

Supplementary information for

## **Air-through-Precursor Suction-Augmented Replica Molding for Fabrication of Anisotropic Microparticles in Gas-impermeable Molds**

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## Experimental

### Materials

Sylgard 184 poly(dimethylsiloxane) (PDMS) was purchased from Dow Corning, USA. NOA 81 was purchased from Norland Products Inc., USA. Epoxy resin was purchased from Epoxiset Inc., USA. Poly(ethylene glycol)diacrylate (PEGDA) with molecular weights of 250 and 700 Da; poly(ethylene glycol) (PEG) with a molecular weight 200 Da; Tween 20; trichloro(1H,1H,2H,2H-perfluorooctyl)silane; azobisisobutyronitrile (AIBN); 2-hydroxy-2-methylpropiophenone (darocur 1173); 1-vinyl-2-pyrrolidinone; and dichlorotris(1,10-phenanthroline) ruthenium(II) hydrate were purchased from Sigma Aldrich, USA. Sera-Mag magnetic carboxylate-modified particles (hydrophilic, 744 nm, 50 mg/mL) was purchased from Thermo Fisher Scientific, USA. Phosphate buffered saline (PBS) was purchased from Welgene, KR. Methacryloxyethyl thiocarbonyl rhodamine B was purchased from Polysciences Inc., USA. Acrylate-PEG-Fluorescein (FITC) was purchased from Nanocs Inc., USA. Poly(vinyl alcohol) (PVA) was purchased from Aladdin, CN. Rhodamine B was purchased from Junsei Chemical, JP. Toluene and methanol were purchased from Daejung, KR.

### Fabrication of gas-impermeable molds

The micromolds composed of NOA 81 or epoxy resin were produced through a sequential replica molding (REM) process. To commence, a positively patterned silicon master template was fabricated employing SU-8 photoresist. Subsequently, PDMS was poured over the master mold and subjected to vacuum degassing, effectively eliminating any entrapped air bubbles within the micropatterns. The PDMS curing process involved placing the master template in an oven set at 70 °C for a duration of 8 hours. Following this step, the negatively patterned PDMS mold, containing the inverse of the original master template patterns, was separated from the master template. The PDMS mold was then subjected to air plasma treatment and subsequently coated with a silane layer by exposure to a trichloro(1H,1H,2H,2H-perfluorooctyl)silane solution within a vacuum chamber for a period of 30 minutes. Subsequently, a fresh batch of PDMS was poured onto the negatively patterned PDMS mold and underwent vacuum degassing to ensure the removal of any residual air bubbles. After curing, the PDMS mold with positive patterns was detached. Then, NOA 81 or epoxy, dispensed onto a microscopic glass slide, was imprinted using the PDMS mold to generate gas-impermeable molds. Following a 10-minute interval, the NOA 81 and epoxy were subjected to curing via exposure to 365 nm UV radiation for a duration of 10 minutes. For fluorosilane deposition, the gas-impermeable mold underwent air plasma treatment and were subsequently exposed to a trichloro(1H,1H,2H,2H-perfluorooctyl)silane solution within a vacuum chamber for 30 minutes.

### Measurement of contact angles

The hydrophilic precursor was formulated with a composition comprising 20% (v/v) PEGDA 700, 74% (v/v) deionized (DI) water, 5% (v/v) darocur 1173, and 1% (v/v) 10 mg/ml rhodamine B. Conversely, the hydrophobic precursor was composed of 20% (v/v) PEGDA 250, 74% (v/v) toluene, 5% (v/v) darocur 1173, and 1% (v/v) 10 mg/ml acrylated rhodamine B. To ascertain contact angles, 20 µl of the respective precursor solutions were dispensed onto NOA 81 surfaces, both treated with silane and left untreated. These samples were subsequently captured using a Canon EOS camera. Subsequently, the contact angles were determined through the analysis of the acquired images utilizing the ImageJ computer software, specifically version 1.48.

### Particle recovery from gas-impermeable micromolds

Following the curing of the precursor, the microparticles residing within the microwells were subjected to an ethanol wash to effectively eliminate any impurities. Subsequently, a 200 µl volume of a PVA solution, possessing a concentration of 5% (w/v), was uniformly distributed onto the mold. This PVA-laden mold was then subjected to a 20-minute degassing process within a vacuum desiccator, ensuring the thorough diffusion of the PVA solution into the cured particles. The formation of a PVA film was accomplished through the evaporation of the solution in a 70 °C oven, after which the film was gently detached from the mold. The microparticles that had adhered to the PVA film during its formation were successfully recovered through the solubilization of the film. This dissolution process took place in a 1xPBS buffer solution with 0.05% (v/v) Tween 20 (1xPBST), and was conducted at a temperature of 70 °C. In order to ensure the comprehensive removal of any remaining impurities within the particle structures, a thorough rinsing procedure was implemented. This procedure encompassed a series of five cycles involving centrifugation, supernatant removal, and the subsequent addition of 1xPBST buffer solution. These repeated rinsing steps effectively eliminated any residual impurities from the recovered microparticles.

### **Particle synthesis through thermal curing**

The precursor employed for thermal curing consisted of 50% (v/v) PEGDA 250, 45% (v/v) AIBN solution (12 wt. % in acetone), and 5% (v/v) acrylated FITC solution. To introduce the precursor into the NOA 81 mold, a degassed PDMS cover was utilized. The PDMS cover was subjected to degassing for a duration of 1 hour, during which it was immersed in acetone. After dispensing 20  $\mu$ l of the precursor solution onto the cover, it was subsequently covered by the PDMS cover, and a pressure of approximately 30 kPa was applied using a weight. Upon the successful loading of the precursor, the hot plate's temperature was adjusted to 90 °C, and the precursor was subjected to curing at this temperature for a duration of 1 minute. The subsequent particle recovery procedure was conducted in the same manner as previously described for photopolymerization.

### **Synthesis of ruthenium-containing composite microparticles**

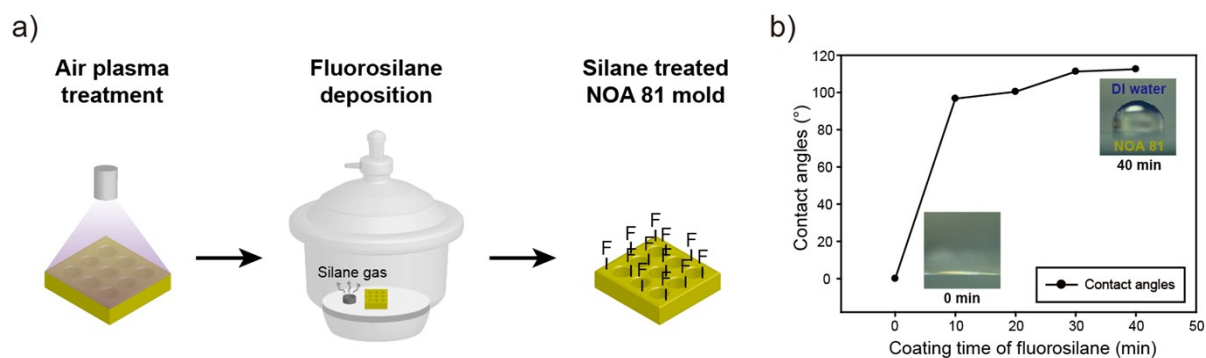
The precursor solution was composed of 84% (v/v) PEGDA 250, 10% (v/v) darocur 1173, 1% (v/v) 1-vinyl-2-pyrrolidone, and a 5% (v/v) ruthenium complex solution. The ruthenium complex solution was prepared by dissolving dichlorotris(1,10-phenanthroline) ruthenium(II) hydrate in a mixture of methanol and toluene (4:1) at a concentration of 15 mg/ml. To introduce the precursor into the micromold, a degassed PDMS cover was employed. Subsequently, a pressure of approximately 30 kPa was applied to minimize the residual precursor layer. For the curing of the loaded precursor, an exposure of 160 mW/cm<sup>2</sup> of UV light was applied to the loaded precursor for a duration of 200 milliseconds. The recovery of particles adhering to the mold followed the procedures as outlined in Figure 1.

### **Oxygen sensing utilizing ruthenium-containing microparticles**

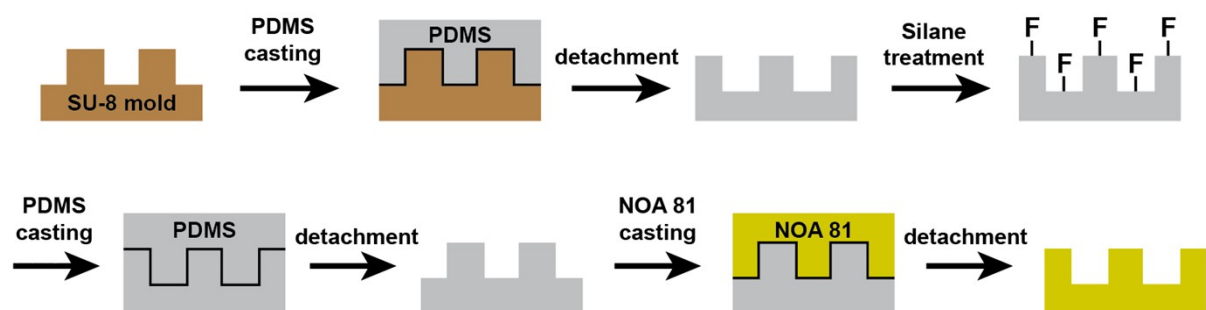
To assess the oxygen-sensing capabilities of ruthenium-containing microparticles, the emission spectra of these particles were scrutinized utilizing a microplate reader (Molecular Devices, USA). The oxygen concentration of the particle solution was systematically controlled through a degassing process conducted within a vacuum desiccator, employing varying levels of degassing pressure. For instance, to diminish the oxygen concentration to 1/5 of the atmospheric level, the air pressure in the vacuum desiccator was set to 0.2 atm. To approach an oxygen concentration level near zero, argon gas was continuously introduced into the vacuum desiccator while simultaneously evacuating the gas. Subsequent to this controlled manipulation of oxygen levels, the emission spectra were promptly measured within the wavelength range of 555 nm to 700 nm. This measurement was conducted immediately after extraction from the vacuum desiccator, following exposure to 470 nm light.

### **Synthesis of magnetic nanoparticles-containing microparticles**

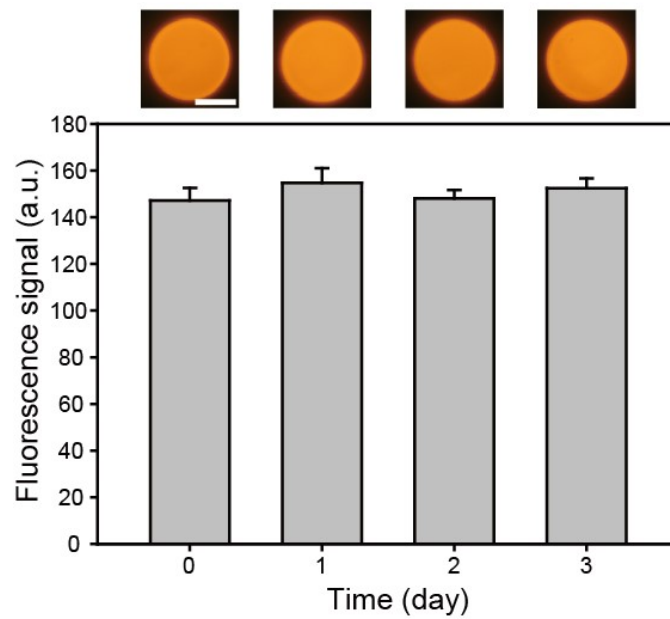
The precursor solution was comprised of 75% (v/v) toluene-based ferrofluid, 14% (v/v) PEGDA 250, 5% (v/v) toluene, 5% (v/v) darocur 1173, and 1% (v/v) 1-vinyl-2-pyrrolidone. In order to mitigate the evaporation of toluene and prevent the aggregation of iron oxide nanoparticles during the APS REM process, the PDMS cover underwent degassing while being immersed in toluene for a duration of 1 hour. Subsequently, the precursor was loaded into the micromold using the degassed PDMS cover. A pressure of approximately 30 kPa was applied to the cover and the mold. The curing of the loaded precursor was executed by exposing it to UV light with a power density of 160 mW/cm<sup>2</sup> for a duration of 8 seconds. Subsequent to this step, the recovery of particles adhering to the mold followed the procedures as outlined in Figure 1.



**Figure S1** Schematic and optimization of fluorosilane treatment on a NOA 81 mold. a) The fluorosilane treatment process for a NOA 81 mold involved initial air plasma treatment of the mold, followed by the deposition of fluorosilane through vapor deposition in a vacuum desiccator. b) The coating time for fluorosilane was determined by monitoring changes in contact angle with respect to the coating time. The optimization process identified 30 minutes as the optimal coating time when the contact angle reached a constant value.



**Figure S2** Schematic of NOA 81 mold fabrication through sequential replica molding. Polydimethylsiloxane (PDMS) was cast onto a SU-8 master template featuring a positive pattern. Following this, the PDMS was cured in a 70°C oven for a duration of 3 hours. Subsequently, deliberate detachment of the PDMS mold from the master template was carried out. Silane treatment was then applied to the PDMS mold. A layer of PDMS was cast onto the silane treated PDMS mold, and upon curing, a positively patterned PDMS mold was obtained. To create a negatively patterned NOA 81 mold, NOA 81 was cast onto the positively patterned PDMS mold. The NOA 81 mold was obtained by curing it through 365 nm UV light exposure for a period of 10 minutes.



**Figure S3** Change in fluorescent intensities emitted by ruthenium-embedded composite microparticles. Following the fabrication of ruthenium-embedded composite microparticles, fluorescent intensities were measured daily for up to the third day (number of measured particles = 7). Scale bar indicates 100  $\mu\text{m}$ .