iCVD를 통해 합성된 양전하 공중합체를 이용한 핵산 추출 및 정제

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Extraction and purification of nucleic acid using a positively-charged copolymer synthesized via iCVD

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Abstract

The rapid and sensitive diagnostics of viral infection through nucleic acid-based methods, such as PCR, have been significant as the viruses are spreading globally at an increasingly alarming rate. However, the detection methods rely on extracting the nucleic acid from the patients, which requires multiple liquid handling and centrifugation steps. We develop a single-step RNA extraction method using charge interaction between the nucleic acid and positively charged copolymer synthesized via initiated chemical vapor deposition (iCVD). A tertiary aminated dimethylaminomethyl styrene (DMAMS) monomer was copolymerized with varying degrees of epoxy-containing glycidyl methacrylate (GMA) incorporation to design the copolymer. Successful copolymerization was confirmed by FTIR, XPS, and contact angle measurement. The series of copolymer films were directed deposited on the inner surface of the PCR tube and applied to bind and isolate nucleic acid through the charge interaction. The capturing efficiency of the copolymer was comparable to the pDMAMS in a range of 60-80 %. The contaminants, including proteins and cellular components, are expected to react with GMA and assist in nucleic acid purification from the specimen.

1. 연구 배경

The detection of the virus at the early stage, when the viral concentrations are still low in number, has become essential to prevent the wide spread-out of infectious diseases.

PCR-based diagnostic has been widely used to detect viral nucleic acids (NAs). However, as a pre-processing step, the PCR-based diagnostic requires extraction of the NAs from the clinical samples. However, extracting the NAs requires multiple steps for purifying and recovering NAs, which need experts to handle and analyze the data, and require toxic organic solvents such as chloroform.

Therefore, we devised a single-step NA extracting polymer, poly(Glycidyl Methacrylate-co-Dimethylaminomethyl Styrene) (p(GMA-co-DMAMS)). iCVD process has been utilized to polymerize p(GMA-co-DMAMS) using glycidyl methacrylate (GMA) and dimethylaminomethyl styrene (DMAMS). iCVD process could make a thin film on soft substrates such as the PCR tube, which we chose to deposit.

Also, GMA is known to have very stable covalent linkages with different protein groups by an epoxy group. DMAMS has (+) charged amine group absorbing (-) charged molecules by ionic interaction. We supposed that coated tube would covalently capture the protein group, and RNAs could be released for RT-PCR or other PCR methods even though it is a clinical sample.

2.연구 방법

Fabrication of the Single-step tubes using initiated chemical vapor deposition: Single and 8-strip PCR tubes (Bio-Rad Laboratories, Inc., USA) were coated with functionalized p(GMA-co-DMAMS) in a vapor phase. The synthesis of copolymers proceeded with a custom-built iCVD reactor (Daeki High-Teck, Korea). Monomers and initiators~~. Flow rates of GMA, DMAMS and tert-butyl peroxide (TBPO) initiator were managed for 3:1:1, 1.7:1:1, 1:2:1, 1:3:1 respectively.

Chamber pressure and substrate temperature were maintained at 120 mTorr, and 40 °C, respectively. The filament temperature was maintained at 120 °C to trigger.

The polymerization of p(GMA-co-DMAMS) was analyzed using the FTIR spectrophotometer (Alpha, Bruker Optics) in the wavenumber region of 400–4000 cm^{-1} at a resolution of 1.4 cm^{-1}.

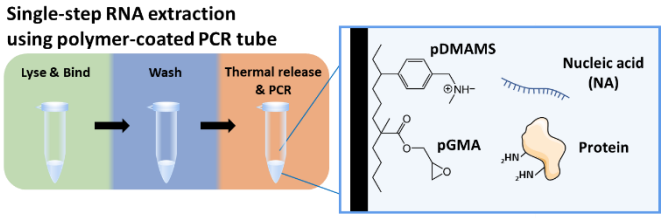
For the XPS analysis (Sigma Probe, Thermo VG Scientific Inc.), pGMA, pDMAMS, p(GMA-co-DMAMS)-coated films were prepared.

3 μL of DNA (Conc. 10 ng μL^{-1} to 1 pg μL^{-1}), which was prepared by tenfold serial dilutions using 10 ng μL^{-1} gDNA, was added into the tubes to measure the amount of captured DNA was measured by real-time PCR.

25 μL PCR cocktail was prepared with the following elements; 1 μL of gDNA, 1.25 μL of GoTaqDNA polymerase (Promega, USA) and SFCgreen I (BIOFACT Co., Ltd., South Korea), 2 μL of 0.8 μM forward and reverse primers and 0.2 mm dNTPs (Promega, USA), 5 μL of 5 X buffer (Promega, USA), and 10.5 μL of ultrapure DNase/RNase-free distilled water (Introgen Therapeutics Inc., USA).

Then, PCR amplification was conducted using CFX Connect Real-Time System (Bio-Rad, USA) under the conditions of initial denaturation at 95 °C for 5 min, 30–35 thermal cycle of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s and a final extension at 72 °C for 5 min.

Subsequently, the quantity of the captured DNA was calculated by subtracting the amount of uncaptured DNA from the injected DNA. The DNA-captured tube was rinsed with deionized water three times and used for PCR afterward to quantify the amount of captured DNAs. All data are presented as mean ± SD of triplicated experiments.



3. 연구 결과

Fig 1. Scheme of Single-step RNA extraction using polymer-coated PCR tube

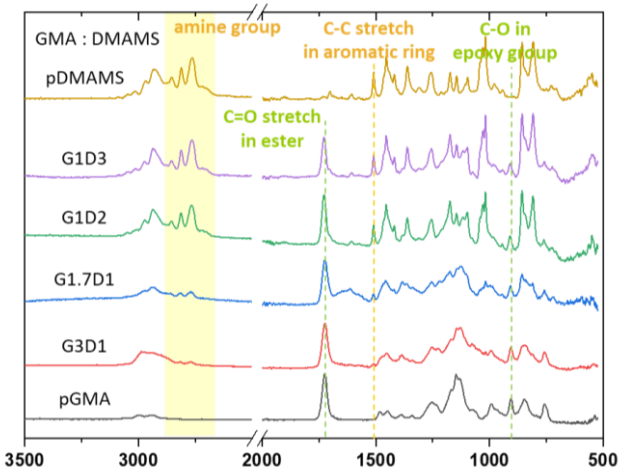


Fig 1. Polymerization Confirmation of Copolymer via FT-IR

Successful copolymerization via the iCVD process from FT-

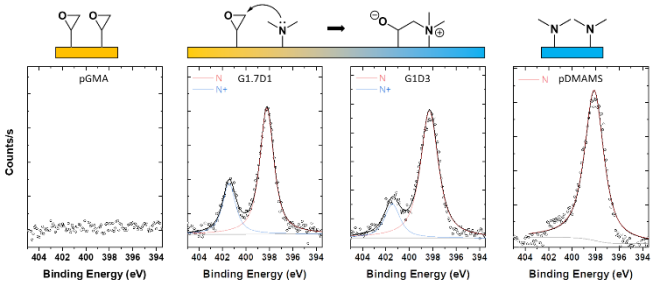


Fig 3. N+ Moiety Change in Different Ratios of Flow rate by XPS (N1s)

IR was confirmed (Fig 2) while remaining controlled ratios of epoxy and amine groups. Also, the water contact angle showed well-preserved amine moiety from the DMAMS monomer after the copolymerization.

According to the XPS data (Fig 3), the gradual increase of the N+ peak was observed as the GMA monomer was added, which will be critical for capturing nucleic acids.

As proof of concept (Fig 4), the series of copolymer films were deposited directly on the PCR tube's surface, and subsequent DNA capturing efficiency was confirmed via PCR.

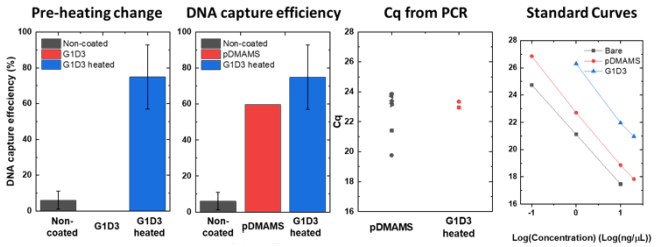


Fig 4. DNA Capture Efficiency of Coated Tubes by qPCR

As a result, the capturing efficiency of the copolymer was comparable to the pDMAMS in a range of 60-80 %, which would be ideal for capturing nucleic acid from the clinical samples without a purification step.

4. Acknowledgements

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