

# A facile approach to patterning pollen microparticles for *in situ* imaging

Soohyun Park<sup>a</sup>, Hokyun Chin<sup>a</sup>, Youngkyu Hwang<sup>a</sup>, Teng-Fei Fan<sup>a</sup>, Nam-Joon Cho<sup>a,b,\*</sup>

<sup>a</sup>School of Materials Science and Engineering, Nanyang Technological University, 50 Nanyang Avenue, 639798, Singapore

<sup>b</sup>Engineering in Translational Science, Nanyang Technological University, 62 Nanyang Drive, 637459, Singapore

## ARTICLE INFO

### Article history:

Received 11 April 2020

Revised 10 May 2020

Accepted 12 May 2020

### Keywords:

Pollen

Bio-inspired material

Patterning

Stimuli-responsive biopolymer

In situ imaging

## ABSTRACT

Pollen is one of nature's most resilient materials and widely used as chemically stable environmental markers and in drug delivery. Recent findings show that a simple alkali treatment, similar to traditional soapmaking, can convert hard pollen grains into soft microgel particles, which exhibit stimuli-responsive changes in size and mechanical properties. Surface-based measurement approaches offer excellent potential to monitor these stimuli-responsive behaviors, however, such methods are encumbered by weakly adsorbing, free-flowing microparticles and a tendency for the microparticles to aggregate on substrates. Here, we demonstrate a facile approach to pattern soft pollen microparticles and track stimuli-responsive behaviors *in situ* based on a combination of chemical functionalization and scanning probe manipulation of individual microparticles. First, we prepared an amine-functionalized glass substrate that can covalently attach to carboxylic acid functional groups on the microparticle surface, tethering the particles stably. Second, we used a scanning probe cantilever to pick up and move individual microparticles to specific locations on the functionalized glass surface. Various patterns of well-separated, tethered microparticles were fabricated, including aligned arrays, hearts, and stars, and we could track the stimuli-responsive behavior of individual microparticles with *in situ* imaging. Taken together, this patterning concept can be broadly utilized to manipulate various classes of intelligent microparticles across fields such as diagnostics, biosensors, drug delivery, and chemo-mechanical actuators.

© 2020 Elsevier Ltd. All rights reserved.

## 1. Introduction

Pollen is known to be practically indestructible [1] and plays a critical role in plant reproduction, protecting the internal cellular materials and transferring them between different reproductive parts of plants [2, 3]. As a part of plants' life cycle, even the stunning diversity in shape and structure, pollen grains are renewably produced in abundant amounts [4]. For each plant species, a particular type of pollen grain is produced that is composed of a hollow microcapsule structure with homogenous size and function-driven shape and ornamental architecture [5]. In all cases, the microcapsule structures possess a robust outer layer, which is mainly composed of sporopollenin role of protecting genetic material within. Sporopollenin is often regarded as the diamond of biopolymers, possessing extreme durability, mechanical strength, and chemical stability to pollen grains [6]. Such properties have led pollen to be regarded as practically indestructible, and pollen

is widely used as stable, content-rich markers for archaeological, ecological, and forensics applications [5, 7-9].

Although there have been long efforts to chemically extract sporopollenin exine capsules (SECs) from the natural pollen grains to study its chemical and physical structure [10-12], the development of exploiting pollen as a class of natural material is relatively recent [13-16]. Along with the growing interest to explore pollen for applications including a template for materials fabrication [17], composite materials [18], and drug delivery vehicles [19], the extraction of pollen shells has been modified and improved together. Our and other groups have developed acid/base processing strategies to produce pollen-derived capsules, in effect removing sensitive biological materials such as proteins, polysaccharides, and nucleic acids while preserving the physical and chemical properties of the sporopollenin shell [20, 21]. Nowadays, the extraction of SECs from defatted pollen grains (after removal of pollenkitt) can be done within a day [20, 22]. In the course of developing various acid/base treatments, we recently reported that an extended incubation of defatted, cytoplasm-removed pollen grains in a strong alkaline solution facilitates microgel assembly with natural pollen shells [23]. Unlike SECs, the pollen material in this case included

\* Corresponding author.

E-mail address: [njcho@ntu.edu.sg](mailto:njcho@ntu.edu.sg) (N.-J. Cho).

both intine and exine layers so that the polysaccharides components such as pectin can react in strong alkaline conditions and become the de-esterified form, pectin acid, which facilitates intermolecular charge repulsion and corresponding changes in packaging and mechanical properties. Further, a simple casting process transformed the microgel particles to assemble into pollen-based paper form, which can reversibly absorb/desorb water due to the exposed hydrophilic functional groups on the de-esterified pollen surface [24].

Similar to other known stimuli-responsive polymers with the acidic functional group [25–27], the pollen-derived microgel particles also showed chemo-mechanical responsiveness based on modulating the pH condition and bridging interactions with di/multivalent cations [23]. These findings are significant because stimuli-responsive polymers can be applied in various fields, including diagnostics, biosensors, drug delivery systems, and chemo-mechanical actuators [28, 29]. Production of synthetic microparticles that can be utilized in sensitive environments is challenging due to the quality control of their size, shape, and morphology [30–32]. The control over physical characteristics is often accomplished by tuning polymer synthesis (e.g., solvents, concentrations or ratios, molecular weights, etc.) or microparticle formulation steps (e.g., spray drying, solvent evaporation, coacervation, microfluidics, etc.), involving a large number of parameters to control [33–35]. Also, natural polymer-based microgels tend to be less thermally stable and have irregular particle shapes and heterogeneous particle sizes [36–38]. These limitations hamper the utility of existing natural materials used for microparticles. As the natural pollen particles are uniform, thermally and mechanically stable, pollen-derived microgel particles are thus a promising candidate to be used as a stimuli-responsive material that can replace synthetic plastics or other inorganic materials.

## 2. Results and discussion

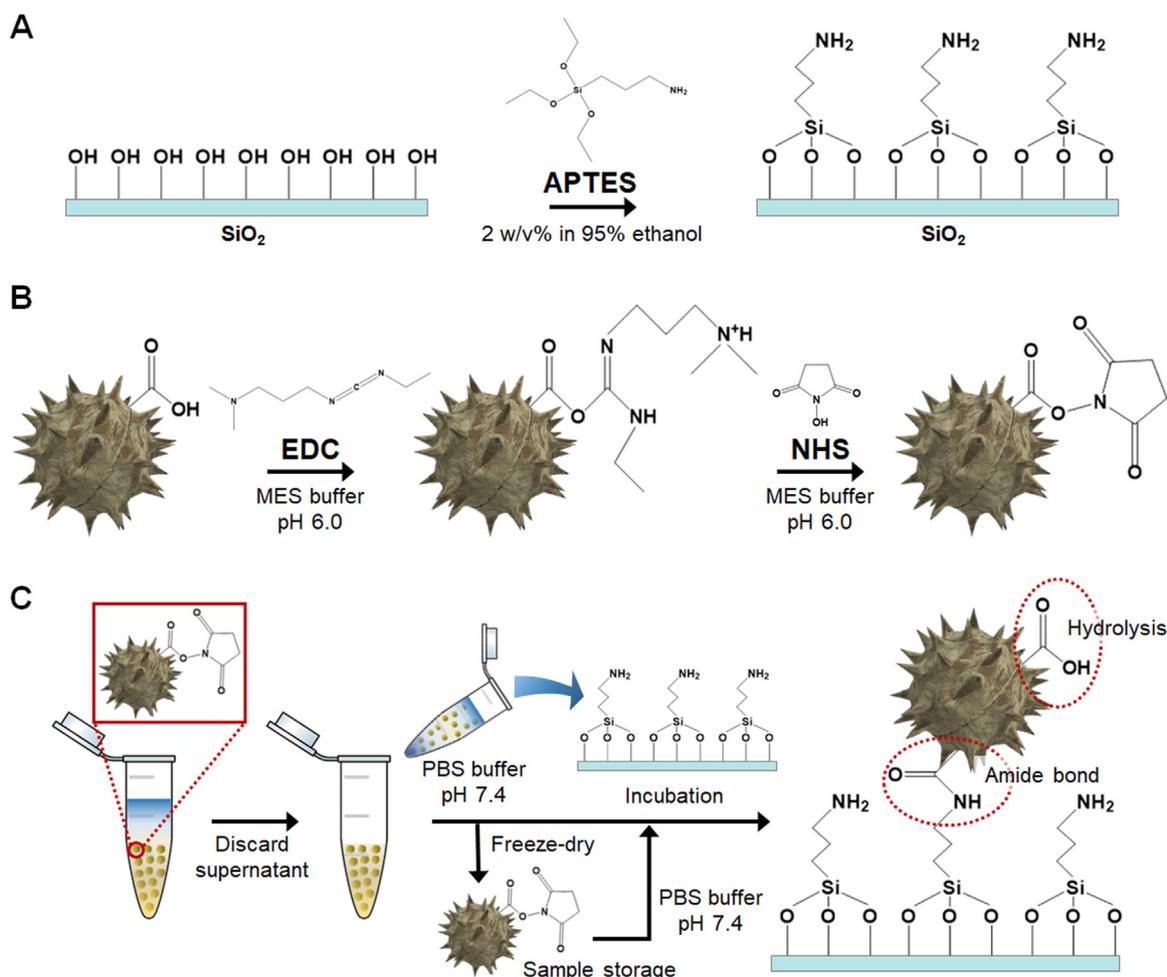
To utilize the stimuli-responsive behavior in sensing applications, it would be ideal to conduct surface-based measurements on the pollen microgel particles, although there are challenges such as the weakly adsorbing, free-flowing nature of the microparticles and a tendency for the microparticles to aggregate on substrates. To overcome these challenges, we have developed an effective method to pattern the soft pollen microgel particles and track stimuli-responsive behaviors of individual particles on solid surfaces. Our *in situ* imaging method is based on a combination of chemical functionalization and scanning probe manipulation of individual microparticles. First, we prepared an amine-functionalized glass substrate that can covalently attach to carboxylic acid functional groups on the microparticle surface. The schematic illustrations in Fig. 1 outline the process. As the primary objective is to visualize pollens using microscopy, the glass coverslip was chosen for the substrate. The clean glass coverslip was soaked in a 2 w/v% solution of (3-aminopropyl) triethoxysilane (APTES) to functionalize the surface as an amine-reactive film (Fig. 1A). Both defatted pollen (acetone and diethyl ether-treated to remove pollen cement) and de-esterified pollen (defatted pollen undergoing strong alkali-treatment) particles possess carboxyl groups on their surface. Due to the de-esterification of pectin to pectate, de-esterified pollens exhibit much higher content of carboxyl groups than defatted pollens. This functional group was activated by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide (NHS) and resulted in an amine-reactive-NHS ester (Fig. 1B). Note that the buffer used in this step is 2-(N-morpholino) ethanesulfonic acid (MES) buffer, pH 6.0, to optimize the reaction. Then, the sample suspension was centrifuged to remove the supernatant containing unreacted EDC/NHS, and subsequently was re-suspended in phosphate-buffered saline (PBS),

pH 7.4 solution (Fig. 1C). As NHS ester can quickly undergo hydrolysis, the activated pollen sample was either used immediately or stored after freeze-drying. Meanwhile, APTES-treated glass coverslip was enclosed in a microfluidic chamber so that the suspension could be injected and incubated. Finally, the covalent amide bond was formed between pollen and APTES-treated glass coverslip.

Next, we performed a control experiment without any chemical attachment between pollens and glass coverslip. As expected, pollens floated and were washed away with the flow, which hampered a static observation during the rapid changes in solution pH (Fig. 2A, Video S1). In marked contrast, the formation of covalent amide bonds between pollens and glass enabled real-time monitoring during the pH change as observed by time-lapsed optical microscopy (Fig. 2B, Video S2). Also, this strategy was tested using other pollen species from the eudicot clade. All tested pollen samples were successfully immobilized onto the surface while going through pH changes (Fig. 2C, Videos S3–7). In all cases, when the solution pH was changed from 12 to 2, the pollen particles underwent fast de-swelling or compression while the extent of area swelling ratio varied from 63% (lotus) to 82% (camellia) with a tendency that relatively bigger particles (e.g., lotus) took longer to de-swell compared to smaller ones (e.g., baccharis) (Fig. 2D). Also, we assessed the influence of covalent bonds during swelling and de-swelling of pollen microgel by comparing the size of pollen grains with or without covalent bonds. The free-pollen particles in solution-based were characterized by dynamic imaging particle analysis (DIPA), which captures time-lapse images while flowing free-particles to produce statistics including equivalent spherical diameter (ESD) based on hundreds or thousands of individual particles. The ratio of de-swelled radius over swelled radius indicated that the immobilization affects the behavior of some pollen microgel particles by restricting the extent of either or both swelling and de-swelling processes (Table S1). This observation can be further studied by controlling the concentration of reagents (EDC/NHS) and pollen species.

In order to apply this tethering strategy to fabricate arrays of pattern, a scanning probe cantilever was used to control the location of individual pollen particles carefully. In Fig. 3A, the schematic of the process is presented. A single activated pollen particle was picked up by a glycerol-covered cantilever, which is to facilitate the viscous surface to attach the pollen particle to cantilever weakly. Then, the cantilever probe was moved to a designated XY position of the APTES-treated glass coverslip and was approached to the surface to make direct contact and form covalent bonds between the pollen and the glass. By repeating the procedure, pollens were placed onto the desired spot, and the glass coverslip was later on enclosed in the microfluidic chamber to monitor the pH-responsiveness of de-esterified pollen particles. A resulted full view of  $5 \times 7$  arrays is presented in Figure S1, observed by confocal laser scanning microscopy (CLSM). In Fig. 3B and C, immobilized and aligned pollen particles were observed during the pH changes from pH 2 to pH 12 and from pH 12 to pH 2. In a low pH of 2, the size of pollen particles ranged  $35 \mu\text{m}$ , and they swell upon the increase of pH to 12, whereby the maximum diameter was over  $50 \mu\text{m}$ . The whole swelling/de-swelling process was fully reversible.

Next, a heart-shape patterned pollen array was characterized by epifluorescence microscopy (Fig. 4A). As natural pollen particles exhibit multispectral autofluorescence characteristics due to the presence of chemical compounds such as phenolics and terpenoids [18], pollens were visualized by green channel (Ex 480/40, Em 535/50). The line fluorescence intensity (F.I.) profile from data (A) overtime was characterized and presented in Fig. 4B. The F.I. was normalized by  $t = 0$ , and the minimum F.I. was observed around 17 s to 1.0 and 0.0 a.u., respectively. When pH increased to



**Fig. 1.** Schematic illustration of pollen immobilization strategy. (A) The glass coverslip was soaked in a 2 w/v% solution of (3-aminopropyl) triethoxysilane (APTES) to functionalize the surface as an amine-reactive film. (B) Both natural pollen and de-esterified pollen particles possess carboxyl groups on their surface, which can be activated by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide (NHS) and resulted in an amine-reactive-NHS ester. Note that the buffer used in this step is 2-(N-morpholino) ethanesulfonic acid (MES) buffer, pH 6.0, to optimize the reaction. (C) The suspension was centrifuged to remove the supernatant containing unreacted EDC and NHS. Then, the activated pollen samples were re-suspended in phosphate-buffered saline (PBS), pH 7.4 solution to be either used immediately or stored after freeze-drying. After incubation, a covalent amide bond was formed between pollen and APTES-treated glass coverslip. Of note, unreacted NHS esters on pollen surface quickly undergo hydrolysis and return to its original form, carboxyl group.

12, or in other words, as the sizes of pollen grains increased, the F.I. dropped because the pollen particles were out of focus as they swell. The observation is then numerically analyzed in the surface plot with normalized F.I. (Fig. 4C). Likewise, the physical changes of tethered pollen microparticles can be read out to certain signals, which can be potentially applied in diagnostics or biosensors.

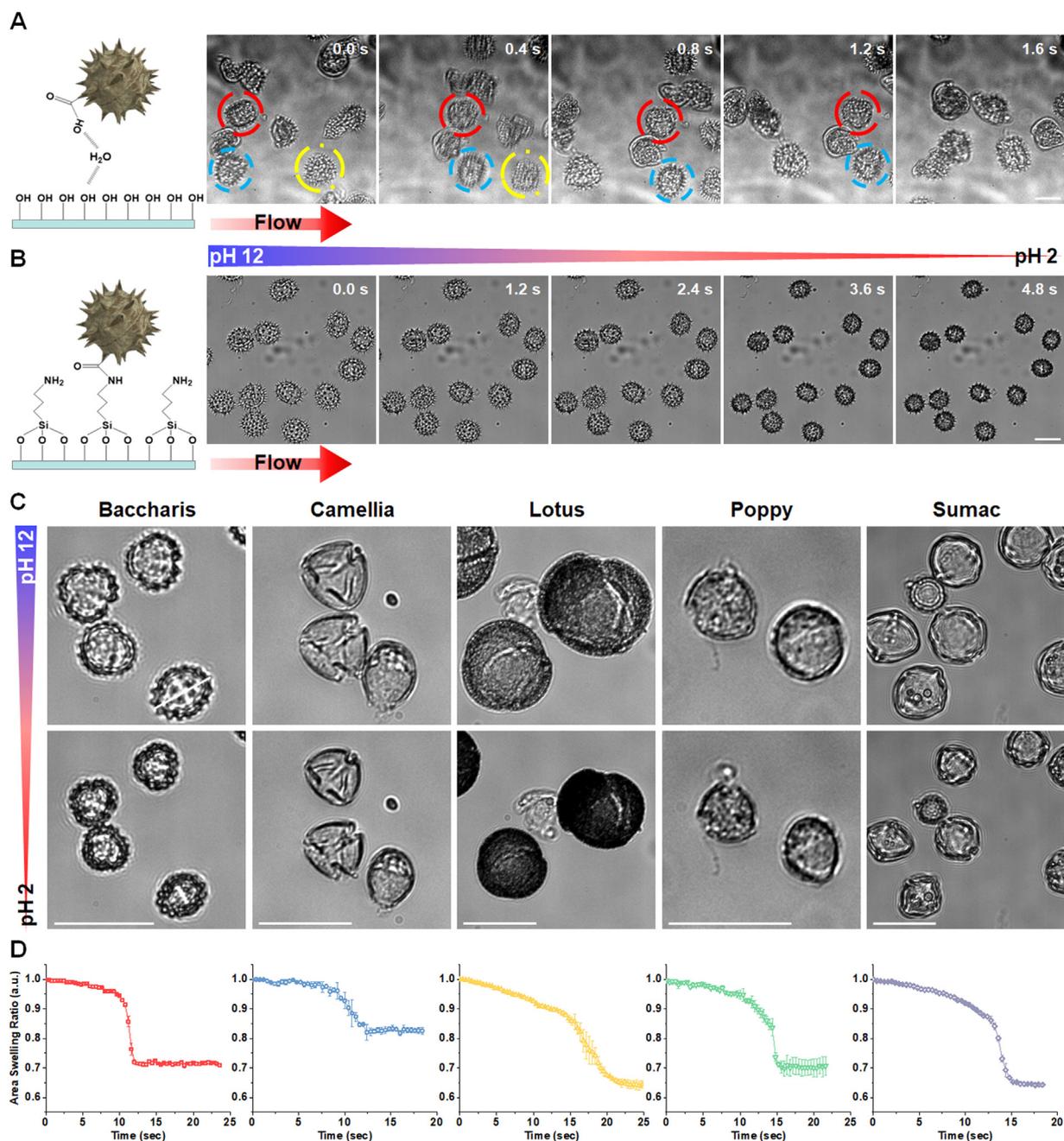
### 3. Conclusion

Taken together, we have demonstrated a facile approach to fabricate tethered arrays of well-separated, pollen-derived microgel particles and directly observe environmentally triggered stimuli-responsive behavior *in situ*. Our tethering strategy involving chemical functionalization is simple and broadly applicable to various classes of polymeric microparticles at solid-liquid interfaces. This colloidal strategy is also compatible with microprinting to create on-demand patterns of well-defined shape and density. Since pollen-derived microgel particles can be utilized as stimuli-responsive smart materials that might replace synthetic polymers, our findings also open the door to create new classes of bio-friendly sensors and actuators based on intelligent microparticle designs.

## 4. Materials and methods

### 4.1. Pollen materials and defatting

Defatted sunflower (*Helianthus annuus* L.) and baccharis (*Baccharis halimifolia* L.) pollen grains were purchased from Greer Labs (Lenoir, NC, USA). Non-defatted, bee pollen granules of camellia (*Camellia Sinensis* L.), lotus (*Nelumbo nucifera*), poppy (*Papaver rhoeas*), and sumac (*Rhus chinensis*) were purchased from Yuen-sun Biological Technology Co., Ltd. (Xi'an, Shaanxi, China) (camellia), Peffer Industrial Co., Ltd. (Zhengzhou, Henan, China) (lotus and poppy), and Fengzhixiang Apiculture Co. Ltd. (Zhengzhou, Henan, China) (sumac), respectively. The bee pollens went through the defatting process to remove pollenkitt, which is an oily layer covering the pollen grain as previously described [39–41]. First, 250 g of bee pollens were refluxed in 500 mL of acetone for 3 h under magnetic stirring (220 rpm) at 50 °C. Then, acetone was decanted, and 1 L of 50 °C deionized water was added to the sample and stirred for 1 h. The pollen suspension was passed through 300 μm diameter pores nylon mesh and subsequently passed through 6 μm diameter pores filter paper in a vacuum filtration system. Next, the pollen sample was hydrated with 1 L of 50 °C deionized water with magnetic stirring for 30 min and then vacuum filtered again.



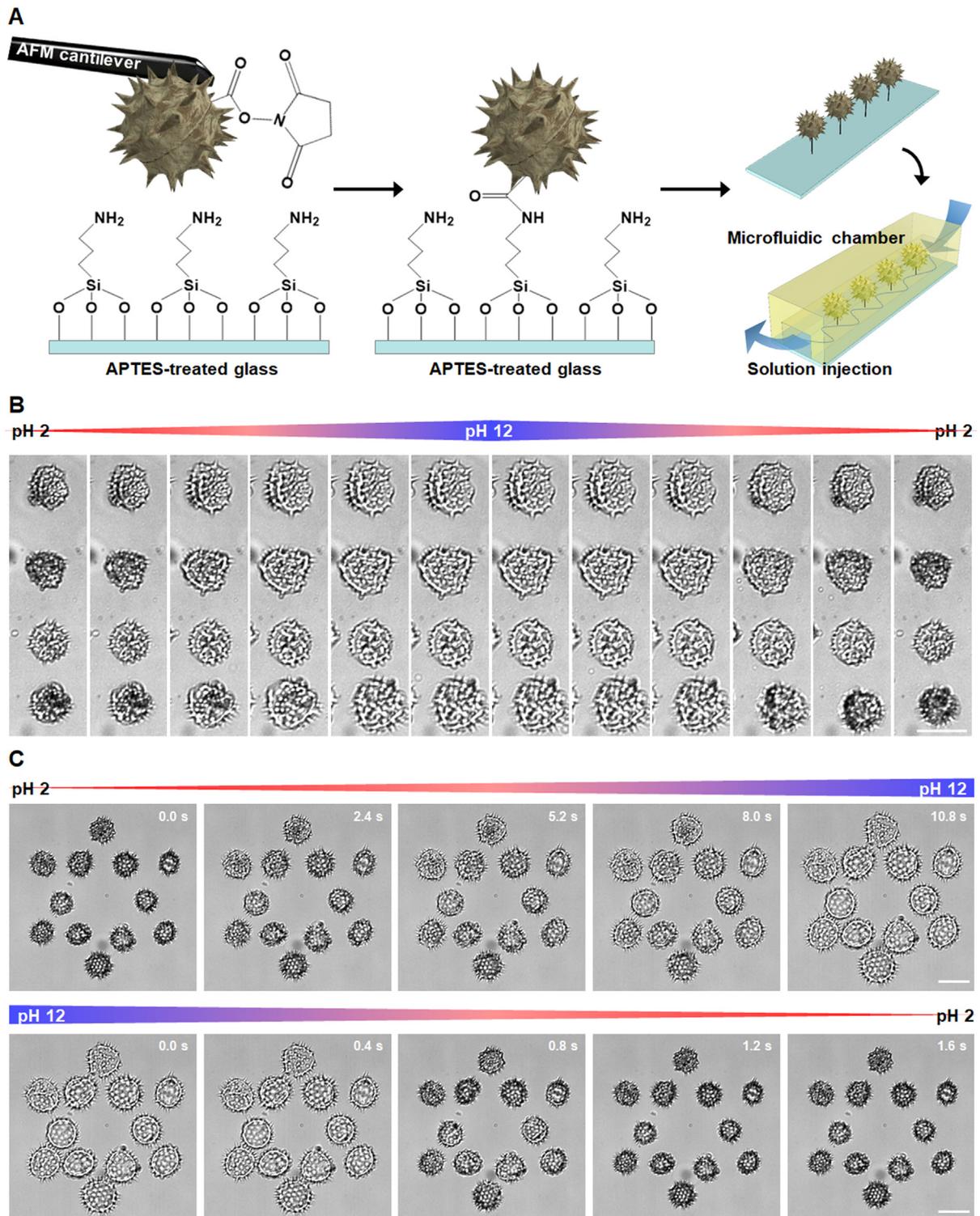
**Fig. 2.** Time-lapsed optical microscopy observation of stimuli-responsive de-esterified pollen particles immobilized on the surface. (A) The sunflower pollen particles without covalent tethering onto the surface. In the absence of covalent bonds, pollen grains floated and were washed away with the flow. (B) After the formation of covalent amide bonds between pollens and glass, pollens were firmly attached to the surface and could be monitored under flow conditions. (C) Other pollen species from the eudicot clade were also tested to be tethered onto the surface following the same procedure as well as to monitor the pH-responsive behavior. All tested pollen samples were successfully immobilized onto the surface while going through pH changes and swelling/deswelling processes. All scale bars are 50  $\mu\text{m}$  (note the differences in each case). (D) Several quantitative comparisons of pH-induced pollen de-swelling behavior. Mean  $\pm$  s.d. are reported from  $n = 3$  particles, and the area swelling ratio was normalized by the initial area at pH 12.

Then, the resulting pollen particles were refluxed in 0.5 L of 50  $^{\circ}\text{C}$  acetone with magnetic stirring (400 rpm) for 3 h. Afterward, acetone was removed by vacuum filtration, and pollen samples were transferred to a glass petri dish and left to dry in a fume hood. After 12 h, dried pollen sample (20 g) was dispersed in 0.25 L of diethyl ether with magnetic stirring (400 rpm) at room temperature for 2 h. This diethyl ether defatting step was done twice, and fresh diethyl ether was used in each cycle. Removal of diethyl ether was done by vacuum filtration. After washing with diethyl ether twice, the pollen sample was dispersed in fresh diethyl ether and left to stir (400 rpm) overnight at room temperature. Diethyl ether

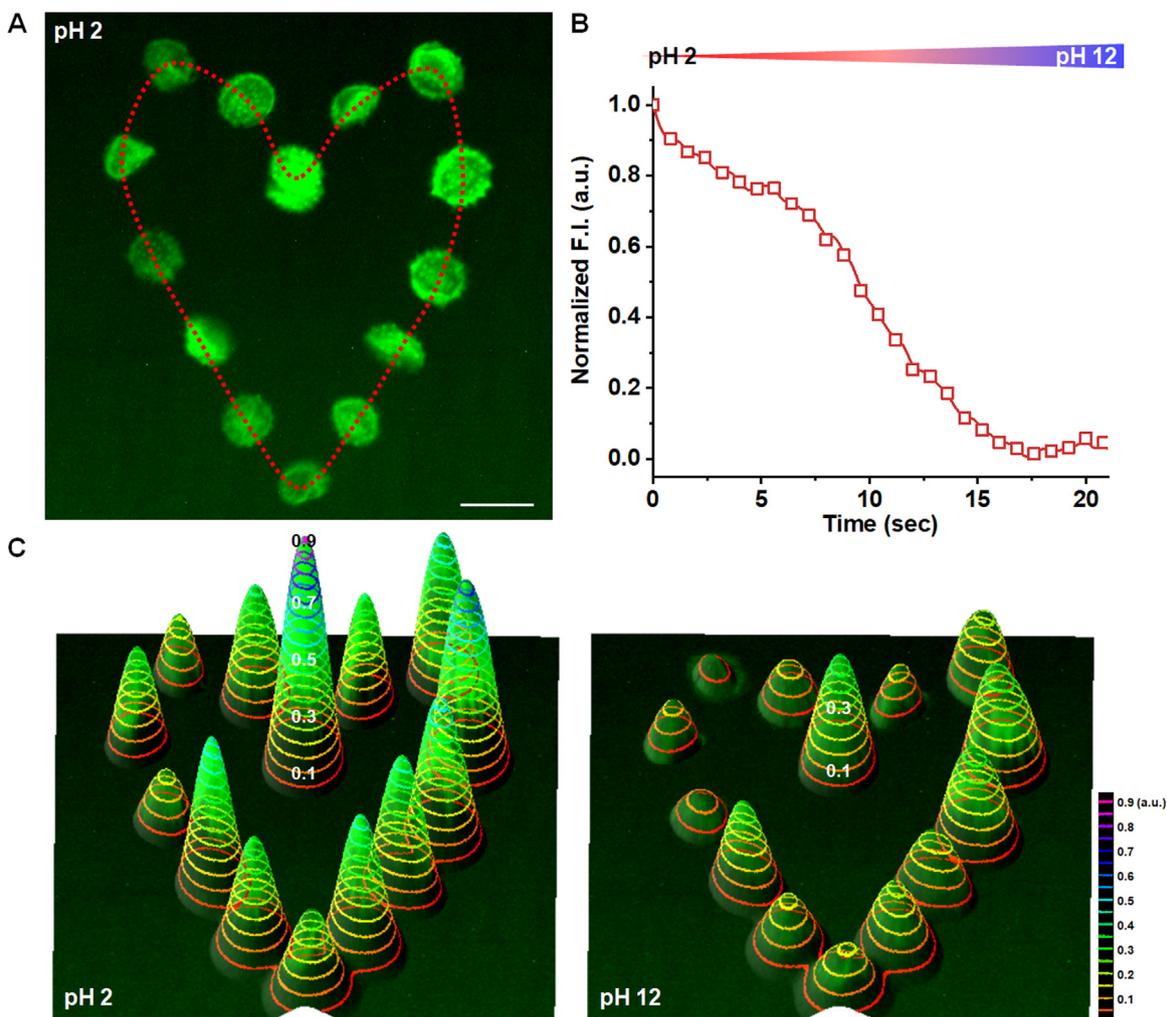
was then removed by vacuum filtration, and the pollen sample was transferred to a petri dish and air-dried in a fume hood for 12 h.

#### 4.2. Preparation of de-esterified pollen particles

The detailed information of pollen materials and defatting process can be referred to Supporting Information. The pollen shell extraction was done by mixing 2 g of defatted pollen with 20 mL of 10 w/v% KOH under magnetic stirring at 400 rpm. The pollen suspension was refluxed (2 h, 80 $^{\circ}\text{C}$ ) with stirring at 200 rpm, then was centrifuged (5 min, 4500 rpm) to remove the super-



**Fig. 3.** Schematic illustration and optical microscopy observation of sunflower pollen arrays fabricated using AFM cantilever. (A) An AFM was employed to manipulate pollen particles. The individual pollen particles were picked up by glycerol-soaked cantilever and placed onto the desired location, thus subsequently immobilized by covalent bonds. After the whole process, the glass coverslip was enclosed within a microfluidic flow-through chamber, and then the liquid solution was introduced to monitor the stimuli-responsiveness. (B) Individual pollen particles were manipulated by AFM cantilever to form an alignment. Then, pH-dependent behavior was observed during the solution pH changes from pH 2 to 12, and from pH 12 to 2. The pollen particles swelled and de-swelled upon increasing and decreasing pH conditions, respectively. (C) The same technique was used to fabricate the star-shaped array. All scale bars are 50  $\mu\text{m}$ .



**Fig. 4.** Epifluorescence observation of heart-shape patterned de-esterified pollen grains exhibiting pH-responsive behavior. (A) The heart-shaped pattern was fabricated and observed using epifluorescence microscopy. Natural pollens exhibit multispectral autofluorescence characteristics due to the presence of chemical compounds such as phenolics and terpenoids. The scale bar is 50  $\mu\text{m}$ . (B) The line fluorescence intensity (F.I.) profile from data (A) over time. The F.I. was normalized by  $t = 0$ , and the minimum F.I. observed around 17 s to 1.0 and 0.0 a.u., respectively. When pH increased to 12, the F.I. dropped as pollen grains' sizes increased. (C) Surface plot of the pollen grains at pH 2 and 12.

natant, which includes cytoplasmic materials. Then, fresh 40 mL of 10 w/v% KOH was added, and the mixture was vortexed (2 min), followed by centrifugation (5 min, 4500 rpm). After repeating this strong alkali washing step for 5 times, the supernatant was finally decanted. As for the final step, the weakly de-esterified, cytoplasm-removed pollen particles were incubated in 10 w/v% KOH conditions (6 h, 80°C) without any stirring. Then, the pollen particles were separated by centrifugation (5 min, 4500 rpm), then repeatedly washed with 40 mL of deionized water to reach its pH level to 7.5. The resulting pollen suspension was collected by the last step of centrifugation and supernatant removal and then stored at 4 °C for further usage. Notably, fully de-esterified pollen particles in water showed gel-like properties.

#### 4.3. EDS/NHS activation of pollen particles

The carboxyl acid functional groups on the surface of pollen particles were activated by treatment with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, Sigma-Aldrich) and N-hydroxysuccinimide (NHS, Sigma-Aldrich). First, pollen samples (~20 mg) was dispersed in 0.5 mL of 100 mM 2-(N-morpholino) ethanesulfonic acid (MES, Sigma-Aldrich) buffer (0.5 M NaCl, pH 6.0). Then, EDC (5 mg) and NHS (15 mg) were

added to the pollen suspension and quickly mixed with vortexing. After an incubation period of 30 min on a rocking platform shaker, the suspension was centrifuged (1500 rpm, 5 min) in order to remove the supernatant containing unreacted EDC and NHS. Then, 0.5 mL of PBS (pH 7.4) was added to re-suspend the pollen. Immediately after resuspension, the pollen samples were either used immediately or frozen by liquid nitrogen and placed in a freeze-dryer under 0.008 mbar vacuum pressure for 4–6 h. Freeze-dried pollen samples were stored in a dry cabinet until further use.

#### 4.4. APTES-treated glass coverslips

Before the experiment, glass coverslips (25 mm  $\times$  75 mm, Ibidi GmbH, Germany) were sequentially rinsed with water and ethanol, dried with a stream of nitrogen gas, and treated with oxygen plasma for 1 min using an Expanded Plasma Cleaner (PDC-002, Harrick Plasma, USA). After cleaning, the glass coverslips were immediately soaked into a 2 w/v% solution of (3-Aminopropyl) triethoxysilane (APTES, Sigma-Aldrich) in 95% ethanol to functionalize the surface. After 30 min, the coverslips were sequentially rinsed with deionized water and 95% ethanol for a total of 3 times before drying with a stream of nitrogen gas.

#### 4.5. Controlled manipulation of pollen particles using a scanning probe

An NX-10 atomic force microscope (AFM) (Park Systems, Suwon, South Korea) was employed to immobilize chemically activated pollen particles to an APTES-coated glass coverslip surface. An aluminum reflex-coated PPP-NCHR silicon cantilever (Nanosensors, Neuchâtel, Switzerland) with a spring constant of  $\sim 42$  N/m was used. First, the cantilever was rinsed with water and ethanol and dried with a stream of nitrogen gas. Then, to render the surface of cantilever hydrophilic, it was treated with UV light (PSD-UV4, Novascan) for 2 h. Then, the cantilever was immediately dipped into an aqueous solution of 10 v/v% glycerol for 90 min. Afterward, the activated pollen particles were dispersed on a bare glass coverslip, and individual particles were picked by using the cantilever scanning probe. Using an optical microscope with the objective lens focused on the glass coverslip surface, the cantilever tip was placed in direct contact with individual pollen particles to facilitate noncovalent attachment, which was aided by glycerol. After moving the XY stage of the measurement platform to the APTES-treated coverslip, the single pollen particle was covalently immobilized by approaching the cantilever probe to the surface. Once the pollen particle was firmly immobilized on the functionalized coverslip and the cantilever probe was released, the XY coordinates were recorded in order to facilitate spatially controlled immobilization of subsequent particles following the same procedure.

#### CRedit author statement

Soohyun Park: Conceptualization, Methodology, Data analysis, Writing- Original draft preparation and Reviewing. Hokyun Chin: Patterning pollen particles using AFM. Youngkyu Hwang and Teng-Fei Fan: Pollen microgel preparation. Nam-Joon Cho: Supervision, Writing- Reviewing and editing.

#### Data availability

All the data supporting the findings of this study are available from the corresponding authors upon reasonable request.

#### Declaration of Competing Interest

N.-J.C. is a co-inventor on patent no. WO2019147190A1. The other authors declare no competing interests.

#### Acknowledgments

The National Research Foundation of Singapore supported this work through a Competitive Research Programme grant (NRF-CRP10-2012-07).

#### Appendix A. Supplementary data

Supplementary data on the full view of  $5 \times 7$  patterned array observed by CLSM is presented as Figure S1. The comparison of average diameters of free- and immobilized pollen particles is presented as Table S1.

#### References

- [1] H.J.B. Birks, H.H. Birks, B. Ammann, The fourth dimension of vegetation, *Science* 354 (2016) 412–413.
- [2] K. Faegri, L. Van der Pijl, *Principles of Pollination Ecology*, Elsevier, 2013.
- [3] J.M. Tylianakis, The global plight of pollinators, *Science* 339 (2013) 1532–1533.
- [4] T. Ariizumi, K. Toriyama, Genetic regulation of sporopollenin synthesis and pollen exine development, *Annu. Rev. Plant Biol.* 62 (2011) 437–460.
- [5] G. Erdtman, Pollen morphology and plant taxonomy: angiosperms, *Brill Archive* (1986).
- [6] G. Mackenzie, A.N. Boa, A. Diego-Taboada, S.L. Atkin, T. Sathyapalan, Sporopollenin, the least known yet toughest natural biopolymer, *Front. Mater.* 2 (2015) 66.
- [7] M.B. Davis, R.G. Shaw, Range shifts and adaptive responses to Quaternary climate change, *Science* 292 (2001) 673–679.
- [8] V.M. Bryant, G.D. Jones, Forensic palynology: current status of a rarely used technique in the United States of America, *Forensic Sci. Int.* 163 (2006) 183–197.
- [9] J.F. van Leeuwen, C.A. Froyd, W.O. van der Knaap, E.E. Coffey, A. Tye, K.J. Willis, Fossil pollen as a guide to conservation in the Galápagos, *Science* 322 (2008) 1206–1206.
- [10] F. Zetzsche, K. Huggler, Untersuchungen über die Membran der Sporen und Pollen. I. 1. *Lycopodium clavatum* L. *Justus Liebigs Ann. Chem.* 461 (1928) 89–109.
- [11] J. Brooks, G. Shaw, Sporopollenin: a review of its chemistry, palaeochemistry and geochemistry, *Grana* 17 (1978) 91–97.
- [12] J.S. Watson, W.T. Fraser, M.A. Sephton, Formation of a polyalkyl macromolecule from the hydrolysable component within sporopollenin during heating/pyrolysis experiments with *Lycopodium* spores, *J. Anal. Appl. Pyrolysis* 95 (2012) 138–144.
- [13] A. Diego-Taboada, S.T. Beckett, S.L. Atkin, G. Mackenzie, Hollow pollen shells to enhance drug delivery, *Pharmaceutics* 6 (2014) 80–96.
- [14] I. Sargin, M. Kaya, G. Arslan, T. Baran, T. Ceter, Preparation and characterisation of biodegradable pollen-chitosan microcapsules and its application in heavy metal removal, *Bioresour. Technol.* 177 (2015) 1–7.
- [15] W. Florkiewicz, A. Sobczak-Kupiec, B. Tyliczszak, Acrylic composite materials modified with bee pollen for biomedical application, *Eng. Biomater.* (2018) 21.
- [16] R.C. Mundargi, M.G. Potroz, S. Park, H. Shirahama, J.H. Lee, J. Seo, N.J. Cho, Natural sunflower pollen as a drug delivery vehicle, *Small* 12 (2016) 1167–1173.
- [17] I.J. Gomez, W.B. Goodwin, D. Sabo, Z.J. Zhang, K.H. Sandhage, J.C. Meredith, Three-dimensional magnetite replicas of pollen particles with tailorable and predictable multimodal adhesion, *J. Mater. Chem. C* 3 (2015) 632–643.
- [18] O.O. Fadiran, N. Girouard, J.C. Meredith, Pollen fillers for reinforcing and strengthening of epoxy composites, *Emergent Mater.* (2018) 1–9.
- [19] M.G. Potroz, R.C. Mundargi, J.J. Gillissen, E.L. Tan, S. Meker, J.H. Park, H. Jung, S. Park, D. Cho, S.I. Bang, N.-J. Cho, Plant-based hollow microcapsules for oral delivery applications: toward optimized loading and controlled release, *Adv. Funct. Mater.* 27 (2017) 1700270.
- [20] R.C. Mundargi, M.G. Potroz, J.H. Park, J. Seo, E.-L. Tan, J.H. Lee, N.-J. Cho, Eco-friendly streamlined process for sporopollenin exine capsule extraction, *Sci. Rep.* 6 (2016) 19960.
- [21] P. Gonzalez Cruz, M.J. Uddin, S.U. Atwe, N. Abidi, H.S. Gill, A chemical treatment method for obtaining clean and intact pollen shells of different species, *ACS Biomater. Sci. Eng.* (2018).
- [22] J.H. Park, J. Seo, J.A. Jackman, N.-J. Cho, Inflated sporopollenin exine capsules obtained from thin-walled pollen, *Sci. Rep.* 6 (2016) 1–10.
- [23] T.-F. Fan, S. Park, Q. Shi, X. Zhang, Q. Liu, Y. Song, H. Chin, M.S.B. Ibrahim, N. Mokrzecka, Y. Yang, H. Li, J. Song, S. Suresh, N.-J. Cho, Transformation of hard pollen into soft matter, *Nat. Commun.* 11 (2020) 1–10.
- [24] Z. Zhao, Y. Hwang, Y. Yang, T. Fan, J. Song, S. Suresh, N.-J. Cho, Actuation and Locomotion Driven by Moisture in Paper Made with Natural Pollen, *Proc. Natl. Acad. Sci. U. S. A.* 117 (2020) 8711–8718.
- [25] J. Zhang, M. Zhang, K. Tang, F. Verpoort, T. Sun, Polymer-Based Stimuli-Responsive Recyclable Catalytic Systems for Organic Synthesis, *Small* 10 (2014) 32–46.
- [26] R.R. Costa, C.A. Custódio, F.J. Arias, J.C. Rodríguez-Cabello, J.F. Mano, Layer-by-layer assembly of chitosan and recombinant biopolymers into biomimetic coatings with multiple stimuli-responsive properties, *Small* 7 (2011) 2640–2649.
- [27] G. Li, S. Song, L. Guo, S. Ma, Self-assembly of thermo- and pH-responsive poly (acrylic acid)-b-poly (N-isopropylacrylamide) micelles for drug delivery, *J. Polym. Sci., Part A: Polym. Chem.* 46 (2008) 5028–5035.
- [28] C. De las Heras Alarcón, S. Pennadam, C. Alexander, Stimuli responsive polymers for biomedical applications, *Chem. Soc. Rev.* 34 (2005) 276–285.
- [29] M. Wei, Y. Gao, X. Li, M.J. Serpe, Stimuli-responsive polymers and their applications, *Polym. Chem.* 8 (2017) 127–143.
- [30] M. Jafari-Nodoushan, J. Barzin, H. Mobedi, Size and morphology controlling of PLGA microparticles produced by electro hydrodynamic atomization, *Polym. Adv. Technol.* 26 (2015) 502–513.
- [31] A. Ghosh, C. Yoon, F. Ongaro, S. Scheggi, F.M. Selaru, S. Misra, D.H. Gracias, Stimuli-responsive soft untethered grippers for drug delivery and robotic surgery, *Front. Mech. Eng.* 3 (2017) 7.
- [32] Y. Xia, D.W. Pack, Uniform biodegradable microparticle systems for controlled release, *Chem. Eng. Sci.* 125 (2015) 129–143.
- [33] G. Subedi, A.K. Shrestha, S. Shakya, Study of effect of different factors in formulation of micro and nanospheres with solvent evaporation technique, *Open Pharm. Biosci. J.* (2016) 3.
- [34] D. Dendukuri, K. Tsoi, T.A. Hatton, P.S. Doyle, Controlled synthesis of nonspherical microparticles using microfluidics, *Langmuir* 21 (2005) 2113–2116.
- [35] P. Korteso, M. Ahola, M. Kangas, M. Jokinen, T. Leino, L. Vuorilehto, S. Laakso, J. Kiesvaara, A. Yli-Urpo, M. Marvola, Effect of synthesis parameters of the sol-gel-processed spray-dried silica gel microparticles on the release rate of dexmedetomidine, *Biomaterials* 23 (2002) 2795–2801.

- [36] T. Bhattacharjee, S.M. Zehnder, K.G. Rowe, S. Jain, R.M. Nixon, W.G. Sawyer, T.E. Angelini, Writing in the granular gel medium, *Sci. Adv.* 1 (2015) e1500655.
- [37] T.J. Hinton, Q. Jallerat, R.N. Palchesko, J.H. Park, M.S. Grodzicki, H.-J. Shue, M.H. Ramadan, A.R. Hudson, A.W. Feinberg, Three-dimensional printing of complex biological structures by freeform reversible embedding of suspended hydrogels, *Sci. Adv.* 1 (2015) e1500758.
- [38] T.J. Hinton, A. Hudson, K. Pusch, A. Lee, A.W. Feinberg, 3D printing PDMS elastomer in a hydrophilic support bath via freeform reversible embedding, *ACS Biomater. Sci. Eng.* 2 (2016) 1781–1786.
- [39] T.-F. Fan, Y. Hwang, M.G. Potroz, K.-L. Lau, E.-L. Tan, M.S. Ibrahim, E. Miyako, N.-J. Cho, Degradation of the sporopollenin exine capsules (SECs) in human plasma, *Appl. Mater. Today* 19 (2020) 100594.
- [40] E.L. Tan, M.G. Potroz, G. Ferracci, J.A. Jackman, H. Jung, L. Wang, N.J. Cho, Light-induced surface modification of natural plant microparticles: toward colloidal science and cellular adhesion applications, *Adv. Funct. Mater.* 28 (2018) 1707568.
- [41] E.-L. Tan, M.G. Potroz, G. Ferracci, L. Wang, J.A. Jackman, N.-J. Cho, Hydrophobic to superhydrophilic tuning of multifunctional sporopollenin for microcapsule and bio-composite applications, *Appl. Mater. Today* 18 (2020) 100525.