Fabrication of PEG-Plasticized Epoxy Resin-Based Microfluidic Chips by Casting over PMMA Mold for PCR Applications: Influence CO_2 Laser-Ablation Parameters of Mold

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***Abstract*—Despite dimethyl siloxane (PDMS) being one of the most used microfluidics chips, its micromachining techniques are infeasible for the mass production of commercial devices. This study proposed Epoxy Resin (ER) as a promising, cost-effective alternative to PDMS in lab-on-chip applications, including Polymerase Chain Reactions (PCR). Moreover, a novel, rapid and non-photolithographic approach has been developed for micromachining epoxy microfluidic chips. A new method for fabricating epoxy resin and polyethylene glycol (PEG) hybrid (ER-PEG) microchannels was developed by casting replication over PMMA molds. A PMMA piece was engraved with a CO_2 laser in the raster mode to produce the mold for the ER-PEG casting first. A positive mold was fabricated from a polymethyl methacrylic acid (PMMA) sheet (6mm) by CO_2 laser ablation. The microfluidic chips with the negative pattern were replicated by casting thermoset epoxy resin onto the micro-machined PMMA mold. The entire process, from device design conception to working device, can be completed in minutes. Herein, to enhance the surface roughness (Ra) of the ablated mold surface, different Distances to Focus (DF) were tried. The low Ra in the mold results in a low Ra in the replica. Also, the performance of ER-PEG in PCR was evaluated to verify the compatibility of the chip material to the PCR test.**

***Index Terms*—Polymethyl Methacrylic Acid (PMMA), CO_2 laser ablation, z-axis, positive mold, epoxy resin, casting**

I. Introduction

For the accurate processing of liquid at the micro/ nanoscale, microfluidic technology has been extensively used in the biological [1], medical [2], chemical [3], and environmental [4] fields. First, microfluidics devices were made with glass or silicon materials with MEMS (micro-electro-mechanical systems) fabrication technologies [5], which typically involved complicated and expensive photolithography and chemical/physical etching processes [1]. The microfabrication techniques for polymer-based microfluidics have been widely investigated by researchers worldwide, including replica molding [6], laser ablation [7], hot embossing [8], injection molding [9], and 3D printing [10]. The laser micromachining technique presents a promising alternating approach for microfluidic devices' fast and inexpensive production [11]. Tuning the laser parameters (speed, power, and focal distance) allows geometry depth and width control, highlighting the technique's versatility [12]. The major drawback of laser ablation procedures is the poor optical condition of the engraved surface [13]. The incident laser beam causes the material to melt, vaporize, and eject, promoting the emergence of pores and the deposition of material residue. This results in high surface roughness and limits the applicability of laser-ablated devices [14]. Ablation includes vaporization and melts ejection from the focal area [15]; by increasing the distance to focus (DF), the laser intensity decreases, which results in better engraving quality. The effect of DF has been studied to investigate its effect on the microchannel properties such as width and depth [12]. There is no study -according to our knowledge- that has been investigated to study the effect of different DF on mold fabrication and the resultant surface roughness.

Epoxy resin has the potential to be a valuable material for microfluidic devices because of its transparency and low price. However, epoxy resin-based materials have some drawbacks, including brittle mechanical properties and production flexibility [16]. Various epoxy chemical

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or physical modifications have been considered to solve some of these problems, including incorporating plasticizers and nanofillers. The most modifiers used are low-molecular-weight liquid rubbers, inorganic nanofillers, and engineering thermoplastics. Of these modifiers, low-molecular-weight liquid polyethylene glycol (PEG) is because of its compatibility with oligomer. Therefore, PEG was considered an appropriate plasticizer for toughening brittle epoxy [17].

It was observed that incorporating polyethylene glycol (PEG) with low-molecular weight into the epoxy matrix in small quantities (up to 10%) enhances the fracture and impact properties of the thermosetting epoxy polymer significantly. At the same time, it does not influence tensile properties [18].

The polymerase chain reaction (PCR) is a thermal cycling method used to amplify target DNA [19]. Concerns about PCR biocompatibility with materials have been raised since the invention of the first PCR microdevices [20]. Currently, various materials are used in producing microfluidic chips, such as silicon, glass, several plastics, and many others. After these materials encounter biomolecule's reaction components, they adsorb and inhibit biomolecules. Adsorption and inhibition are both problems that should be avoided because the reaction will fail even if one of the components is inhibited [19].

Therefore, this study aimed to develop a rapid casting onto laser ablated polymethyl methacrylic acid (PMMA) mold approach for epoxy resin-based microfluidics and explore the impact of PEG_{400} on the structural properties of epoxy resin and the demolding step. The profile of the fabricated microchannels, along with the surface quality of mold and replica, was also discussed in this study.

II. Methodology

A. Fabrication of PMMA-Positive Molds

The positive molds based on PMMA were machined by CO_2 laser ablation on PMMA sheets with a thickness of 6mm using a commercial benchtop CO_2 laser system with a wavelength of 10.6μm (Universal Laser System, VLS 3.5, USA) with a maximum power of 30W and a z-adjustable stage. Based on the designed pattern of microfluidics, using CorelDrawX5 2010 and laser system software associated with a computer-aided design (CAD) plotter, which provides the feature to specify the processing parameters, including laser power and the scanning speed, a positive pattern was created, and imported to the laser system. Based on our preliminary study, the optimum power (P) and scanning speed (U) were 80W and 20mm/s, respectively. At the same time, the focal distance was varied to investigate the effect of distance-to-focus (DF). This programmed software was used to alter the laser's power, speed, and cutting stage's z-position to control the laser's parameters. For PMMA, the distance-to-focus (DF) is calculated as the distance between the stage and the laser's focal point (6mm, 10mm, 15mm, 20mm, and 40mm; a focused laser setup requires a minimum distance of 6mm) [12].

B. Preparation of Epoxy Resin with PEG_{400}

Epoxy resin (diglycidyl ether of bisphenol-A (DGEBA) with epoxide equivalent mass of the resin (185-190) and curing agent (modified cyclo-aliphatic polyamine) were purchased from Green Build Chemical Company (Egypt). Polyethylene glycol (PEG) with a molecular weight of 400 was acquired from Loba Chemie Pvt. Ltd. Based on our preliminary research, the mixed ratio of epoxy resin (ER) and curing agent (hardener) was 2:1.25. After mechanically stirring for 20 minutes, the mixture was degassed in a vacuum oven at 25°C for 20 minutes to remove bubbles before being cast into silicone molds [16]. The cast resin was left at 60°C for 30 minutes to complete the curing process. The epoxy resin was mixed with 10% PEG_{400} at room temperature for 15 minutes before adding the hardener to make the epoxy/PEG hybrid (ER-PEG). The curing process is the same as the control sample. The addition of the PEG showed better processability as by adding PEG, the demolding process was achievable compared to epoxy resin which has high adhesion to the mold.

C. The Casting of PEG_{400}-Plasticized ER over a Positive Mold

CO_2 laser ablated positive molds were cleaned; PMMA was washed with isopropanol to remove any ablated powder. The mold was placed on the bottom of the silicon mold, then ER-PEG was cast carefully over the mold, then cured at 60°C for 30 minutes. The casted ER-PEG was demolded from the mold. A 3D laser microscope measured the surface roughness of the parts cast over the ablated mold.

D. Characterization of Epoxy Resin and Its Based Chips

The chemical structure of cured epoxy resin and samples with different concentrations of PEG were analyzed by Fourier transform infrared (FT-IR) spectral analysis. FTIR spectra were recorded on a Shimadzu FTIR-8400S spectrometer with a resolution of $4cm^{-1}$ and 32 scans in the range of $400cm^{-1}$ to $4000cm^{-1}$. The samples were analyzed in KBr pellets. Also, the mold and replica surface roughness was measured using a 3D profile measurements laser microscope (KEYENCE VK-x100), as shown in Fig. 1.

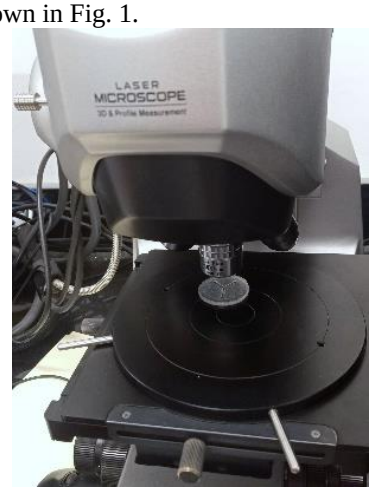

Fig. 1. 3D laser microscope measurement of epoxy resin replica.

E. PCR Compatibility Test

This test studied the effect of exposing a polymerase chain reaction (PCR) mixture to cure ER-PEG. DNA samples (kindly provided by Sarah Hassanein). DNA purity and quantification measurements were done using NanoDrop (Thermo Scientific™ Nanodrop 2000c, USA) and were used as well to quantify the DNA present in all the samples. The entire length of the epidermal growth factor receptor (EGFR) exon 19 gene was amplified using the oligonucleotide primers Ex19 Fw (5′-AGCATGTGGCACCATCTCAC-3′) and Ex19 Rv (5′-ATGAGAAAAGGTGGGCCTGA-3′). The reaction mixtures volume was 25µl containing 0.1ng of sample DNA, 12.5µl 2x PCR FastGene Taq 2x Ready Mix (Nippon Genetics, Germany), a 200 nM concentration of both primers, and 2.5 µl bovine serum albumin (BSA). BSA weight (1µg/µl) dissolved in freshly prepared phosphate buffer saline (PBS) solution (pH 7.4) [21]. PCR cycling was performed in a Lab cycler (Senso Quest GmbH, Germany) with the following parameters: one cycle of 5 minutes at 95°C, followed by 35 cycles of denaturation for 40 seconds at 94°C, annealing for 40 seconds at 63°C, extension for 40 seconds at 72°C and final extension at 72°C for 10 minutes [22]. The reaction mixture was added to a PCR tube containing a piece of ER or ER-PEG10% to test its effect on the PCR test efficiency.

F. Gel Electrophoresis

PCR amplification products were visualized by running in 1.0 % agarose gel in a 0.5X TBE buffer system with 90V for 35 minutes and in horizontal gel electrophoresis apparatus (Cleaver Scientific, UK), followed by staining with ethidium bromide nucleic acid staining solution and pictured on UV Transilluminator, Nippon Genetics, Germany. Fragment sizes were approximated using eco in Action (ready-to-use) 100 bp DNA Ladder (H3 RTU).

III. Results and Discussion

A. FTIR Spectra

Fourier transform infrared (FT-IR) spectral analysis reveals the chemical structure of the investigated samples. FT-IR spectra of the cured epoxy resin samples were recorded by (Shimadzu FTIR-8400 S, Japan) in the 400–4000cm^{-1} wavenumber range. The curing process can be indicated from Carbon-Nitrogen group vibration at 1108cm^{-1} [16], [23], NH_2 vibration absorption at 3340cm^{-1} to 3200cm$^{-1,}$ and C–OH at 3400cm^{-1} to 3500cm^{-1} [24]. The addition of the PEG does not affect the curing process because ER-PEG10% and ER-PEG20% show the previously mentioned bands at the same frequency level as ER, as shown in Fig. 2. ER-PEG 10% was selected because it demonstrated better processability in the demolding step compared with ER-PEG20%. The max strain of ER-PEG20% was 43.732%, leading to deformation of the casted pattern; however, ER-PEG10% has a max strain of 11.192%.

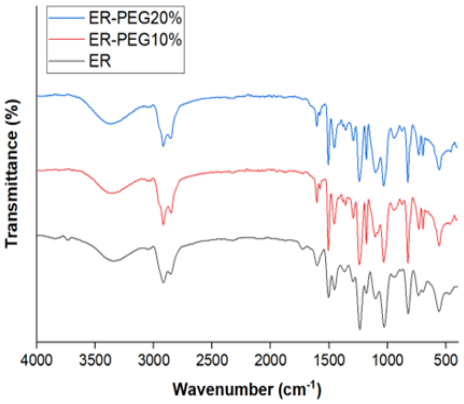

Fig. 2. FT-IR spectra of neat epoxy resin (ER), ER-PEG10%, and ER-PEG20%

B. 3D Laser Microscopy

To enhance the surface roughness of laser-ablated parts of the mold, different distance-to-focus (DF), i.e., the distance between the focal point of the laser and the substrate, were applied. As a result, this manufacturing method is extremely versatile, allowing for rapid iterative prototyping and sharing microfluidics designs. PMMA with a thickness of 6mm was CO_2 laser ablated to make a positive mold for ER-PEG10% replica casting. A 3D laser microscope examined both the mold and the replica. By increasing the distance-to-focus (z-axis) above the thickness of 6 mm PMMA, a considerable decrease in the ablated surface roughness was observed, as shown in Fig. 3; from the previous results, *Z*=15 mm was selected for ER-PRG10% replica casting because at this distance the lowest Ra was recorded (4.246±1.554µm). Distance-to-focus represented the distribution or spread of the energy. Higher DF increases energy distribution over a wider region and decreases the laser's energy density [12], resulting in a smoother and clearer ablated surface.

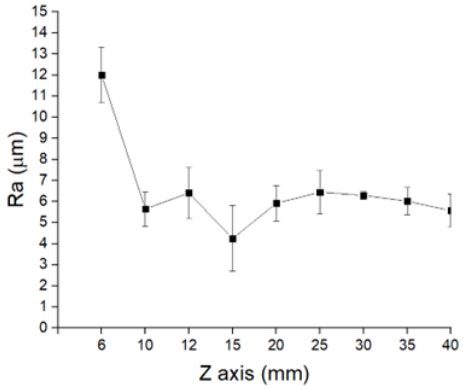

Fig. 3. Surface roughness (Ra) of the ablated PMMA surface at different z-axis distances.

Focused laser configurations (low DF) generated lower surface roughness than highly unfocused configurations with higher surface roughness (12±1.3256µm). As demonstrated in Fig. 4A, at *Z*=6 mm, the ablation process resulted in a very deformed channel compared with *Z*=15 mm, as shown from the 3D optical images. At *Z*=6mm (the same thickness as the PMMA sheet), the non-ablated surface was affected by the high laser intensity due to low

DF. However, at high DF (*Z*=15mm), the laser intensity was lower, no heat defects were observed on the non-ablated surface, and low surface roughness was measured at the ablated surface. The same results were demonstrated in Fig. 4-B and 4-C, where the images show the difference in the mold at *Z*=6 (unclear and full of debris) compared with *Z*=15mm (clear and smooth surface). The low Ra in the mold means low Ra in the replica. Ablated surface Ra= 4.246µm at *Z*=15mm, where epoxy replica facing the ablated surface Ra= 4.132µm. Fig. 4-D demonstrated a 3D laser image of the epoxy replica channel with surface roughness=1.250µm, which was cast over the non-ablated surface (Ra=2.254µm).

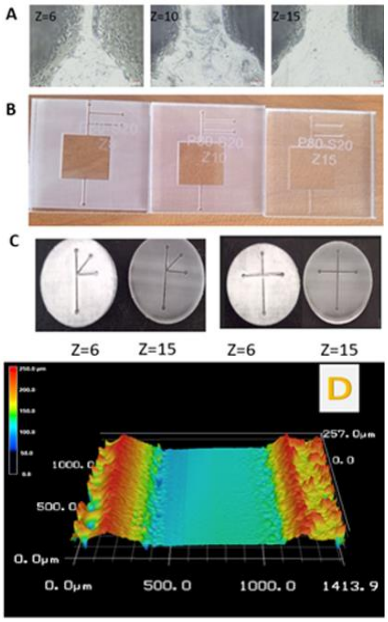

Fig. 4. Positive PMMA laser ablated at P= 80W, U= 20 mm/s, and different z-axis distances in mm; A: 3D laser optical images of the protruded channel, B: Images of the whole mold, C: Images of the mold at different designs, and D: 3D laser image of epoxy replica channel with Ra=1.250µm.

C. PCR Compatibility Test

PCR is a thermal cycling procedure to amplify target DNA. Agarose gel electrophoresis represents the most efficient separation technique for separating PCR products ranging in size from 100bp to 25kb. The phosphate backbone of the DNA molecules that are negatively charged can be separated through an agarose matrix with an electric field according to their size, where shorter molecules migrate faster than longer ones because of the gel-sieving effect. The electrophoresis was performed for each PCR product with a horizontal electrophoresis apparatus [25]. Silicon, various types of silicon oxide, glasses, plastics, adhesives, and wax, are among the common materials employed in fabricating microfluidic chips and were evaluated for PCR compatibility [19]. However, no PCR compatibility test was conducted with cured epoxy resin or its composites. Material-based PCR inhibition can occur through two mechanisms: ions/inhibitory components from the material may leach into the PCR mixture, or critical PCR components may adsorb onto the material surface. [26]. The first mechanism is not happening because a specific and overall migration has been done in our previous study and proved no unreacted monomers were migrated to the solute [16]. Therefore, the second mechanism is why no PCR products appeared in lanes 7 and 8. BSA is thought to compete with polymerase for adsorption at material surfaces, improving PCR yields [19]. Taq polymerase[27] and BSA protein [28], [29] adsorption mechanisms have been extensively investigated. In inhibitor chelation, BSA competes with Taq polymerase [30]. Furthermore, BSA promotes primer annealing, stabilizes both the DNA and the polymerase, and acts as an osmoprotectant-compatible solute. [27]. Gel electrophoresis lanes from 3 to 6 show the effect of adding BSA; the bands are apparent due to the addition of BSA. In lane 2, BSA was added to the control PCR mixture to assure there was no adverse effect of adding it, as demonstrated in Fig. 5.

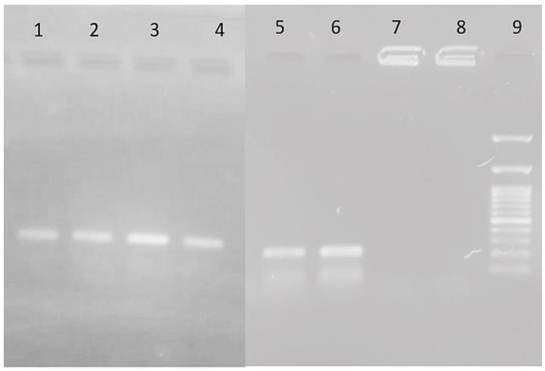

Fig. 5. Agarose gel electrophoresis image of the PCR products: lane 1: PCR control, lane 2: PCR+BSA, lane 3,4: PCR+BSA+ER, Lane 5,6: PCR+BSA+ER-PEG10%, lane 7: PCR+ER, lane 8: PCR+ER-PEG10% and lane 9: the labeled marker set (100 DNA H3 RTU).

IV. Conclusion

Transferring microfluidics fabrication techniques from research labs to the mass production and industrial scale still needs a lot of optimizations and reduced costs. The methods described here enable low-cost transformation from research ideas to prototypes and low-requirement fabrication of devices, facilitating new research groups' acceptance of microfluidic tools. CO_2 laser ablation is a very cost-effective technique and casting of an inexpensive polymeric material such as epoxy resin. The positive PMMA mold must be a smooth surface to reduce the surface roughness of the epoxy replica. Different DF was tested to achieve this aim, and Z=15 mm was the optimum parameter that resulted in Ra= 4 in both mold and replica. Casting ER over PMMA mold was very difficult to demold and caused cracks in the replica. Adding PEG to ER by 10% has enhanced the demolding step and the processability of the fabrication technique. ER-PEG 10% was PCR compatible, introducing a new hybrid material to be used in microfluidic fabrication techniques for biomedical applications and point-of-care (POC) applications.

Conflict of Interest

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

A. M. Fath El-Bab and E. A. Soliman elaborated on the concept of the study. Heba Mansour performed and analyzed the experimental investigations with the support of the other authors. Heba Mansour wrote the manuscript in consultation with E. A. Soliman, and A. Abdel-Mawgood participated in the design of the study and its coordination.

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