Supplementary Information

Automating Multi-Step Tests Using Integrated Layering of Reagents

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Supporting Figures

Schematic diagram of the approach for creation pH pullulan film, explained in the experimental section, is illustrated in Figure S1 below.

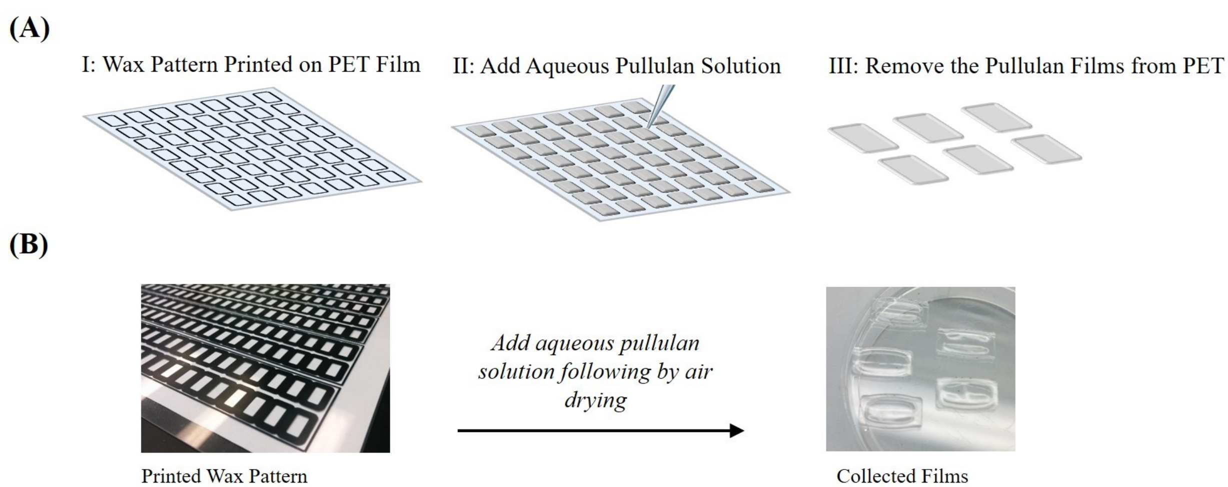

Fig S1. (A) Schematic diagram and (B) actual image of creating pH pullulan film

The creation of disc-shaped or other desired shaped pullulan films is illustrated in Figure S2 below.

Fig S2. Schematic of how to make preloaded pullulan films in (A) a disc shape or (B) other desired shapes, with various dimensions, by pipetting the pullulan mixture on surface of a flexible transparency PET sheet followed by air drying. The desired patterns are created with PowerPoint and printing these on the PET sheet using a wax printer. (C) Examples of various shapes created using this method.

Release Kinetics

To study the release kinetics of the pullulan film, Allura Red was added to the pullulan solution and cast into films as described in the methods section. For sample analysis, serial dilutions of Allura Red dye were prepared to create a standard curve using a spectrometer (Tecan infinite® M1000 plate reader). The absorbance of each sample was then recorded, and the corresponding concentration was calculated using the standard curve.

Figure S3 shows the total mass of Allura Red released from the pullulan film as a function of time for different film thicknesses.

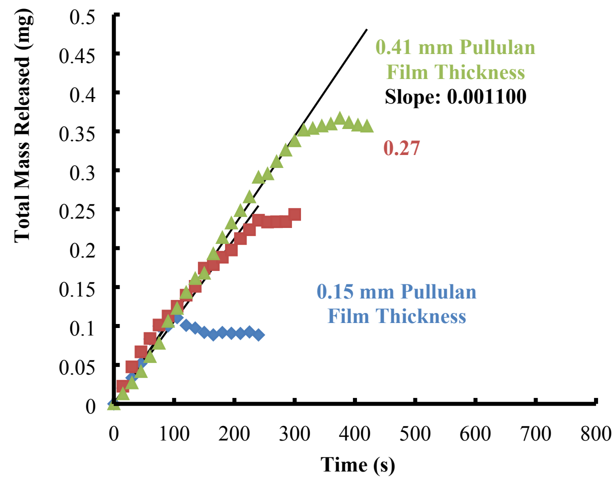

Fig S3. Release Kinetics for pullulan film. Zero order kinetic shown by the total mass of Allura Red release as a function of time.

Furthermore, as depicted in Figure S4, similar kinetic study is done for CMC (Sodium carboxy methyl cellulose, MW 250,000), MC (Methyl cellulose), PVA (Polyvinvyl amine, MW 125,000, 87-89% hydrolysis), and HEC (Hydroxylethyl Cellulose). Depends on the application, different polymers may be combined with pullulan films to prolong the releasing process.

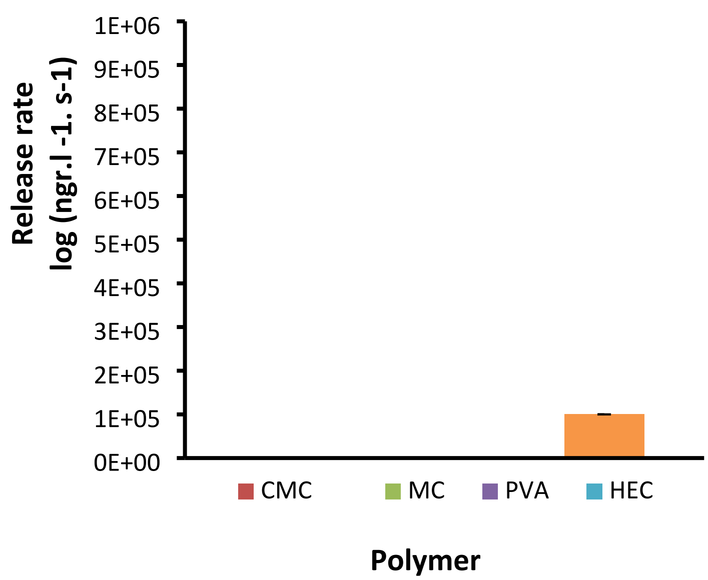

Fig S4. Release Kinetics for different polymeric films.

The delivery of multiple reagents from pullulan films was initially observed by using dyed-pullulan-films, as shown in Figure S5. Pullulan solutions with different dyes were cast and allowed to dry for pattern construction. Placing two films with different dye colors side-by-side on top of Whatman and wetting of the paper by capillary action, allowed for un-mixed dye release on distinct sides of the channel (Figure S5A). Other pullulan film patterns were also constructed, in addition to the side-by-side configuration. Figure S5B shows a 'checkered' pattern film, where different colored films were cut and adhered to a plain film backing. Figure S5C shows an sample of a layer-by-layer stacking pattern. The ease of assembly ensures that these patterns can be easily manipulated and that the pullulan film system can be used for multiple reagent delivery.

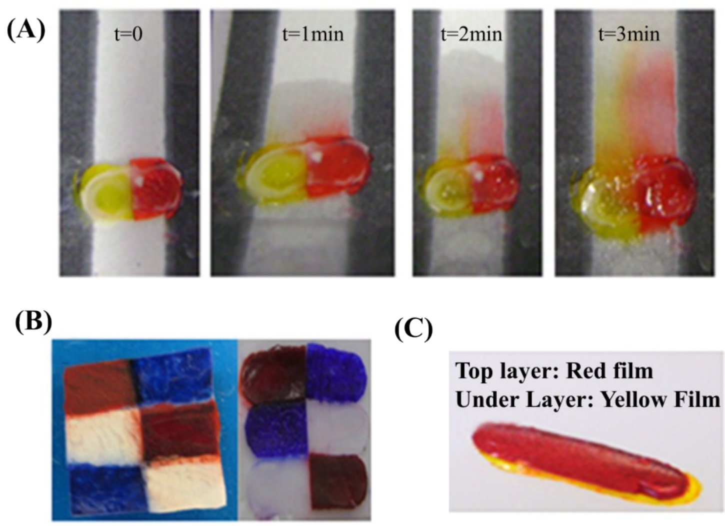

Fig S5. (A) Pictures of dye being released from a 2-tone/side-by-side red and yellow bridge. (B) Examples of Pullulan bridge formations and a 'checkered' pattern. (C) Stack pattern.