactants (Zoukei-mura, Japan) and stirred thoroughly. Blank samples were prepared by adding uncolored ethanol/ethylene glycol to PDMS. Liquid polymer was placed in custom PDMS molds, degassed using a vacuum pump (Hitachi, Japan) for 30 mins and then cured at 65 °C overnight. Gradient flexibility samples were prepared by dividing the mold into four sections, with each section containing a different ratio of oligomer/crosslinker, ranging from 10:1 to 10:4. After the degassing period the barriers between each section were removed and the sample was cured as described above.

2.2. Fluorescence measurement and image analysis

Optical characterization of dye-containing PDMS was done with a V-770 spectrometer (Jasco, Japan) and an RF-5300PC spectrofluorometer (Shimadzu, Japan). PDMS strips were stretched on a custom-made configuration made of two 1-axys manual translation stages (Sigma Koki, Japan). Degree of stretching was monitored using digital calipers (Niigata Seiki, Japan).

For imaging measurements, a Ricoh projector was used for illumination (PJ K111, 243 W, Japan), with light passing through a 450 nm bandpass filter (Edmund Scientific, Japan). Irradiation was done at 30° while images were taken with a Nikkon camera (D300, Japan) with a Tokina macro lens (ATX 100mm, Japan). 2 filters were used for image acquisition: 500 and 550 nm longpass filters (Sigma Koki, Japan). Images taken with the 550 nm filter were attributed to RB emission, while the Fl channel was obtained by subtracting the RB image from the one taken with the 500 nm filter. All image processing was done using ImageJ software (NIH, USA). 5 Images were taken for each measurement, then averaged, cropped, converted to greyscale and had the blank images subtracted from them. Ratiometric images were attained by amplifying the Fl channel, dividing it by the RB one and attributing each value with an appropriate color using the Lookup table (LUT) editor. Intensity was also plotted graphically using the “plot profile” tool.

2.3. Young’s modulus

PDMS samples with or without dyes in EG/EtOH were prepared to the size of 50.8 X 10 X 1.4 mm. Samples were stretched using an Instron system (Model 4442, USA) at a rate of 5 mm/min under ambient conditions (23 °C). Stress (σ) is defined as the ratio of force (F) to cross-sectional area (A) as follows:

\[ \sigma = \frac{F}{A} \]  

While strain (ε) is calculated by dividing the elongation (ΔL) by the original length (L₀).

\[ \varepsilon = \frac{\Delta l}{l_0} \]  

True stress (σ_T) and true strain (ε_T) were calculated using the assumption of preservation of volume, dictated by a Poisson’s ratio of 0.5.

\[ \sigma_T = \sigma (1 + \varepsilon) \]  

\[ \varepsilon_T = \ln(1 + \varepsilon) \]

Finally, true stress was plotted against true strain, and by fitting a third order polynomial, Young’s Modulus was differentiated.

3. Results and discussion

3.1. Optical properties of dye-doped PDMS

The elastomer PDMS was chosen for this research due its ease of use, inert nature and its biocompatibility which enables it to be used in many biomedical applications [11]. It is a hydrophobic transparent silicone rubber with high flexibility, compressibility and stability over a wide range of temperatures (−100 ~ 100 °C). The dyes chosen as an ET couple are fluorescein (Fl) and rhodamine B (RB) due to their well-established compatibility and wide use [12]. In order to test the optical properties of each dye in PDMS, Fl and RB were dissolved in ethanol (EtOH) and each one was mixed thoroughly into the polymer reagents (10% V/V), followed by curing as per the manufacturer’s instructions. It can be seen in Fig. 1 that RB showed similar fluorescence intensity in solid PDMS, compared to solution. On the other hand, when Fl was incorporated into the polymer, significant quenching was observed. This can be rationalized by the fact that while RB is amphiphilic, FL is hydrophilic and shows significant quenching upon integration into non-polar medium [13].

It is known that Fl forms dianionic species in alkaline environment, which have higher quantum yield compared to the neutral and anionic forms [14]. In a further effort to increase the polar nature of the PDMS environment, ethylene glycol (EG) was mixed into alkaline ethanol (EtOH, 10 mM NaOH) at different ratios with Fl, before mixing into the polymer reagents. EG has a high boiling point.
Fig. 1. Fluorescence spectra of 1.2 µM RB (A) and Fl (B) in ethanol and PDMS (solid and dashed lines, respectively).

Fig. 2. Evolution of the Fl peak as a factor of EG content. (197 °C), which ensures it does not evaporate during polymerization, resulting in pockets of polar surroundings inside the elastomer. Figure 2 shows the effect of increasing the EG to EtOH ratio added to PDMS on the intensity of the Fl peak. It is evident that at 1:1 mixture rate (overall 5% EG V/V in PDMS) the fluorescence intensity almost tripled compared to neat alkaline EtOH. Overall, addition of alkaline 1:1 EG/EtOH resulted in a peak with 45% of the intensity of the liquid solution.

One of the factors complicating the measurement of strain in elastic materials is the Poisson effect. It is defined as the ratio of transverse strain to axial strain, as manifested by the volume change due to deformation [15]. In the case of a polymer like PDMS which has a Poisson factor of 0.5, elongation in one axis will result in a contraction of half the magnitude in the transverse directions, with an overall volume change of zero [16]. Because this 3-dimensional compression can effectively cancel out the effect of elongation and result in no net change in donor-acceptor distance, it is desirable to minimize the contribution of fluorophores from one of the orthogonal axes. To that end we set out to increase the opacity of the PDMS blend, thus ensuring fluorescence mostly from the surface, effectively dictating a 2-dimensional behavior of the polymer. The increase in opacity was achieved by the presence of EG in the PDMS matrix, as the two have different refractive indices (1.432 and 1.407, respectively) [17,18]. Figure 3 shows the rise in optical loss due to scattering and absorption as EG content is increased in 1 cm-thick samples. For example, at the FRET excitation wavelength of 450 nm, polymers prepared with 5% EG show an increase of 26.5% in optical loss, compared to 0% EG.

Fig. 3. Optical loss as a function of ethylene glycol (EG) content. Absorption spectra of 1 cm-thick PDMS discs containing 0, 2.5 and 5% V/V EG (black, dotted grey and dashed light grey lines, respectively).

Finally, the feasibility of energy transfer between Fl and RB in PDMS was evaluated by mixing Fl with or without RB at different concentrations into the liquid polymer. Figure 4A shows the change in absorption spectral profile of dye-doped PDMS as increasing amounts of RB are added to 1.5 µM Fl, suggesting that the absorption of both molecules is not hampered by the polymer environment. Figure 4B displays the ET behavior of Fl-containing elastomer as RB is added. It can be seen that as RB content is increased, its emission increases, accompanied by a decline of the Fl peak. This indicates that energy transfer between the two fluorophores is possible inside PDMS.

In order to calculate the efficiency ($E$) of ET the following formula was used, where $F_{DA}$ represents the intensity of the acceptor’s (Fl) emission in the presence of the donor (RB), and $F_D$ without it [19]:

$$ E = 1 - \frac{F_{DA}}{F_D} $$

The calculations show that the addition of the lowest amount of RB yields a 7% efficiency, which
gradually increases to 30%. At these concentrations and efficiencies, it would seem that the mechanism of ET is not FRET, but rather a radiative one.

3.2. EG effect on elastic modulus

The elastic properties of PDMS have been characterized in depth and are known to be highly sensitive to factors such as: sample thickness, oligomer/cross-linker ratio and curing temperature, among others [15]. The main quality that dictates the behavior of an elastomer when forces are applied to it is called Young’s Modulus, or Elastic Modulus, which is often calculated by plotting the slope of the stress/strain response of a sample. Since the dye-doped PDMS used in this research contained traces of liquid EG, we set out to measure the effect they might have on the polymer’s elastic properties. PDMS samples prepared according to the manufacturer’s instructions, without any additives had an Elastic Modulus of 1.60 MPa (± 0.07 MPa) which is slightly lower than that reported for the widely-used Sylgard 184 PDMS [15,20]. Addition of 5% EG slightly increased the material’s elasticity to a value of 1.76 MPa (± 0.06 MPa) which is still lower than common values for PDMS. The Stress-strain curves are given in Fig. 5.

3.3. Mechano-optical properties

Strips containing the fluorophores were made to the size of 30 × 8 × 1.5 mm and their emission spectra upon stretching was evaluated. While optimal ET favors a low D/A ratio, for practical reasons, a ratio between 1-2 is often used in biomedical systems [21]. For example, Karthikeyan and Sijbesma used a 2:1 ratio for their system [10]. However, since Fl is quenched in PDMS, it was decided to use a 4:1 ratio (5:1.25 µM) and test its performance against controls made with Fl or RB alone. The Mechano-optical behavior of strips prepared with only Fl or RB was compared to that of samples prepared with both, as they are stretched and the spectrum was recorded. The intensity of each fluorophore was integrated and the Fl/RB ratio was calculated and plotted in Fig. 6.

Fig. 4. (A) Absorption spectra of 1.5 µM Fl in PDMS as RB is added: 0 µM (black, bold line), 0.3 µM (grey, solid), 1.5 µM (grey, dashed) and 3 µM (light grey, dotted). (B) changes in 0.75 µM Fl (grey circles) and RB (black triangles) intensity at different concentrations of RB upon excitation with 450 nm light.

Fig. 5. (A) Absorption spectra of 1.5 µM Fl in PDMS as RB is added: 0 µM (black, bold line), 0.3 µM (grey, solid), 1.5 µM (grey, dashed) and 3 µM (light grey, dotted). (B) changes in 0.75 µM Fl (grey circles) and RB (black triangles) intensity at different concentrations of RB upon excitation with 450 nm light.

Fig. 5. Stress/strain curves. The Young’s modulus was calculated from the above curves that represent neat PDMS prepared according the manufacturer’s instructions (black line) and PDMS mixed with Fl and RB dissolved in an alkaline EG/ethanol mixture (1:1 10% V/V, grey dashed line).

Fig. 6. Change in Fl/RB peak ratio in PDMS strips prepared with either Fl (grey triangles), RB (light grey squares) or a mix of both (black circles) upon stretching and irradiating with 430 nm light.

Strips prepared with Fl or RB alone show almost no change in the FL/RB ratio as the polymer is stretched. Additionally, values for Fl are relatively high, as there is little emission around the 570 nm peak and conversely, RB values are low due to absence of emission from Fl. On the other hand, the Fl/RB mix shows a marked increase, from a starting value of 1.3, the ratio increases by 15%, to 1.5 upon extension of 40%. It was assumed that as the strips are stretched, the distance between donor and acceptor increases and the ET efficiency is decreased, resulting in a rise in the Fl/RB emission.
ratio. This demonstrates that even though PDMS’s Poisson value of 0.5 dictates no overall change in volume as the elastomer is stretched, this effect is minimized by a sample with high opacity that fluoresces mostly from the surface.

Figure 7 shows the spectral evolution of the Fl-RB-doped polymer upon stretching. It is noted that the Fl peak is at about 520 nm, indicating the presence of mostly the dianionic species which is characterized by a high quantum yield and better FRET efficiency with RB [14]. In the unstretched state, the FL and RB peaks are almost equal in height, but as the strip is being elongated, the Fl peak increases relative to the RB one. This suggests a decreased efficiency of ET as strain increases.

3.4. Imaging of strain

In order to visualize the strain experienced by the polymer, samples prepared with Fl and RB (4:1) were stretched gradually, while being irradiated with 450 nm light, and images were taken upon each step. The two fluorophores were distinguished from one another by acquiring images with either a 500, or a 550 nm longpass filter. The images acquired were averaged for each channel and converted to greyscale using the ImageJ software. The image taken with the 550 nm filter was assigned to RB and was then subtracted from the one taken with the 500 nm image to attain the Fl channel. For each state – relaxed and stretched, the intensity of the Fl channel was divided by the RB one and plotted, seen in Fig. 8A. While in the relaxed state the Fl/RB ratio is slightly above 1, at 40% elongation the ratio dramatically increases to about 1.8. This method allows to quantitively analyze strain across a selected area in the sample. To better visualize the strain distribution in the sample, ratiometric images were produced by dividing the Fl image by the RB one. Next, grey shades or colors were artificially assigned to the resulting greyscale image, where black represents low Fl/RB, gradually changing to light grey for higher ratios. Comparison of the images for the unstretched and stretched states (Figs. 8B and 8C, respectively. Color figures are available in the Supplementary Data Fig. S1) reveals a stark difference between the two. The substantially high Fl/RB ratio in the stretched samples allows for easy identification of uniform strain exerted upon an elastic polymer. It is noted that the textures of both images are not completely smooth, with many specks that represent small differences in Fl/RB ratio. These small variations might not be necessarily due to topographical changes in strain, but might be due to the unsmooth surface of the polymer or perhaps because of ununiform distribution of dye.

Finally, to demonstrate the ability to detect strain which is distributed in an ununiform fashion in...
elastomers, PDMS strips were fabricated with gradients in cross-linking and thus gradients in flexibility. Samples were prepared by adjusting oligomer/cross-linker ratio from 10:1 to 10:4, left to right, which should decrease flexibility in the same direction [15]. It was demonstrated before that such flexibility gradients in PDMS result in a gradual change in strain across the polymer [22]. Indeed, upon uniform stretching of the gradient sample, a change in the Fl/RB ratio can be seen, with the highest value of 1.8 in the flexible side decreasing to about 1.4 in the stiffer part, as seen in Fig. 9A (Color figures are available in the Supplementary Data Fig. S2). In contrast, the unstrained sample retains a uniform Fl/RB ratio. To visualize the change in strain across the samples, ratiometric pictures were produced using the same method described before. The differences between the two states are clearly visible, with the stretched sample displaying a gradual change in color, from regions of light grey on the left, to darker shades on the other end. The unstretched sample retains a fairly uniform black color across its entirety.

4. Conclusion
A method for evaluating and visualizing strain in elastic polymers using ET was developed. It was shown that ET can occur between Fl and RB in PDMS, resulting in a noticeable change in the emission profile of the two visible range dyes. This change was used to detect strain in samples that were stretched in a uniform fashion. Furthermore, by using simple image-acquiring and processing techniques, strain was also visualized clearly. The advantages of this method are that it relies on inexpensive, bio-compatible materials and a simple DSLR camera, with minimal image processing.

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