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PDMS aqueous leachates cause acute toxicity in *C. elegans*

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Polydimethylsiloxane (PDMS), often assumed to be biocompatible, is widely used in microfluidic devices and biomedical research. Here, we systematically assess the organismal effects of PDMS network components and their leachates using *Caenorhabditis elegans* as a whole-animal model. We demonstrate that uncrosslinked vinyl-terminated PDMS (v-PDMS) chains, which comprise the majority of a PDMS network and are known to diffuse into aqueous environments, cause acute, environmentally-dependent toxicity. Low-molecular-weight v-PDMS (6 kDa) caused mild lethality in nutrient-rich S-medium (SM) but significantly higher mortality in minimal S-buffer (SB), showing that media composition strongly influences toxic effects. Adding cholesterol, calcium, or magnesium notably reduced v-PDMS-induced lethality, whereas trace metals increased it. Using a DAF-16::GFP reporter strain, we show that cholesterol influences organismal stress responses to v-PDMS exposures. Progeny from starved parents showed full resistance to v-PDMS, suggesting transgenerational stress memory plays a role in reducing PDMS toxicity. We also find that linear siloxanes cause modest but significant lethality, whereas cyclic siloxanes do not. The crosslinker TDSS, however, provides partial protection when present with v-PDMS, revealing diverse biological effects among PDMS network precursors. Overall, these results show that PDMS-derived components are not universally harmless and that susceptibility depends greatly on environmental conditions, sterol levels, and physiological history. Our findings emphasize the importance of carefully evaluating PDMS formulations for biomedical use and offer a framework for assessing polymer leachate toxicity in living organisms.

1. Introduction

Biomaterials play a crucial role in the fabrication of medical devices, drug delivery systems, and microfluidics, where biocompatibility is a key concern. Polydimethylsiloxane (PDMS) is a widely used silicone-based polymer with unique chemical and physical properties that enable diverse uses. PDMS oils are also used as lubricants,[1] water barriers,[2] and in cosmetic products such as topical creams and shampoos.[3] PDMS can also be found in food, due to its anti-foaming properties.[3] PDMS can be cured into a solid network by using a crosslinking agent, often in the presence of a catalyst. These PDMS networks result in a transparent, soft elastomer often described as biocompatible, resistant to many solvents, chemically inert, and thermally stable.[4] These characteristics expand the material's applications to include coatings,[4,5] medical implants,[6,7] lenses,[8] and drug delivery systems.[9,10] PDMS is also the most commonly used material for fabricating microfluidic devices.[11,12]

Although PDMS elastomers are considered chemically stable, it is well documented that unreacted PDMS components can leach under certain conditions.[13–15] Breast implants have been reported to "bleed", meaning that short molecular weight silicone molecules leach out of the network into the patient's tissues.[7,15] In microfluidic devices, PDMS leaching has also been shown to influence cell culture outcomes, raising concerns about its potential biological effects.[14,16,17] These phenomena have been reviewed in the context of microfluidic culture, highlighting absorption of hydrophobic molecules, leaching, and altered cellular responses.[18] While PDMS is often regarded as biocompatible,[4] recent studies suggest that certain components in PDMS formulations can interact with biological molecules, modifying proteins and altering metabolic functions in studied organisms, including barnacle larvae and mammalian cells.[14,16,19–22] Despite its widespread use in the medical, cosmetic, and food industries (where PDMS has undergone safety and biocompatibility testing), the biological effects of PDMS-derived leachates under aqueous conditions, particularly at the whole-organism level, remain underexplored. Little is known about the impact of PDMS and its components on simple model organisms, which limits our understanding of the broader ecological and physiological consequences of their use.

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To address this knowledge gap, we investigated potential biological interactions between PDMS components and *Caenorhabditis elegans* (*C. elegans*), a widely used model organism in toxicology and developmental biology.[23–25] *C. elegans* is particularly well-suited to assess biomaterial toxicity due to its short lifecycle, transparency, and well-characterized metabolic and stress response pathways.[26] The organism's ability to respond to environmental stressors such as temperature, oxidants, xenobiotics, and osmotic challenges makes it an ideal system for evaluating how unreacted PDMS components affect development, behavior, and survival.[26,27] Additionally, *C. elegans* is frequently studied in PDMS-based microfluidic devices, making it particularly relevant for investigating PDMS interactions.[28–30] Here, we investigate the interactions between *C. elegans* and PDMS components leached into the culture medium. We assess toxicity using survival assays across different media backgrounds and test activation of the key transcription factor DAF-16, which participates in the stress response.[31,32] Our results reveal that these interactions are formulation-dependent and vary across PDMS components, with some exhibiting toxic effects while others appear protective. Moreover, we find that the composition of the culturing medium significantly modulates these outcomes, with nutrient-rich environments either mitigating or exacerbating the observed toxicity.

2. Experimental

2.1. Preparation of aqueous and growth media

S-Medium, S-buffer, M9, and nematode growth medium (NGM) were prepared according to standard *C. elegans* protocols, as described in WormBook.[33] S-Buffer was prepared by mixing 1 L of deionized water with 5.85 g NaCl, 1 g K_2HPO_4, and 6 g KH_2PO_4, then autoclaving. S-Medium was prepared by supplementing 1 L of S-buffer with 10 mL of 1 M potassium citrate, 10 ml of trace metals solution (1.86 g disodium EDTA, 0.69 g of $FeSO_4{\cdot}7H_2O$, 0.2 g $MnCL_2{\cdot}4H_2O$, 0.29 g $ZnSO_4{\cdot}7H_2O$ and 0.025 g $CuSO_4{\cdot}5H_2O$ per liter of deionized water), 3 mL of 1 M $CaCl_2$, 3 mL of 1 M $MgSO_4$ and 1 ml cholesterol (5 mg mL in ethanol). The M9 buffer was prepared by dissolving 3 g of KH_2PO_4, 6 g of Na_2HPO_4, 5 g of NaCl, and 1 mL of 1 M $MgSO_4$ in 1 L of deionized water, then sterilizing it by autoclaving. NGM was prepared by dissolving 3 g of NaCl, 17 g of agar, and 2.5 g of peptone in 975 ml of water, then autoclaving to sterilize. After cooling to at least 55 °C, the medium was supplemented with 1 mL of cholesterol (5 mg mL^{-1} in ethanol), 1 mL of 1 M $MgSO_4$, and 25 mL of 1 M KPO_4 buffer while vigorously stirring. The medium was then poured into individual presterilized Petri dishes using a peristaltic pump under sterile conditions.

2.2. Preparation of leachate solutions

Three distinct PDMS elastomer networks were prepared using DOW Sylgard-184 by mixing the elastomer base (part A) and curing agent (part B) at weight ratios of 5 : 1, 10 : 1, and 20 : 1 (A : B). Mixtures were vigorously stirred and subsequently degassed in a vacuum chamber for approximately 30 minutes at room temperature. The degassed mixtures were then poured into polystyrene Petri dishes and cured at 65 °C for 4 hours, following the manufacturer's recommendations. After curing, the solid PDMS networks were allowed to return to room temperature.

To enhance reproducibility and sensitivity, cured PDMS was crushed using a stainless-steel spice grinder before incubation, thereby increasing the polymer–water interfacial area relative to intact PDMS geometries. This was done to generate a high-surface-area leachate system, enabling assessment of responses under conditions of enhanced leaching. 10 g of each cured formulation were transferred into 15 mL conical centrifuge tubes, to which 5 mL of S-buffer was added. The mixtures were allowed to equilibrate at room temperature for 24 hours. Following incubation, the liquid was separated by filtering through a Nylon Corning cell strainer with 70 μm pores into clean tubes and used immediately for subsequent toxicity assays without further dilution.

Fragments of the shredded PDMS networks were measured by spreading them on the scanning surface of an Epson perfection V600 scanner at maximum resolution. The images were processed in ImageJ by removing noise with a band-pass filter and adjusting contrast and brightness to obtain individual and full fragments. The scale was set by scanning a 3 inch glass slide alongside the fragments. Then we used the "analyze particles" function of ImageJ to obtain a list of particle areas with a minimum size of 0.01 in ref. 2, as this was the threshold we identified as best for discriminating true particles from background noise (Fig. S3). Our studies on PDMS leachates have shown that analyzing hydrophobic silicone cyclic oligomers in aqueous media requires GC-MS. That leachate composition is dynamic, making it difficult to analyze. However, we have identified that the major components of PDMS leachates consistently include cyclic silicones (primarily D5 and D6), and linear silicones (broadly distributed fragments with either α,ω-hydroxy or α-hydroxy terminations).

2.3. Preparation of oil mixture solutions

All tested oil-based substances were prepared using a standardized equilibration protocol. In 15 mL conical centrifuge tubes, the aqueous medium (S-buffer or S-medium) and the oil phase were combined at a 10 : 1 (aqueous : oil) volume ratio. For mixed formulations such as TDSS/v-PDMS and the cyclic siloxane blend, the oils were first pre-mixed by gentle stirring before being added to the aqueous phase. All mixtures were then vigorously vortexed and sonicated for 3 minutes to enhance oil dispersion.

This procedure was applied identically to all experimental and control samples. Following mixing, the tubes were left undisturbed at room temperature for 24 hours to allow for phase separation. After incubation, the aqueous phase was

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carefully transferred to a new centrifuge tube, taking care to avoid any carryover of undissolved oil droplets. The resulting equilibrated media were used immediately for the assays.

2.4. Nematode culture and growth

For all survival experiments, we used *C. elegans* worms from the Bristol N2 wild-type strain. Worms were age-synchronized using a standard bleaching solution (2 parts 1 M NaOH, 1 part sodium hypochlorite, 1 part deionized water) and cultured on NGM agar seeded with *E. coli* OP50 at 20 °C for approximately 50 hours, or until they reached the L4 stage.[34] To prevent activation of epigenetic stress-response pathways, all worms used in these experiments were maintained well-fed for at least 5 generations (unless specified otherwise) by regular transfer to freshly seeded NGM plates every 2 days, ensuring continuous access to abundant bacterial food. Some strains were provided by the Caenorhabditis Genetics Center (CGC), which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440).

2.5. Toxicity assays

Once worms reached the L4 stage, they were washed off the plate with M9 buffer containing 0.01% Triton X-100 into a 1 mL conical tube, allowed to settle, and then transferred into a 24-well plate by pipetting 5 μL from the bottom of the tube. Each well contained 300 μL of either the oil equilibrated medium or the leachate solution of interest (Table 1). Each experiment included approximately 100 worms per biological replicate, distributed across three wells, with three biological replicates per experiment. In all leachate experiments, worms were exposed to the leachate solution alone, so that each well contained only the media pre-incubated with the indicated PDMS formulation, without further dilution or mixing. Worms were incubated on a rocker at 30 rpm for 24 hours to maintain constant agitation, after which survival was assessed. Worm viability was assessed with a standard gentle-touch assay; worms that failed to exhibit any movement in response to a gentle touch stimulus with a platinum wire pick were recorded as dead.[35,36]

2.6. DAF-16 imaging

To assess DAF-16 activation, we used the ASM10 strain, which has a GFP insertion at the 3′ end of the genomic *daf-16* locus (*daf-16*(del2[*daf-16::GFP-C1^3xFlag*])),[31] in an otherwise wild-type genetic background. Worms were cultured on NGM plates until the L4 stage, then transferred to a liquid suspension in 24-well plates 6 hours before imaging. After exposure, worms were collected into microcentrifuge tubes, allowed to settle, and transferred using a micropipette. A drop of worms was placed on 2% agarose pads on glass slides, and immobilization was achieved by adding a drop of 2 mM tetramisole in M9 buffer.

Images were collected on a Leica DMi8 microscope fitted with a CrestOptics X-light V2 spinning disk unit and a Hamamatsu Orca-Fusion camera, using a 63× objective lens. Illumination was provided by an 89 North LDI laser diode system. Acquisition parameters were kept identical across all samples: 60 ms exposure and 30% laser power. Image stacks consisted of 20 optical sections acquired at 1 μm intervals, and maximum-intensity projections of these *z*-stacks were used for analysis.

ASM10 expresses DAF-16::GFP in all tissues; therefore, the DAF-16::GFP fluorescence was quantified as area-normalized mean intensity. A binary mask corresponding to the worm body was generated for each image, and the sum of the pixel intensities within the mask was divided by the total number of "worm pixels", yielding an average fluorescence intensity per unit of worm area.

2.7. Chemicals

α,ω-Terminated polydimethylsiloxanes with molecular weights 6.0, 28.0, 49.5, and 62.0 kDa (v-PDMS),

Table 1 Summary of PDMS-derived formulations and exposure conditions used in this study

Condition name	Material equilibrated	Aqueous medium	Equilibration conditions	Purpose	Figure
Control	None	S-Buffer or S-medium	N/A	Baseline survival	Fig. 1–5
PDMS 5 : 1 leachate	Cured Sylgard 184 (5 : 1 A : B)	S-Buffer	2 g_{PDMS} mL^{-1}, 24 h	PDMS network leachate toxicity	Fig. 1a
PDMS 10 : 1 leachate	Cured Sylgard 184 (10 : 1 A : B)	S-Buffer	2 g_{PDMS} mL^{-1}, 24 h	PDMS network leachate toxicity	Fig. 1a
Sylgard A	Sylgard 184 part A	S-Buffer	10 : 1 aqueous : oil, 24 h	Component toxicity	Fig. 1b
Sylgard B	Sylgard 184 part B	S-Buffer	10 : 1 aqueous : oil, 24 h	Component toxicity	Fig. 1b
v-PDMS (6 kDa)	v-PDMS (6 kDa)	S-Buffer	10 : 1 aqueous : oil, 24 h	MW & Media-dependent toxicity	Fig. 2d
v-PDMS (28, 49.5, 62 kDa)	v-PDMS (28, 49.5 or 62 kDa)	S-Buffer or S-medium	10 : 1 aqueous : oil, 24 h	MW-dependent toxicity	Fig. 2b and e
v-PDMS + cholesterol	v-PDMS (6 kDa)	S-Buffer + cholesterol	10 : 1 aqueous : oil, 24 h	Cholesterol protection	Fig. 3a and c
v-PDMS + salts	v-PDMS (6 kDa)	S-Buffer + salts	10 : 1 aqueous : oil, 24 h	Ionic effects	Fig. 3a
v-PDMS + trace metals	v-PDMS (6 kDa)	S-Buffer + trace metals	10 : 1 aqueous : oil, 24 h	Metal interaction	Fig. 3a
Linear Siloxanes	MM/MDM/MD2M	S-Buffer	10 : 1 aqueous : oil, 24 h	Small-molecule toxicity	Fig. 4
Cyclic Siloxanes	D3 : D4 : D5 (1 : 8 : 1)	S-Buffer	10 : 1 aqueous : oil, 24 h	Cyclic siloxane toxicity	Fig. 4
TDSS	TDSS	S-Buffer	10 : 1 aqueous : oil, 24 h	Crosslinker effects	Fig. 5b
v-PDMS + TDSS	v-PDMS + TDSS	S-Buffer	10 : 1 aqueous : oil, 24 h	Crosslinker interactions	Fig. 5c

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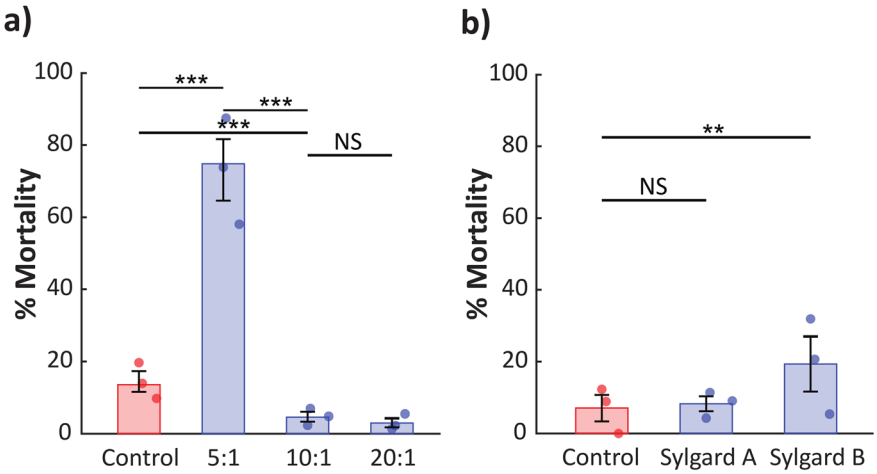

Fig. 1 Mortality of *C. elegans* larvae exposed to Sylgard 184 leachates. (a) Leachates from cured PDMS prepared at different base : curing agent ratios (5 : 1, 10 : 1, 20 : 1). (b) Media equilibrated with Sylgard 184 part A or part B. Data shown as mean ± SEM, with weighted averages indicated by star symbols. Significance determined by Fisher's exact test ($p < 0.05$, $*p < 0.01$, $**p < 0.001$). Approximately 100 worms per replicate per condition.

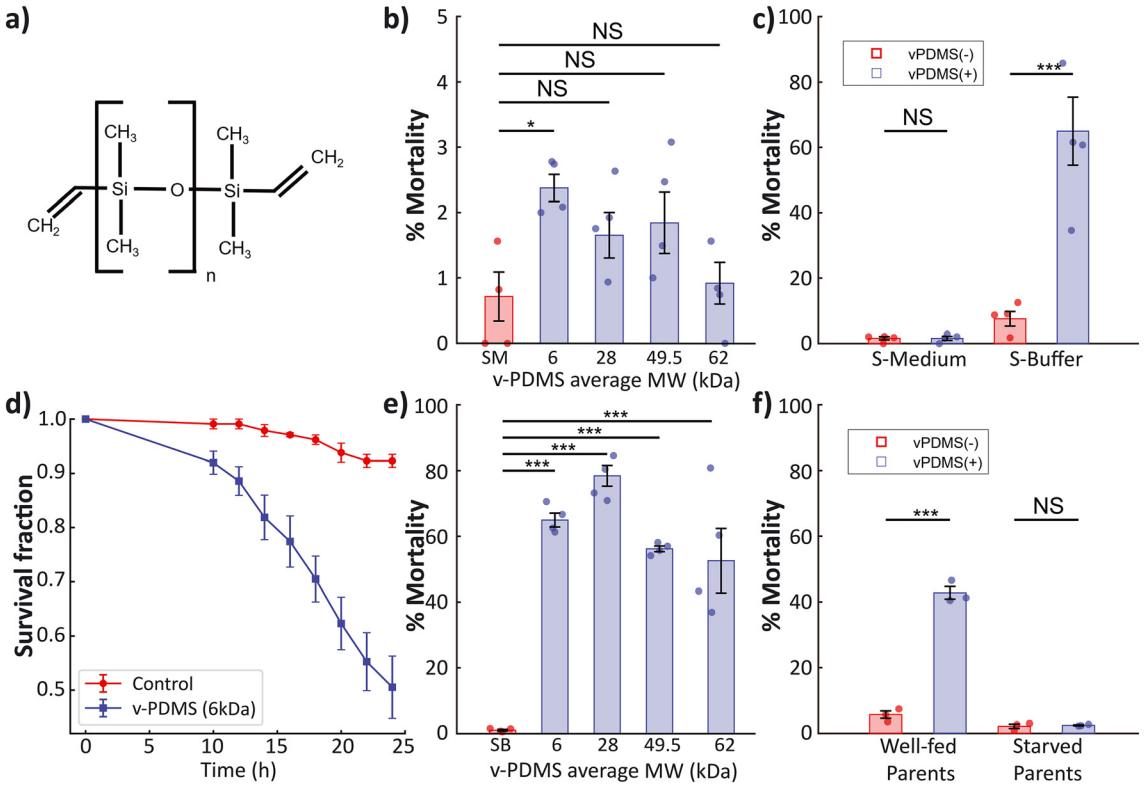

Fig. 2 Survival of *C. elegans* exposed to v-PDMS oils under different conditions. (a) Chemical structure of (α,ω-vinyl-terminated polydimethylsiloxane) v-PDMS. (b) Mortality with v-PDMS of different molecular weights when cultured in S-medium, was significant only for 6 kDa chains. (c) Increased lethality of 6 kDa v-PDMS in S-buffer compared to S-medium. (d) Survival curves of L4 cultured in S-buffer with and without v-PDMS. (e) All tested chain lengths induce mortality in S-buffer. (f) Progeny of starved worms show full resistance to 6 kDa v-PDMS. Data as mean ± SEM, weighted averages indicated by stars. Fisher's exact test with Bonferroni correction.

tetrakisdimethylsiloxysilane (TDSS), hexamethyldisiloxane (MM), octamethyltrisiloxane (MDM), decamethyltetrasiloxane (MD2M), hexamethylcyclotrisiloxane (D3), octamethylcyclotetrasiloxane (D4), and decamethylcyclopentasiloxane (D5) were purchased from Gelest Inc., and used as received.

3. Results

3.1. Toxicity of Sylgard 184 formulation

To assess whether unreacted components from a PDMS network leach into aqueous solutions at levels that might be

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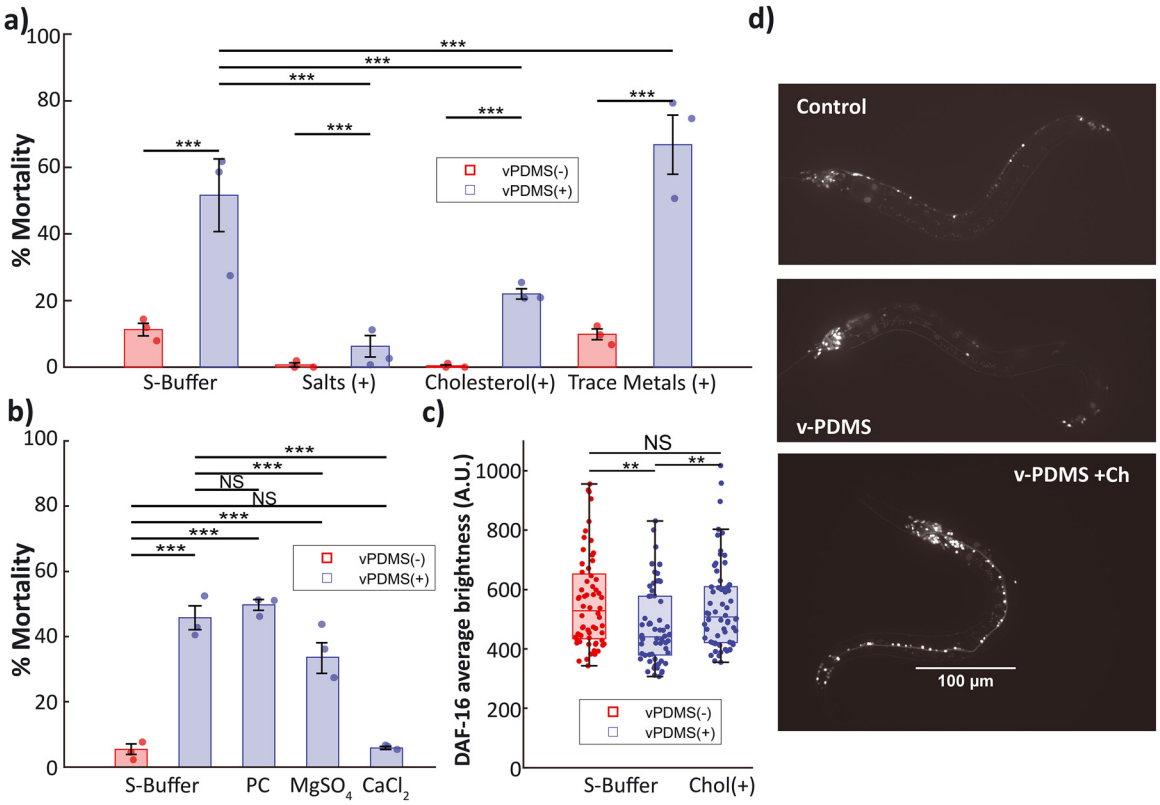

Fig. 3 Media components modulate v-PDMS toxicity. (a) Cholesterol and salt supplementation reduce mortality, while trace metals increase it. (b) Addition of individual salts: potassium citrate (PC) shows no effect; magnesium is partially protective; calcium is fully protective. Mean ± SEM; Fisher's exact test with Bonferroni correction. (c) DAF-16::GFP expression is reduced after v-PDMS exposure in the absence of cholesterol. Data analyzed by two-way ANOVA. (d) Example images of DAF-16::GFP expression.

toxic, we exposed *C. elegans* to aqueous leachates from cured Sylgard 184 at three different base-to-curing agent (A : B) ratios: 5 : 1, 10 : 1, and 20 : 1. Sylgard 184 is a silicone commercial formulation that contains 30–60 wt% of fumed silica to improve mechanical properties of cured elastomer films. The A : B = 10 : 1 ratio is recommended by the manufacturer.[37] Henceforth, we refer to Sylgard 184 as PDMS and ignore the effects of fumed silica and other fillers, as well as processing improvement components. The alternative formulations were selected for two reasons: (1) to simulate improperly mixed elastomers with a localized excess of one component, and (2) to reflect the common practice of modifying PDMS ratios to adjust mechanical properties, with higher curing agent content yielding stiffer features[38,39] and higher base content yielding more flexible ones.[40–42] The cured elastomers were shredded to average sizes of 3.72 ± 0.08 mm, 3.68 ± 0.08 mm, 3.24 ± 0.08 mm for 5 : 1, 10 : 1, and 20 : 1 PDMS compositions respectively (Fig. S3), and incubated in a buffer solution suitable for handling worms (S-buffer) at a ratio of 2 g of PDMS per 1 mL of buffer for 24 hours at room temperature. After incubation, the aqueous phase was filtered to remove any residual particulate matter. The resulting leachate solutions were then used as culture media for *C. elegans* L4 larvae, simulating the conditions the animals might encounter during exposure to PDMS-based microfluidic devices. Worm viability was assessed 24 hours post-exposure. Because survival was assessed after 24 h of exposure, all mortality measurements reported here reflect acute toxicity rather than longer-term or chronic effects. Surprisingly, we found that L4 *C. elegans larvae* cultured in S-buffer pre-incubated with 5 : 1-cured PDMS, exhibited

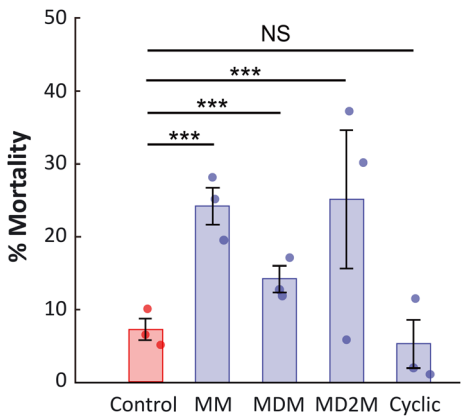

Fig. 4 Toxicity of short siloxanes. Exposure to linear siloxanes (MM, MDM, MD2M) slightly but significantly increases mortality, while cyclic siloxanes (D3 : D4 : D5, 1 : 8 : 1) do not differ from controls. Mean ± SEM. Fisher's exact test with Bonferroni correction.

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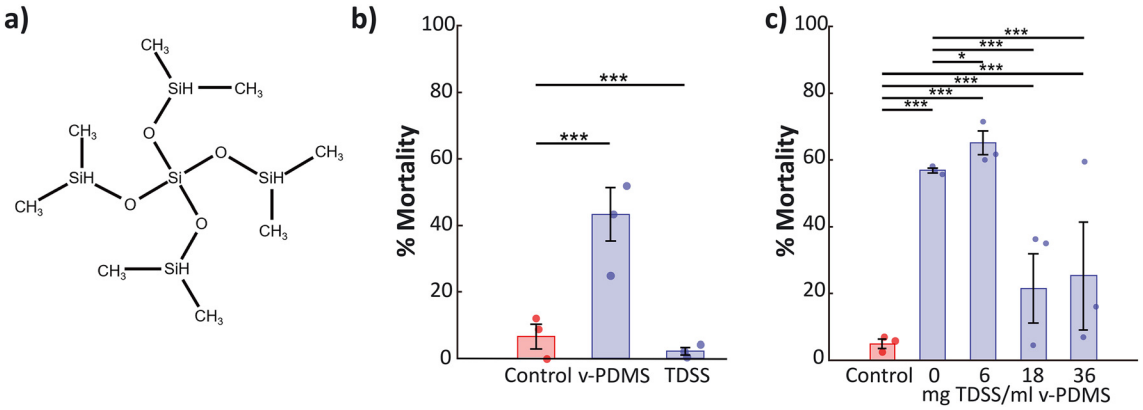

Fig. 5 Effects of TDSS crosslinker on *C. elegans* survival. (a) Chemical structure of tetrakis-(dimethylsiloxy)-silane (TDSS). (b) TDSS alone reduces mortality compared to S-buffer control. (c) TDSS attenuates v-PDMS toxicity in a dose-dependent manner. Mean ± SEM, with star symbols for weighted averages. Fisher's exact test with Bonferroni correction.

significant mortality. In contrast, no mortality was observed in worms exposed to leachates from PDMS cured at 10 : 1 or 20 : 1. In fact, survival was higher than in control conditions (Fig. 1a).

These results suggest that the toxic leachates observed in PDMS cured with excess curing agent originate from components present in Sylgard 184 component B, as only the condition with elevated proportions of component B resulted in increased worm mortality. Conversely, the improved survival observed in groups exposed to leachates from PDMS cured with excess elastomer base suggests that protective components responsible for this effect are likely present in Sylgard-184 part A. To test whether the individual components of Sylgard 184 could induce similar effects to those of the leachates from cured elastomers, each component was mixed separately with the aqueous medium and incubated for 24 hours. The aqueous phase was then collected and used to culture *C. elegans* larvae. Consistent with the findings from the cured PDMS leachate experiments, exposure to media incubated with Sylgard 184 elastomer base (part A) did not induce mortality in *C. elegans*, and survival rates were comparable to those of the control group. However, exposure to part B resulted in a significant increase in lethality; unexpectedly, the effect was less pronounced than that observed with leachates from the 5 : 1-cured PDMS network (Fig. 1b). These differences may indicate that specific components in the uncured mixtures become bioactive only upon network formation.

3.2. v-PDMS toxicity depends on molecular weight and media components

(α,ω-Vinyl-terminated polydimethylsiloxane) v-PDMS chains (Fig. 2a) constitute the main ingredient in PDMS formulations. In Sylgard 184, these chains make up more than 60% of part A, and about 15–40% of the mass of part B,[43] and have been shown to leach into culture media from microchannels.[14] To evaluate the potential bioactivity of v-PDMS leachates in *C. elegans*, we exposed L4-stage larvae to aqueous media (S-medium) that had been equilibrated with v-PDMS of varying average molecular weights, following the same mixing preparation protocol described in the previous section. Initial results showed that exposure to low-molecular-weight v-PDMS (6 kDa) induced mild toxicity in worms cultured in S-medium. In contrast, higher-molecular-weight variants produced no significant effects (Fig. 2b). To determine whether components of the used aqueous media could contribute to v-PDMS toxicity, we compared S-medium and S-buffer as aqueous solutions. S-Buffer is a minimal medium typically used for short-term handling or stress assays that contains potassium phosphate buffer and NaCl. S-Medium contains S-buffer in addition to cholesterol, potassium citrate, and other salts, as well as trace metals that support bacterial growth and worm development. We hypothesized that culturing worms under more nutritionally limited conditions and higher temperatures could heighten their sensitivity to v-PDMS. Increasing the temperature to 25 °C did not significantly affect survival (Fig. S1). However, substituting S-medium with S-buffer resulted in a marked increase in v-PDMS-induced lethality (Fig. 2c). Time-course assays further confirmed that 6 kDa v-PDMS causes a progressive decline in worm survival, with significant mortality observed within 24 hours of exposure (Fig. 2d). Given the results of these assays, all experiments in this study were performed using S-buffer as the culture medium unless otherwise specified. To further investigate the effect of v-PDMS molecular weight, we repeated the exposures using S-buffer as the culture medium. Under these conditions, all tested v-PDMS molecular weights exhibited substantially higher toxicity than in experiments with S-medium. However, a clear relationship between molecular weight and lethality was no longer observed (Fig. 2e).

While performing these experiments, we observed that all worms derived from recently thawed populations survived exposure to v-PDMS in biological replicates. Given that *C. elegans* exhibits transgenerational stress resistance following starvation,[44,45] we hypothesized that differences in developmental history could explain differences in mortality

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between populations. To test this idea, we compared two populations: one well-fed for 5 generations and another derived from a population that had experienced starvation during the L1 stage. We found that the progeny of starved worms exhibited complete resistance to v-PDMS toxicity (Fig. 2f). This finding suggests that the harmful effects of v-PDMS can be attenuated through endogenous stress-resistance pathways that can be activated *via* transgenerational epigenetic inheritance. To ensure consistency, and based on this finding, all the other experiments presented here were conducted using worm populations that had been well-fed for at least five generations.

3.3. Calcium, magnesium, and cholesterol account for the media-dependent resistance to v-PDMS

To determine the cause of the increased toxicity observed in S-buffer compared to S-medium, we conducted toxicity experiments using modified variations of S-buffer supplemented with different S-medium components. S-Buffer serves as the base solution for S-medium but lacks essential components necessary for the normal development of *C. elegans* and the growth of its bacterial food source, *E. coli.*[33] To identify which components of S-medium contribute to the reduced toxicity observed relative to S-buffer, we supplemented S-buffer individually with each of the major S-medium additives (cholesterol, essential salts, and trace metals) and assessed worm survival following exposure to media incubated with 6 kDa v-PDMS using the same preparation and exposure protocol described previously. Since the base formulation for all liquid media used here is S-buffer, all have similar pH (Table S1). When the worms were cultured in S-buffer supplemented with cholesterol, a significant decrease in toxicity was observed, suggesting that cholesterol protects against v-PDMS-induced toxicity (Fig. 3a). However, the presence of trace metals in S-medium had the opposite effect, enhancing the toxicity of v-PDMS leachates rather than mitigating it (Fig. 3a). S-Medium contains magnesium sulfate and calcium chloride, essential minerals for *C. elegans* long-term viability,[33] and potassium citrate as a chelating agent and buffer. The added salts reduced the toxicity to a minimum, indicating that the lack of essential salts is the primary factor contributing to the increased sensitivity to v-PDMS observed in S-buffer compared to S-medium (Fig. 3a). To further assess the individual contributions of each salt, we tested them separately. As expected, due to its minor contribution to metabolic support, the addition of potassium citrate to S-buffer did not change mortality.

In contrast, magnesium sulfate partially increased survival, whereas calcium chloride provided complete protection against the toxic effects of v-PDMS (Fig. 3b). As described earlier, cholesterol also emerged as a key protective factor. In addition to being crucial for development and reproduction, cholesterol plays an important role in *C. elegans* by enabling the synthesis of sterol-derived hormones required for activation of DAF-16, a central modulator of the stress response.[46,47] Because cholesterol is required for the activation of DAF-16 we then asked whether the protective effect of cholesterol against v-PDMS involved activation of DAF-16 signaling. We hypothesized that the absence of exogenous cholesterol impairs the activation of a DAF-16 response to v-PDMS exposure. To further investigate how cholesterol confers this protective effect, we used a transgenic *C. elegans* strain expressing fluorescently labeled DAF-16 protein fused to Green fluorescent protein (GFP) and quantified its abundance under different conditions. We found that worms exposed to v-PDMS without cholesterol supplementation showed a decrease in overall DAF-16::GFP reporter levels compared to control groups with either cholesterol or no v-PDMS exposure (Fig. 3c and d). This reduction in reporter signal may reflect altered DAF-16 activity or stability under sterol-limited conditions. However, we did not observe significant differences in nuclear translocation of DAF-16::GFP between these groups (Fig. S2), suggesting that the observed effect is more likely related to reporter abundance rather than canonical nuclear localization dynamics.

3.4. Toxicity of short PDMS oligomers and cyclic siloxanes

PDMS elastomer formulations can include a variety of small linear and cyclic organosilicone compounds, which differ in their potential leachability and bioactivity. Among the short linear siloxanes, formulations may contain hexamethyldisiloxane (MM), octamethyltrisiloxane (MDM), and decamethyltetrasiloxane (MD2M), which vary in chain length and solubility. Additionally, cyclic siloxanes, such as hexamethylcyclotrisiloxane (D3), octamethylcyclotetrasiloxane (D4), and decamethylcyclopentasiloxane (D5), are often present.[20] These compounds are products of the synthesis process[3,4] or are added as processing aids and plasticizers, and have been identified as a cause of cellular death.[22]

To assess the potential biological effects of additional PDMS components, we tested the effects of linear and cyclic siloxanes. The siloxanes were equilibrated in S-buffer following the same protocol used in previous sections for v-PDMS exposures. Linear siloxanes were tested independently, while the cyclic siloxanes (D3, D4, and D5) were evaluated as a mixture. Cyclic siloxanes were tested as mixtures because D3 is not liquid at room temperature and requires dissolution, making it impractical to test in isolation. Additionally, these cyclic siloxanes are commonly present in a 1 : 8 : 1 molar ratio (D3 : D4 : D5) in PDMS formulations.[20] By maintaining this ratio, we aimed to replicate the typical composition of cyclic siloxanes present in elastomer formulations and assess their collective impact on *C. elegans*.

The results revealed that the linear siloxanes tested exhibited statistically significant toxicity compared to the control group, suggesting that these compounds may

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contribute to the overall negative biological impact of PDMS leachates (Fig. 4). In contrast, exposure to the cyclic siloxane mixture (D3 : D4 : D5, 1 : 8 : 1 molar ratio) did not result in any observable difference in survival compared with the control, indicating that these compounds, under the tested conditions, do not contribute to the toxic effects observed with other PDMS components.

3.5. Protective effects of tetrakis-(dimethylsiloxy)-silane crosslinker

Another essential component of a PDMS network is the crosslinker.[11,43,48] Previous studies have reported that the crosslinker tetrakis-(dimethylsiloxy)-silane (TDSS) (Fig. 5a) exhibits acute toxicity in other organisms, particularly when combined with v-PDMS oils.[20] To evaluate its potential bioactivity in *C. elegans*, we equilibrated TDSS in S-buffer using the same protocol described for other PDMS components and exposed L4-stage larvae to the resulting solution. Surprisingly, worms exposed to TDSS alone consistently exhibited lower mortality rates than control groups cultured in plain S-buffer (Fig. 5b), suggesting a possible protective role for the compound under these conditions. Intrigued by this outcome, we proceeded to mix v-PDMS with TDSS (which do not react in the absence of a catalyst) at increasing TDSS concentrations. Remarkably, we observed a dose-dependent protective effect, where higher concentrations of TDSS were associated with increased worm survival, even in the presence of v-PDMS (Fig. 5c).

These findings suggest that TDSS may counteract or neutralize some of the toxic effects induced by low-molecular-weight v-PDMS chains, though the underlying mechanism remains unclear. One possibility is that TDSS interacts physically or chemically with toxic species in solution, reducing their bioavailability or altering their uptake. Alternatively, TDSS itself could activate a protective response pathway in *C. elegans*, as we observed with calcium chloride and cholesterol, especially given that the TDSS-treated group consistently outperformed the control group in survival. To determine whether TDSS acts through sequestration of toxic species, modulation of their uptake, or induction of a protective response, further mechanistic studies are required.

4. Discussion

PDMS is widely used in biomedical applications, microfluidics, and consumer products. Yet, its assumed biocompatibility remains largely untested in whole-organism models, such as *C. elegans*. Our findings reveal that v-PDMS chains that dissolve in aqueous solutions induce acute toxicity in *C. elegans* larvae. When the media provides access to all essential nutrients (S-medium), larvae do not experience significant detrimental effects from PDMS leachates. However, when cultured in a suboptimal medium (S-buffer), leachates from an undercrosslinked PDMS (5 : 1) networks can induce death in nematode larvae, suggesting that environmental factors play a critical role in modulating PDMS toxicity. Supplementation with $CaCl_2$ and $MgSO_4$ significantly improved survival rates. Since NaCl accounts for >99% of the ionic concentration in S-buffer,[49] the differences are unlikely to come from osmotic strength. The protective role of Ca and Mg is likely to stem from their biological roles in maintaining neuronal function,[50] muscle activity,[51] enzymatic reactions, and cellular homeostasis.[52,53] Proper signaling that requires Ca and Mg enables responses to environmental changes, including locomotion, feeding, and avoidance. Additionally, calcium signaling is important for activating stress response gene expression programs and maintaining cellular homeostasis.[54,55] Additionally, magnesium is crucial for cellular metabolism and stabilizes membrane integrity and ion channels,[52] which could help worms regulate osmotic balance and maintain normal physiological function in varying environmental conditions.

We observed that cholesterol supplementation led to increased resistance to v-PDMS. Cholesterol is indispensable for *C. elegans* development and reproduction.[46,56] Sterol-derived hormones regulate stress-responsive gene networks of the DAF family,[57,58] and cholesterol is essential for activation of the master stress regulator DAF-16.[59] We found that v-PDMS exposure can activate DAF-16, an effect that is significantly reduced in the absence of cholesterol. The transgenerational resistance observed in worms whose ancestors experienced early-life starvation further supports a role for DAF-16 mediated stress adaptation against PDMS toxicity, as this pathway is involved in transgenerational inheritance of hormetic stress.[31,45,60] Alternatively, cholesterol deprivation may alter membrane integrity and permeability,[56] facilitating the entry of hydrophobic PDMS-derived molecules into cells and thereby amplifying toxicity. The effects of cholesterol deprivation may be further exacerbated by the sequestration of sterol-derived hormones by PDMS present in the medium.[14]

Trace metals are essential for cellular function[61] but can be toxic homeostatic regulation is disrupted.[62,63] In our assays, trace metals significantly enhanced the toxicity of PDMS leachate. Under standard culture conditions, worms are exposed to trace metals in the presence of bacterial food, which could reduce the bioavailability of these species. Our food-free exposure setup could lead to elevated free trace metal concentrations, thereby exacerbating physiological stress and increasing susceptibility to v-PDMS-induced toxicity. Interestingly, when worms were cultured in v-PDMS-equilibrated S-medium, which contains the same trace metal concentration as supplemented S-buffer, they did not exhibit increased mortality. This suggests that the protective components of S-medium ($CaCl_2$, $MgSO_4$, and cholesterol) are sufficient to counteract the combined effects of trace metals and v-PDMS.

Aside from the functionalized v-PDMS, we identified that short linear siloxanes induced a small but statistically significant increase in lethality. Cyclic siloxanes (D3, D4, D5) have been shown to induce cell death in human cell lines,[22]

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yet no significant toxicity was observed in *C. elegans* under the conditions tested here. The protective effect of TDSS was unexpected, and the mechanisms underlying this phenomenon remain unclear. While previous studies have reported the acute toxicity of TDSS in other organisms, particularly when combined with v-PDMS (M_w = 17 kDa),[20] our results show that TDSS alone is not toxic to *C. elegans* and instead enhances survival. Future research should explore whether TDSS influences PDMS bioavailability, adsorption properties, or interactions with biological membranes, which could help explain the observed increase in survival.

Overall, our study provides new insights into the biological interactions between *C. elegans* and PDMS-derived compounds, revealing that while v-PDMS and certain linear siloxanes exhibit toxicity, their effects are modulated by environmental conditions and access to essential nutrients. Perhaps most unexpectedly, TDSS displayed a protective effect against v-PDMS toxicity, highlighting the complexity of PDMS-related interactions in biological systems. It is important to note that the toxic effects identified here are specific to leachates produced by contact with high-surface-area PDMS particles. The effects could be milder or stronger in actual microfluidic applications, as the leachate composition will depend directly on contact time and surface area. Given the widespread use of PDMS in biomedical and consumer applications, these results underscore the need for further research into the long-term effects of PDMS exposure and the mechanisms underlying its interactions with biological systems. Future studies should investigate whether these findings extend to other organisms, explore potential metabolic disruptions beyond acute toxicity, and determine the broader implications of PDMS leachate exposure in real-world settings.

Conflicts of interest

There are no conflicts to declare.

Data availability

Data for this article, including numbers for all bar plots, are available at Dryad at 10.5061/dryad.1zcrjdg6k.

Supplementary information (SI) is available. See DOI: https://doi.org/10.1039/d6lc00017g.

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