Heat-Shrunken Hierarchical Silica Nanomembrane for Solid Phase DNA Extraction

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Introduction: DNA extraction from blood, serum, or plasma, is a critical step for downstream genetic analysis. Conventionally, liquid phase extraction techniques, such as Phenol/Chloroform precipitation, are widely used. Although this approach yields DNA of better quality, it is laborious, time-consuming and highly operator-dependent. Solid phase extraction techniques, such as spin columns and silica magnetic particles, are gaining popularity these days. These porous matrices and micro/nano particles, although can enlarge surface area for nucleic acid binding, induce high shear during flow and mixing, resulting in DNA of relatively integrity. In this paper, we present a simple strategy to fabricate hierarchical silica nanomembrane by thermally inducing surface wrinkling of heat-shrinkable polyolefin (PO) film deposited with silica. The resulting membrane exhibits overlaying hierarchical structures from nano to micro scale depending on the thickness of silica layer deposited. These nanostructures significantly enlarge the total surface area of silica on the membrane with exceptional binding capacity for nucleic acids as a novel substrate for solid phase extraction. DNA isolated with the proposed nanomembrane shows higher recovery yield, purity and better integrity comparing with commercial columns and particles, as well as comparable performance with gold standard Phenol/Chloroform precipitation.

Materials and Methods: For the fabrication of the silica nanomembrane, silica (Kurt J. Lesker Company) was first deposited onto both sides of the PO film (Uline Inc.) using electron beam physical vapor deposition. Then the silica-coated film was baked at 300°F for 3 min to induce surface wrinkling. The resulted shrunk film is smaller than 10% of its original size and exhibits hierarchical nanostructures under scanning electron microscope. For solid phase DNA extraction, cultured cells were lysed in 50µL proteinase K (10mg/mL, Invitrogen) and 200µL Buffer AL (Qiagen) at 55 °C for 2 hrs. 200µL isopropanol and one piece of silica nanomembrane (ø6mm) were added and the mixture was rotated at room temperature for 30 mins. After discarding all the liquid waste in the tube, nanomembrane was rinsed in 700µL buffer AW1 (Qiagen) once and 500µL buffer AW2 (Qiagen) twice. Then all the liquid waste was discarded completely, and 100µL Buffer AE (Qiagen) was added to elute DNA by incubating at 70°C for 20 mins. Finally, the eluent was transferred into a new tube.

Results and Discussion: Variation in thickness of the deposited silica is mainly responsible for the distinctive hierarchical structures of the nanomembrane. With 2nm silica, the membrane displays only micro-scale ridges rising slightly from the surface. As silica layer gets thicker, micro-ridges become taller and more densely packed. When silica layer reaches 100nm, a large number of nano-flakes emerge and interweave with the ridges, forming overlaying hierarchical nanostructures. Once the silica layer exceeds 150nm, these nano-scale flakes interweave to form secondary structures on micro scale replacing those ridges completely. These hierarchical structures significantly enlarge the specific surface area, enhancing DNA absorption capability on the nanomembrane. We compared the performances of the nanomembrane and commercial particles for control DNA re-isolation in three aspects: recovery yield, purity and integrity. With 4µg genomic DNA input, we were able to recover about 3.2 µg (80%) using 200nm silica nanomembrane, while commercial silica magnetic particles only recovered about 20% DNA under the same condition. DNA shearing induced by commercial particles was also observed by running re-isolated DNA in gel electrophoresis, and DNA isolated using small particles (about 100nm in diameter) were sheared much more significantly compared with using large particles (about 5um in diameter). In contrast, DNA isolated using the silica nanomembrane retained their integrity. We performed DNA extraction from cultured human cells utilizing our silica nanomembrane. We were able to extract 11±4 µg of genomic DNA from about 2×10^6 cells, and gel electrophoresis result proved that the yield DNA had high molecular weight DNA, over 23kb, comparable with those extracted by phenol/chloroform method in both yield and quality. Moreover, with the same amount of cultured cells as input (about 3million), the amount of DNA extracted by spin-column and magnetic particles are only about 42% and 56% of that by our nanomenbrane respectively.

Conclusions: In summary, we have demonstrated a novel method to fabricate silica nanostructures based on self-wrinkling induced by thermal shrinkage. The formation of overlaying hierarchical nanostructures on the membrane is closely related to the thicknesses of deposited silica layer. These micro- and nano-scale structures have vastly enlarged the specific area of silica, thus enabling us to implement the nanomembrane in DNA solid phase extraction. We applied our silica nanomembrane for DNA extraction and demonstrated better performance than commercial columns and particles, as well as comparable performance with gold standard Phenol/Chloroform precipitation in terms of DNA yield and quality. Therefore, we expect this hierarchical nanomembrane to be widely adapted in various DNA analyses.