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Inhibition of polymerase chain reaction by hydrogel monomers

Nguyen Anh Nhung Tran [b], Chansik Oh [a], Isaac Jang [a], Seung Hyun Shin [a], Sangmin Lee [a], Bhargav Krishna Pullagura [c], Bumsang Kim [a], Dohyun Kim [c,*], Minsub Chung [a,*]

[a] Department of Chemical Engineering, Hongik University, Mapo-gu, Seoul 04066, South Korea
[b] Department of Biochemistry, Can Tho University of Medicine and Pharmacy, Can Tho, Viet Nam
[c] Department of Mechanical Engineering, Myongji University, Yongin-si, Gyeonggi-do 17508, South Korea

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ABSTRACT

Hydrogels are increasingly being integrated into polymerase chain reaction (PCR)-based diagnostic platforms because of their biocompatibility and ability to be compartmentalized at the microscale. However, the direct effect of hydrogel monomers on PCR performance is not fully understood. As such, we systematically evaluated the inhibitory effects of commonly used hydrogel monomers, including acrylamide, poly(ethylene glycol) dimethacrylate (PEGDMA), ethylene glycol diacrylate (EGDA), ethylene glycol dimethacrylate (EGDMA), and gelatin methacryloyl (GelMA), on Taq polymerase activity and amplification efficiency. Our results revealed that even low concentrations of PEGDMA and acrylamide strongly inhibited the PCR, whereas GelMA and EGDMA minimally interfered with PCR. The results of mechanistic studies suggested that the α,β-unsaturated carbonyl groups in monomers inactivate the polymerase through covalent interactions with nucleophilic amino acids. Various PCR enhancers were evaluated to address this issue. Nonionic surfactants with low critical micelle concentrations, such as Tween 20, Tween 80, and NP-40, successfully restored PCR amplification under PEGDMA-rich conditions. In contrast, additives such as dimethyl sulfoxide and Triton X-100 were ineffective. Using excess Taq polymerase mitigated the acrylamide-induced inhibition, supporting direct monomer–enzyme interactions. These findings provide molecular insights into hydrogel–PCR compatibility and can be used to guide the development of strategies for developing robust hydrogel-integrated PCR systems for diagnostics and genomics.

1. Introduction

Polymerase chain reaction (PCR) was developed by Kary Mullis in 1985. PCR is a molecular biology technique that exponentially amplifies specific DNA sequences from small amounts of template DNA [1]. PCR is applied in numerous fields such as clinical diagnostics, environmental monitoring, forensics, and molecular biology. Combining PCR with solid-phase or compartmentalized environments to enhance throughput, reaction isolation, and compatibility with downstream analysis has received considerable interest as PCR platforms have become miniaturized and integrated [2].

Hydrogels are composed of hydrophilic polymer networks with tunable mechanical and chemical properties, showing promise as candidates for embedding PCR in microscale formats. Synthetic hydrogels based on poly(ethylene glycol) diacrylate (PEGDA), polyacrylamide, gelatin methacryloyl (GelMA), and methacrylated hyaluronic acid (MeHA) provide three-dimensional semipermeable matrices that support localized amplification, increase signal retention, and minimize the diffusion of PCR products [3–5]. Choi et al. developed a PEGDA-based micropost array system that enabled spatially separated quantitative PCR (qPCR) reactions for multiplexed miRNA detection, which was highly specific without cross-reactivity [5]. Yi et al. designed nanoporous PEG hydrogels for directly performing digital PCR on untreated samples through physically excluding inhibitors while allowing the diffusion of the target DNA and reagents [4]. Polyacrylamide gels have also been used for solid-phase PCR, where immobilized primers allow the amplification of single DNA molecules into discrete “polonies” within the gel matrix, enabling digital genotyping and haplotyping [3]. Systems such as EpicPCR encapsulate single cells in acrylamide-based microgels to link taxonomic markers with functional genes via *in situ* amplification [6]. Despite these promising applications, the inhibitory interactions between hydrogel components and PCR reagents,

* Corresponding authors.
E-mail addresses: dohyun.kim@mju.ac.kr (D. Kim), minsubc@hongik.ac.kr (M. Chung).

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particularly polymerases, remain poorly understood and underexplored.

Hydrogel monomers, such as acryl- and methacryl-functionalized compounds, possess electrophilic α,β-unsaturated carbonyl groups that react with nucleophilic sites in enzymes or nucleotides via Michael-type addition [7]. This reaction results in polymerase inactivation or template modification, which completely inhibit amplification. The surfaces of materials such as polydimethylsiloxane (PDMS), glass, and SU-8 varied in their adsorption behavior of Taq polymerase; however, these findings were not validated with thermal cycling [8]. Our study focused on the inhibitory effects of hydrogel materials, although various materials have been tested for PCR compatibility [8]. Moreover, additives such as bovine serum albumin (BSA) and betaine as well as surfactants such as Tween 20 increase amplification efficiency through stabilizing polymerases or minimizing reagent adsorption [9,10].

We systematically evaluated the inhibitory effects of hydrogel monomers including acrylamide, PEGDMA, EGDA, EGDMA, GelMA, and MeHA on PCR amplification. We investigated the mitigating roles of various enhancers, such as BSA, DMSO, and nonionic surfactants, with different critical micelle concentrations (CMCs) in these effects. Our findings demonstrate the structure-dependent impact of monomers on the PCR yield and can be used to develop strategies to overcome this inhibition, offering insights into the design of hydrogel-integrated PCR platforms for diagnostics and molecular analysis.

2. Materials and methods

2.1. Materials and reagents

Deionized water (18.2 MΩ·cm) was used to prepare the solutions (Model LA621, Intertek). The monomers, including 40 % acrylamide (product number #A4058), 90 % EGDA (#2214–11–5), 98 % EGDMA (#97–90–5), PEGDMA (average Mn ~550, #409510), MeHA (#914,568), GelMA with a 40 % substitution degree (#900,629), PCR enhancers such as BSA (#A2153), gelatin from porcine skin (#G2500) and ethidium bromide solution (#E1510), were purchased from Sigma-Aldrich. GelMA with a 75 %–80 % substitution degree (#SKU0010) was obtained from Gelomics (Australia). The lambda DNA template (#D-2510), Taq polymerase (#E-2011–1), Pfu polymerase (#E-2015–1), 100 bp DNA ladder (#D-1030), 1 kbp DNA ladder (#D-1040), and 6x agarose gel loading buffer (#C-9020) were purchased from Bioneer (Daejeon, Korea). The forward and reverse lambda DNA primers (GAAGCGTTTATGCGGAAGAG and TGACTCCTGTTGATAGATCCAGT, respectively) were synthesized by Macrogen (Korea). Certified molecular biology agarose (#1613102), a wide mini-sub cell GT horizontal electrophoresis system, 15 × 10 cm tray, and Powerpac basic power were supplied by Bio-Rad (USA). The bands in the gel were detected with DuxGeldoc (Biomedux). DNA was amplified using a SimpliAmp Thermal Cycler (Thermo Fisher Scientific).

2.2. PCR condition

The PCR mixture was prepared following the standard protocol provided by Bioneer (Daejeon, Korea) using Taq polymerase (#E-2011–1) and a lambda DNA template (#D-2510). Each 50 μL reaction contained 5 U of Taq polymerase, 250 μM dNTPs, 10 mM Tris–HCl (pH 9), 40 mM KCl, 1.5 mM $MgCl_2$, 1 ng of lambda DNA template, and 20 pmol of each primer. A forward (5′-GAAGCGTTTATGCGGAAGAG) and a reverse (5′-TGACTCCTGTTGATAGATCCAGT) primer were used (Macrogen, Korea).

The PCR was amplified using a SimpliAmp Thermal Cycler (Thermo Fisher Scientific). The thermal cycling conditions included an initial denaturation at 95 °C for 3 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s. A final extension step at 72 °C for 3 min was performed at the end of amplification. The PCR products were analyzed with agarose gel electrophoresis on 1 % agarose gels, for which 4 μL of 6x agarose gel loading buffer was loaded with 20 μL of amplification products. Gels were stained with 2 μL of ethidium bromide, visualized on a UV transilluminator (DuxGeldoc), and documented with a Sony CMOS camera (IMX179).

The PCR reagents included Tween 20 (#9005–64), Tween 80 (#9005–65–6), NP-40 (NP40S), BSA (#A2153), and (ditothreitol (DTT); #43,816), all of which were purchased from Sigma-Aldrich (USA). DMSO (#67–68–5) was purchased from Samchun (Korea).

2.3. Preparing hydrogel monomer solutions and photopolymerization conditions

A 40 % acrylamide solution (#A4058) was used as the acrylamide monomer. The crosslinker N,N′-methylenebisacrylamide (MBAA, #146,072, Sigma-Aldrich, USA) was prepared by dissolving the powder in DMSO at 5 % (w/v). The acrylamide and MBAA were mixed in a 19:1 ratio, which produces the smallest average pore size among the common acrylamide gels [11]. PEGDMA was prepared by dissolving the powdered monomer in distilled water at a concentration of 50 % (w/v). GelMA was dissolved in distilled water at 20 % (w/v) and heated in a 50 °C water bath for 5 minutes prior to use. Hydrogel precursor solutions were formulated by dissolving monomers in distilled water at concentrations adjusted according to each experimental condition, followed by the addition of a photoinitiator (LAP, lithium phenyl-2,4,6-trimethylbenzoylphosphinate, #900889) at a final concentration of 0.1 %. In experiments conducted to evaluate inhibition-relieving effects, Tween 20 was additionally incorporated into the formulation at a final concentration of 4 % (v/v).

3. Results and discussion

3.1. PCR inhibition by hydrogel monomers

Four representative hydrogels (acrylamide, EGDA, EGDMA, and GelMA) were evaluated by including each monomer at a final concentration of 5 % (v/v) in the PCR mixture to assess the inhibitory effects of these hydrogel monomers on PCR [12–18]. The chemical structures of the monomers are shown in Fig. 1a. The PCR was amplified under standard thermal cycling conditions using lambda DNA as the template. The resulting amplicons were analyzed using agarose gel electrophoresis (Fig. 1b).

The positive control, which contained no monomer (lane 2), produced a clear and intense DNA band, confirming successful amplification. In contrast, the reactions containing 5 % acrylamide (lane 3) or 5 % EGDA (lane 4) yielded no detectable amplicons, indicating complete PCR inhibition. The absence of bands in these lanes suggested that EGDA and acrylamide strongly interfered with polymerase activity, likely because of their reactive acrylate or amide functionalities, which covalently modify the nucleophilic residues of the enzyme or sequester essential cofactors.

A moderate-intensity DNA band was observed when 5 % EGDMA was included (lane 5), indicating partial PCR inhibition, but amplification was detectable. This suggests that although EGDMA, a dimethacrylate monomer, possesses electrophilic groups similar to those of EGDA, the bulkier structure or steric hindrance of EGDMA may reduce its reactivity with PCR components, resulting in milder inhibition compared with the inclusion of the other hydrogel monomers.

The inclusion of 5 % GelMA (lane 6) did not observably inhibit the PCR. The resulting band intensity was comparable to that of the positive control, indicating that the PCR efficiently proceeded in the presence of this methacrylated biopolymer. The relatively benign behavior of GelMA can be attributed to its partially substituted methacrylate groups and protein-based backbone, which sterically shield the reactive sites or provide a more biocompatible environment for enzymatic activity.

These results emphasize that the inhibitory effects of hydrogel monomers on PCR strongly depend on their structures. Highly reactive

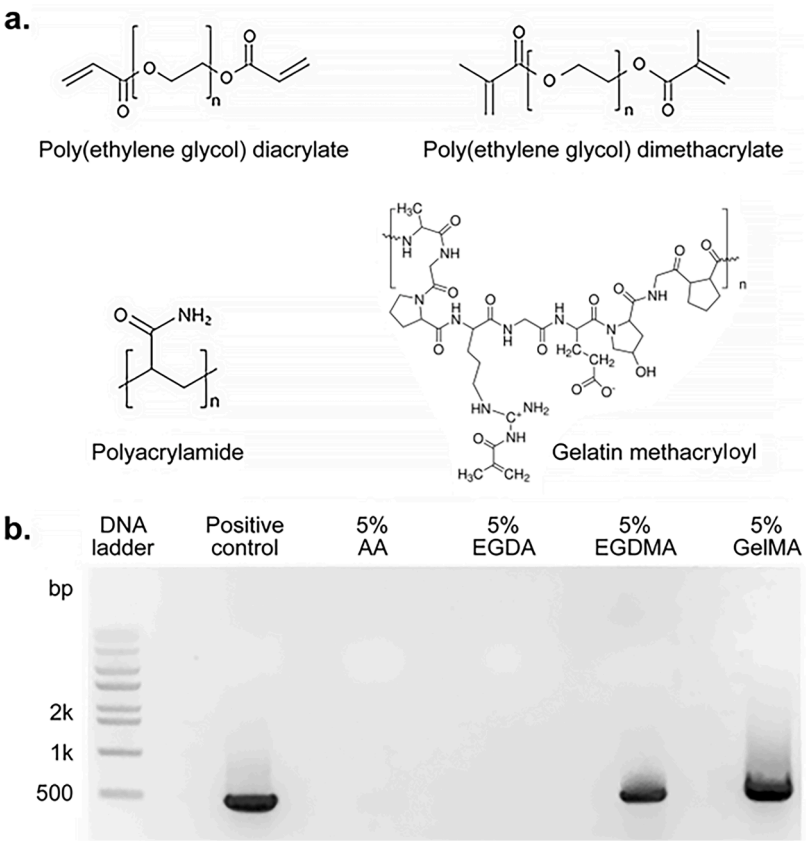

Fig. 1. Effect of various hydrogel monomers on PCR amplification. (a) Chemical structures of hydrogel monomers evaluated in this study: acrylamide, EGDA, EGDMA, and GelMA. (b) Agarose gel electrophoresis of PCR products after amplification in the presence of 5 % (v/v) monomer. Lane 1: DNA ladder; Lane 2: positive control (no monomer); Lane 3: 5 % acrylamide; Lane 4: 5 % EGDA; Lane 5: 5 % EGDMA; Lane 6: 5 % GelMA. PCR efficiency was strongly inhibited by acrylamide and EGDA, partially inhibited by EGDMA, and the least affected by GelMA.

diacrylates, such as EGDA, completely inhibit PCR; amplification is normal when macromolecular or sterically hindered monomers, such as GelMA, are included. Thus, monomers must be carefully selected and characterized when developing PCR-compatible hydrogel systems for molecular diagnostics.

3.2. *Effect of methacrylate functional groups on PCR amplification*

We focused on the ratio of methacrylate groups in the PCR because relatively appropriate PCR amplification results were observed for the monomers containing methacrylate groups, as shown in Fig. 1b We investigated the amplification outcomes with the inclusion of methacrylated hydrogel components; GelMA with different degrees of methacrylation; and two methacrylate-based crosslinkers, EGDMA and PEGDMA, to evaluate the influence of methacrylate groups on PCR performance. The results are shown in Fig. 2.

GelMA with 80 % and 40 % methacrylation was tested at a 0.5 % (w/v) (Fig. 2a). The positive control (lane 1) contained a strong amplicon band at approximately 500 bp. PCR amplification efficiently proceeded with a band intensity comparable to that of the positive control when 80 % methacrylated GelMA was added (lane 2), indicating that high methacrylation proportions did not inhibit the reaction. In contrast, amplification was almost completely suppressed when 40 % methacrylated GelMA was used (lane 3), with only a faint or negligible band observed. These results suggest that the presence of methacrylate groups, the degree of methacrylation, and the overall chemical environment of the polymer matrix strongly influence the compatibility with PCR. Higher methacrylation proportions may reduce the availability of reactive residues or limit the unmodified gelatin domains, which could interfere with polymerase activity.

We amplified the PCR in the presence of EGDMA and PEGDMA at concentrations ranging from 0.5 % to 5 % to assess the concentration-dependent effects of the methacrylated monomers (Fig. 2b). The band intensity for EGDMA (lanes 2–5) remained relatively consistent across all tested concentrations, indicating that EGDMA minimally inhibited PCR, even at 5 % (v/v). This result is notable given the electrophilic acrylate groups of EGDMA, suggesting that EGDMA did not effectively interact with or disrupt the activity of DNA polymerase under the tested conditions.

In contrast, PEGDMA concentration-dependently inhibited. The PCR amplification at 0.5 % PEGDMA (lane 6), was comparable to that of the control. However, no visible PCR product was detected above a 1 % PEGDMA inclusion (lanes 7–9), indicating complete amplification inhibition. The inhibition may have arisen from the higher hydrophilicity and extended chain length PEGDMA compared with the other hydrogel monomers, which promoted stronger interactions with DNA or enzyme surfaces or sequestered essential cofactors such as Mg^{2+}. Alternatively, the higher viscosity and potential to form micelle-like structures of PEGDMA may have hindered enzyme–substrate interactions at higher concentrations.

These findings collectively indicate that the influence of methacrylate-containing materials on PCR compatibility is complex, structural, and concentration-dependent. Some methacrylated compounds, such as EGDMA, minimally impacted PCR amplification; others, such as PEGDMA, completely inhibited PCR at concentrations above 1 %. EGDMA inhibited PCR much less than PEGDMA, despite EGDMA having a higher methacrylate group content per unit mass owing to its molecular weight (198.22 g/mol) being smaller than that of PEGDMA (~550 g/mol). EGDMA contains approximately 2.8 times more methacrylate groups than PEGDMA at equal mass concentrations. This result

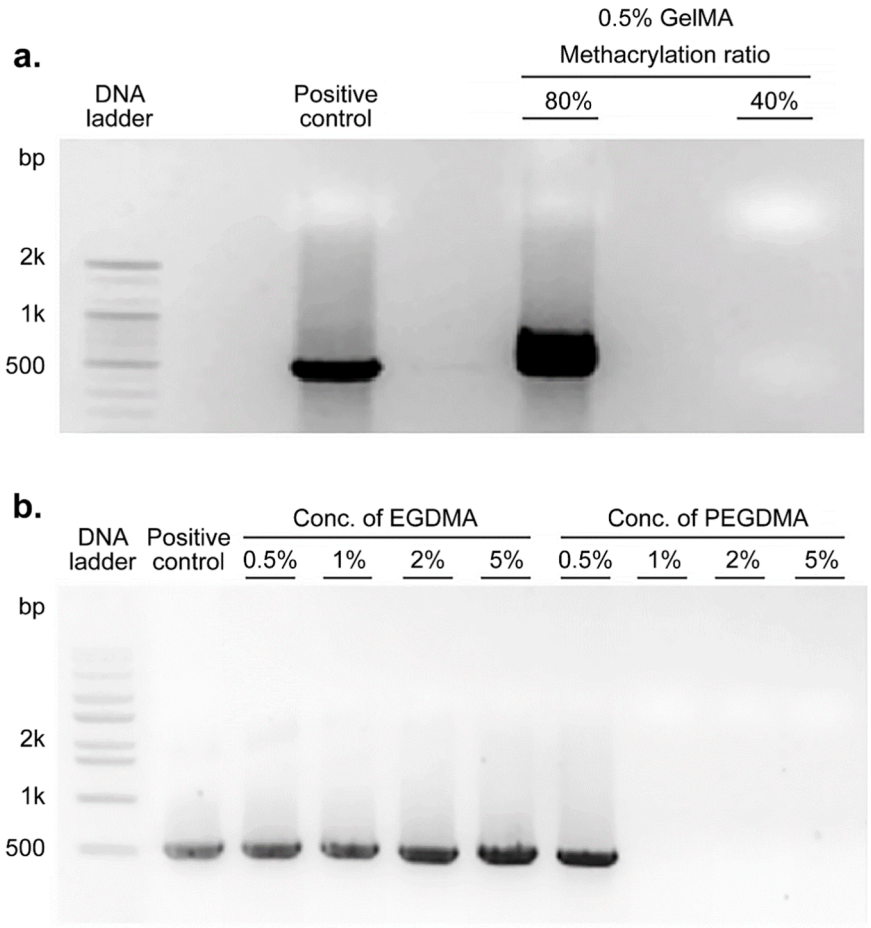

Fig. 2. Effect of the inclusion of methacrylate-functionalized hydrogel components on PCR amplification. (a) PCR was performed with the inclusion of 0.5 % (w/v) GelMA with different degrees of methacrylation. Lane 1: positive control (no GelMA); lane 2: 80 % methacrylated GelMA; lane 3: 40 % methacrylated GelMA. Amplification was efficient with the inclusion of highly methacrylated GelMA, whereas 40 % GelMA resulted in near-complete amplification inhibition. (b) PCR amplification in the presence of various concentrations (0.5 %–5 %) of methacrylated crosslinkers. Lanes 1–5: positive control and 0.5 %, 1 %, 2 %, and 5 % EGDMA (v/v), respectively; lanes 6–9: positive control and PEGDMA at 0.5 %, 1 %, 2 %, and 5 % (v/v), respectively. EGDMA minimally inhibited amplification regardless of concentration, whereas PEGDMA strongly inhibited amplification above 1 %, with no detectable amplicons at 2 % and 5 %.

suggests that the inhibition of PCR is not solely dependent on the absolute number of methacrylate groups but also on other factors such as steric hindrance, molecular conformation, or interactions with polymerase and cofactors. These findings imply that monomers with a higher methacrylate density inhibit PCR than bulkier, less-functionalized analogs, potentially because of reduced accessibility or interactions with PCR components. Therefore, monomers must be rationally selected based on their chemical structure and functional group density when developing hydrogel formulations compatible with PCR-based applications.

3.3. *Effect of enhancers*

Various additives have been used to increase the efficiency and robustness of PCR, particularly under conditions where amplification is challenged by inhibitory components. Among the most widely used additives is BSA, which increases the thermal stability of DNA polymerase and prevents the nonspecific adsorption of PCR reagents to the walls of reaction tubes, thereby increasing the overall PCR yield and reproducibility [19–21]. Nonionic surfactants such as Tween 20, Tween 80, and NP-40 also enhance PCR performance through destabilizing secondary DNA structures, reducing the stability of double-stranded helices, and competitively interacting with other PCR inhibitors, thereby mitigating their effects [22–25]. Dimethyl sulfoxide (DMSO) also increases PCR efficiency by lowering the melting temperature of DNA and suppressing the formation of stable double-stranded structures [26–28]. In this study, we evaluated the performance of these enhancers under the strong inhibitory conditions induced by 4 % acrylamide. Acrylamide is a hydrogel monomer commonly used in molecular biology (e.g., SDS-PAGE) and completely suppresses PCR amplification (Fig. 1). Therefore, we selected acrylamide as an inhibitory monomer to assess the extent to which common PCR enhancers restore amplification efficiency under these conditions.

No DNA bands were detected at any DMSO concentration as shown in lanes 2–6, in which 0.2 % BSA and varying concentrations of DMSO were added to the acrylamide-containing PCR mixture. The combination of BSA and DMSO ineffectively restored PCR amplification in the presence of acrylamide. Although BSA is commonly used to reduce polymerase adsorption onto surfaces and DMSO to facilitate strand separation or relieve secondary structure formation, the combination of DMSO and BSA insufficiently counteracts the strong inhibition of PCR amplification caused by acrylamide monomers.

Amplification partially recovered in lanes 7 and 8, for which 4 % Tween 20 was used with 0 % and 2.5 % DMSO, respectively. The PCR band was faint but detectable in lane 7 and weaker in lane 8. However, amplification was fully suppressed as the concentration of DMSO increased to 5 % or more (lanes 9–11). These results suggested that Tween 20 mitigated the effects of acrylamide toxicity, possibly by reducing nonspecific interactions or altering micelle formation, which influenced local monomer availability. However, this effect was limited

and negated by the addition of higher DMSO concentrations.

Overall, these findings indicate that neither the BSA + DMSO nor Tween 20 + DMSO combinations fully restored PCR activity in the presence of 4 % acrylamide. The partial benefit observed with Tween 20 suggested that the surfactant-based shielding of inhibitory monomer-enzyme interactions offered limited protection, but additional strategies, such as prepolymerization, chemical blocking, or selecting less-reactive monomer systems, would be necessary to ensure PCR compatibility in acrylamide-rich environments Fig. 3.

3.4. Reduction in PEGDMA-induced PCR inhibition using nonionic surfactants

We conducted experiments using PEGDMA, a dimethacrylate compound that strongly inhibits PCR, even at concentrations below 1 %, to evaluate whether nonionic surfactants could mitigate the PCR inhibition caused by methacrylate-containing monomers (Fig. 2b). The aim in this study was to determine whether commonly used PCR enhancers could restore amplification efficiency in the presence of PEGDMA. The selected additives, Tween 20, Tween 80, NP-40, Triton X-100, and DMSO, were tested at concentrations effective for enhancing PCR [23, 26]. Each enhancer was mixed with 10 % PEGDMA and combined with the PCR master mix. PCR was performed under standard thermal cycling conditions, and the amplification products were analyzed using agarose gel electrophoresis (Fig. 4b).

Tween 20, Tween 80, and NP-40 restored PCR amplification in the presence of 10 % PEGDMA. The positive control (lane 1) produced a strong band, whereas amplification was completely inhibited in the negative control containing only PEGDMA (lane 2; Fig. 4b). The addition of Tween 20 (lane 3), NP-40 (lane 4), or Tween 80 (lane 6) produced strong amplification bands comparable to those of the control, indicating that these surfactants effectively suppressed the PEGDMA-induced inhibition. In contrast, the addition of Triton X-100 (lane 5) or DMSO (lane 6) failed to restore the amplification, where no detectable bands were observed.

The ability of these surfactants to mitigate PCR inhibition may correlate with their CMC. Tween 20, Tween 80, and NP-40 have relatively low CMC values at room temperature of 0.06, 0.012, and 0.12 mM, respectively; however, Triton X-100 has a higher CMC (0.9 mM), according to the manufacturer. The CMC values suggest that low-CMC surfactants more effectively form micellar environments that shield Taq polymerase from reactive methacrylate groups or sequester inhibitory monomers, preserving enzyme activity. The lack of effect of Triton X-100 may be attributed to its short hydrophobic tail, which limits its protective interactions with polymerase.

We conducted a dose–response experiment using increasing concentrations of PEGDMA (30 %, 40 %, 50 %, and 60 %), while fixing each surfactant concentration at 4 % to further investigate the concentration threshold at which the surfactants remained effective in protecting against PEGDMA-induced PCR inhibition. The amplification bands were strong for all surfactants in 30 % PEGDMA (lanes 3–5; Fig. 4c). Amplification was maintained with Tween 80 at 40 % PEGDMA (lanes 6–8); Tween 20 and NP-40 yielded weaker bands. A 40 % PEGDMA solution corresponds to approximately 0.73 M, which is a substantially higher concentration than the enhancer concentration used (~0.03 M). Therefore, the three enhancers likely did not neutralize PEGDMA monomers directly but rather restored PCR efficiency by protecting the fixed-concentration Taq polymerase from inhibitory interactions with the monomer [29,30]. Only a faint band was observed for Tween 80 at 50 % PEGDMA (lanes 9–11), and no bands were detected for the other surfactants. No amplification was detected with any surfactant with 60 % PEGDMA (lanes 12–14).

These results suggest that the surfactant-assisted protection of the polymerase is dose-dependent and limited by the concentration of the inhibitory monomer that is present. Tween 80 showed the strongest resistance to inhibition among the tested agents, possibly because of its low CMC and larger hydrophobic domain, which may increase the efficiency of shielding of the enzyme–monomer interactions. These differences may be attributed to the structural and physicochemical properties of the surfactants. Tween 20, Tween 80, and NP-40 have relatively low CMC values and strong amphiphilic character, allowing them to form micelles that may sequester PEGDMA or shield the enzyme surface. Tween 80, with its long oleate tail, likely offers stronger hydrophobic interaction and steric protection, explaining its superior performance at higher PEGDMA concentrations. NP-40, though non-PEG-based, similarly mitigates inhibition through effective surface association. In contrast, Triton X-100 has a higher CMC and a shorter hydrophobic tail, limiting its ability to form protective micellar structures under PCR conditions. These results highlight that both hydrophobic domain length and micellization efficiency are critical for

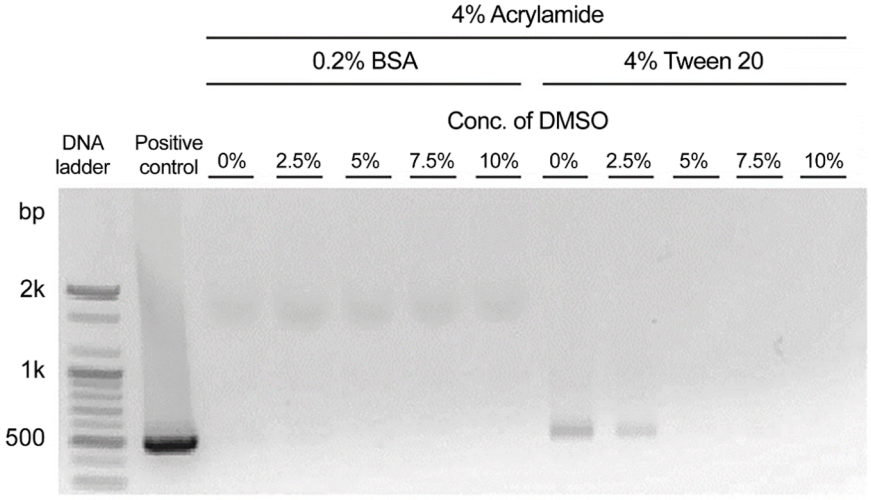

Fig. 3. Evaluation of the effects of adding various combinations of enhancers on reducing the PCR inhibition caused by acrylamide. PCR was amplified in the presence of 4 % (w/v) acrylamide to assess the ability of commonly used enhancers to restore amplification efficiency. Lane 1: positive control (no acrylamide); lanes 2–6: 4 % acrylamide + 0.2 % BSA with increasing concentrations of DMSO (0 %, 2.5 %, 5 %, 7.5 %, and 10 %, respectively); lanes 7–11: 4 % acrylamide + 4 % Tween 20 with increasing concentrations of DMSO (0 %, 2.5 %, 5 %, 7.5 %, and 10 %, respectively). Lanes 2–6 contained no amplification bands, indicating that the BSA+DMSO combination did not overcome the acrylamide-induced inhibition. Weak bands were detected in lanes 7 and 8, suggesting partial recovery using Tween 20 at low DMSO concentrations; however, no amplification was observed at higher DMSO levels (lanes 9–11).

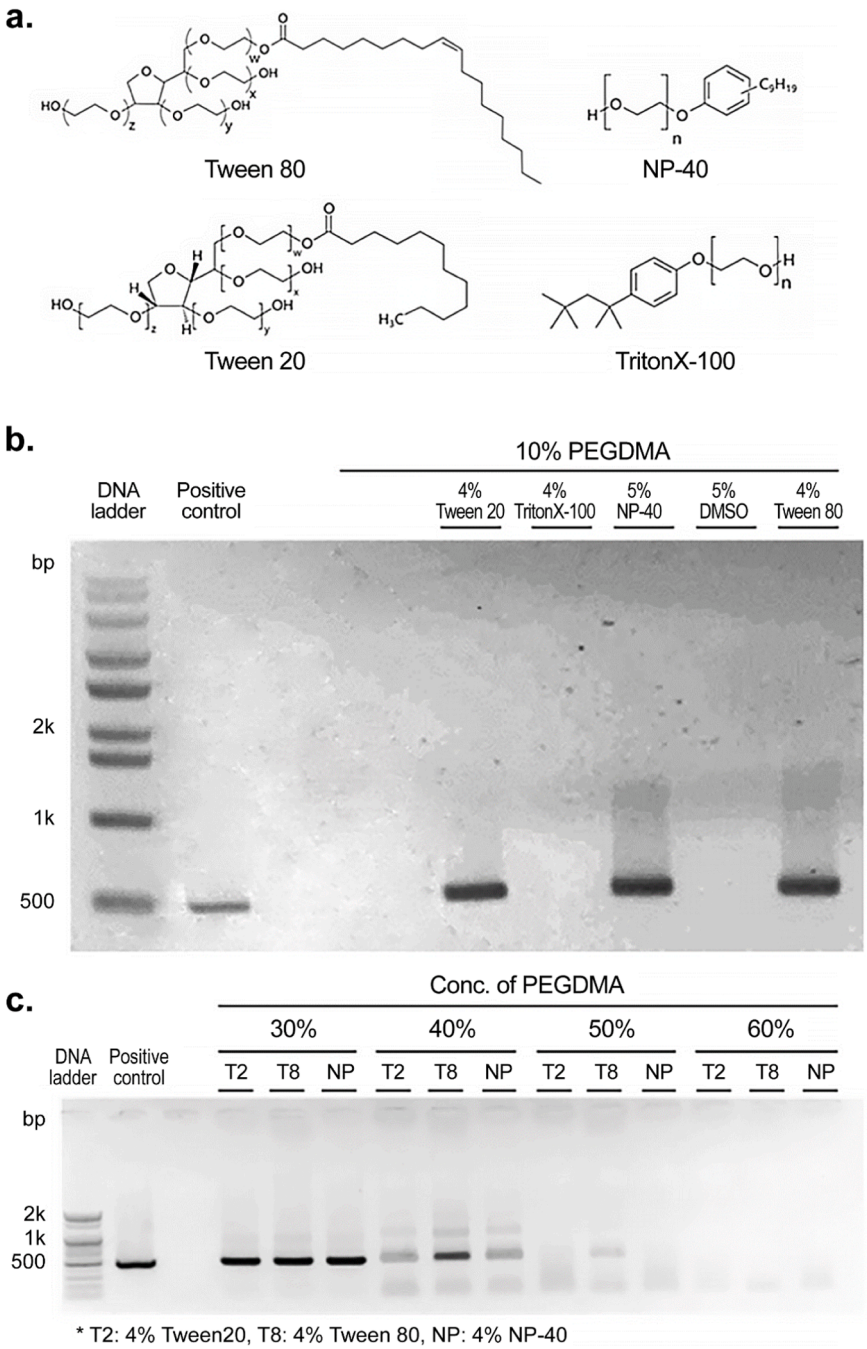

Fig. 4. Results of using nonionic surfactants for mitigating PEGDMA-induced PCR inhibition. (a) Chemical structures of nonionic surfactants: Tween 20, Tween 80, NP-40, Triton X-100. (b) PCR amplification in the presence of 10 % PEGDMA with or without selected surfactants. Lane 1: positive control (no PEGDMA); lane 2: 10 % PEGDMA only (PCR inhibition); lane 3: +4 % Tween 20; lane 4: +4 % NP-40; lane 5: +4 % Triton X-100; lane 6: +5 % DMSO; lane 7: +4 % Tween 80 (c) PCR amplification with increasing PEGDMA concentrations (30–60 %) in the presence of fixed 4 % surfactant. Lanes 3–5: 30 % PEGDMA + Tween 20, Tween 80, NP-40; lanes 6–8: 40 % PEGDMA; lanes 9–11: 50 % PEGDMA; lanes 12–14: 60 % PEGDMA. Lane 1: positive control; lane 2: negative control (no polymerase).

surfactant-mediated protection of Taq polymerase. Overall, these findings highlight the potential of using specific nonionic surfactants, particularly those with low CMC values, to extend the usable concentration range of methacrylated hydrogels for PCR-compatible applications.

3.5. Reduction in acrylamide-induced PCR inhibition via competitive protection by excess polymerase

We examined the competitive interactions between acrylamide monomers and essential amino acid residues in DNA polymerase to investigate the mechanism underlying acrylamide-induced PCR inhibition. Acrylamide is neurotoxic and carcinogenic; the covalent interactions of acrylamide with biological macromolecules, especially nucleophilic amino acids such as cysteine, valine, lysine, and histidine, have been widely studied in toxicology and food sciences [31,32]. These interactions occur via the formation of stable adducts that inactivate proteins. Such reactions likely interfere with the catalytic function of the polymerase in PCR by modifying the residues essential for enzymatic activity. Acrylamide is an α,β-unsaturated compound that readily undergoes Michael addition with thiol-containing nucleophiles, further supporting its ability to chemically inactivate enzymes such as Taq

polymerase (Fig. 5a) [33,34].

Although all tested monomers possess α,β-unsaturated carbonyl groups, the degree of PCR inhibition varied significantly. This suggests that inhibition is not solely due to covalent modification of nucleophilic residues in the polymerase active site. For example, PEGDMA may cause steric interference or noncovalent adsorption onto the enzyme surface due to its long, hydrophilic chain structure. Acrylamide has also been reported to interact with biological proteins through hydrogen bonding and noncovalent association prior to covalent adduct formation [35,36]. These combined effects may amplify the inhibitory behavior of specific monomers such as PEGDMA and acrylamide beyond their chemical reactivity alone.

We thus hypothesized that increasing the amount of Taq polymerase would competitively buffer or neutralize the inhibitory effects of acrylamide on PCR through shifting the balance between the active and modified enzymes. PCR reactions were performed with varying concentrations of acrylamide (0.2–0.4 M) and increasing units of Taq polymerase (5–20 U per reaction) to test this hypothesis. The amplification products were analyzed using agarose gel electrophoresis (Fig. 5b).

The amplification band was strong for the positive control (lane 2), whereas the band was moderate for PCR with 5 U of Taq polymerase and 0.2 M acrylamide (lane 3), indicating partial inhibition. Increasing the acrylamide concentration to 0.3 and 0.4 M (lanes 4 and 5, respectively) more strongly and completely inhibited amplification, respectively, with no detectable bands for the latter (Fig. 5b).

The band intensity recovered to nearly that of the positive control when the amount of Taq polymerase was 10 U in the 0.2 M acrylamide condition (lane 6), indicating that the enzyme excess fully compensated for the inhibition of amplification. Similarly, 15 and 20 U of Taq polymerase in the presence of 0.3 and 0.4 M acrylamide (lanes 7 and 8, respectively) restored amplification to moderate levels, although the bands were weaker than those of the positive control.

These results support the hypothesis that acrylamide inhibits PCR by interacting with the polymerase and that this inhibition is mitigated by increasing the enzyme concentration. This competitive protection mechanism could be applied in designing acrylamide-tolerant PCR systems, particularly for hydrogel-based platforms in which acrylamide monomers are used as precursors. However, the protective effect appears to be concentration-dependent, and complete recovery may not be achievable under high monomer concentrations owing to irreversible polymerase modification or other nonspecific interactions.

4. Conclusions

We systematically investigated the inhibitory effects of hydrogel monomers on PCR amplification and proposed practical strategies to overcome this inhibition. Monomers containing acrylate or methacrylate groups, particularly PEGDMA and acrylamide, strongly and structurally dependently inhibited PCR, likely through covalent interactions with the nucleophilic residues on the polymerase. PCR inhibition did not

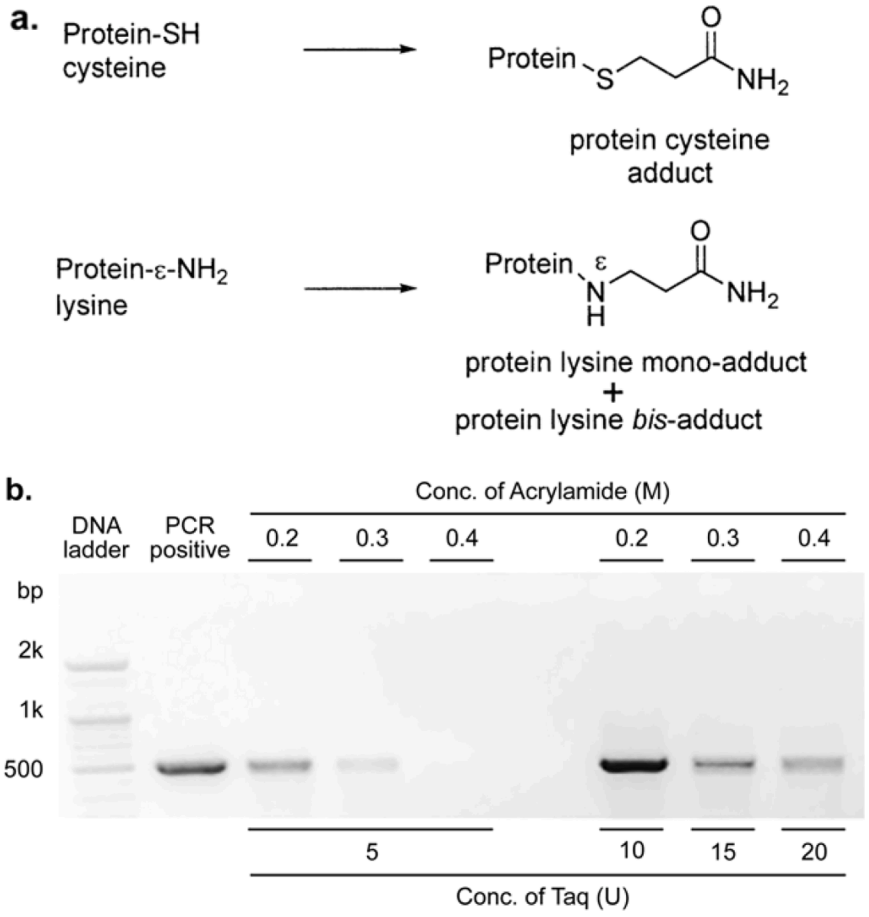

Fig. 5. Reduction in acrylamide-induced PCR inhibition using excess polymerase. (a) Plausible chemical reaction of carbonyl monomers (e.g., acrylamide or methacrylate) undergoing Michael addition with a nucleophilic thiol or amine group, such as those found on cysteine or lysine residues in the polymerase. (b) PCR was amplified in the presence of various acrylamide concentrations (0.2–0.4 M) and amounts of Taq polymerase and/or nonionic surfactant. Lane 1: DNA ladder; lane 2: PCR positive control (no acrylamide); lane 3: 0.2 M acrylamide + 5 U Taq polymerase; lane 4: 0.3 M acrylamide + 5 U Taq polymerase; lane 5: 0.4 M acrylamide + 5 U Taq polymerase; lane 6: 0.2 M acrylamide + 10 U Taq polymerase; lane 7: 0.3 M acrylamide + 15 U Taq polymerase; lane 8: 0.4 M acrylamide + 20 U Taq polymerase.

simply correlate with methacrylate content, as the highly substituted GelMA and EGDMA minimally interfered with PCR, whereas even low concentrations of PEGDMA strongly suppressed amplification.

We found that certain nonionic surfactants, Tween 20, Tween 80, and NP-40, substantially restored PCR amplification in the presence of PEGDMA, particularly when used below their CMCs. Other additives, such as Triton X-100 and DMSO, were ineffective under the same conditions. We found that the addition of excess Taq polymerase mitigated the effects of acrylamide-induced PCR inhibition, supporting the hypothesis of direct enzyme–monomer interactions via Michael-type addition.

These findings are directly relevant to the development of hydrogel-integrated PCR platforms for real-world applications, including point-of-care diagnostics, microfluidic PCR, and digital PCR. The compatibility of hydrogels with PCR can be substantially increased by selecting the appropriate monomer structures and incorporating protective additives, enabling the broader application of hydrogel systems in molecular diagnostics and bioanalytical technologies. In these systems, monomeric or partially cured hydrogels are frequently used for immobilizing reagents, isolating compartments, or supporting thermal and structural stability. Understanding which monomers inhibit PCR and how to mitigate that inhibition using low-CMC surfactants or enzyme buffering strategies allows researchers to tailor polymer formulations for compatibility with amplification-based assays. As such, the results of this study provide practical design guidance for integrating hydrogel systems into next-generation nucleic acid testing platforms.

CRediT authorship contribution statement

Nguyen Anh Nhung Tran: Writing – original draft, Methodology, Formal analysis. **Chansik Oh:** Formal analysis, Data curation. **Isaac Jang:** Methodology, Investigation, Formal analysis, Data curation. **Seung Hyun Shin:** Methodology. **Sangmin Lee:** Methodology, Conceptualization. **Bhargav Krishna Pullagura:** Resources. **Bumsang Kim:** Funding acquisition, Conceptualization. **Dohyun Kim:** Writing – review & editing, Funding acquisition, Conceptualization. **Minsub Chung:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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