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Ultra-rapid and low-cost fabrication of centrifugal microfluidic platforms with active mechanical valves

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A. Blood Groups

For assembling a PSA and a PVC film, first the unwanted cut- outs and the top cover of the PSA are removed (see Figure 1.ci). The PSA is then partially covered with a square/rectangular PSA cover (see Figure 1.cii). It should be noted that the bottom side of the square PSA cover does not stick to PSA as, according to manufacturer i.e. FLEXcon, it is coated with silicone, and it is ultra-smooth. A PVC disc is next aligned visually (or/and with the aid of two alignment bars) and partially stuck to the PSA (see Figure 1.ciii). To prevent bubble formation between the PSA and PVC film during assembly, the square cover is gradually removed while the PVC disc is stuck on the PSA layer using a flat object (see Figure 1.civ). To facilitate the fabrication of microfluidic discs with more than three layers that contain sequential PSA and PVC layers of the same design, it is possible to cover one or both sides of the PVC with PSA and then cut the layers all at once. These multiple-layer stacks provide deeper channels and cavities of consequently larger volumes.

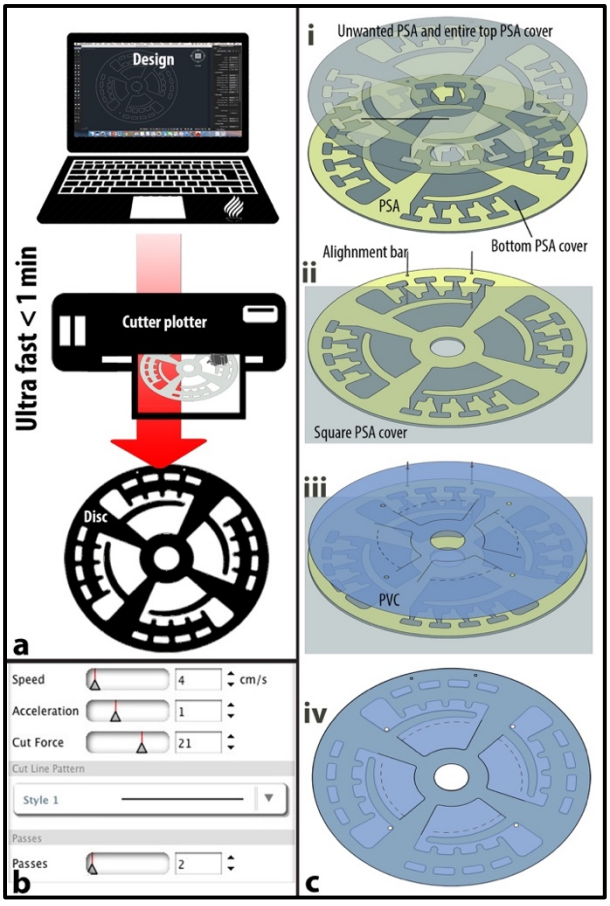

Figure 1. **(a)** Ultra rapid disc fabrication process with cutter plotter. **(b)** The plotter software settings for cutting PVC film. **(c)** Assembly of microfluidic layers: i removing the front PSA cover and unwanted cut- outs, ii covering the PSA with a rectangular PSA cover, iii aligning and placing a PVC layer over the PSA disc. Iv replacing the PSA cover with the PVC disc.

B. PEGylation

PEGylation refers to covalent binding of one or several chains of polyethylene glycol (PEG) to a targeted biomolecule (mainly a therapeutic protein, peptide and antibody).[1] PEG is a non-immunogenic and non-toxic polymer that can improve pharmacokinetic behavior of medicines i.e., decrease immunogenicity and increase solubility of drugs.[2, 3] In general, PEGylation reduces dosing frequency of a drug by increasing its retention time and stability, and reducing its proteolysis and renal excretion. However, the resultant solution after PEGylation is a homogeneous pollution of modified (i.e., mono and di-PEG) and unmodified biomolecules; their separation is mainly achieved through several chromatographic steps and is one of the major challenges in the field. The second fluidic disc is was designed to mimic a protocol for purification of monoPEGylated ribonuclease (RNase) that is recently reported by Hernández-Martínez and Aguilar.[4] They employed a chemically modified resin to separate different PEGylated species in a single chromatographic step employing Hydrophobic Interaction Chorography. In that study, a chemically modified support was used as an alternative for the separation of PEGylated RNase A; the covalent addition of PEG 5000 to Sepharose-6B with a step gradient optimization, showed an improvement of the separation of monoPEGylated RNase with a purity and yield of 85% and 96%, respectively.[4] Thus, the design is composed of a column for Sepharose 6B and five source chambers for washing solution, the sample, 100%, 61%, 48%, and 0% phase A, and a collection chamber. The cartridge's fluidic design contains five source chambers with M-valve for sample, washing and eluent buffers, a protein separation intake/column and a collection chamber.

i. Materials and Sepharose 6B PEGylation

Sepharose 6B, PEG 5000 g/mol, 1,4-butanediol diglycidyl-ether, sodium borohydride, iodine, barium chloride, from Sigma–Aldrich (St. Louis, MO. USA). Ribonuclease A monoPEG were donated by the ITESM Biotechnology, Sodium hydroxide from DEQ (Monterey, Mexico). Ammonium sulfate [$(NH_4)_2SO_4$], monobasic and dibasic potassium phosphate, and water HPLC grade were obtained from VWR (West Chester, PA. USA). All experiments were performed at room temperature except PEGylation reactions as indicated below.

Briefly, 10 g of Sepharose 6B was mixed with 5 mL of 1,4-butanediol diglycidyl ether and 5 mL of 0.6 M sodium hydroxide solution with 2 mg of sodium borohydride per milliliter, for 8 h at 25 °C. Then 16 g of PEG 5000, was dissolved in 50 mL distilled water at 40 °C. The pH was adjusted to 12.5–12.8. After this, 5 g of epoxy-activated Sepharose 6B was added and kept on a rotary shaker for 18 h at 40 °C. Modified Sepharose 6B was treated with NaOH 1.0 M overnight at room temperature to inactivate possible free epoxy sites. Modified resin, referred as Sepharose 6B-PEG5000 were stored at 4 °C for further use.

REFERENCES

1. F. M. Veronese and A. Mero, BioDrugs, 2008, 22, 315-329.
2. N. Nischan and C. P. Hackenberger, The Journal of organic chemistry, 2014, 79, 10727-10733.
3. C. Zhang, X.-l. Yang, Y.-h. Yuan, J. Pu and F. Liao, BioDrugs, 2012, 26, 209-215.
4. A. Hernández-Martínez and O. Aguilar, Separation and Purification Technology, 2014, 136, 190-198.