



PCR Amplification of HPRT1 Gene Within Photo-Crosslinkable Gelatin Methacrylate Hydrogel for Molecular Diagnostics

Hyerin Nam¹ · Ajith Mohanasundaran¹ · Jeongha Yoo² · Hansol Lee¹ · Jongsung Kim¹

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Abstract

This work investigates the potential of GelMA hydrogels to serve as a supportive medium for PCR amplification, presenting a promising substitute for traditional solution-based PCR methods. Liquid-phase PCR often faces issues like evaporation, handling errors, and contamination. Embedding PCR reagents in a hydrogel matrix offers a stable, enclosed environment that helps overcome these challenges and supports efficient amplification. This study investigates the use of GelMA hydrogels crosslinked with the lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) photo initiator for thermal PCR amplification of the HPRT1 gene. GelMA hydrogels were characterized by SEM, EDX, ¹HNMR, FTIR, XRD, UV–Vis. spectroscopy, and rheological analysis (loss and storage modulus) to confirm successful synthesis and tunable network formation. Amplification succeeded in uncrosslinked and UV-treated GelMA without LAP but failed when PCR components were present during UV-induced crosslinking with LAP, which may be due to radical-induced DNA or enzyme damage. Notably, loading PCR reagents after crosslinking restored amplification, demonstrating reagent accessibility within the hydrogel network. Increased LAP concentrations affected crosslink density and amplification efficiency. These results demonstrate that GelMA hydrogels can serve as effective in-gel PCR platforms, combining enhanced stability with lower contamination risk, holding promise for advanced biosensing and diagnostic applications.

Keywords DNA amplification · GelMA · LAP · Thermal PCR

Introduction

Advancements in DNA-based diagnostic methods have impacted modern healthcare by enabling precise, fast, and economical detection of serious illnesses. The ability to identify specific genetic sequences has enabled the development of personalized medicine approaches, early disease detection systems, and point-of-care diagnostic tools. The key component of many molecular diagnostic strategies is polymerase chain reactions (PCR) allowing for

quick amplification of specific nucleic acid targets [1–3]. The hypoxanthine-guanine phosphoribosyltransferase 1 (HPRT1) gene is widely used as a housekeeping gene due to its stable and uniform expression across human tissues. It encodes a critical enzyme in the purine salvage pathway and is considered a reliable reference for normalization in molecular biology applications, including studies related to cancer, metabolic disorders, and pharmacogenomics. In molecular diagnostics, HPRT1 DNA amplification serves both as a functional control for assay validation and as a model system for evaluating the performance of emerging amplification platforms [4, 5]. Its well-characterized sequence and consistent amplification efficiency make it particularly valuable as a proof-of-concept target in the development of novel PCR-based diagnostic methods.

The traditional DNA diagnostic methods are typically performed in liquid-phase, homogeneous mixtures within standard reaction tubes or plates. These approaches require complex laboratory infrastructure and trained personnel, limiting their applicability in resource-limited settings [6]. Moreover, challenges such as reagent instability,

✉ Hansol Lee
hlee22@gachon.ac.kr

✉ Jongsung Kim
jongkim@gachon.ac.kr

¹ School of Chemical, Biological, and Battery Engineering, Gachon University, 1342 Seongnam Daero, Seongnam-Si 13120, Gyeonggi-Do, Republic of Korea

² Wells Bio Inc, 16, Magokjungang 8-ro 1-gil, Seoul 07795, Republic of Korea

contamination risk, and inefficient thermal transfer can compromise amplification efficiency and assay reproducibility. Advancements in biomaterial research have created new opportunities to integrate molecular diagnostic techniques with biocompatible systems, which provides the way to overcome existing limitations while maintaining diagnostic accuracy and reliability [7–9]. Hydrogels have gained significant attention as active matrices in molecular diagnostics due to their hydrophilic, three-dimensional polymer networks, which retain large amounts of water and provide a biocompatible, mechanically supportive environment. Hydrogel-embedded PCR can provide enhanced spatial control of reactions, improved stability of biological components, and reduced risk of contamination through physical containments [10]. Their porous structure can also concentrate reactants and shield them from inhibitors, potentially improving amplification efficiency. As a result, photocrosslinkable hydrogels are increasingly being investigated as effective materials for creating durable and user-friendly diagnostic tools. In addition, the hydrogel matrix minimizes evaporation during thermal cycling, maintaining a stable microvolume environment throughout the amplification process. It also ensures better confinement of reagents within defined regions, thereby improving reaction uniformity and minimizing variability between runs. By serving as a semi enclosed and contamination resistant environment, the hydrogel-based PCR platform ensures reliable and reproducible amplification, effectively addressing major challenges associated with conventional liquid phase PCR setups.

Materials such as agarose, polyvinyl alcohol (PVA), and polyethylene glycol (PEG) have been investigated for embedding molecular reagents or supporting amplification reactions [11]. However, many of these hydrogels lack one or more critical features needed for integration into diagnostic systems, such as ease of functionalization, rapid gelation under mild conditions, mechanical stability, or compatibility with biological reagents. Among these hydrogels, gelatin methacrylate (GelMA) has emerged as a prominent semi-synthetic material that combines the bioactivity of gelatin with the customizable chemical properties by methacryloyl functionalization [12]. GelMA hydrogels can be rapidly photo-crosslinked using ultraviolet (UV) light in the presence of photo initiator such as lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP), enabling the formation of stable, porous networks with adjustable mechanical properties and degradation profiles [13]. Importantly, GelMA exhibits tuneable porosity, stiffness, and degradation rates, which can be tailored by adjusting the degree of methacrylation or polymer concentration [14]. These properties facilitate the diffusion of small biomolecules such as dNTPs and primers, while potentially preserving enzyme activity and

protecting embedded nucleic acids. Furthermore, the ability of GelMA to form thin films or droplets allows its application in compact and miniaturized formats, essential for portable diagnostic systems. Compared to more inert synthetic hydrogels like PVA, PEG, or thermally gelling materials like agarose, GelMA offers a combination of mechanical robustness, UV-curability, and biological compatibility that is uniquely advantageous for supporting biochemical reactions such as PCR. The GelMA based PCR platform offers advantages for portable and point-of-care diagnostics over conventional PCR methods. The GelMA hydrogel forms a stable, UV-crosslinked matrix that maintains structural integrity and supports efficient DNA amplification during transport. When combined with a simple agarose medium, the amplified DNA can be easily visualized, enabling rapid, on-site detection without complex instrumentation. The combination of stability, biocompatibility, and ease of handling makes GelMA a promising material for portable PCR-based diagnostics.

In this study, we explore the feasibility of thermal PCR amplification of the HPRT1 gene within a UV-crosslinked GelMA hydrogel matrix. By embedding DNA templates, primers, and amplification reagents directly into GelMA prior to crosslinking, we aim to evaluate whether the hydrogel environment supports efficient gene amplification without inhibiting the function of DNA polymerase. The amplified product is subsequently analyzed using agarose gel electrophoresis to determine yield and specificity. This work aims to demonstrate that GelMA, beyond its applications in tissue engineering and drug delivery, can serve as a functional matrix for hydrogel-based molecular diagnostics. Establishing the compatibility of GelMA with thermal PCR could pave the way for novel biosensing technologies and integrated diagnostic platforms capable of performing localized, stable, and multiplexed nucleic acid amplification *in situ*.

Experimental

Materials

Chemicals of analytical quality were all utilized directly without additional purification. Gelatin was obtained from DaeJung, while methacrylate and lithium phenyl-2,4,6-trimethylbenzoylphosphinate were sourced from Sigma-Aldrich. A dialysis membrane with a molecular weight cutoff of 500–1000 Da was purchased from Spectrum Laboratories, Inc. PCR reagents including Taq DNA polymerase, dNTPs, 10× PCR buffer with MgCl₂, and nuclease-free water were procured from Bioneer (South Korea). Custom-designed primers targeting the HPRT1 gene, along with its

DNA template, were also supplied by Bioneer. Ethidium bromide was obtained from Sigma-Aldrich. Agarose powder, 50× TAE buffer, and a DNA ladder (100 ng/μL) were additionally obtained from Bioneer.

Instruments and Measurements

Multiple techniques were employed to characterize the GelMA hydrogel and assess DNA amplification performance. Fourier-transform infrared (FTIR) spectroscopy was carried out using a Bruker Vertex 70 (USA) equipped with an ATR module. The spectra were collected within the 4000–400 cm^{-1} range, with a resolution of 1 cm^{-1} , and averaged over four scans to identify functional groups and bonding features specific to GelMA [15]. To investigate the optical characteristics and monitor absorbance behavior of LAP-incorporated GelMA, UV–Vis spectroscopy was performed using a Jasco V-770 spectrophotometer [16]. X-ray diffraction (XRD) analysis was conducted with a Rigaku MPA-2000 system (Gachon university), utilizing a 4 kW X-ray source. The diffraction data were collected over a 2θ scan range of 0° to 80° to examine the crystalline nature

of the material. Furthermore, the viscoelastic behavior of GelMA was evaluated by measuring the storage modulus (G') and loss modulus (G'') using a rheometer. Surface morphology was observed using field-emission scanning electron microscopy (FE-SEM) with a Hitachi SU8600 (Japan). Prior to SEM imaging, samples were coated with a thin Au-Pd layer to enhance conductivity and mounted on conductive adhesive Table [17].

Preparation of the GelMA

GelMA was synthesized by dissolving 10 g of gelatin in 100 mL of carbonate-bicarbonate buffer (CB) at 50 °C with continuous stirring at 700 rpm for 1 h (Fig. 1a). Subsequently, 5 mL of methacrylic anhydride was slowly added to the gelatin solution to modify the lysine residues. The reaction mixture was then stirred at 50 °C and 700 rpm for an additional 2 h. After completion of the reaction, the solution was dialyzed against deionized water using a 500–1000 Da dialysis membrane for 7 days at 40 °C to remove unreacted components. The dialyzed solution was subsequently filtered

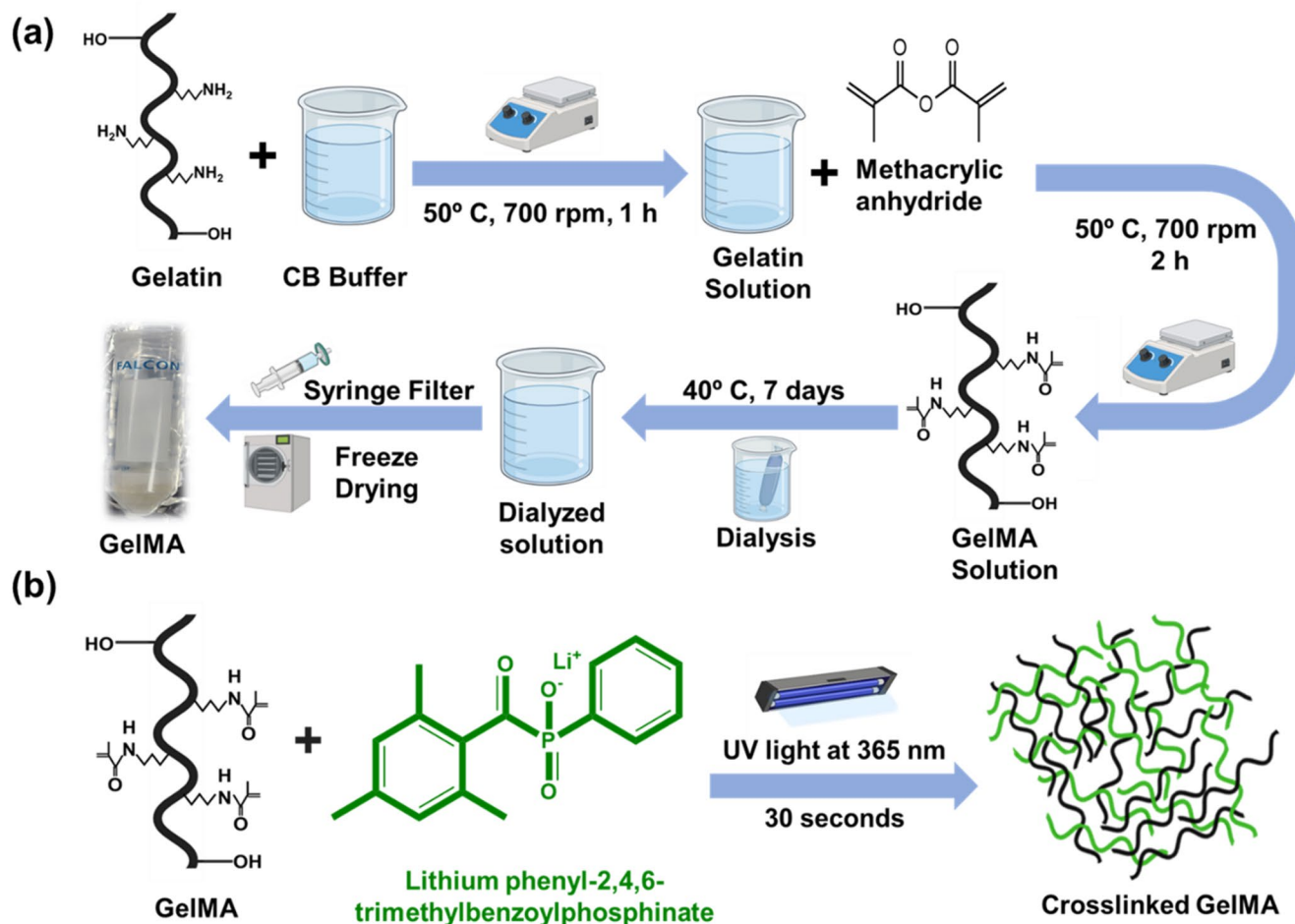


Fig. 1 Synthesis methodology of (a) GelMA and (b) Crosslinked GelMA

through a 0.22 μm syringe filter and lyophilized, after which the resulting product was stored at $-80\text{ }^{\circ}\text{C}$ [18, 19].

Preparation of the Crosslinked GelMA

Crosslinked GelMA was prepared by dissolving 10 mg of lyophilized GelMA in 1 ml CB buffer at $60\text{ }^{\circ}\text{C}$ with continuous stirring for 1 h (Fig. 1b). After complete dissolution, varying concentrations of the photo initiator LAP (2.5%, 5%, and 7.5% w/v) were gradually added to the solution under dark conditions and the samples were named as GEL-LAP2.5, GEL-LAP5, GEL-LAP7.5 which was illustrated on Table 1. Stirring was continued until the LAP was fully dissolved. The LAP-Gelma mixture (L-GelMA) was then exposed to ultraviolet (UV) light at 365 nm for 10 s to initiate crosslinking, resulting in the formation of crosslinked GelMA hydrogels [19–22].

Amplification of HPRT1 DNA

To assess the compatibility of UV-crosslinked GelMA hydrogels with thermal DNA amplification, a model PCR targeting the HPRT1 gene was conducted within the hydrogel matrix. The PCR reaction mixture was prepared using 2 μL of HPRT1 DNA template (50 ng), 1 μL each of forward and reverse primers (100 pM/ μL), and 10 μL of a commercial PCR master mix containing 10 mM dNTPs, Taq DNA polymerase, MgCl_2 -buffer, and adjusted with the final reaction volume to 20 μL using nuclease-free water. The complete reaction mixture was loaded into an thermal cycler (EdvoCycler 2.0, Edvotek) and subjected to amplification under the following conditions: an initial denaturation at $95\text{ }^{\circ}\text{C}$ for 5 min; 30 cycles of denaturation at $95\text{ }^{\circ}\text{C}$ for 30 s, annealing at $60\text{ }^{\circ}\text{C}$ for 30 s, and extension at $72\text{ }^{\circ}\text{C}$ for 60 s; followed by a final extension at $72\text{ }^{\circ}\text{C}$ for 5 min and a hold at $4\text{ }^{\circ}\text{C}$ [23, 24]. PCR products were analyzed by 1% agarose gel electrophoresis, stained with ethidium bromide, and visualized under UV illumination. Positive control reactions were included, and a negative control (lacking DNA template) was also performed. To evaluate the influence of GelMA on amplification, control reactions were carried out by adding GelMA directly to the PCR mixture and following the same amplification protocol. Similarly, liquid GelMA (L-GelMA) was added to a separate tube containing

the PCR mixture. In another condition, L-GelMA mixed with PCR reagents was exposed to UV light at 365 nm for 10 s prior to amplification. Additionally, a setup was tested where L-GelMA was first UV-crosslinked for 10 s, and then the PCR mixture was added into the pre-formed hydrogel. All experimental groups underwent thermal cycling under identical conditions to the positive control. A DNA ladder (100–2000 bp) was used as a molecular marker to confirm the size of the PCR amplicons. The Real time PCR was also conducted by following the above procedures. Image J (National Institutes of Health) software was employed to quantitatively analyze the color intensity of each DNA band, using positive control as a reference standard.

Results and Discussion

Characterization of GelMA

Chemical and physical characteristics of the synthesized hydrogel by using SEM, EDX, FTIR, XRD, UV, ^1H NMR and rheological analysis. The microstructural features of lyophilized GelMA hydrogels were visualized using scanning electron microscopy (SEM), as shown in Fig. 2a. These images reveal a highly porous, interconnected three-dimensional network characteristic of freeze-dried hydrogels. The overall architecture exhibits a homogeneously distributed porous matrix, with pore sizes in the range of hundreds of nanometers. The structural uniformity suggests consistent gelation and crosslinking throughout the bulk material [25]. The walls of the pores appear smooth and well-defined, indicative of successful methacrylation. The formation of such well-developed pores is essential for efficient mass transport and diffusion of biomolecules such as primers, DNA templates, and enzymes during molecular amplification processes. Moreover, the open porous network may contribute to improved reagent retention and reduced evaporation [26]. The porous morphology also facilitates mechanical compliance and water uptake, providing an environment that can mimic soft biological tissues. This is particularly beneficial for biosensing and diagnostic assays where hydrogel deformation under thermal cycling could otherwise influence amplification fidelity. The consistent porosity and interconnected channels observed across samples further confirm the reproducibility of the GelMA synthesis and fabrication protocol.

To examine the surface structure and elemental composition of the synthesized hydrogel, SEM–EDX analysis was performed. Elemental mapping was conducted to evaluate the distribution of carbon, oxygen, and nitrogen throughout the matrix [27]. The EDX elemental mapping images revealed that carbon (Fig. 2c), oxygen (Fig. 2d), and nitrogen

Table 1 Composition of GelMA hydrogels used.

Sample Name	GelMA Conc. (mg)	LAP Conc. (mg)	UV exposure (Sec)
GEL-LAP0	10	0	10
GEL-LAP2.5	10	2.5	10
GEL-LAP5	10	5	10
GEL-LAP7.5	10	7.5	10

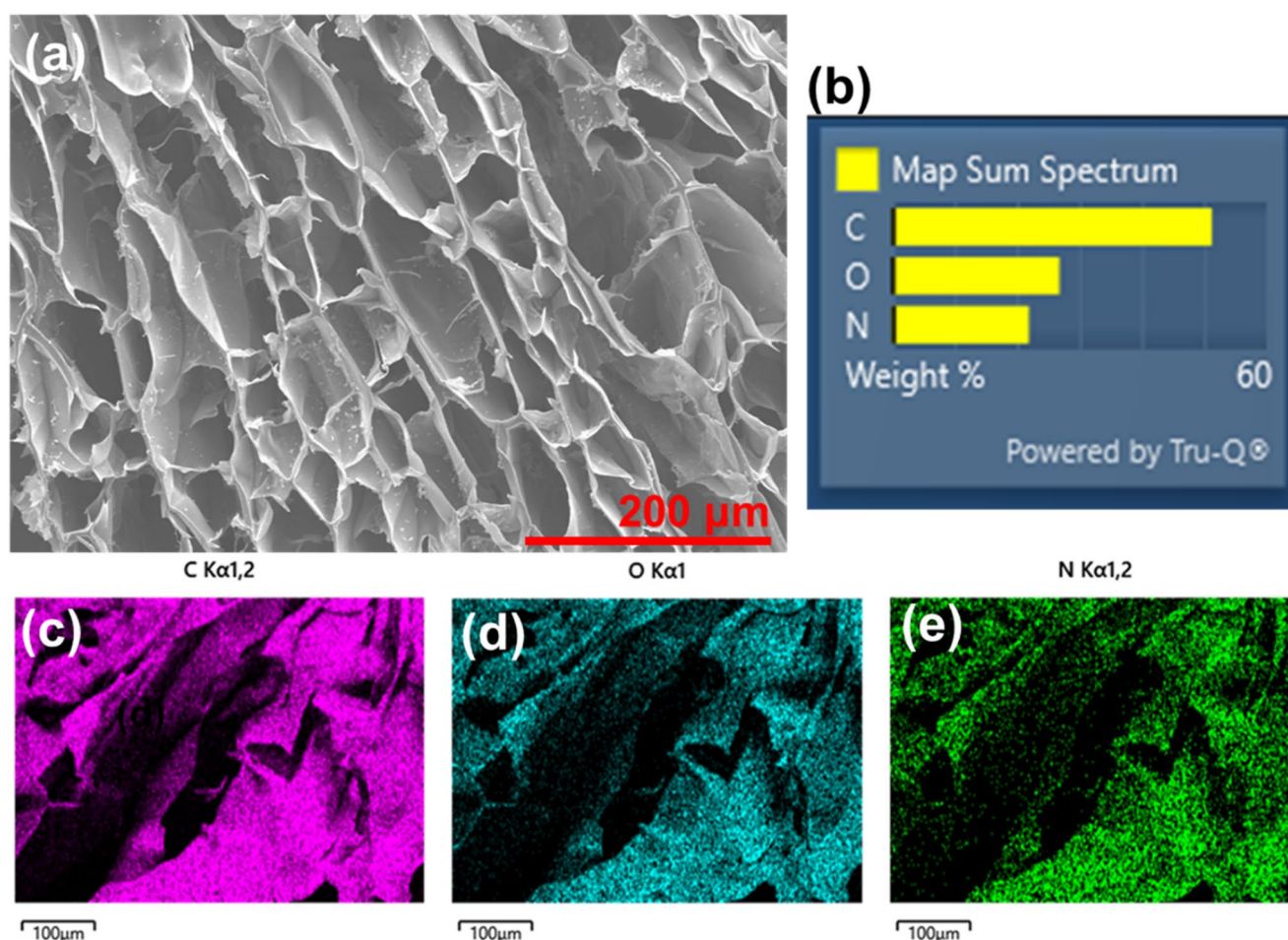


Fig. 2 (a) SEM images of the GelMA (b) SEMEDX elemental composition analysis, Elemental mapping images of (c) Carbon, (d) Oxygen, (e) Nitrogen

(Fig. 2e) were evenly distributed throughout the GelMA which suggests the uniform elemental dispersion across the hydrogel. The consistent detection of nitrogen highlights the protein-based nature of the gelatin backbone, while the presence of carbon and oxygen aligns with the functional groups such as hydroxyl, carboxyl, and methacrylate. The EDX spectrum supported the elemental analysis by showing distinct peaks for carbon, oxygen, and nitrogen, with no notable signals from contaminants. Quantitative data presented in the inset bar graph indicated that carbon was the most abundant element, followed by oxygen and nitrogen, as illustrated in Fig. 2b. This elemental profile reflects successful methacrylation and confirms that the purification process effectively removed unreacted components during GelMA synthesis.

To confirm the successful methacrylation of gelatin, proton nuclear magnetic resonance (^1H -NMR) spectroscopy was performed (Fig. 3a). The ^1H -NMR of GelMA shows significant signals in the 5–6 ppm region (a + b) which correspond to the vinyl protons of methacryloyl groups, absent

in unmodified gelatin, confirms the incorporation of methacrylate groups [28]. Additionally, a distinct resonance around 1.8 ppm (d), attributed to the methyl protons of the methacryloyl moieties, was evident in the GelMA spectrum. These spectral features indicate that the methacrylation primarily occurs at the primary amine and hydroxyl functional groups of gelatin [29, 30]. The presence of these vinyl protons is critical because they are the reactive sites for UV-induced photopolymerization during hydrogel formation. The presence of characteristic signals from the original gelatin backbone in the GelMA spectrum indicates that the methacrylation process does not significantly alter or degrade the fundamental structure of gelatin. This preservation of the native structure ensures that the biocompatibility and intrinsic biological functions of gelatin remain intact. Such a controlled, partial modification is beneficial, as it allows the material to retain its favorable biological characteristics while introducing photo-crosslinkable features.

Fourier-transform infrared (FTIR) spectroscopy was conducted to investigate chemical bonding changes before and

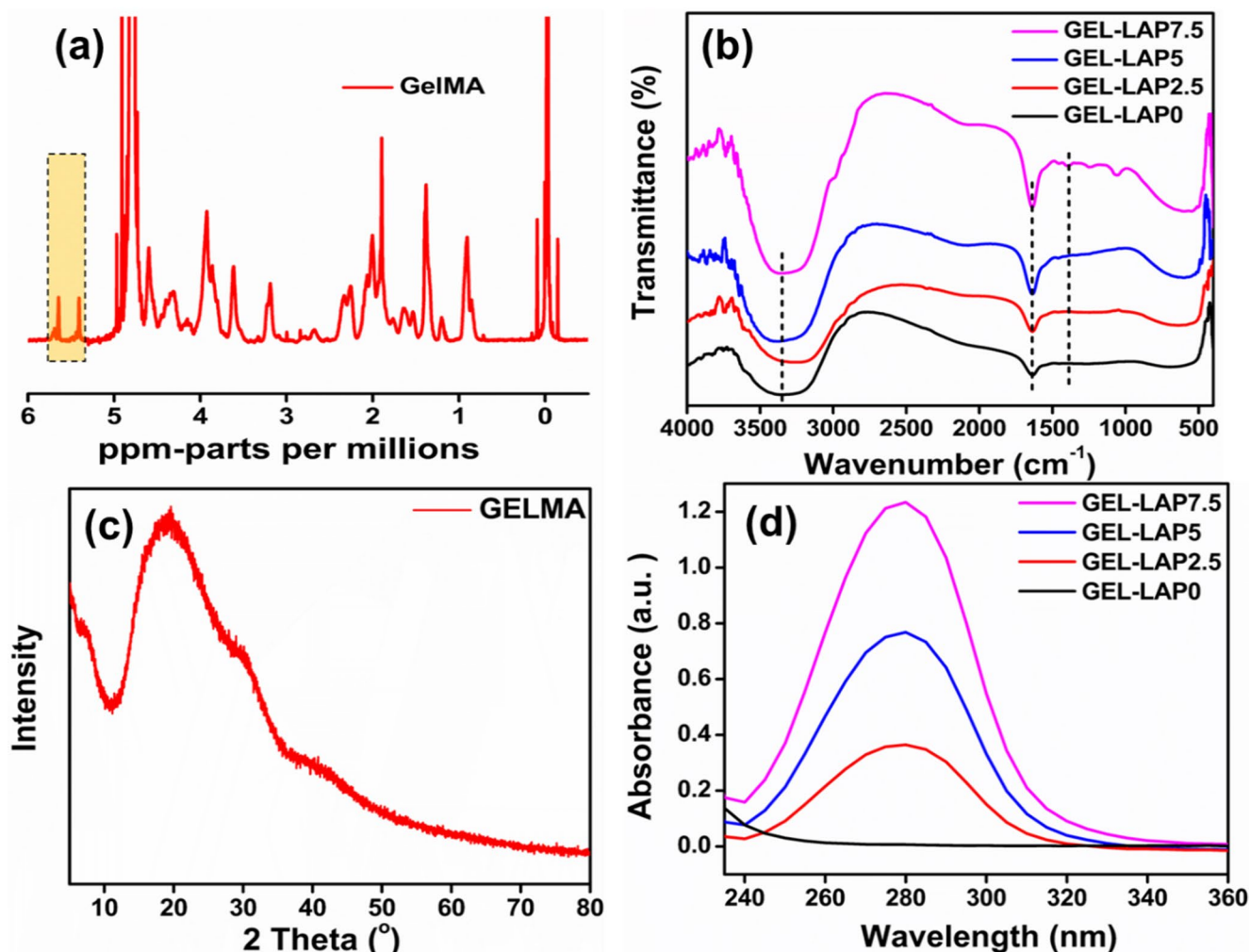


Fig. 3 (a) ^1H -NMR spectra of GelMA, (b) FTIR spectra of GelMA with different concentration of LAP, (c) XRD pattern of the GelMA, (d) UV-Vis. absorption spectra of different concentration of LAP

after LAP incorporation in GelMA (Fig. 3b). The spectra show characteristic peaks around 1630 cm^{-1} (C = O stretching from amide I), which is retained across all samples, indicating preserved peptide bonds [15, 16]. An increase in intensity and slight shifts in the -OH and -NH stretching vibrations ($\sim 3200\text{--}3500\text{ cm}^{-1}$) are observed with increasing LAP concentration, suggesting enhanced hydrogen bonding and network density in the photo-crosslinked L-GelMA. X-ray diffraction (XRD) analysis (Fig. 3c) further confirms the structural transformation of gelatin to GelMA. GelMA exhibits a broad, less intense diffraction pattern compared to the peaks of native gelatin, indicating a transition from a semi-crystalline to a more amorphous structure [31]. This loss of crystallinity is consistent with the introduction of methacrylate groups, which disrupts the regular packing of gelatin chains [32, 33].

UV-Vis. absorption spectra (Fig. 3d) illustrate the optical behavior of GelMA solutions with varying LAP concentrations. A strong absorbance peak is observed around 280 nm,

corresponding to the aromatic components of LAP [34, 35]. As LAP concentration increases, the absorbance intensity increases significantly, confirming the successful incorporation of LAP and its potential to initiate efficient photo-cross-linking. The results suggest that higher LAP concentrations enhance the hydrogel's UV reactivity, which is crucial for forming stable crosslinked networks. The most pronounced increase in absorbance was observed in the GEL-LAP7.5 sample. This upward trend in absorbance intensity is linked to the higher concentrations of LAP, which contains aromatic and conjugated structures capable of absorbing UV radiation in this region. Notably, LAP demonstrates strong UV absorption near 280 nm, owing to its benzophenone-based framework, which plays an essential role in initiating the free-radical polymerization process under UV illumination.

The viscoelastic properties and mechanical strength of GEL-LAP2.5, GEL-LAP5, and GEL-LAP7.5 were evaluated to assess their rheological performance following

UV-induced crosslinking. The temporal evolution of the storage modulus (G') and loss modulus (G'') is illustrated in the dynamic time sweep curves shown in Fig. 4a. The G' characterizes the elastic, solid-like nature of the hydrogels, while the G'' represents their viscous, fluid-like characteristics. Rheological analysis was initiated prior to UV irradiation and carried on throughout the gelation phase, enabling real-time monitoring of the crosslinking dynamics. For all LAP concentrations tested, a pronounced rise in the G' was detected at approximately 60 s, aligning with the onset of UV exposure. This indicates swift gel formation and effective initiation of the photopolymerization process, resulting in the development of a crosslinked polymer network [36, 37]. Among all the formulations, GEL-LAP7.5 exhibited the highest G' , signifying the development of a more robust and elastic hydrogel network attributed to its higher LAP content. Conversely, GEL-LAP5 and GEL-LAP2.5

showed a gradual decrease in G' values, implying that lower amounts of photo initiator yield softer gels with reduced mechanical strength. Among the tested samples, the G' remained substantially greater than the G'' , confirming that the hydrogels exhibit primarily elastic, solid-like behavior in post-crosslinking. Although G'' did increase during the gelation process, it stayed markedly lower than G' , underscoring the formation of a stable and rigid hydrogel network. Moreover, the ratio of storage to loss modulus (G'/G'') rose with increasing LAP concentration, reinforcing the idea that higher photo initiator levels enhance the mechanical strength and stiffness of the hydrogel. The plateau in the modulus values between 60 and 90 s indicates that the crosslinking process reaches completion rapidly. This rapid gelation is an important feature for applications such as bio-fabrication, injectable hydrogels, or hydrogel systems compatible with thermocycling processes like PCR.

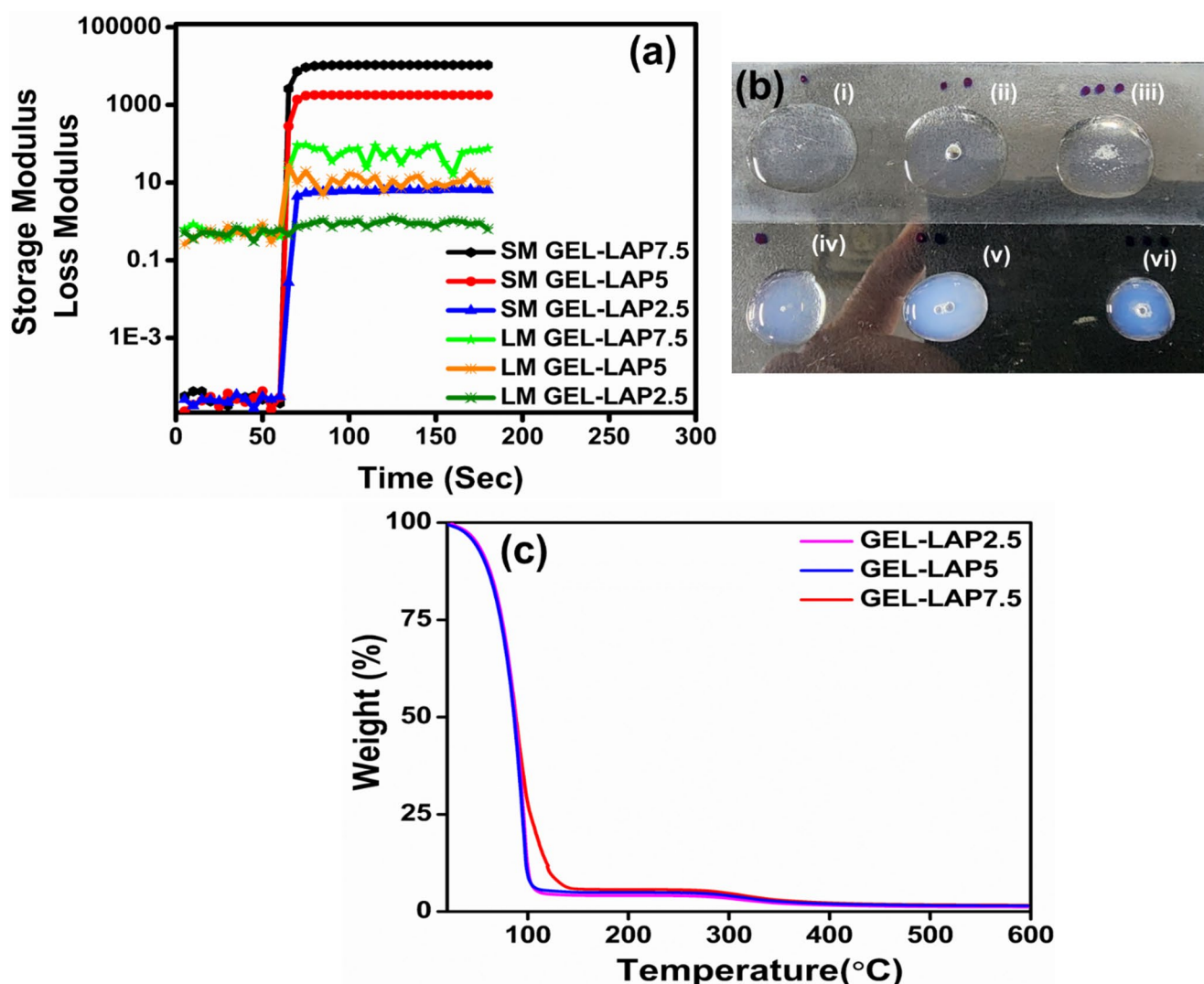


Fig. 4 (a) Viscoelastic properties of GEL-LAP2.5, GEL-LAP5, and GEL-LAP7.5. (b) Photographic documentation captured before (i-iii) and after (iv-vi) UV exposure. (c) TGA of GEL-LAP2.5, GEL-LAP5, and GEL-LAP7.5

The crosslinking efficiency and physical transformation of GEL-LAP2.5, GEL-LAP5, GEL-LAP7.5 hydrogels were examined through photographic documentation captured before and after UV exposure, as illustrated in Fig. 4b. Figure 4b(i-iii) display the images of GEL-LAP2.5, GEL-LAP5, and GEL-LAP7.5 samples before UV exposure. Prior to the UV exposure, all hydrogels appeared transparent and shows a semi-liquid gel which reflects the uncrosslinked state of the hydrogel. No noticeable differences in transparency were observed between the various formulations, suggesting that varying LAP levels had no impact on the appearance of the samples before crosslinking. Figure 4b(iv-vi)) shows the images of GEL-LAP samples with 2.5%, 5%, and 7.5% LAP concentrations, respectively, after UV irradiation. Post-photoirradiation, a noticeable change in the overall appearance is evident. The hydrogels become opaquer, exhibit better structural integrity, and display increased cloudiness. This transformation results from the photoinitiated crosslinking of methacrylate groups, which leads to the creation of a dense, three-dimensional polymer network that scatters light and enhances the material's mechanical strength. The GEL-LAP2.5 sample (Fig. 4b-iv) appears mildly cloudy with a loosely held shape, indicating a low level of crosslinking. GEL-LAP5 (Fig. 4b-v) presents a more consistent and visibly denser structure than GEL-LAP2.5, reflecting improved formation of the polymer network. In the case of GEL-LAP7.5 (Fig. 4b vi), the material shows a clearly defined, compact, and highly opaque appearance, suggesting the highest crosslinking density and rigidity among all tested samples. These visual findings are consistent with the rheological analysis, supporting the observation that greater LAP content enhances the structural integrity and mechanical strength of the hydrogel. The

thermal stability of all the hydrogels was assessed through TGA analysis, as illustrated in Fig. 4c. All samples exhibited an initial weight loss in the range of approximately 40–100 °C, which is attributed to the evaporation of free and bound water within the hydrogel network. No polymer backbone degradation occurred in this region. The thermal decomposition of the organic components commenced only around 270–280 °C, indicating that all the hydrogels remain structurally stable. Since PCR thermocycling involves maximum temperature steps at 95 °C, and this is well below the decomposition onset, the hydrogels preserve their structural and chemical integrity throughout PCR processing, with only minor water evaporation, which can be minimized by performing reactions under sealed conditions.

Amplification of DNA

The suitability of GelMA-based hydrogels as a platform for thermal DNA amplification was assessed by performing PCR targeting the HPRT1 gene under a range of experimental conditions. The objective was to assess the effects of GelMA, the LAP photo initiator, and UV-induced crosslinking on the efficiency of DNA amplification and the accessibility of the polymerase enzyme. After PCR amplification, the resulting products were analyzed using 1% agarose gel electrophoresis, treated with ethidium bromide for staining, and observed under UV illumination, as depicted in Fig. 5 [38–40]. The Fig. 5a(i) shows the DNA ladder, used as a reference for determining molecular sizes. The sharp, distinct bands indicate high-quality gel performance and enable precise sizing of amplified fragments in the test lanes. The regular spacing between bands reflects consistent

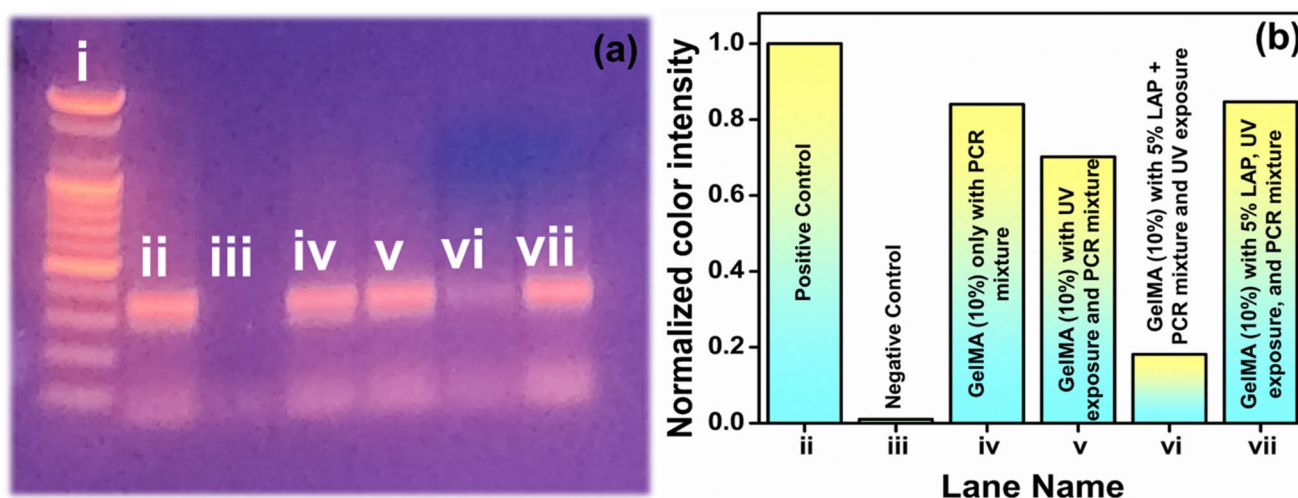


Fig. 5 (a) Gel electrophoresis image showing DNA amplification profiles: (i) DNA ladder, (ii) positive control, (iii) negative control, (iv) GEL-PCR mixture, (v) GEL-UV expose-PCR mixture, (vi) GEL-

LAP5-PCR mixture-UV expose, (vii) GEL-LAP5-UV expose-PCR mixture, (b) Quantitative analysis of normalized band color intensity for each lane

migration during electrophoresis and confirms the overall reliability of the gel run. Figure 5a(ii) represents the positive control, providing a baseline for evaluating successful PCR amplification under conventional conditions. This lane includes the full PCR mixture without the incorporation of any hydrogel components. A distinct and well-defined band of the expected size, corresponding to the HPRT1 gene, is clearly visible, confirming optimal reaction setup, active enzyme function, accurate primer binding, and high-quality DNA template. This control is crucial for assessing how the presence of hydrogel matrices influences amplification efficiency in the experimental groups. Figure 5a(iii) shows the negative control, which includes all PCR reagents except for the HPRT1 DNA template. This control is used to verify the absence of contamination and non-specific primer amplification. As expected, no visible band is present in this lane, demonstrating the high specificity of the primers and the absence of unintended DNA within the reagents. This validates that the positive amplification signals in the other lanes are solely due to the target DNA.

In Fig. 5a(iv), the PCR was performed using a mixture that included GelMA without the addition of LAP and without undergoing UV curing. This setup was intended to examine how non-crosslinked GelMA affects the DNA amplification process. A clear band representing the HPRT1 gene was successfully detected, showing that using GelMA in its uncrosslinked form doesn't interfere with the PCR reaction. Even though the material is thick and gel-like, it still allows the enzymes, primers, and nucleotides to move freely enough for the amplification process to work effectively. In addition, the PCR thermal cycling process worked smoothly in this environment, suggesting that unmodified GelMA is well-suited for enzyme-based DNA amplification. Its high-water content and ability to attract and retain moisture contribute to its compatibility with PCR. This allows it to function as a soft, gel-like medium that does not hinder enzyme activity or disrupt heat transfer during thermal cycling. Figure 5a(v) illustrates the results of a sample in which GelMA, without the photo initiator (LAP), was first exposed to UV light before introducing the DNA, primers, and PCR reagents. A clear amplification band corresponding to the HPRT1 gene is observed, confirming that the PCR proceeded successfully. This finding indicates that UV exposure alone, without LAP, does not initiate crosslinking or lead to any significant changes in the GelMA structure. Because no polymer network is formed, the material remains soft and porous, allowing PCR component such as enzymes and nucleotides to move freely. As a result, amplification is not hindered, and the hydrogel continues to support efficient DNA replication.

Figure 5a(vi) presents the outcome of a condition where L-GelMA was blended with the full PCR mixture, including

DNA template, primers, buffer, dNTPs, and Taq polymerase and then exposed to UV light at 365 nm to induce photocrosslinking of the hydrogel matrix. After crosslinking, the sample was subjected to thermal cycling. Interestingly, the agarose gel electrophoresis showed no visible amplification band, indicating that PCR did not occur successfully under these conditions. This outcome suggests that initiating UV crosslinking while PCR reagents are present may interfere with the reaction. The free radicals generated during the photopolymerization process may damage the DNA template. These radicals can also inactivate the Taq polymerase enzyme, both of which are essential for successful DNA amplification. These findings highlight a major limitation of simultaneous crosslinking and PCR setup, emphasizing the need to separate the two processes when working with photopolymerizable hydrogels [41]. In the experiment shown in Fig. 5a(vii), L-GelMA hydrogel was first exposed to UV light to form a stable, three-dimensional network. Once this crosslinking was complete, the full PCR mixture was added to the hydrogel. The clear amplification band shows that the crosslinked GelMA structure allows the PCR reagents to penetrate and function effectively. This means that even though the hydrogel becomes more rigid after crosslinking, DNA amplification can still happen successfully if the reagents are introduced afterward. Based on these findings, we conducted additional tests using different LAP concentrations during crosslinking. The aim was to assess the effect of LAP concentration through changes in crosslink density and radical exposure on the efficiency of DNA amplification within the hydrogel matrix.

Figure 5b illustrates the quantitative evaluation of normalized color intensity for each amplification band obtained from the agarose gel, analyzed using ImageJ software. The intensities of all bands were quantified and normalized to the positive control to compare PCR efficiency under various experimental conditions. As expected, the positive control in lane ii exhibited the highest intensity, confirming optimal PCR performance, while the negative control in lane iii showed an almost undetectable response, confirming the absence of nonspecific products. The samples consisting of GelMA without LAP, both untreated and UV-exposed (lanes iv and v), exhibited moderate band intensity, suggesting that the non-crosslinked GelMA matrix allows sufficient diffusion of PCR reagents and supports the polymerase function effectively. In contrast, when crosslinking was initiated in the presence of the PCR mixture, as in lane vi, the band intensity decreased markedly, reflecting inhibition of amplification by radicals generated during photopolymerization. Notably, LAP-integrated GelMA exposed to UV light prior to adding the PCR mixture, as shown in lane vii, demonstrated substantially higher intensity comparable to the positive control, confirming that sequential

crosslinking followed by PCR preserves enzymatic functionality. Overall, the quantitative data in Fig. 5b are consistent with the qualitative results in Fig. 5a, highlighting that pre-crosslinked GelMA hydrogels can effectively support thermal DNA amplification when polymerization and PCR are performed as separate steps.

Exploring LAP Concentration Effects on HPRT1 Amplification

The compatibility of GelMA hydrogels were investigated with different concentrations of the photo initiator LAP under UV treatment conditions. The gel electrophoresis image in Fig. 6a shows the amplification profiles obtained from various experimental groups, and the corresponding band color intensities were quantitatively analyzed and presented in Fig. 6b. Figure 6a(i) contains a DNA ladder, which functions as a reference standard to estimate the size of the amplified PCR products. Figure 6a(ii) and 6b(iii) together establish the reliability of the PCR conditions in the study. No detectable band appeared in the negative control (Fig. 6a(ii)), which lacked DNA template, indicating that the system was clean and free from any contamination or unintended reactions. On the other hand, the positive control (Fig. 6a(iii)) which contains the complete PCR mix without any hydrogel, shows a clear band confirms that the reaction performs as expected under standard conditions. Figure 6a(iv), 6a(v), and 6a(vi) represent GEL-LAP2.5, GEL-LAP5, GEL-LAP7.5 respectively. In these setups, both the L-GelMA solution, and the PCR mix were exposed to UV light at the same time before running the reaction.

Interestingly, none of these lanes show visible amplification bands. This indicates that exposing the full PCR mixture to UV light in the presence of LAP can interfere with the reaction. The free radicals generated during the photo-crosslinking process may have damaged the DNA or compromised the activity of the polymerase enzyme, which prevents the successful amplification.

Figure 6a(vii) illustrates the PCR outcome for GEL-LAP2.5, where the hydrogel underwent UV crosslinking prior to introducing the amplification mixture. A distinct band appears, confirming successful DNA replication. This result indicates that pre-crosslinking the scaffold before adding PCR components limits radical-induced damage and maintains enzyme functionality. Similarly, Fig. 6a(viii), corresponding to GEL-LAP5 processed under identical conditions, shows a visible amplification band. This band appears slightly more intense than that in 6a(vii) and remains clearly visible without any special equipment. Conversely, 6a(ix) displays the GEL-LAP7.5 sample, which was UV-crosslinked before PCR reagent addition but shows no amplified product. This suggests that a higher concentration of LAP, even in the absence of direct UV exposure to the PCR reagents, may leave residual radicals or hinder diffusion within the matrix, thereby impeding amplification. Furthermore, Fig. 6a(x) depicts the control sample consisting of GelMA and PCR mixture without LAP or UV treatment, where amplification occurs, demonstrating that the hydrogel alone does not obstruct the reaction. Figure 6a(xi) shows GelMA containing LAP mixed with PCR reagents but without UV activation, and amplification is still observed. This confirms that LAP remains non-inhibitory

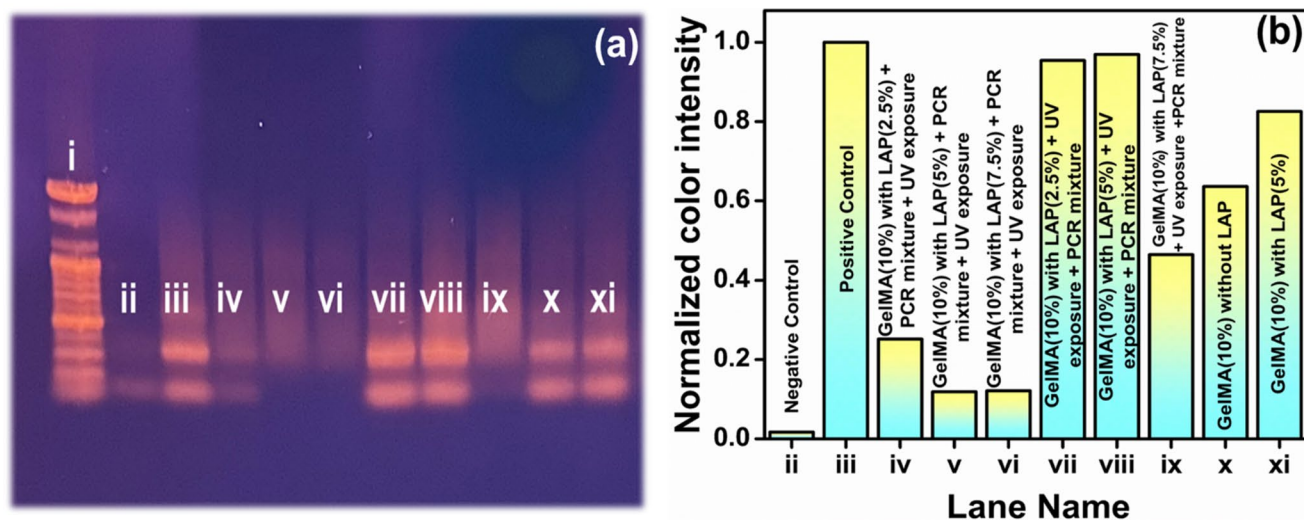
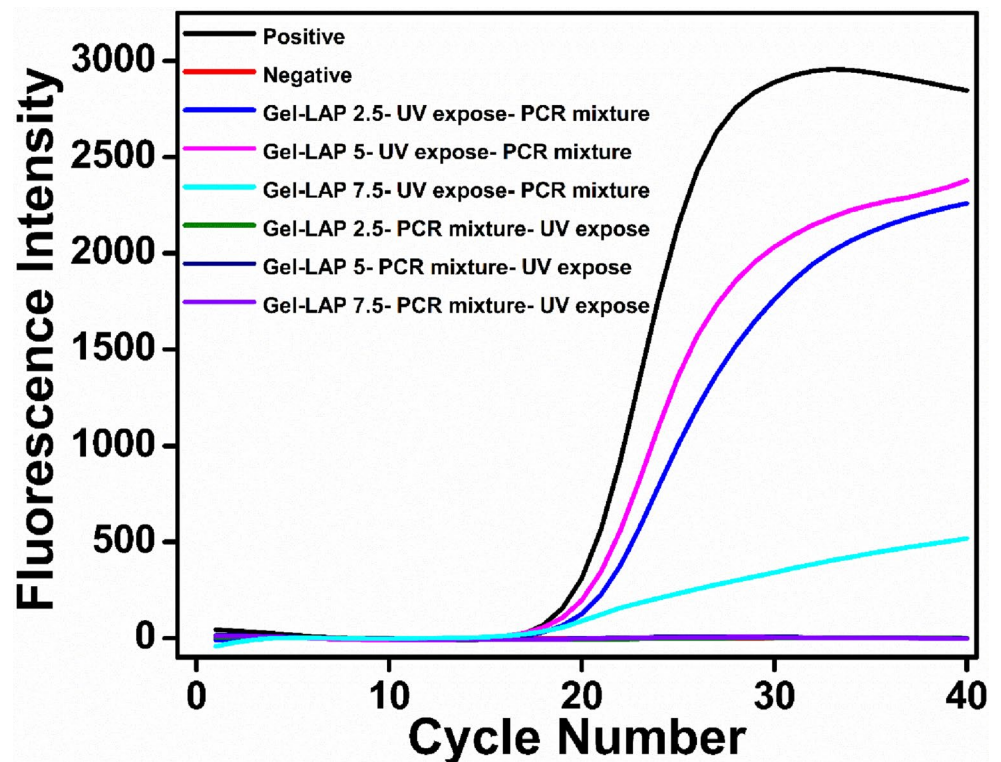


Fig. 6 (a) Gel electrophoresis image showing PCR amplification profiles of different experimental groups: (i) DNA ladder, (ii) negative control, (iii) positive control, (iv) Gel-LAP 2.5-PCR mixture-UV expose, (v) Gel-LAP5-PCR mixture-UV expose, (vi) Gel-LAP 7.5-PCR mixture-UV expose, (vii) Gel-LAP 2.5-UV expose-PCR mixture, (viii) Gel-LAP5-UV expose-PCR mixture, (ix) Gel-LAP7.5-UV expose-PCR mixture, (x) Gel only-PCR mixture, (xi) Gel-LAP5-PCR mixture; (b) Quantitative analysis of normalized band color intensity for each lane

ture, (viii) Gel-LAP5-UV expose-PCR mixture, (ix) Gel-LAP7.5-UV expose-PCR mixture, (x) Gel only-PCR mixture, (xi) Gel-LAP5-PCR mixture; (b) Quantitative analysis of normalized band color intensity for each lane

Fig. 7 RTPCR amplification curves for different Gel-LAP hydrogel conditions



unless photoactivated. Considering these findings, GelMA formulated with 5% LAP emerges as the most suitable candidate for DNA amplification, balancing increased crosslink density with efficient HPRT1 gene replication. Among all concentrations evaluated, this formulation offers the best compromise between structural robustness and amplification efficacy. The graph of normalized color intensity versus lane mirrors the visual patterns observed in Fig. 6b. Samples of pre-crosslinked GEL-LAP2.5 and GEL-LAP5 (lanes vii and viii) exhibit high intensity values comparable to the positive control, indicating efficient DNA amplification. Notably, lane viii shows a slightly stronger signal than the others, closely matching the positive control. In contrast, samples exposed to UV during PCR or containing excessive LAP (lanes iv–vi, ix) display very low intensity, demonstrating significant inhibition. Overall, the graph offers a clear, numerical comparison of amplification efficiency across all conditions, confirming that pre-crosslinked GelMA with 5% LAP achieves the most effective PCR results.

The RTPCR profiles presented in Fig. 7 quantitatively validate the amplification trends observed in the thermal PCR analysis (Fig. 6). The positive control (Black curve) containing the complete PCR mixture without any hydrogel components, shows a strong amplification curve, confirming that the reaction proceeds efficiently under standard conditions. In contrast, negative control (Red curve), exhibits no detectable signal, demonstrating the absence of contamination or nonspecific amplification. When the

PCR mixture and GelMA containing 2.5%, 5%, and 7.5% LAP were simultaneously exposed to UV during photo-crosslinking (Olive, Navy, Purple), no amplification was detected. This aligns with the thermal PCR results and indicates that direct UV exposure in the presence of LAP generates free radicals that damage the DNA template or impair polymerase activity, thereby abolishing amplification. In the subsequent set, where GelMA–LAP hydrogels were UV-crosslinked prior to adding the PCR reagents, a distinct improvement in amplification performance was observed. The GEL–LAP2.5 (Blue) shows a clear amplification curve, demonstrating that pre-crosslinking preserves both DNA integrity and enzyme functionality. GEL–LAP5 (Magenta) exhibits a slightly higher fluorescence signal than GEL–LAP2.5, suggesting more efficient amplification; however, the difference between the two is minimal. Conversely, GEL–LAP7.5 (Cyan) displays a marked reduction in signal intensity despite pre-crosslinking, implying that higher LAP concentrations may retain residual radicals or restrict diffusion within the matrix, ultimately suppressing amplification. Overall, the RTPCR data confirm that GEL–LAP5 provides the most favorable hydrogel environment for efficient HPRT1 DNA amplification.

Conclusion

This study demonstrated that GelMA hydrogels are promising matrices for thermal PCR amplification of the HPRT1 gene. Among the tested formulations, GEL-LAP5 exhibited an optimal balance of crosslinking density and mechanical strength, as confirmed by rheological analysis, supporting efficient DNA amplification. Structural characterizations using SEM, EDX, ^1H NMR, FTIR, XRD, and UV–Vis. spectroscopy verified the successful synthesis and functionalization of GelMA, while rheological data emphasized the effect of LAP concentration on gel stiffness and elasticity. PCR results showed that uncrosslinked GelMA and UV-exposed GelMA without LAP supported successful amplification, whereas simultaneous crosslinking with LAP and PCR reagents inhibited amplification. Notably, loading PCR reagents after crosslinking restored amplification, highlighting reagent accessibility within the hydrogel matrix. Interestingly, GEL-LAP2.5, with a lower LAP content, exhibited weak gelation and lacked the mechanical stability required for reliable handling. In contrast, GEL-LAP7.5, with a higher LAP content, resulted in over-crosslinking, which likely hindered reagent permeability and enzyme function, ultimately suppressing DNA amplification. Only GEL-LAP5 facilitated successful PCR, indicating that LAP concentrations above 5% adversely affect the system, possibly due to increased radical generation that compromises biomolecular stability. Notably, the RTPCR results further supported these findings, showing that GEL-LAP5 yielded the highest fluorescence intensity and maximum DNA amplification compared with all other hydrogel formulations. Thus, GEL-LAP5 emerged as the most suitable hydrogel, combining effective crosslinking with high amplification efficiency. Its semi-solid nature offers advantages over conventional liquid PCR by enhancing stability and ease of handling, making it readily transportable. This hydrogel material holds great potential for developing advanced diagnostic devices capable of trapping and amplifying multiple DNA analytes within different hydrogel platforms simultaneously, enabling multiplexed, contamination-resistant molecular assays.

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Declarations

Conflict of 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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