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BRIEF TECHNICAL NOTE

A Speckling Technique for DIC on Ultra-Soft, Highly Hydrated Materials

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Abstract

Background Digital image correlation is a useful tool in many engineering disciplines to measure and visualize deformation fields. Noteworthily, its successful application is critically dependent on a high-quality surface speckle pattern. While there are numerous standard techniques to apply such patterns, some materials require special techniques because of their unique surface properties.

Objective The goal of our technical brief is to introduce a speckling technique for ultra-soft and highly hydrated materials for which standard speckling techniques may not be suitable. We chose blood clot as our primary sample material.

Methods We identified polymer granules as an easy, fast, and inexpensive speckling material. To test its efficacy and applicability, we patterned blood clot with a 50:50 mix of black and white granules. Next, we conducted pure shear and mode-I fracture experiments to determine whether these granules produce a high-quality DIC pattern and whether their application alters the material's behavior.

Results We found that applying a 50:50 mix of black and white granules produced high-quality speckle patterns as evaluated via the mean image gradient and a digital image correlation simulator. Additionally, we found that applying granules to the samples' surfaces does not alter their material properties as measured via the material's stiffness, strength, work-to-fracture, and fracture toughness. We confirmed that our technique also works for other ultra-soft and highly hydrated materials by applying it to gelatin.

Conclusion In conclusion, we provide an easy, fast, and inexpensive speckling technique for ultra-soft, highly hydrated materials, such as blood clot and gelatin, which does not alter the materials' mechanical properties.

Keywords Digital image correlation · Blood clot · Soft tissues · Mechanical testing

Introduction

Digital image correlation (DIC) is an image processing technique used to capture and visualize solids' full-field deformations [1, 2]. Areas of application are plentiful and include civil engineering, mechanical engineering, aerospace engineering, and biomedical engineering [3, 4]. In these disciplines, DIC has proven useful across many spatial scales, for many strain rates, and for a highly diverse set of materials [5]. Regardless of the application, a key challenge is to create or apply a random, superficial speckle pattern to the material of investigation. It is through the successful correlation of this superficial surface pattern that the material's deformation is identified and visualized. To create such surface patterns, the experimentalist can choose between numerous standard techniques such as printing [6, 7], laser patterning [8], powder particle deposition [9, 10], and spray painting [11]. However, sometimes material-specific speckling techniques are required. For example, Scrivens et al. have used vapor thin-film rearrangement to apply a nano-scale speckle pattern [12]. In another example, Quino et al. have used tattoo paper and stamps to apply speckle patterns resilient to impact testing [13]. For an excellent review on the use of DIC, specifically with biological materials, we direct the reader to work by Palanca et al. [14].

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To study the impact of material heterogeneity on the mechanics of blood clot, we have recently turned to DIC to provide full-field deformation maps. However, as we did so, we faced the challenge of speckling our samples. That is, blood clot is a biological material arising from coagulating blood, akin to a hydrogel [15, 16]. As such, it is ultra-soft with shear moduli on the order of a few kPa. Additionally, it is very fragile with fracture toughness on the order of a few J/m^2 [17–19]. To make matters more challenging, it is also highly hydrated, with a water content of approximately 50% [20]. Because of its softness and highly hydrated state, none of our standard speckling techniques appeared appropriate. For example, applying ink speckles led to a diffuse pattern on the samples' wet surface, while other methods would likely damage or artificially stiffen the material. Thus, we began a quest for an inexpensive, easy-to-apply, and, most importantly, high-quality patterning technique that could be used to conduct DIC on ultra-soft and highly hydrated materials.

In our current work, we introduce such a patterning technique. Specifically, we show that superficially "sprinkling" our material of interest with a mix of black and white polymeric granules leads to a high-quality speckle pattern as evaluated through computing the mean image gradient (MIG) and a DIC simulator by Estrada et al [21]. We also demonstrate its usefulness using pure shear and mode-I fracture experiments with blood clot. Finally, we show that applying these polymeric granules does not alter the mechanics of blood clot; we do so by comparing the tangent stiffness, yield strength, work to fracture, and fracture toughness of blood clot with and without speckling.

Methods & Materials

Sample Preparation We sourced bovine blood from a commercial vendor (Lampire Biological Laboratories, Pipersville, PA). Within 72 hours of receiving the blood, we coagulated the blood into 3D-printed molds by adding calcium chloride ($CaCl_2$) to a final concentration of 20 mM. The molds were lined with Velcro into which the blood coagulates and through which we mounted the samples to our mechanical test equipment. Once mixed and transferred into the molds, we placed the samples into an incubator at 37 °C for 60 minutes. During coagulation, the samples were covered to avoid dehydration. The molds used in our current study yield samples with dimensions 40x10x3 mm^3. Please see extensive details on this process in our published protocols [22]. To evaluate the generalizability of our protocol, we also made pure shear samples with the same molds from 10 wt% gelatin (Sigma-Aldrich Cat #G9391) by dissolving the powder in 37 °C deionized water.

Speckle Selection & Technique Once the blood clot samples were fully coagulated, we removed them from the incubator and placed them on a hydrated surface. We divided the samples into two groups, samples of the first group received a 13 mm pre-cut for mode-I fracture testing, and the second group was not cut for pure shear experiments. Half of each type of sample was then manually speckled with one of three methods: i) a 50:50 mix of two-colored modeling sand (Just Artifacts, Cat# SND160018 and SND160002), ii) 100% white polymer granules (Ranger, Cat# EPJ36678), or iii) a 50:50 mix of black and white polymer granules (Ranger, Cat# EPJ36678 and EPJ37392). The other half of the samples were not speckled as controls. As to the actual speckling technique, we gently blotted the molds with a KimWipe to remove excess moisture without touching the blood clot. We then generously covered the wet sample surfaces with the speckling agent, ensuring that the entire sample was covered. Next, we carefully tilted the sample to remove excess sand/granules. In total, we prepared 35 samples (9 pure shear with speckling, 8 pure shear without speckling, 9 mode-I with speckling, and 9 mode-I without speckling), see Fig. 1. We followed the same protocol to speckle gelatin samples with the above 50:50 mix of black and white polymer granules.

Mechanical Testing Upon concluding the speckling process, we mounted our samples to our tabletop Instron device (Instron 5942, Norwood, MA) equipped with a 10 N load cell. During mechanical testing, we displaced the sample mounts at a rate of 0.1 mm/s, equivalent to a strain rate of 1%/s. For each sample, we collected displacement data, force data, and images at 5 Hz using a 5-megapixel black and white camera (iDS uEye, Stoneham, MA). Please note, that the camera and the Instron device were synchronized through a custom Labview VI (National Instruments, Version 21.0.1, Austin, TX) that we triggered using the Instron's external signal function. To test the adherence of our granules to the samples, we also cyclically loaded an additional set of speckled samples to a displacement of 3 mm (or equivalently, a stretch of 1.3) for a total of 10 cycles. Please note, unless indicated otherwise, we computed "stretch" for all subsequent analyses as $\lambda = h/H$, where h and H are the sample height in the deformed and undeformed configuration, respectively.

Digital Image Correlation During post-processing, we first quantified the speckle pattern quality via each image's MIG using the open-source software Glare [23] and via the open-source DIC simulator by Estrada et al. [21] Additionally, we correlated the images using the open-source DIC software Ncorr [24] as implemented in Matlab (MathWorks, Version R2022a, Natick, MA). For the correlation options, we chose a subset radius of 19 pixels and a subset spacing of 1 pixel. Additionally, we used the default iteration cutoff settings in

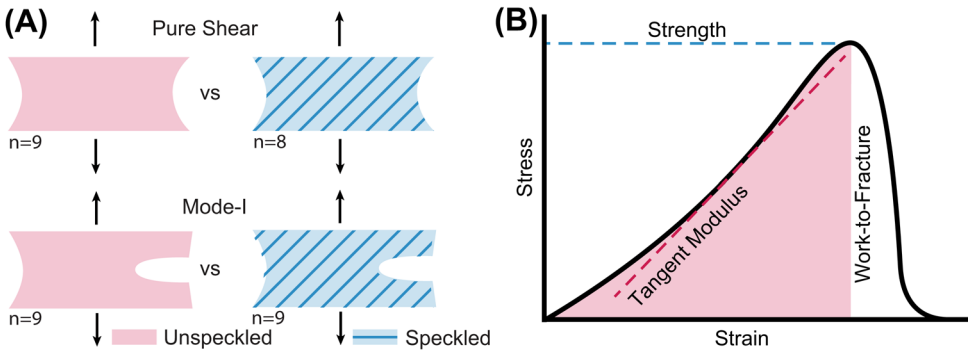

Fig. 1 (**A**) Blood clot sample allocation for pure shear and mode-I fracture experiments. Samples for each test were either speckled or not speckled to identify whether speckling impacted the samples' mechanical behavior. (**B**) Illustration of a stress-strain curve from a typical pure-shear experiment based on which we compute the samples' tangent modulus, strength, and work to fracture (we additionally compare fracture toughness, the computation of which is not illustrated here)

Ncorr (difference vector norm cutoff 1e-6, iteration count cutoff 50) and enabled high-strain analysis due to the large deformation applied to our samples. Lastly, we chose the seed propagation method with automatic propagation enabled and enabled subset truncation to avoid distortion near the crack tip of our notched samples. More details on these settings are available in the Ncorr manual [24].

Statistics To compare mechanical metrics derived from samples that were speckled and those that weren't speckled, we conducted two-tailed independent Student's t-tests in RStudio [25] (Version 2022.02.3). We defined statistical significance at a p-value of 0.05 and reported all metrics as mean ± standard deviation.

Results

Polymer Granules Provide a Reliable, High-quality Speckle Pattern Most importantly, a 50:50 mix of black and white polymer granules created a high-quality speckle pattern on blood clot. That is, it made a pattern with a mean image gradient (MIG) value of 20.13 ± 2.31 (n=9) [26]. We also used the DIC simulator by Estrada et al. [21] to test the quality of our speckle pattern. Thereby, we found that our pattern produces minor, single-digit errors under uniaxial tension, simple shear, and translation. We also found that our speckle pattern had a very low noise floor, as determined by conducting repeated correlations of a static sample and computing the average standard deviations between them. Specifically, across 189 repeated measurements, we found an average standard deviation of 5.0×10^{-4} for the stretch in the horizontal 1-direction and a standard deviation of 6.3×10^{-4} for the stretch in the vertical 2-direction. Additionally, we found that the pattern reliably adhered to the sample surface even under very large deformation (up to a stretch of at least 1.5) and after 10 loading cycles, see next section and Supplementary Fig. 1. Please note, that we also compared the mix of polymer granules to just white granules and to two-colored modeling sand. Neither alternative technique provided equally high MIG values (13.45 ± 0.95, n=5, and 9.63 ± 1.94, n=8, respectively). Please see Fig. 2 for randomly selected examples of speckle patterns from each group. Also, Fig. 3 shows the pure shear and mode-I

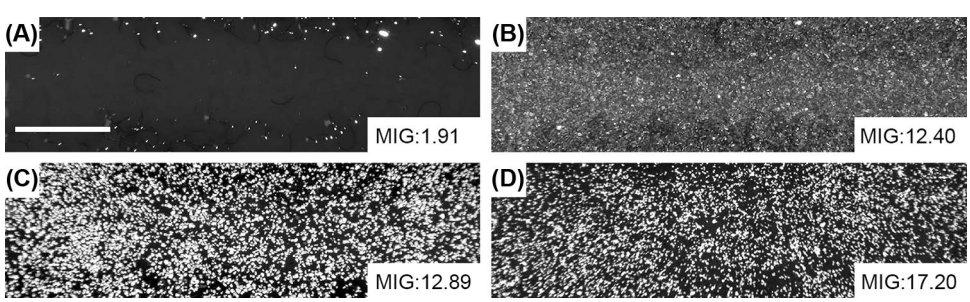

Fig. 2 Example speckle patterns and their mean image gradient (MIG), showing (**A**) bare blood clot without speckling, (**B**) speckling with two-colored sand, (**C**) speckling with white polymeric granules, (**D**) speckling with a 50:50 mix of black and white polymeric granules. Please note, the average diameter of the polymeric granules was 162.45 ± 52.09 µm as determined via optical microscopy

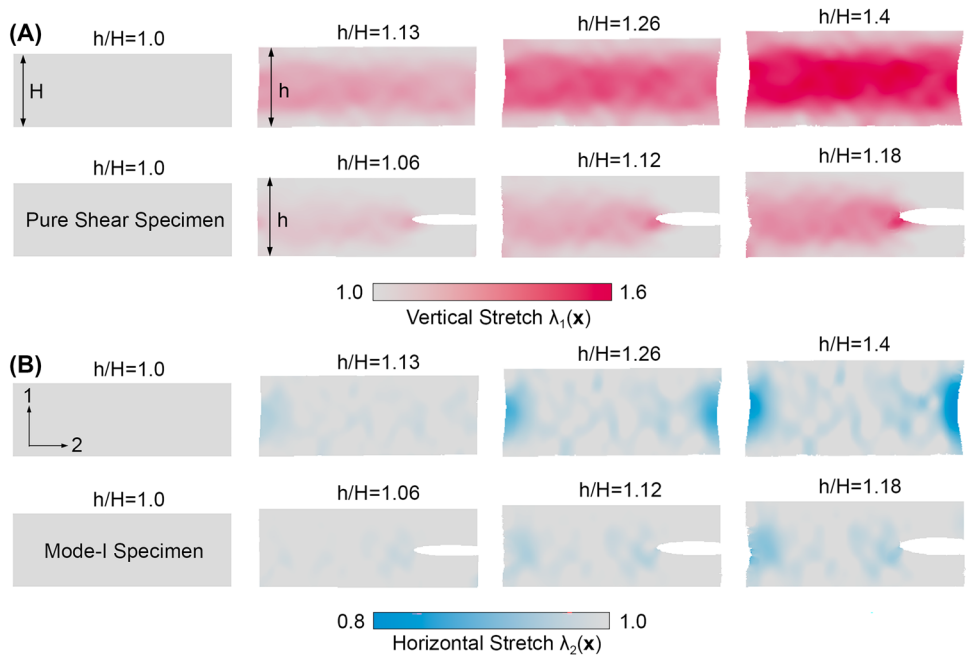

Fig. 3 Pure shear and mode-I deformation of 40x10x3 mm^3 blood clot samples visualized via digital image correlation. (**A**) Under pure shear, blood clot samples showed a mostly homogeneous strain field in the vertical 1-direction. On the other hand, the mode-I case showed high strains near the crack tip in that same direction. (**B**) In the 2-direction, the pure shear case showed some contraction at the outer edges; less so in the mode-I case

deformation fields for blood clot specimens using our speckling technique. Additionally, see Supplementary Fig. 2 for DIC results of gelatin, also under pure shear. Overall, our speckling pattern allowed for visualizing full-field deformation maps using DIC.

Speckling Blood Clot Does Not Affect Its Mechanics Because of the ultra-softness of blood clot, and other highly hydrated materials such as gelatin, our concern was that speckling could alter its mechanical properties. To this end, we conducted a control experiment comparing the mechanics of blood clot as a representative material with and without speckling. Specifically, we conducted pure shear experiments as well as mode-I fracture experiments. In total, we conducted 35 experiments (17 pure shear, 9 with speckling, and 8 without; 18 mode-I, 9 with speckling, and 9 without). Figure 4(A) shows the average stress-stretch curves for each sample group. Notably, before fracture, deviations

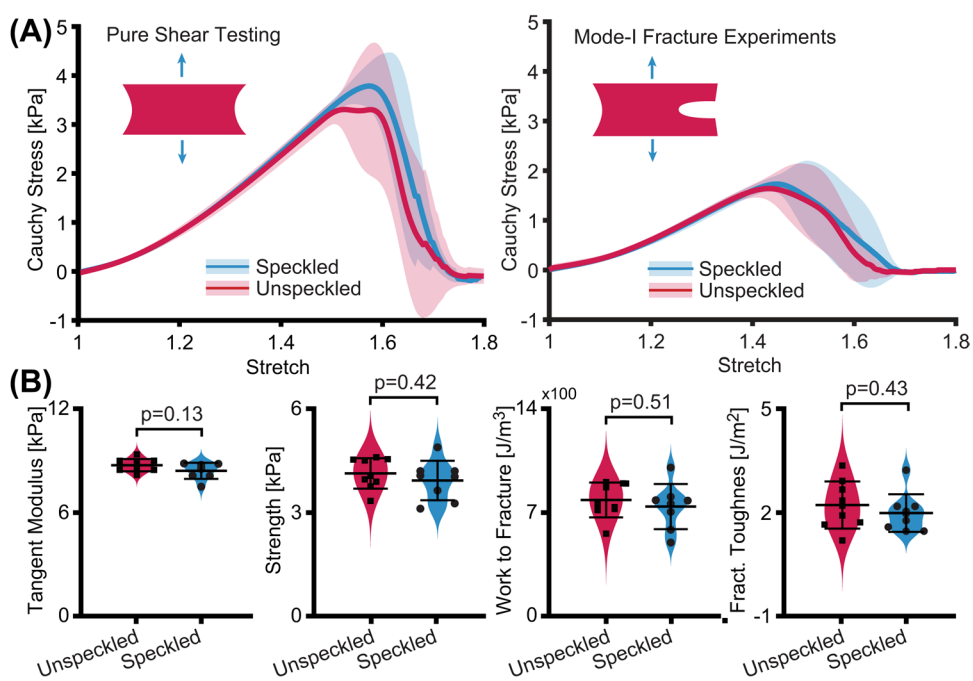

Fig. 4 Speckling blood clot with polymeric granules does not alter its mechanical properties. (**A**) Stress-stretch curves of both unspeckled and speckled samples during pure shear and mode-I fracture experiments. (**B**) Quantitative comparison of four metrics of blood clot mechanics. Please note that we used independent Student's t-tests to compare unspeckled and speckled samples. The violin plots show the mean ± standard deviation and a probability density estimate of the data

between samples were very small (as shown via negligible standard deviations). Once failure became imminent, variability between samples increased. However, qualitatively it appears as if there was no difference between samples, whether speckled or not, during both pure shear and mode-I fracture experiments.

Quantitatively, we compared several metrics derived from the above stress-stretch curves. For example, we compared blood clot's tangent modulus in the pure shear samples at a stretch of 1.35, blood clot's strength as the peak stress before failure, also in pure shear, as well as work to fracture and fracture toughness. To compute the latter, we used an energy-based approach through which we calculated $\Gamma_c = W(\lambda_c)H$, where $W(\lambda_c)$ is the strain energy of the pure shear sample at the fracture stretch λ_c, and H is the sample height in the undeformed configuration. Please note, λ_c was determined as the stretch at which the crack in the mode-I experiments first propagated. In contrast, the strain energy density function $W(\lambda)$ is that determined via separate pure shear experiments. Figure 4(B) shows a comparison between speckled and non-speckled samples with no apparent deviations. In fact, the p-values for Student's t-tests do not indicate that there is a significant difference between the two groups. Overall, no qualitative or quantitative differences appear between the mechanics of speckled and unspeckled samples.

Discussion

The goal of this technical brief was to introduce a simple, quick, and inexpensive speckling technique for ultra-soft, highly hydrated materials. Our work was specifically motivated by use with blood clot, where traditional speckling techniques would likely fail or alter the mechanical properties of our sensitive samples.

We identified thermoplastic granules that easily adhered to the wet surface of blood clots. Please note that we tried many other powders that did not yield satisfying results because they either clumped, wetted, or didn't produce sufficient contrast. Among them, we tested aluminum oxide, magnesium oxide, titanium oxide, and graphite. Using a 50:50 mix of black and white granules, we achieved a relatively high MIG, which we used as our primary quality measure for this experiment. While our actual MIG value fell below those reported using other speckling techniques on more traditional engineering materials, we found it to be highly effective in combination with the DIC software Ncorr. Specifically, we showed that Ncorr successfully correlated deformation fields up to very large deformations. As an additional means of quality control, we used the DIC simulator by Estrada et al. [21] to test our speckle pattern quality. Thereby, we found that our speckle pattern yielded low to negligible errors under simulated uniaxial extension, simple shear, and translation.

Importantly, we also showed that speckling our samples did not alter the mechanical properties of blood clot. While this may not be a concern with traditional engineering materials that tend to be very stiff, blood clot is very soft and fragile. Our concern was that external speckling could alter the samples' surface properties, which can play a critical role in very soft materials where surface energetics play an increased role [27, 28].

Although not the primary interest of our work, we would also like to highlight that our findings on blood clot mechanics closely match those reported by others. For example, our findings on thrombus stiffness match our past work as well as work by others [15, 16, 19]. Similarly, our findings on blood clot fracture properties also match others' work. Specifically, Liu et al. measured fracture toughness values of whole blood clot, again closely matching our findings in this current manuscript [17].

We would also like to briefly mention that we used the opportunity of this work to test multiple image processing techniques to investigate their potential ability to improve the speckle quality and, thus, the correlation quality. We implemented four image processing algorithms in Fiji/ImageJ: "Sharpen," "Contrast-Limited Adaptive Histogram Equalization (CLAHE)," "Gaussian" with a radius 0.25, and "Gaussian" with a radius 0.5 [29]. We found that while the MIG of processed images improved (up to a 1.5-fold increase), the runtime of the DIC algorithm worsened substantially (up to a 2-fold increase). For this reason, we chose to run DIC on our raw images.

While we tested our technique only on blood clot and gelatin, we don't see why it wouldn't work on other ultra-soft, highly hydrated materials. For example, we could see our technique finding use with synthetic materials such as hydrogels and other biological materials such as brain tissue that are very similar in softness and hydration state to blood clot and gelatin.

In conclusion, we introduced a simple speckling technique that enabled DIC on blood clot and gelatin as representatives for ultra-soft, hydrated materials. We also showed that this technique does not alter the mechanical properties of blood clot. Thus, we recommend others interested in conducting DIC on ultra-soft, hydrated materials to test our proposed methods.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11340-023-00938-x.

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Data Availability Our DIC images and mechanical raw data have been uploaded to https://dataverse.tdl.org/dataverse/bloodclotDIC and are openly available to the interested reader.

Declarations

Conflict of Interest Dr. Rausch has a speaking agreement with Edwards Lifesciences. The other authors have no conflicts to declare.

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