iScience

CellPress
OPEN ACCESS

Article

Soap Bubble Pollination

Xi Yang, Eijiro Miyako

e-miyako@jaist.ac.jp

HIGHLIGHTS

Developed soap bubbles exhibit various biological and physicochemical properties

The soap bubbles allow effective flower pollination

A flying robot equipped with a bubble maker can be used for autonomous pollination

Yang & Miyako, iScience *23*, 101188
June 26, 2020
https://doi.org/10.1016/j.isci.2020.101188

iScience

CellPress
OPEN ACCESS

Article

Soap Bubble Pollination

Xi Yang[1] and Eijiro Miyako[1,2,*]

SUMMARY

Natural and artificial flower pollination are critical processes in the life cycle of flowering plants. Declines in the number of global pollinator insects, the heavy labor of conducting artificial pollination manually, and the rising cost of pollen grains are considered to be significant worldwide problems. Here we show that chemically functionalized soap bubbles exhibit effective and convenient delivery of pollen grains to the targeted flowers thanks to their stickiness, softness, high flexibility, and enhancement of pollen activity. By exploring the physicochemical properties of functional soap bubbles, we could prepare mechanically stabilized soap bubbles capable of withstanding the windmills produced by robotic pollination. An unmanned aerial vehicle equipped with a soap bubble maker was autonomously controlled to pollinate flowers. Such technology of automatic intelligent robotic pollination with functional soft materials would lead to innovative agricultural systems that can tackle the global issues of pollination.

INTRODUCTION

Pollination by bees and other insects is one of the natural and essential biological processes for about three-quarters of global crop species (Abrol, 2012; Barth, 1991; Real, 1983). Pesticides, land clearing, and climate change have caused serious declines in the number of many of these living creatures (Goulson et al., 2015). Hand pollination with a cotton swab or a small brush is an effective method that has been used since ancient times as it allowed operators to apply pollen grains directly to flowers (Abrol, 2012; Barth, 1991; Real, 1983); however, this method required heavy labor to manually apply the pollen grains to all flowers within a farm in a timely manner. Machine pollination methods such as pollen blowers, dusters, and spray dispensers have been alternatively used recently to reduce the human labor and the reliance on insect pollination (Razeto et al., 2005; Williams et al., 2019); however, the expenses incurred from these conventional machine pollination methods have largely increased owing to the cost of pollen grains. In fact, these approaches produce a large number of inefficient pollen grains, especially those scattered from machines, which are not directly targeted toward the flowers. Alternatively, robotic crop pollination has attracted significant attention because of its potential advantageous performance in terms of individual flower detection, autonomous operation ability, and utilization of biomimetic strategies (Abutalipov et al., 2016; Elamvazhuthi and Berman, 2015; Pottsa et al., 2018). As such, the notion of robotic pollination might address the problem of reduction of natural insect pollinators as an alternative way. Moreover, developments in the field of robotic pollination seem to be directed at paying attention to reducing the pollination workload of farmers engaged in the agricultural business. Very recently, there appeared a study on a materially engineered artificial pollinator equipped with sticky ionic liquid gel coated by vertically aligned animal horse hair, which can work as a biomimetic honeybee to transport pollen grains among flowers (Chechetka et al., 2017). However, our previous pollination using a small toy drone lacked autonomous controlling system and the sticky animal hairs on a drone were needed to physically scrabble onto the flowers for delivering pollen grains. Operability of the technology itself was unfortunately impractical, and flowers were seriously damaged. Bearing fruit was thus not achieved by our previous technique due to these critical problems. Nonetheless, there remain huge demands for the development of material-engineering-based intelligent robotic pollination for effectively and efficiently pollinating crops. In addition, developing and designing of intelligent functional robots is attractive as an emerging technology and promising for autonomous precision robotic pollination (Ohi et al., 2018; Strader et al., 2019).

For centuries, scientific studies on soap bubbles have fascinated people of all ages mainly because of their beautiful rainbow colors and thin-film-based geometric structures based on simple scientific principles (Behroozi et al., 2008; Bird et al., 2010; Cantat et al., 2013; Salkin et al., 2016). However, despite the immense use of soap bubbles for entertainment, scientific, and educational purposes, establishing methodologies

[1]Graduate School of Advanced Science and Technology, Japan Advanced Institute of Science and Technology, 1-1 Asahidai, Nomi, Ishikawa 923-1292, Japan

[2]Lead Contact

*Correspondence: e-miyako@jaist.ac.jp

https://doi.org/10.1016/j.isci.2020.101188

and applications using soap bubbles as a functional material remains substantially unaddressed. We believe that the steady liquid membrane and the large surface area of soap bubbles are suitable media for delivering microscopic lightweight pollen grains for pollination and that the easy degradation and the low cost of the eco-friendly ingredients of soap bubbles are attractive and unique features. In addition, flowers do not sustain substantial physical damage from directly shooting soap bubbles because soap bubbles are lightweight, soft, and highly flexible.

With this aside, a large number of pollen grains are easily scattered in orchards through conventional artificial pollination with a brush and a machine pressure sprayer (Razeto et al., 2005; Williams et al., 2019). Unfortunately, the scattered pollen grains non-specifically attach to superfluous flowers, which often disturb the bearing and harvest of fruits. In fact, the inefficient fruits produced from such non-specific attachments are eventually thinned out, accompanied by heavy human labor resources for maintaining fruit development and tree vigor (Ouma, 2012). High controllability of the directional flying of soap bubbles by bubble-making devices is useful for simply shooting soap bubbles directly onto the target flowers so as to systematically reduce the workload of thinning out superfluous fruits. Moreover, scattering of pollen grains can be physically restrained as they are tightly confined to the liquid membrane of the soap bubbles. Thus, we are confident that soap bubbles are an ideal material for delivering pollen grains through robotic artificial pollination.

In this study, we demonstrate that (1) chemically functionalized soap bubbles exhibit unique properties, such as delivering pollen grains to the targeted flowers in a simple manner, reducing the usage of pollen grains, effectively attaching soap bubbles on the pistils of the targeted flowers using the high stickiness of the soap bubble membrane, preventing severe damage to delicate flowers using the softness and high flexibility of soap bubbles, and enhancing the pollen activity by promoting germination ratio and length of pollen tube; (2) chemically functionalized soap-bubble-mediated pollination can be used for practical *Pyrus pyrifolia* var. *culta* pollination at orchards aside from its contribution toward the healthy expression of fertility for various pollen grains; (3) mechanically stabilized soap bubbles capable of withstanding windmills due to robotic pollination can be successfully prepared; and (4) an autonomous controllable unmanned aerial vehicle (UAV) equipped with a mechanically stabilized soap bubble maker can fully automatically transport pollen grains to *Lilium japonicum* flowers, thus successfully aiding in plant pollination. The findings and concepts demonstrated in this study will undoubtedly influence the design of next-generation soft-materials-based robotic technologies (Kim et al., 2013; Pfeifer et al., 2007; Rus and Tolley, 2015), including applications in agriculture, material chemistry, biomimetic science, and aviation engineering.

RESULTS

Preparation of Soap Bubble Solutions

Figures 1A and S1 show a schematic illustration of how soap bubbles containing pollen grains are prepared using a bubble gun. At the interface between a soap film and air, the surfactant transforms a molecular bilayer in which the heads are directed toward the aqueous phase and the tails are directed toward the air (Ueno et al., 2016). We thus hypothesize that pollen microparticles are pushed out with the solution and then physically absorbed onto the bilayer of a bubble membrane by mechanical blowing from the fun in the bubble gun. We accidently found that natural pollen grains can be easily incorporated into a soap film and flown in the air using various bubble devices. One interesting device is the battery-and-motor-driven bubble gun, which can produce a lot of soap bubbles (Figures 1A and S2, and Video S1). We used pear pollen grains from *Pyrus bretschneideri* as an experimental model for the hereinafter described practical pollination at orchards and measured the germination ratio and growth of the pear pollen tubes using *in vitro* pollen activity assays (measurements of germination ratio and length of pollen tube) according to instructions from previous works (Fan et al., 2001; Rodriguez-Enriquez et al., 2013).

We initially tested the influence of five different surfactants—lauramidopropyl betaine (AMPHITOL 20AB [A-20AB]), sodium polyoxyethylene lauryl ether sulfate (EMAL E-27C [E-27C]), laurylhydoxysulfo betaine (AMPHITOL 20HD [A-20HD]), sodium polyoxyethylene alkyl ether sulfate (EMAL D3D [E-D3D]), and [N-cocoyl-(2-aminoethyl)-N-(2-hydroxyethyl)-N-sodiumcarboxymethyl] ethylenediamine (AMPHITOL 20YB [A-20YB])—on the pollen activity and bubble formation within the range of 0.2%–2.0% (Figures 1B, 1C, and S3). These surfactants were randomly chosen from among many commercially available products for their foaming ability to produce many soap bubbles by triggering a bubble gun once. The pollen activity assays indicated that the five surfactants showed a dose-dependent inhibition effect on pollen germination and tube growth (Figures 1B and 1C). Pollen germination ratio (G) was calculated as $G = N/N_t \times 100$ (%), where

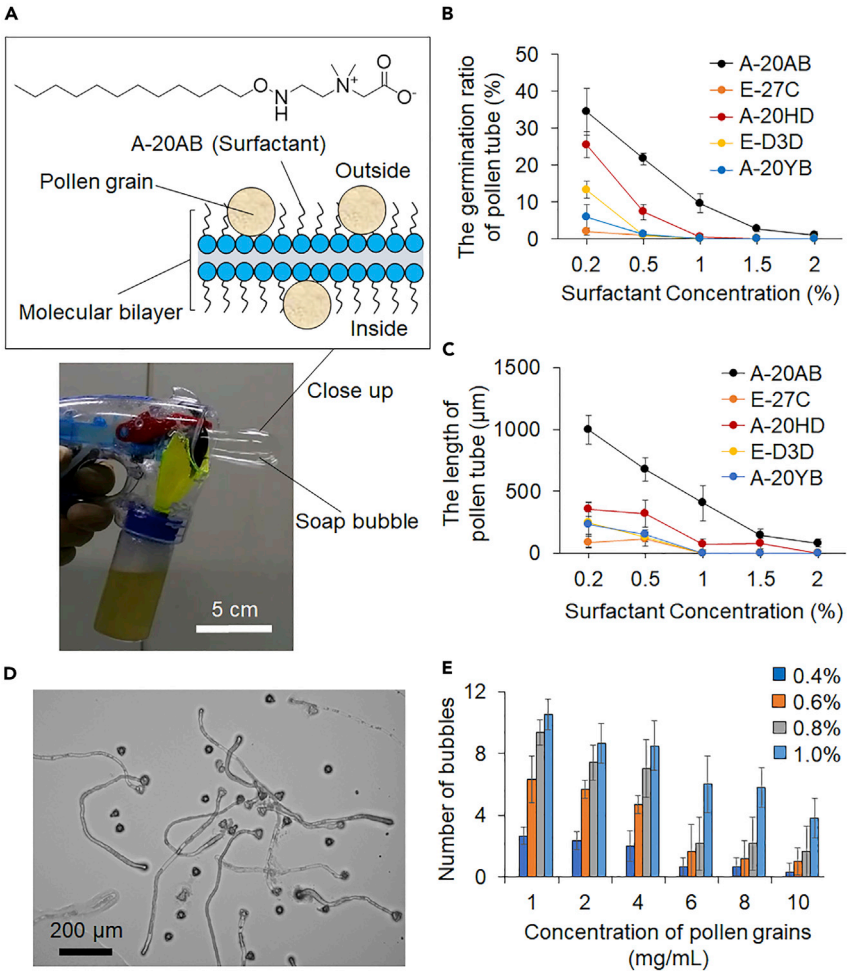

Figure 1. *In Vitro* Soap-Bubble-Mediated Pollination

(A–C) (A) A schematic illustration of soap-bubble-mediated pollination using a bubble gun. Influence of the type of surfactant on the (B) pollen germination ratio and (C) pollen tube growth.

(D) Pollen activity assay on an agar dish after soap-bubble-mediated pollination, at an A-20AB concentration of 0.5%.

(E) Impacts of the addition of different concentrations of A-20AB (0%–1%) and pollen grains (1–10 mg/mL) on the number of bubbles. Soap bubble quantity is increased by increasing the surfactant concentration and reducing pollen concentration. Data are represented as mean ± SEM in (B, C, and E).

N and N_t denote number of observations for pollen tubes by optical microscopy and total number of observations (100), respectively. Besides, the length of pollen tubes was measured by the results of direct observation and ImageJ software. The neutralized surfactant A-20AB demonstrated the highest performance in terms of pollen germination and tube growth compared with the other surfactants. In fact, the generated pollen tubes that were treated with a low A-20AB concentration were healthy after being subjected to soap-bubble-mediated pollination in an agar dish (Figures 1D and S4). In particular, A-20AB possessed the highest soap bubble formation ability among the utilized surfactants (Table S1). As such, we subsequently applied it as a foaming component in pollination solutions to create soap-bubble-carried pollen grains because of its powerful bubble-formation ability and superior capability of pollen germination and tube growth. A-20AB also showed dose- and incubation-time-dependent inhibition effects on pollen germination and tube elongation within the range of 0.0%–1.0% at a smaller interval (Figure S5). Nevertheless, the concentrations of A-20AB and pollen grains had a direct influence on the formation of soap bubbles (Figure 1E). We investigated the effects of different concentrations of surfactant and pollen grains on bubble formation by recording the number of bubbles produced per one trigger of the gun. Generally, higher surfactant concentration can help to make a lot of soap bubbles. Besides, larger number of pollen grains, which often have water-insoluble agglomeration, tends to disturb the formation of bubble

membrane in the nozzle of the gun so that it decreases the number of soap bubbles. For instance, no bubbles could be produced at 0.0% or 0.2% A-20AB with pollen grains in a wide range of 1–10 mg/mL, whereas at least more than one soap bubble could be produced in the case of 0.4%–0.8% A-20AB and pollen grains of no more than 4 mg/mL. Moreover, with 1.0% A-20AB, 4–11 bubbles were formed within a pollen grain concentration of 1–10 mg/mL. In any case, pollen grains could be certainly carried by the formed soap bubbles, and the number of pollen grains loaded on the soap bubble increased with more pollen grains (Figure S6). Considering the above-mentioned inhibition property of surfactants against pollen grains and the available number of soap bubbles formed, we chose 0.4% A-20AB and a pollen grain concentration of 4 mg/mL for subsequent soap-bubble-mediated pollination. In this case, we could load a maximum of approximately 2,000 pollen grains on every soap bubble after triggering the bubble gun once (Figure S6).

To ensure successful pollination, we further optimized the components of the soap bubble solution under various physiological conditions (Figure S7), considering the pH value as an essential influencing factor on pollen germination and tube growth (Rus and Tolley, 2015). The germination ratio reached its highest value (ca. 30.7%) at pH 7.0 (Figure S7A), and the pollen tube length exhibited a similar trend and reached its maximum value (ca. 1,010 μm) at pH 7.0.

Moreover, moderate addition of boron, calcium, magnesium, and potassium promotes pollen germination and tube length through direct or indirect mechanisms (Brewbaker and Kwack, 1963; Nyomora et al., 2000; Wang et al., 2003). Among them, calcium plays an important role in the germination and growth of pollen. It can improve the germination and growth of pollen due to the binding of calcium to pectate carboxyl groups along the pollen wall. Other elements such as boron, magnesium, and potassium can enhance the calcium effect. Here, we added individual concentrations of H_3BO_3, $MgSO_4{\cdot}7H_2O$, $CaCl_2$, and KCl to the solution to improve the pollen activity (Figures S7B–S7E). Although H_3BO_3 (0–60 ppm) showed a negligible effect on promoting pollen germination, we observed an obvious increase in the pollen tube growth at 5 ppm ($p < 0.001$) (Figure S7B). The pollen tube length actually reached 1,187 μm, which is 1.3 times higher compared with the control without H_3BO_3 (Figure S7B). Similarly, $MgSO_4{\cdot}7H_2O$ did not significantly promote pollen germination, but it obviously stimulated tube elongation at a low concentration of 0.1 mM ($p < 0.01$), within which the tube length reached a maximum value of 1,127 μm, which is 1.3 times higher compared with the control without $MgSO_4{\cdot}7H_2O$ (Figure S7C). With this aside, we found that $CaCl_2$ concentrations within the range 0.1–2.0 mM dramatically improve the pollen germination ratio and tube growth (Figure S7D). More specifically, the germination ratio and tube length reached a maximum of 45.7% and 1,152 μm, respectively, at 1.0 mM $CaCl_2$ (Figure S7D), which are approximately 1.6 and 1.3 times higher compared with the control without $CaCl_2$. Moreover, although there was no sign that KCl promoted pollen germination, it otherwise improved the tube elongation significantly ($p < 0.05$) (Figure S7E). Eventually, at a KCl concentration of 1 mM, the pollen tube length reached a maximum value of 1,032 μm. Furthermore, under optimal conditions, the germination ratio and tube length of the pollen grains reached 45.0% and 1,232 μm, respectively, which were approximately 1.8 and 1.4 times higher compared with the control without chemical additives (Figure S7F).

Gelatine is a water-soluble protein that consists of large amounts of glycine, proline, and hydroxyproline, which might play an essential role in pollen germination and tube elongation (Hong-Qi et al., 1982). We added 0.2%–2.0% gelatine to the aforementioned pollination solution including optimized chemicals. Apparently, after treating the solution with 0.8% gelatine, the germination ratio and tube length reached a maximum value of approximately 50% and 1,363 μm, respectively (Figure S8A). We also added 0.0%–0.5% hydroxypropyl methylcellulose (HPMC) (Burdock, 2007) to the solution and investigated its effect on pollen germination and tube elongation. HPMC was chosen to mechanically stabilize the films of the soap bubbles for robotic pollination (as mentioned earlier) and to moisturize the pollen particles in the solution to improve the pollen activity. Interestingly, the presence of HPMC (at a concentration of 0.2%; $p < 0.01$) significantly promoted the elongation of pollen tubes to a maximum value of 1,408 μm, whereas it slightly promoted pollen germination (Figure S8B). As we expected, HPMC also helped make the bubbles more stable, which was helpful in retaining the pollen grains on the thin film of the soap bubbles and transporting them to the targeted flowers.

The membrane thickness of a soap bubble can be determined using the following formula (Lautrup, 2011):

$$\tau = (M \div \rho) \div 4\pi R^2$$

where τ, M, ρ, and R represent the membrane thickness, weight (ca. 7.7 mg), density (ca. 0.99 g/cm), and radius (ca. 1.6 cm) of the soap bubble. If we assume the value of π to be 3.14, then τ is 2.4 μm, which is a reasonable value for a conventional soap bubble within the thickness range of 1–10 μm as calculated using spectroscopic methods (Behroozi, 2008; Rutgers et al., 2001).

Field Work of Pear Flower Pollination

For artificial pollination at an orchard, we selected pears as our model mainly because of their potential large market size, as well as their easy handling during experiments. We initially examined the activity of pear pollen grains in an optimized soap bubble solution during the pollination process for 3 h, for comparison with other methods such as powder pollination and solution pollination, in addition to non-optimized soap bubble pollination. At the beginning of the optimized soap-bubble-mediated pollination, the pollen germination ratio and tube length were 49% (Figure 2A) and 1,221 μm (Figure 2B), respectively, which are about 1.9 and 1.5 times higher compared with a non-optimized soap bubble solution. After 3 h of pollination, the pollen germination ratio and tube length decreased to 28% and 990 μm, respectively, which were about 5.9 and 1.9 times higher compared with a non-optimized soap bubble solution. This result indicated that optimization remarkably improved the pollen activity in the bubble pollination process. More particularly, the pollen activity of the optimized soap-bubble-mediated pollination did not decrease, when compared with that of other pollination methods (powder pollination and solution pollination), after 3 h of pollination. In summary, soap-bubble-mediated pollination exhibited higher pollen activity compared with other conventional pollination methods for at least 3 h after the pollination process.

To demonstrate the feasibility of soap-bubble-mediated pollination, we shot different numbers (0, 1, 2, 5, 10, 20, and 50) of soap bubbles on natural pear flowers (Figure 2C). After overnight incubation at 25°C, the pistils of the flowers were cut and stained with aniline blue. Fluorescence microscopy showed that the pollen grains successfully landed on the pistils and that the growth of pollen tubes became clearly visible after pollination (Figures 2D and S9). In the control group, in which no soap bubbles containing pollen grains were attached, no pollen grains or tubes were present at all (Figure S9). Essentially, the number of pollen grains on each pistil increased as the number of soap bubbles got larger. The number of pollen grains decreased and the tube lengths became shorter after more than 10 bubbles were shot, which could be attributed to the toxicity of the accumulated pollination solution on the pollen grains and/or pistils. Therefore, less than 10 bubbles were subsequently shot onto the pear flowers at the farms.

Here, we pollinated the flowers of *P. pyrifolia* var. *culta* (N = 50) in an orchard using a soap-bubble-mediated technique with an optimized solution (Figures 2E and S10). Surprisingly, after shooting the soap bubbles onto the targeted flowers, young fruits formed after 16 days at a volume that was almost the same as that of conventional hand pollination with a spherical feather brush. The volumes of pear fruits obtained over time increased steadily and exhibited the same swelling tendency as that of flowers pollinated after 16 days. The 2–10 soap bubbles that hit the flowers had no influence on pollination. Anyway, it is an amazing effect because even only a couple of bubbles can effectively achieve bearing fruits. Moreover, control flowers, which did not undergo any kind of pollination, yielded the smallest volume of young fruits, probably because of the influence of naturally pollinating insects as well as the non-specific and imperfect attachment of scattered pollen grains in the wind from other hand pollination work by farmers in the same orchard. Specifically, because of these environmental factors, the fruit-bearing rate of the control that did not undergo any pollination was only about 58% (Figure S11). Moreover, the rates of both soap-bubble-mediated pollination and hand pollination were approximately 95%, and no significant differences were observed between them. Apparently, such results demonstrated that soap-bubble-mediated pollination is effective not only for the expression of fertility of pollen grains but also for the substantial production of pear fruits.

Figure S12 and Table S2 show the number of pollen grains used in different pollination methods. For the tests, we used the inexpensive *Lycopodium clavatum* spore as a model to replace costly natural pollen grains. Conventional hand pollination with a spherical feather brush required approximately 1,747 mg pollen grains for one instance of pollination, whereas machine-based pollination required approximately 165 mg pollen grains. Moreover, a solution hand spray ejected 3.2 mg of pollen grains by one-push spraying. Based on our calculation, the soap-bubble-mediated method consumed only 0.06 mg pollen grains for essential pear pollination via two or three soap bubbles. Thus, the supersizing reduction effect of pollen grains by soap bubble-mediated pollination should be beneficial for practical cost cutting of labor and pollen manufacturing.

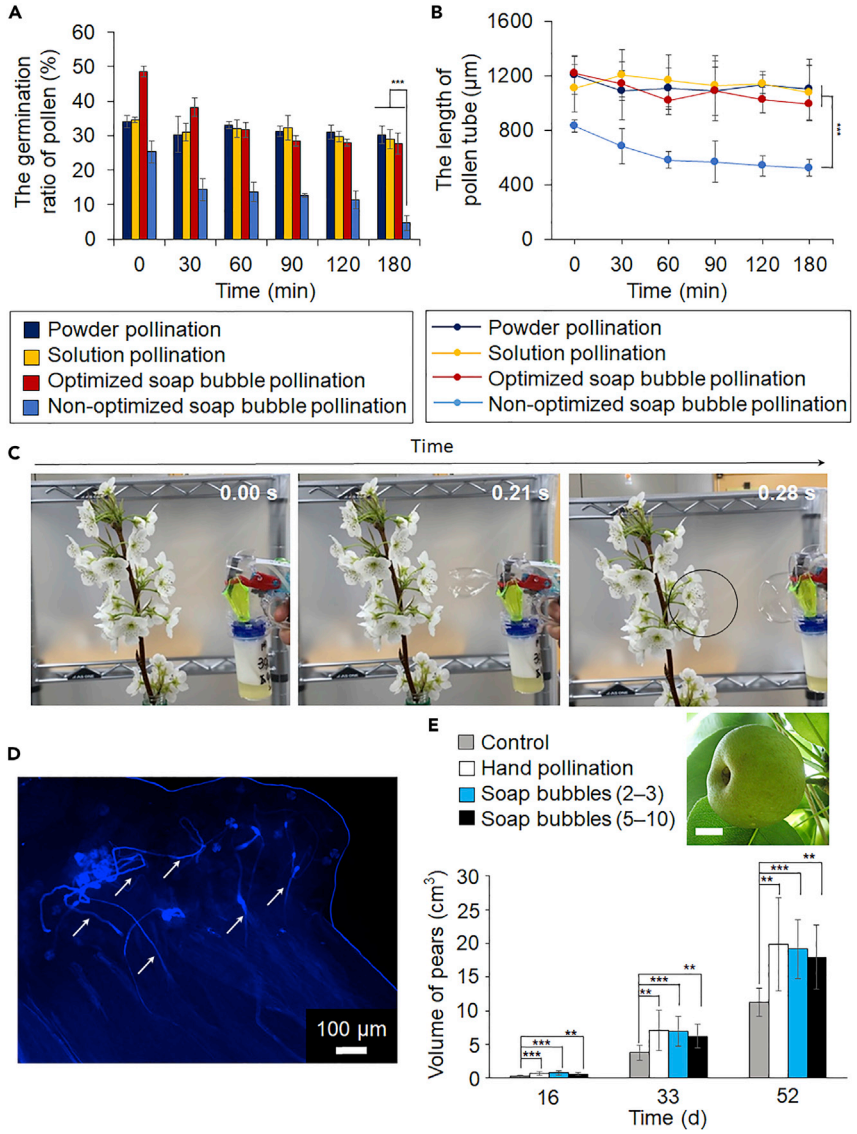

Figure 2. Field Work of Soap-Bubble-Mediated Pollination

(A) Pear pollen germination ratio of various pollination methods during the pollination process for 3 h.

(B) Length of pear pollen tubes after soap-bubble-mediated pollination for 3 h incubation time in solution when compared with different pollination methods.

(C) Photographs of soap-bubble-mediated pollination for pear flowers using a bubble gun.

(D) Fluorescence microscopy images of pollen tube formation after soap-bubble-mediated pollination with two bubbles. Scale bar, 100 μm. The white arrows indicate the pollen tubes.

(E) Formation of young pear fruits by soap-bubble-mediated pollination. The inset image shows young pear fruits formed by 5–10 soap bubbles shot onto a flower after 53 days. Scale bar, 1 cm. **$p < 0.01$, ***$p < 0.001$. Data are represented as mean ± SEM in (A, B, and E).

Robotic Pollination

Our final goal was to perform robotic pollination using a drone and soap bubbles. However, as drones often generate strong downstream wind from their propellers, the soap bubbles should be mechanically stabilized to ensure that they are correctly shot at the flowers while retaining their original structure. Indeed, the above-mentioned normal soap bubbles that were prepared by bubble formation tools vanished quickly. Herein, we loaded 2% HPMC into the solution to physically stabilize the soap film. Interestingly, soap bubbles containing 2% HPMC and 1% A-20AB turned out to be very stable self-standing

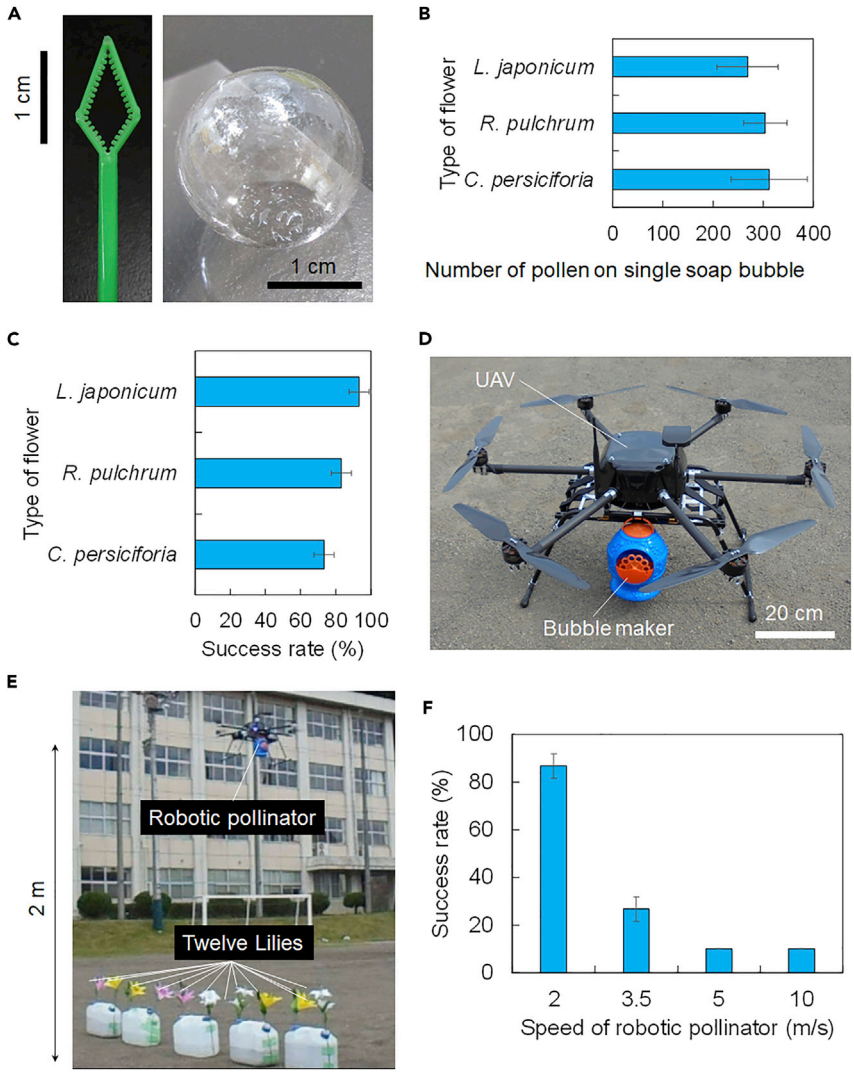

Figure 3. Robotic Pollination Using Mechanically Stabilized Soap Bubbles

(A) A photograph illustrating a commercial bubble wand (left) and a mechanically stabilized soap bubble on a glass slide (right).

(B) Number of adsorbed pollen grains per bubble for various flowers.

(C) Pollination success rate of different types of flowers by attachment of a single soap bubble containing pollen grains.

(D) Image of a robotic pollinator consisting of a UAV and a bubble maker.

(E) Robotic pollination using an autonomous robotic pollinator and *L. japonicum* flowers.

(F) Effect of the speed of a robotic pollinator on the success rate (hitting of bubbles onto flowers) of artificial pollination. Data are represented as mean ± SEM in (B, C, and F).

bubbles with a diameter of approximately 2 cm, prepared using a bubble blowing tool (Figure 3A). Unexpectedly, most of the mechanically stabilized soap bubbles did not quickly vanish for at least 10 min at 25°C. What was more surprising was that some of them maintained their spherical shape for as long as 5 h, and they also could withstand compression as they were recorded to tolerate up to 0.03 N maximum proof compressive load (Figure S13). During the compression test, we heard faint pops as the soap bubbles burst, signifying their HPMC-improved mechanical stability.

Accordingly, mechanically stabilized soap bubbles exhibited a membrane thickness of 4.1 μm, thicker than the above-mentioned conventional soap bubbles (2.4 μm), probably because of the formation of a thick polymer film due to the highly condensed HPMC.

Besides, we confirmed that the pear pollen grains still demonstrated strong activity even after being pollinated with soap bubbles containing 2% HPMC (Figure S14). The kinematic viscosity and density of the prepared soap bubble solution were 7,530 cSt and 1.023 g/cm, respectively. The relatively high viscosity of the solution was also useful for pollen dispersion (Figure S15); in fact, various pollen grains, such as those of *L. japonicum*, *Rhododendron pulchrum*, and *Campanula persicifolia*, were dispersed and suspended well in the highly viscous soap bubble solution (Figure S15A). Moreover, we observed a Tyndall effect (Petrucci et al., 2006) in *L. japonicum* pollen colloidal particles in a quartz cuvette at 650 nm laser irradiation (Figure S15B). Optical microscopy directly revealed that all types of pollen grains were individually and well dispersed in the solution (Figure S16). We counted the number of pollen grains on each soap bubble (*L. japonicum*, ca. 269 particles; *R. pulchrum*, ca. 304 particles; *C. persicifolia*, ca. 312 particles) using optical microscopy after smashing it between two cover glasses (Figures 3B and S17). Subsequently, we confirmed the concentration dependence of spore particles on loading number onto the soap bubble solution (Figure S18). The mechanically stabilized soap bubbles were also observed to stay on the pistil of each flower for a while, ranging from 10 s up to 5 min (Figure S19), and then they vanished depending on the pistil size and the amount of sticky pollenkitt (Amador et al., 2017) on the pistil. More particularly, the soap bubbles vanished shortly after attaching to the largest pistil in the flower of *L. japonicum*, which had lots of viscous pollenkitt when compared with other flowers. Interestingly, even after the pistil of each flower was hit only by one soap bubble containing pollen grains, followed by overnight incubation, we observed the growth of fibrous pollen tubes, indicating successful pollen fertilization (Figure S20). The control flower, which was not hit by any soap bubble including pollen grains, did not show any pollen grain adsorption or pollen tube growth at all, for all types of flowers (Figure S21). In essence, single-soap-bubble-mediated pollination reached a maximum success rate of 90% for the flowers of *L. japonicum* (Figure 3C), which was higher compared with the flowers of *R. pulchrum* and *C. persicifolia*, probably because of the bigger pistil size and large amount of pollenkitt (Figure 3C). Pollination success rate (P_{SR}) was calculated as $PSR = N/Nt \times 100$ [%], where N and N_t denote the number of actual pollen tubes observed under fluorescence microscopy and the total number of observations (10), respectively.

An automatic bubble maker was likewise used in combination with a drone (Figure 3D). This bubble maker continuously produced approximately 5,000 mechanically stabilized soap bubbles per minute (Figure S22 and Video S2). To demonstrate the use of robots in pollination, we utilized a commercially available, fully automatically controllable UAV as a robotic pollinator. Here, a bubble maker was attached to the body of the UAV (Figure 3D). The movement of the robotic pollinator was controlled using a fully automatic operation system (Nonami et al., 2010) equipped with a global navigation satellite system (GNSS) (Figures S23A–S23C). Then, soap bubbles were incessantly shot onto the targeted fake *L. japonicum* flowers from a height of about 2 m at an angle of about 70–80° (Figures 3E and S23D, and Video S3). Apparently, the physical impact caused by the downstream wind from the UAV contributed to the immediate destruction of the soap bubbles after hitting the pistil of the *L. japonicum* flower (Figure S24). The UAV exhibited a downstream wind speed of about 4.5 m/s as it hovered steadily in the air; nonetheless, this downstream wind speed under the UAV increased to 5.8 or 8.7 m/s when it moved at a constant speed of 2 or 4 m/s, respectively. Based on the results of the robotic pollination experiment, the success rate of soap bubbles hitting the flowers depended on the speed of the UAV (Figure 3F). Although the isolated single soap bubble was able to hit a pistil on a lily flower as shown in Figure S24, most soap bubbles were formed from the UAV as a bunch of bubbles seemed like "cluster." Thus, the number of the soap bubble cluster, which could be accurately struck to each flower, was recorded for figuring out the average success rate when the UAV moved over the flowers at a different speed (2–10 m/s). After all, we achieved a higher than 90% success rate at a velocity of 2 m/s; more importantly, we observed the growth of natural pollen tubes of real *L. japonicum* flowers (Figure S25). These results are a clear indication that a robotic pollinator equipped with a soap bubble maker would successfully promote effective flower pollination.

DISCUSSION

In this study, we designed and prepared chemically functionalized soap bubbles for the artificial pollination of flowers using various bubble-making devices. Herein, these chemically functionalized soap bubbles demonstrated interesting properties, such as the delivery of pollen particles, enhancement of pollen activity, excellent mechanical stability, suitable size and geometry of flower pistils, softness and high flexibility to prevent damage to flowers, adhesiveness on flowers for effective pollination, and simple ejection ability on

a large number of soap bubbles from the devices. In addition, we successfully pollinated the flowers of *P. pyrifolia* var. *culta* in an orchard by soap bubbles using a bubble gun, which consequently formed young pear fruits. We also integrated soap bubbles within a drone for fully automatic pollination of *L. japonicum* flowers. This study is the first exploring the unique properties of soap bubbles as a material used for the artificial pollination of flowers using different types of bubble-making tools and an autonomous controllable drone. We expect our multidisciplinary approach combining soap bubbles and drone technology to lead to innovative developments in the field of agricultural engineering. We also assume that our findings will pave the way for discovering artificial pollination methods that can address relevant global issues such as the decline in pollinator insects, the heavy labor involved in artificial pollination, and the soaring costs of pollen grains.

Limitations of the Study

The surfactants used in this study are biocompatible, but their elimination in the environment might cause their accumulation and difficult degradation. Therefore, we are trying to use eco-friendlier and edible surfactants for future practical pollination. Investigations of the soap bubble-mediated pollination using the automated robotic drone at field or orchard scale are also future challenges because the use of a prototype artificial pollinator to spray bubbles caused a lot of waste as most bubbles would miss the flower. Further innovative technologies, such as state-of-the-art localization and mapping, visual perception, path planning, motion control, and manipulation techniques, would be essential for developing autonomous precision robotic pollination.

Resource Availability

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Eijiro Miyako (e-miyako@jaist.ac.jp).

Materials Availability

This study did not generate new unique reagents.

Data and Code Availability

The data that support the findings of this study are available from the corresponding author on reasonable request.

METHODS

All methods can be found in the accompanying Transparent Methods supplemental file.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2020.101188.

ACKNOWLEDGMENTS

This work was supported by a Japan Society for the Promotion of Science (JSPS) KAKENHI Grant-in-Aid for Scientific Research (A) (Grant number 19H00857); JSPS KAKENHI Grant-in-Aid for Scientific Research (B) (Grant number 16H03834); and JSPS KAKENHI Fund for the Promotion of Joint International Research (Fostering Joint International Research) (Grant number 16KK0117). The author also thanks Kao Chemical Co., Ltd. for providing the surfactants. The authors also thank National Institute of Advanced Industrial Science and Technology for their partial support with some experiments.

AUTHOR CONTRIBUTIONS

E.M. conceived and designed the experiments and prepared the manuscript; E.M. and X.Y. performed the experiments and analyzed the data. All the authors discussed the results and contributed to the writing of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: April 20, 2020
Revised: May 9, 2020
Accepted: May 18, 2020
Published: June 26, 2020

REFERENCES

Abrol, D.P. (2012). Pollination Biology: Biodiversity Conservation and Agricultural Production (Springer-Verlag).

Abutalipov, R.N., Bolgov, Y.V., and Senov, H.M. (2016). Flowering plants pollination robotic system for greenhouses by means of nano copter (drone aircraft). in: 2016 IEEE Conf. Qual. Manag. Transp. Inf. Secur. Inf. Technol., IEEE 7–9.

Amador, G.J., Matherne, M., Waller, D., Mathews, M., Gorb, S.N., and Hu, D.L. (2017). Honey bee hairs and pollenkitt are essential for pollen capture and removal. Bioinspir Biomim. *12*, 026015.

Barth, F.G. (1991). Insects and Flowers: The Biology of a Partnership (Princeton Science Library).

Behroozi, F. (2008). Soap bubbles in paintings: art and science. Am. J. Phys. *76*, 1087.

Bird, J.C., de Ruiter, R., Courbin, L., and Stone, H.A. (2010). Daughter bubble cascades produced by folding of ruptured thin films. Nature *465*, 759–762.

Brewbaker, J.L., and Kwack, B.H. (1963). The essential role of calcium ion in pollen germination and pollen tube growth. Am. J. Bot. *50*, 859–865.

Burdock, G.A. (2007). Safety assessment of hydroxypropyl methylcellulose as a food ingredient. Food Chem. Toxicol. *45*, 2341–2351.

Cantat, I., Cohen-Addad, S., Elias, F., Höhler, R., Pitois, O., Rouyer, F., and Saint-Jalmes, A. (2013). Foams: Structure and Dynamics (Oxford University Press).

Chechetka, S.A., Yu, Y., Tange, M., and Miyako, E. (2017). Materially engineered artificial pollinators. Chem *2*, 224–239.

Elamvazhuthi, K., and Berman, S. (2015). Optimal control of stochastic coverage strategies for robotic swarms. in: 2015 IEEE Int. Conf. Robot. Autom., IEEE 1822–1829.

Fan, L.-M., Wang, Y.-F., Wang, H., and Wu, W.-H. (2001). In vitro Arabidopsis pollen germination and characterization of the inward potassium currents in Arabidopsis pollen grain protoplasts. J. Exp. Bot. *52*, 1603–1614.

Goulson, D., Nicholls, E., Botías, C., and Rotheray, E.L. (2015). Bee declines driven by combined stress from parasites, pesticides and lack of flowers. Science *347*, 1255957–1255966.

Hong-Qi, Z., Croes, A., and Linskens, H. (1982). Protein synthesis in germinating pollen of Petunia: role of proline. Planta *154*, 199–203.

Kim, S., Laschi, C., and Trimmer, B. (2013). Soft robotics: a bioinspired evolution in robotics. Trends Biotechnol. *31*, 287–294.

Lautrup, B. (2011). Physics of Continuous Matter: Exotic and Everyday Phenomena in the Macroscopic World, Second Edition (CRC Press).

Nonami, K., Kendoul, F., Suzuki, S., Wang, W., and Nakazawa, D. (2010). Autonomous Flying Robots (Springer Verlag Gmbh).

Nyomora, A.M.S., Brown, P.H., Pinney, K., and Polito, V.S. (2000). Foliar application of boron to almond trees affects pollen quality. J. Am. Soc. Hortic. Sci. *125*, 265–270.

Ohi, N., Lassak, K., Watson, R., Strader, J., Du, Y., Yang, C., Hedrick, G., Nguyen, J., Harper, S., Reynolds, D., et al. (2018). Design of an Autonomous Precision Pollination Robot (IROS).

Ouma, G. (2012). Fruit thinning with specific reference to citrus species: a review. Agric. Biol. J. N. Am. *3*, 175–191.

Petrucci, R., William, H., Herring, F., and Madura, J. (2006). General Chemistry: Principles and Modern Applications, Ninth Edition (Prentice Hall).

Pfeifer, R., Lungarella, M., and Iida, F. (2007). Self-organization, embodiment, and biologically inspired robotics. Science *318*, 1088–1093.

Pottsa, S.G., Neumannb, P., Vaissièrec, B., and Vereecken, N.J. (2018). Robotic bees for crop pollination: why drones cannot replace biodiversity. Sci. Total Environ. *642*, 665–667.

Razeto, B., Reginato, G., and Larraín, A. (2005). Hand and machine pollination of kiwifruit. Int. J. Fruit Sci. *5*, 37–44.

Real, L. (1983). Pollination Biology (Academic Press, Elsevier).

Rodriguez-Enriquez, M.J., Mehdi, S., Dickinson, H.G., and Grant-Downton, R.T. (2013). A novel method for efficient in vitro germination and tube growth of Arabidopsis thaliana pollen. New Phytol. *197*, 668–679.

Rus, D., and Tolley, M.T. (2015). Design, fabrication and control of soft robots. Nature *521*, 467–475.

Rutgers, M.A., Wu, X.L., and Daniel, W.B. (2001). Conducting fluid dynamics experiments with vertically falling soap films. Rev. Sci. Instrum. *72*, 3025.

Salkin, L., Schmit, A., Panizza, P., and Courbin, L. (2016). Generating soap bubbles by blowing on soap films. Phys. Rev. Lett. *116*, 077801.

Strader, J., Nguyen, J., Tatsch, C., Du, Y., Lassak, K., Buzzo, B., Watson, R., Cerbone, H., Ohi, N., Yang, C., and Gu, Y. (2019). Flower interaction subsystem for a precision pollination robot. IEEE/RSJ International Conference on Intelligent Robots and Systems (IROS) 2019, Macau, China.

Ueno, M., Isokawa, N., Fueda, K., Nakahara, S., Teshima, H., Yamamoto, N., Yokoyama, H., Noritsugu, Y., Shibata, K., Miyagawa, K., et al. (2016). Practical chemistry of long-lasting bubbles. World J. Chem. Educ. *4*, 32–44.

Wang, Q., Lu, L., Wu, X., Li, Y., and Lin, J. (2003). Boron influences pollen germination and pollen tube growth in Picea meyeri. Tree Physiol. *23*, 345–351.

Williams, H., Nejati, M., Hussein, S., Penhall, N., Lim, J.Y., Jones, M.H., Bell, J., Ahn, H.S., Bradley, S., Schaare, P., et al. (2019). Autonomous pollination of individual kiwifruit flowers: toward a robotic kiwifruit pollinator. J. Field Robot. *37*, 1–17.

iScience, Volume *23*

Supplemental Information

Soap Bubble Pollination

Xi Yang and Eijiro Miyako

Supplementary Data For

Soap Bubble Pollination

Xi Yang[1] and Eijiro Miyako[1,2,*]

[1]Graduate School of Advanced Science and Technology, Japan Advanced Institute of Science and Technology, 1-1 Asahidai, Nomi, Ishikawa 923-1292, Japan
[2]Lead Contact
*Correspondence: e-miyako@jaist.ac.jp

Table of contents:

Transparent Methods

Pollen activity assays. We determined the pollination activity of purified pear pollen grains from *Pyrus bretschneideri* (Kobayashi Bag Mfg. Co., Ltd., Nagano, Japan) using *in vitro* pollen germination and pollen tube growth assays, which were performed on an agar medium in a gamma-sterilised Petri dish (AS ONE, Osaka, Japan) consisting of 10% sucrose (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) and 1% agar (FUJIFILM Wako Pure Chemical Corporation). We adjusted the pH value of our medium to 6.5 before autoclaving at 121℃ for 15 min (LSX-300; TOMY, Tokyo, Japan). The prepared medium was kept at 25℃ until just before use. Afterwards, we inoculated the pear pollen grains onto the medium. After incubating the pollen grains at 25℃ for 24 h, we observed their shapes under a microscope (Olympus IX73; Olympus, Tokyo, Japan) equipped with an objective lens with 10× magnification (UPLFLN10X; Olympus) at 25℃. We then analysed the germination properties of the pear pollen grains from 100 pollen particles and then measured the lengths of the pollen tubes using ImageJ software (Fiji). The pear pollen grains were stored at −80℃ (long-term storage) and incubated at 25℃ overnight just before use.

Next, we investigated the effects of five different surfactants (A-20AB, E-27C, A-20HD, E-D3D and A-20YB) on the pollen viability and bubble formation ability in order to develop high-foamed, low-toxic soap bubble pollination. Briefly, the pear pollen grains were dispersed in a 10% (w/v) sucrose solution at a concentration of 2 mg mL^{-1}. After incubating the mixtures for 1 h at 25℃, we added the surfactant at a final concentration of 0–2%. We then immediately put each mixture into a glass vial in a bubble gun (Uni Enterprise, Yamaguchi, Japan). Nozzle and trigger made of vulnerable polystyrene in an original gun were replaced to robust metallic parts which were prepared by a three-dimensional printer (ProX200; 3D SYSTEMS, Rock hill, SC, US). We then captured 10 soap bubbles containing pollen grains (generated from the gun) on an agar dish and then incubated them overnight at 25℃. For soap solutions with no bubble formation, we cautiously dropped 20 μL of soap solution supplemented with 2 mg mL^{-1} pollen grains onto an agar dish before uniformly spreading it on the agar using a disposable spreader (Thomas Scientific, Waltham, MA, USA) and then performed the pollen activity assays. We investigated the effects of different surfactants on bubble formation by recording the number of bubbles produced per one trigger of the gun. The surfactant that displayed the best bubble formation ability and lowest toxicity was selected for further tests.

In order to decide which parameter in the foaming ingredients was the most important in soap-bubble-mediated pollination, we further investigated the effect of the surfactant on the pollen activity within a surfactant concentration range of 0–1% during 0–3 h of pollination. Additionally, we estimated the effect of pollen grain concentration (1–10 mg mL^{-1}) on the number of soap bubbles produced by a gun. The number of pollen grains loaded on a single soap bubble was also counted using optical microscopy.

In order to evaluate the effect of the pH value of the solution on the pollen activity, we prepared a 10% (w/v) sucrose solution with pH values varying from 6.0 to 8.5. Pear pollen grains were dispersed in the solution at a concentration of 4 mg mL^{-1}. After incubating the mixtures for 1 h at 25℃, we added

the surfactant at a final concentration of 0.4%. We then used the mixtures immediately to generate soap bubbles from the gun. The pollen grains were inoculated on an agar medium once 10 bubbles have been captured on the agar dish, to check the pollen activity by optical microscopy.

We determined the effects of various chemicals, such as boron, magnesium, calcium and potassium, on pollen germination and pollen tube growth as follows. Briefly, we mixed a 10% sucrose solution with H_3BO_3 (0–60 ppm), $MgSO_4 \cdot 7H_2O$ (0–2 mM), $CaCl_2$ (0–2 mM) and KCl (0–2 mM) (all from FUJIFILM Wako Pure Chemical Corp.). The solution's pH value was adjusted to 7.0 using 0.1 N HCl or 0.1 N NaOH (both from FUJIFILM Wako Pure Chemical Corp.). Here, the pollen grain concentration was 4 mg mL^{-1}. In order to measure the pollen grain activity, we inoculated 10 bubbles containing pollen grains onto an agar dish. We then assessed the pollen activity and performed a comparison with the control group without the above chemicals. Moreover, we investigated the activity of pollen grains in the optimised pollination solution at fixed time intervals of 0, 30, 60, 90, 120 and 180 min.

In order to further improve the pollen activity in the soap bubble solution, we investigated the effects of gelatine (FUJIFILM Wako Pure Chemical Corp.) and hydroxypropyl methylcellulose (HPMC) (Alfa Aesar, Haverhill, MA, USA) on pollen germination using a pollen activity assay at a concentration range of 0–2% for these chemicals.

For powder pollination, we carefully put the powder of *Pyrus bretschneideri* pollen grains on an agar dish (**Fan et al., 2001; Rodriguez-Enriquez et al., 2013**). For solution pollination, we prepared a solution with 4 mg mL^{-1} *P. bretschneideri* pollen grains in a 10% sucrose solution including 0.1% agar (**Hopping et al., 1982; Yano et al., 2017**). We then ejected the prepared solution onto an agar plate using a hand spray (Cainz Home, Saitama, Japan). Pollen activity assays for powder and solution pollination were conducted in a manner similar to that of the soap-bubble-mediated pollination above.

Fertility assay of pear flowers. We collected natural *P. pyrifolia* var. *culta* flowers from an orchard in advance and kept them at room temperature until just before the experiment. We prepared a pollination solution by mixing Milli-Q water with 10% sucrose, 5 ppm H_3BO_3, 0.1 mM $MgSO_4 \cdot 7H_2O$, 1.0 mM $CaCl_2$, 1.0 mM KCl, 0.8% gelatine and 0.2% cellulose. Pear pollen grains from *P. pyrifolia* var. *culta* were then dispersed in the solution at a concentration of 4 mg mL^{-1}. We obtained a purified powder of pear pollen grains from farmers. After 1 h of incubation, the surfactant A-20AB was added to the solution at a final concentration of 0.4%. Afterwards, we mixed the solutions immediately, set them in a bubble gun and shot different numbers of soap bubbles (0–50) onto blooming *P. pyrifolia* var. *culta* live flowers. Our negative control group was a set of pear flowers with no soap bubbles containing pollen grains attached. Moreover, we kept the flowers at room temperature overnight after pollination with or without soap bubbles. Pollinated pistils were collected and soaked in 1 N NaOH solution and heated at 60°C for 1 h. Shortly thereafter, the pistils were transferred into an aniline blue solution prepared by dissolving 0.01 wt.% aniline blue (FUJIFILM Wako Pure Chemical Corp.) in a 2 wt.% aqueous K_3PO_4 solution (FUJIFILM Wako Pure Chemical Corp.). We observed the formation of pollen

tubes on the stained pistils using a fluorescence microscope (Olympus IX73; Olympus) equipped with an objective lens with 10× magnification (UPLFLN10X; Olympus) at 25℃.

Measurement of pollen consumption. We estimated the pollen grain consumption in different pollination methods using the following procedure. Briefly, we used *L. clavatum* spores (Mitsuwa, Tokyo, Japan) for the tests. For traditional hand pollination, we dipped a spherical feather brush (Amazon, Seattle, DC, USA) directly into a 50 mL beaker with the powder of *L. clavatum* spores (5 g). We then took out the brush from the beaker and put it into a large sampling bag to keep it from coming into contact with the bag. Finally, the brush wrapped in the bag was gently hit onto a fake pear flower (TRIAL, Fukuoka, Japan) only once to simulate the pollination operation. We estimated the consumed pollen grains by measuring the weight of the spores that dropped from the brush in the bag during contact with the flower.

For machine pollination, we sprayed the spores into a sampling bag using the pollination tool (New Pollen Duster; Agri, Saga, Japan) once and measured the weight of the collected spores using a balance.

For solution pollination, we prepared the solution by mixing spores with a concentration of 4 mg mL^{-1} in Milli-Q water. The solution was spouted once into a sampling bag using a hand spray (Cainz Home). Subsequently, we measured the weight of the consumed spores using a balance after completely drying them overnight at room temperature.

For soap-bubble-mediated pollination, a 10 μL bubble solution containing 4 mg mL^{-1} spores and other optimised ingredients was dropped onto a glass slide with grid lines (AGC Techno Glass, Shizuoka, Japan). A cover glass (AGC Techno Glass) was then plated on the slide. Next, we counted the number of spores under a microscope and weighed 10 μL of pollination solution with or without spores using an analytical microbalance (UMT2; Mettler Toledo, Greifensee, Switzerland). The total weight of spores in a single soap bubble (W) was represented by the equality $W = (W_1 - W_2)/N_1 \times N_2$, where W_1, W_2, N_1 and N_2 are the weight of a 10 μL pollination solution with spores, the weight of a 10 μL pollination solution without spores, the number of spores on the slide and the number of spores in a single bubble, respectively.

Pollination at a pear orchard. We used the results of the laboratory pollination to conduct field work in a pear orchard in Japan. Purified pollen grains of *P. pyrifolia* var. *culta* were donated by the orchard's farmer. We randomly selected three trees with similar age, size and branch patterns for pollination in each orchard, with traditional hand pollination by a spherical feather brush (Amazon) as our control. We recorded the shape and size of the pear fruits continuously using a Vernier caliper two or three days a week for two months after pollination.

Mechanically stabilised soap-bubble-mediated pollination. We dissolved 1% A-20AB (Kao Chemicals) and 2 wt.% HPMC (Alfa Aesar) in water by vigorous stirring at room temperature for 5 h. After stirring, the solution was incubated in a vial overnight at room temperature to remove the bubbles.

We determined the kinematic viscosity and density of the obtained soap bubble solution using an automatic kinematic viscosity tester (VMC-352; Rigo, Saitama, Japan) at UBE Scientific Analysis Laboratory, Inc. (Tokyo, Japan).

L. japonicum and *C. persicifolia* flowers were purchased from Kasumi Co., Ltd. (Ibaraki, Japan), and *R. pulchrum* was collected from the Doho Park in Tsukuba, Japan. Using scissors and tweezers, we isolated the anthers from the flowers and then placed them in acetone (10 mL) (FUJIFILM Wako Pure Chemical Corp.). After vortexing for 5 min, excess anther husks were removed from the solution. Pollen grains were centrifuged and washed by fresh acetone three times. We filtered the pellet of pollen grains that we obtained and dried them in a fridge at 4℃ before use. Prepared natural pollen grains (50 mg) or a commercially available dry powder of *L. clavatum* spores (10–100 mg) (Mitsuwa) was added to water (1 mL) and then gently mixed into the soap bubble solution (4 mL) with a rotor for 1 h. For large-scale preparation, the volume ratio between the water containing pollen grains and the soap bubble solution was fixed at 1 : 4. Soap bubbles were generated from a bubble blower (Seria Co., Ltd., Gifu, Japan) or a bubble maker (Super Bubble Machine; Toys“R”Us, Wayne, NJ, USA).

We characterised the dispersion property of pollen grains in the soap bubble solution by observing the Tyndall phenomenon using 650 nm laser irradiation at 1 mW (power density: 50 μW mm^{-2}) (ELA-R40D; Kokuyo, Osaka, Japan).

Moreover, we determined the average number of pollens or spores on each soap bubble under an optical microscope after the bubble was smashed between two cover glasses (Matsunami Glass Ind., Ltd., Osaka, Japan).

The compressive strength of the soap bubbles (N = 5) was measured using a universal testing instrument (AG-100kNX; Shimadzu, Kyoto, Japan) at UBE Scientific Analysis Laboratory, Inc., with uniaxial compression tests performed at a rate of 5 mm min^{-1}.

A soap bubble containing natural pollen grains was made to hit the pistil of an *L. japonicum* flower using a bubble blower for pollination. After this single soap bubble attached to the pistil, the flowers were incubated overnight. We confirmed the formation of pollen tubes using a fluorescence microscope (Olympus IX73; Olympus) equipped with an objective lens with 60× magnification (UPLFLN60X, Olympus) at 25℃. Images were recorded using an EM-CCD camera system (DP80; Olympus). Prior to the observations, we heated the pistils in 1 N NaOH at 60℃ for 1 h and stained them with an aniline blue solution before they were sandwiched between two cover glasses (Iwaki, Tokyo, Japan) for observation.

Robotic pollination. A bubble maker (length: 150 mm, width: 150 mm, height: 200 mm; Toys“R”Us) with a soap bubble solution was attached to the bottom of a UAV (entire length: 1,120 mm, height: 360 mm, weight: 3.5 kg) that was equipped with two rechargeable Li-polymer batteries (22.2 V, 10,000 mAh × 2, weight: 2.6 kg) (ACSL-PF1; Autonomous Control Systems Laboratory, Ltd., Chiba, Japan). All robotic pollination procedures were performed by Autonomous Control Systems Laboratory, Ltd. The movement of the robotic pollinator was controlled using a fully automatic

operation system (ACSL AP2) with GNSS. We designed the soap bubbles in the robotic pollinator to hit replica lilies (TRIAL) or real *L. japonicum* flowers (Kasumi) by a programmed movement with varying speeds (2, 3.5, 5 and 10 m s^{-1}) at a fixed flight altitude (1 or 2 m) to ensure pollination. The robotic pollinator programmatically passes over each two or three bunch of flowers-attached container one time (Fig. S23C). We validated the fertility of the pollen grains as with the fluorescent observation of pollen tube growth as above. Moreover, all robotic pollination procedures were conducted with almost no wind or with no wind at all (0−1 m s^{-1}).

Accordingly, we attached an anemometer (GM816; Floureon, Shenzhen, China) under the UAV to measure the variation in wind speed. In particular, we recorded the wind speed for 30 s as the UAV hovered in the air at a fixed flight altitude of 2 m or as it flew a horizontal distance of 20 m with a speed of 2 or 4 m s^{-1} at a 2 m flight altitude. Note that the wind speed was measured in a calm state (0−1 m s^{-1}).

After the UAV-assisted pollination, we confirmed the fertility of the lily pollen grains in the same manner as in the above mechanically stabilised soap-bubble-mediated pollination.

Statistical analyses. All experiments were repeated at least three times for determination of pollen germination ratio (N = 100), measurement of pollen tube length (N = 100), calculation of average number of bubbles (N = 10), calculation of pollination success rate (N = 10), field works at the pear orchard (N = 50) and robotic pollination (N = 10), respectively. Data were expressed as the mean ± standard deviation of three independent experiments. Student's *t*-test and two-way analysis of variance were the main analysis methods employed for the experimental data. Here, we used *, ** and *** to indicate the significance of the result at $P < 0.05$, 0.01 and 0.001, respectively.

Supplementary Figures and Tables

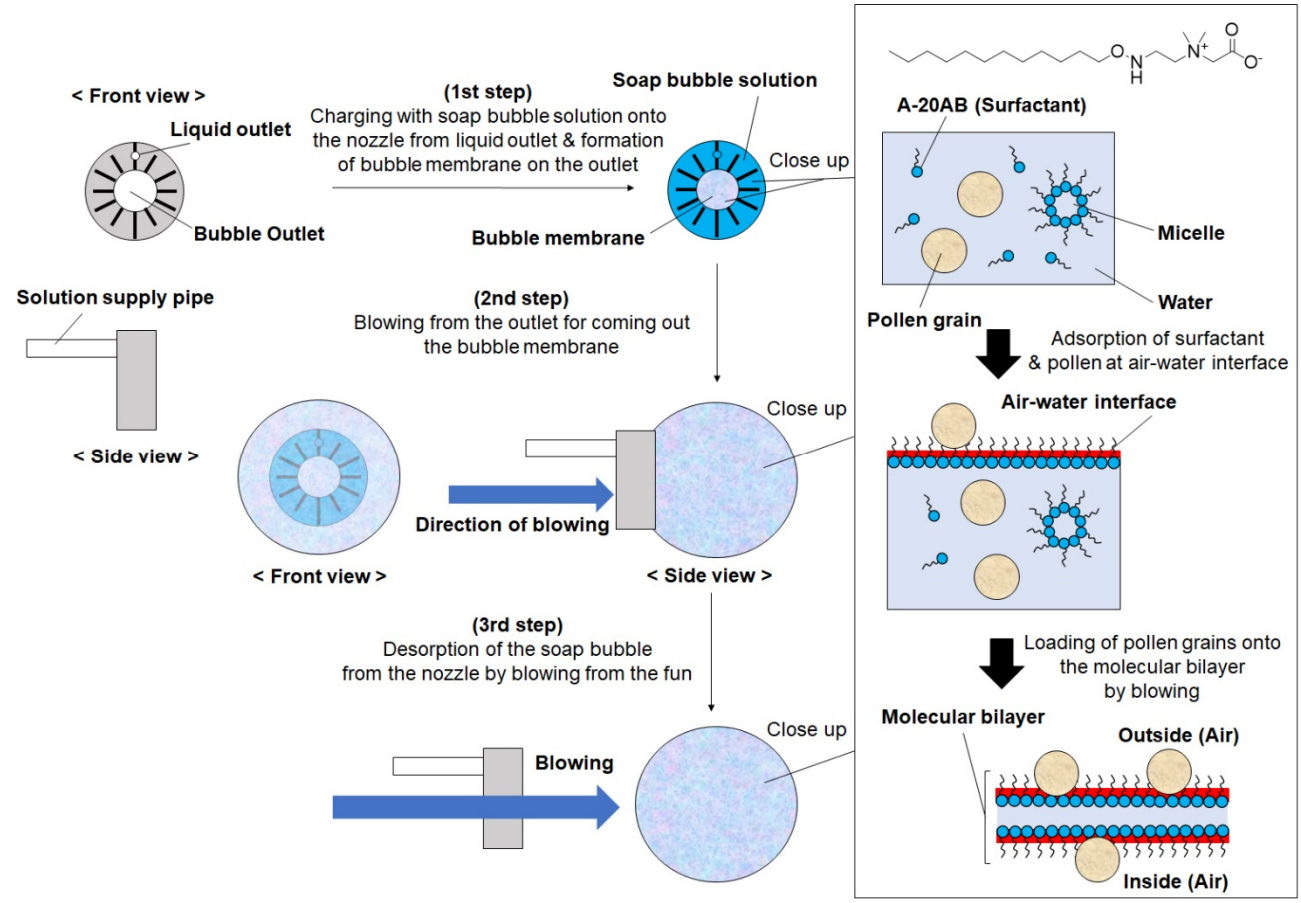

Figure S1. Related to Figure 1. A schematic illustration of loading of pollen grains onto a soap bubble membrane using a bubble gun.

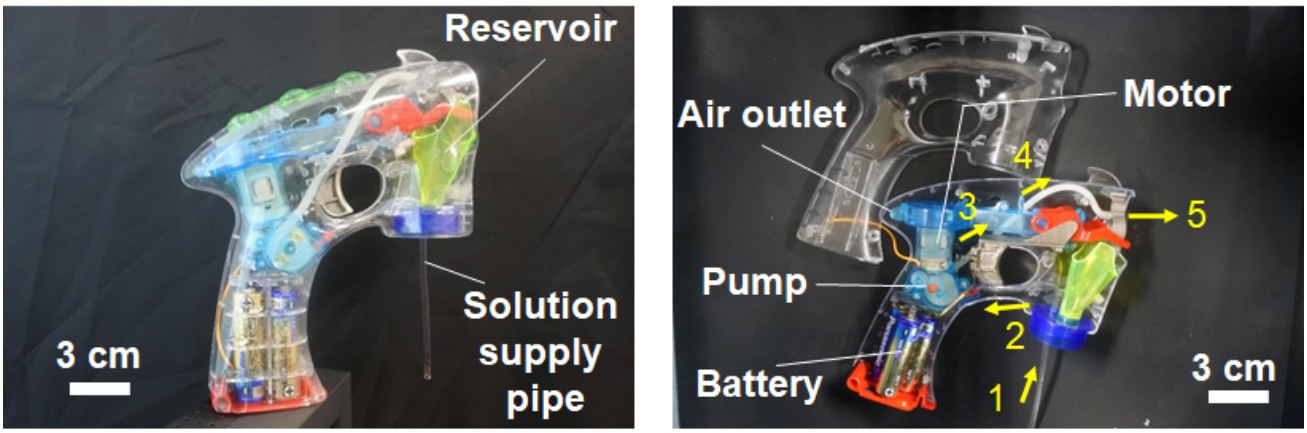

Figure S2. Related to Figure 1. Images of the bubble gun used in the present study. The yellow arrows and yellow numbers indicate the flow direction and transfer order of the soap bubble solution, respectively.

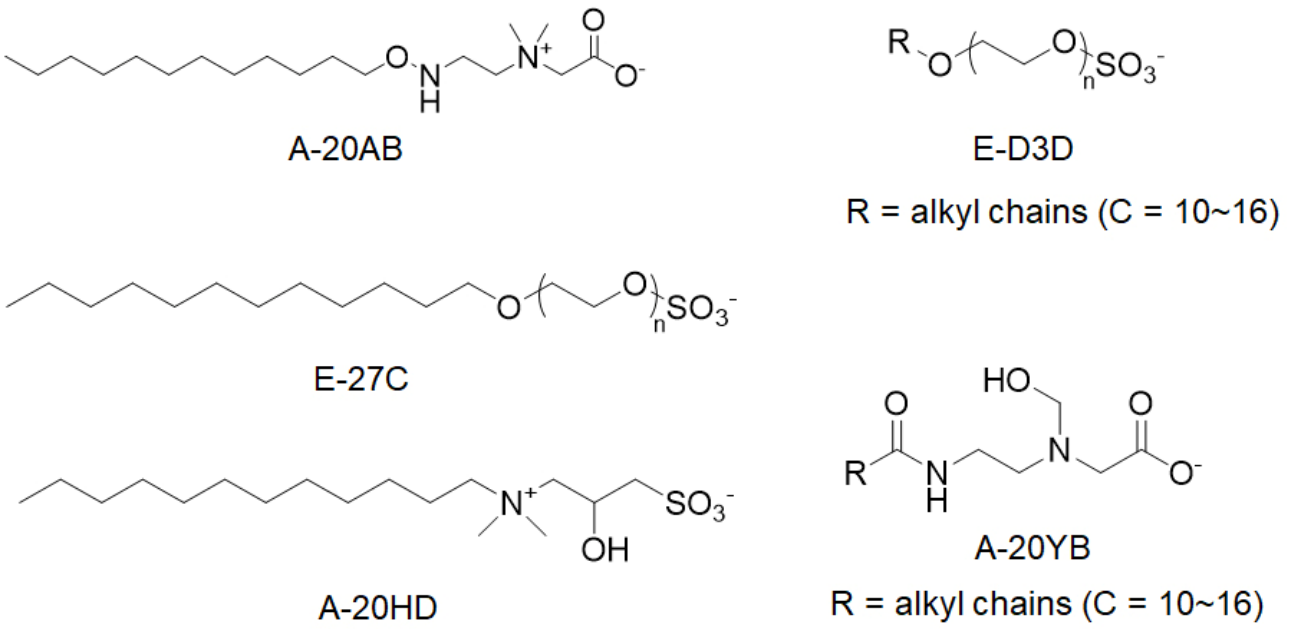

Figure S3. Related to Figure 1. Chemical structures of the surfactants used for soap-bubble-mediated pollination.

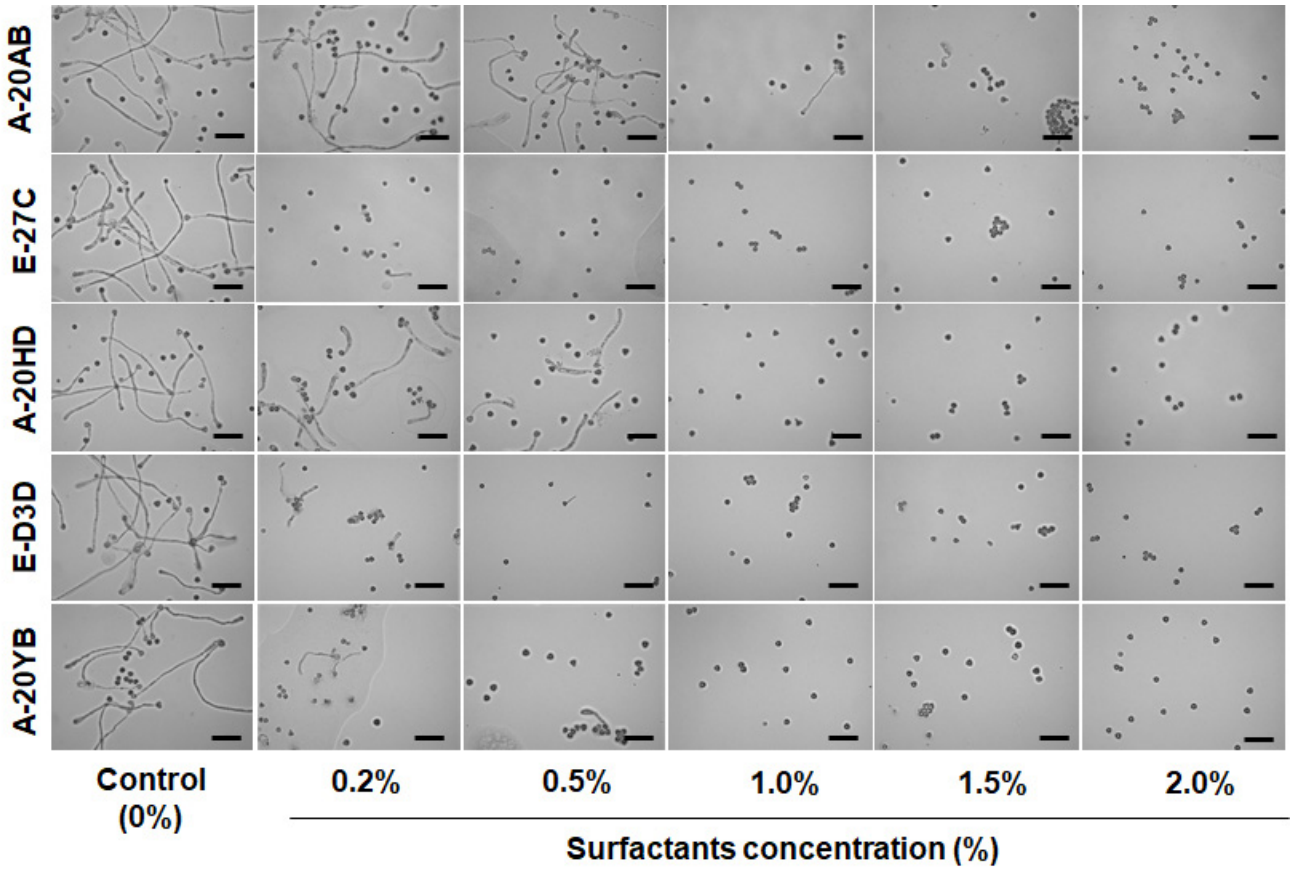

Figure S4. Related to Figure 1. Optical microscopy images of pollen grains in an agar dish after soap-bubble-mediated pollination by different surfactants with various concentrations. Scale bar: 200 μm.

Table S1. Related to Figure 1. Number of bubbles produced by a soap bubble solution containing different types of surfactants with various concentrations.

Concentration (%)	Type of surfactants				
	A-20AB	E-27C	A-20HD	E-D3D	A-20YB
0.2	0.67 ± 0.58	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.5	3.20 ± 1.69	0.56 ± 0.53	1.00 ± 0.95	0.00 ± 0.00	0.83 ± 0.58
1.0	>10	0.77 ± 0.73	3.40 ± 1.88	0.67 ± 0.50	2.88 ± 2.36
1.5	>10	3.69 ± 1.97	>10	2.07 ± 1.44	5.25 ± 2.66
2.0	>10	5.46 ± 2.82	>10	5.46 ± 2.07	>10

The concentration of pear pollen grains was 2 mg mL^{-1} for each soap bubble solution.

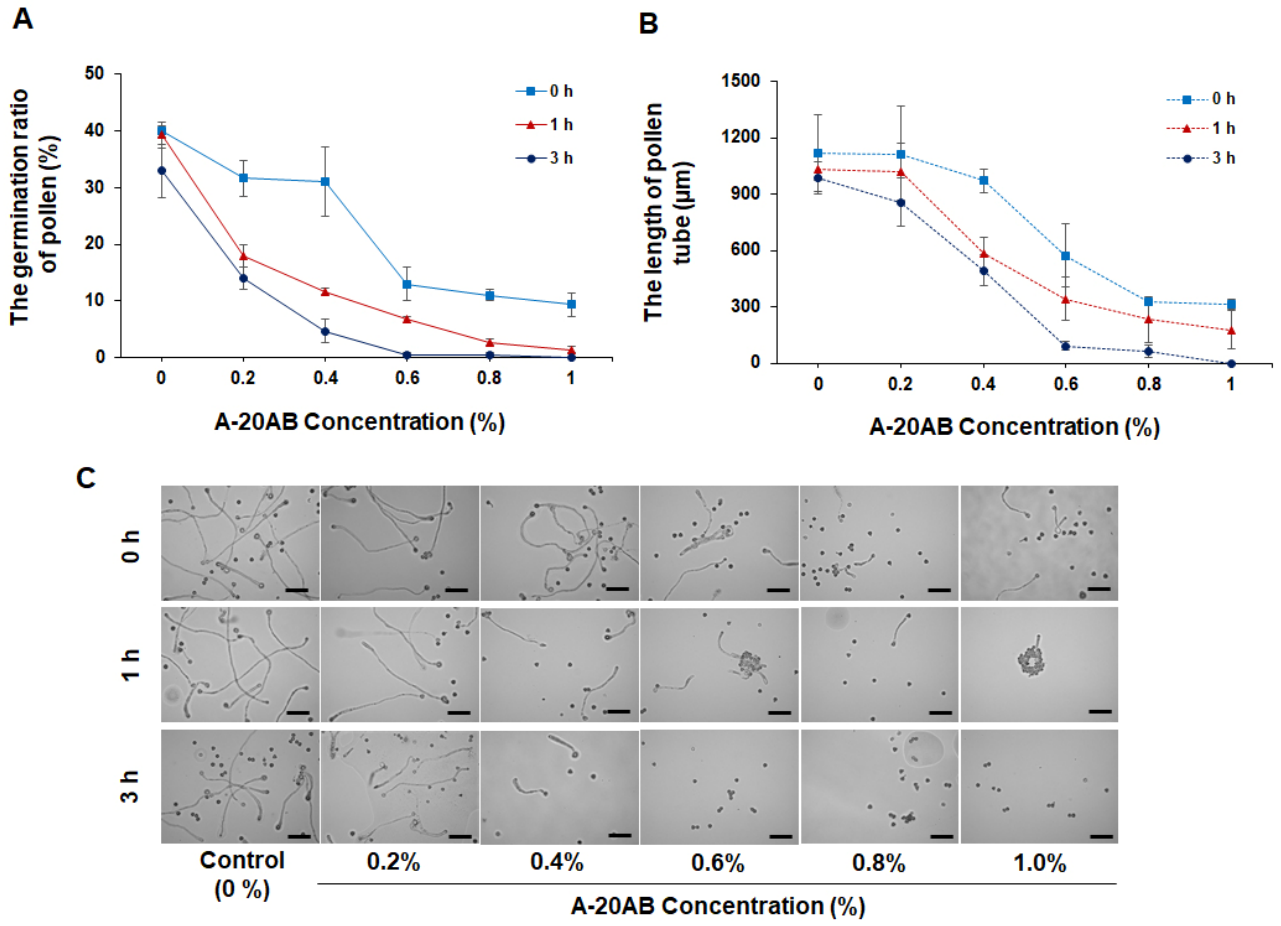

Figure S5. Related to Figure 1. Performance of the A-20AB surfactant in soap-bubble-mediated pollination. (A) Influence of A-20AB concentration (0.0–1.0%) on (A) pollen germination and (B) pollen tube growth after 0, 1 and 3 h of pollination. (C) Optical microscopy images of pollen grains after 0, 1 and 3 h of pollination. The concertation of pollen grains was 4 mg mL^{-1} in soap bubble solutions. Scale bar: 200 μm.

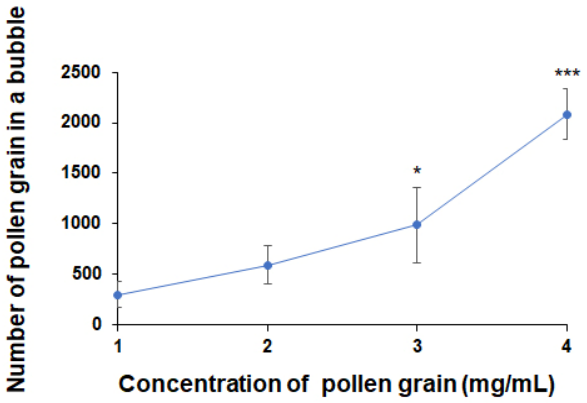

Figure S6. Related to Figure 1. Number of pollen grains loaded on a soap bubble at various concentrations (1–4 mg/mL). The symbols * and *** indicate a significant result at $P < 0.05$ and $P < 0.001$ against the value obtained at 1 mg mL^{-1} of pollen grains, respectively.

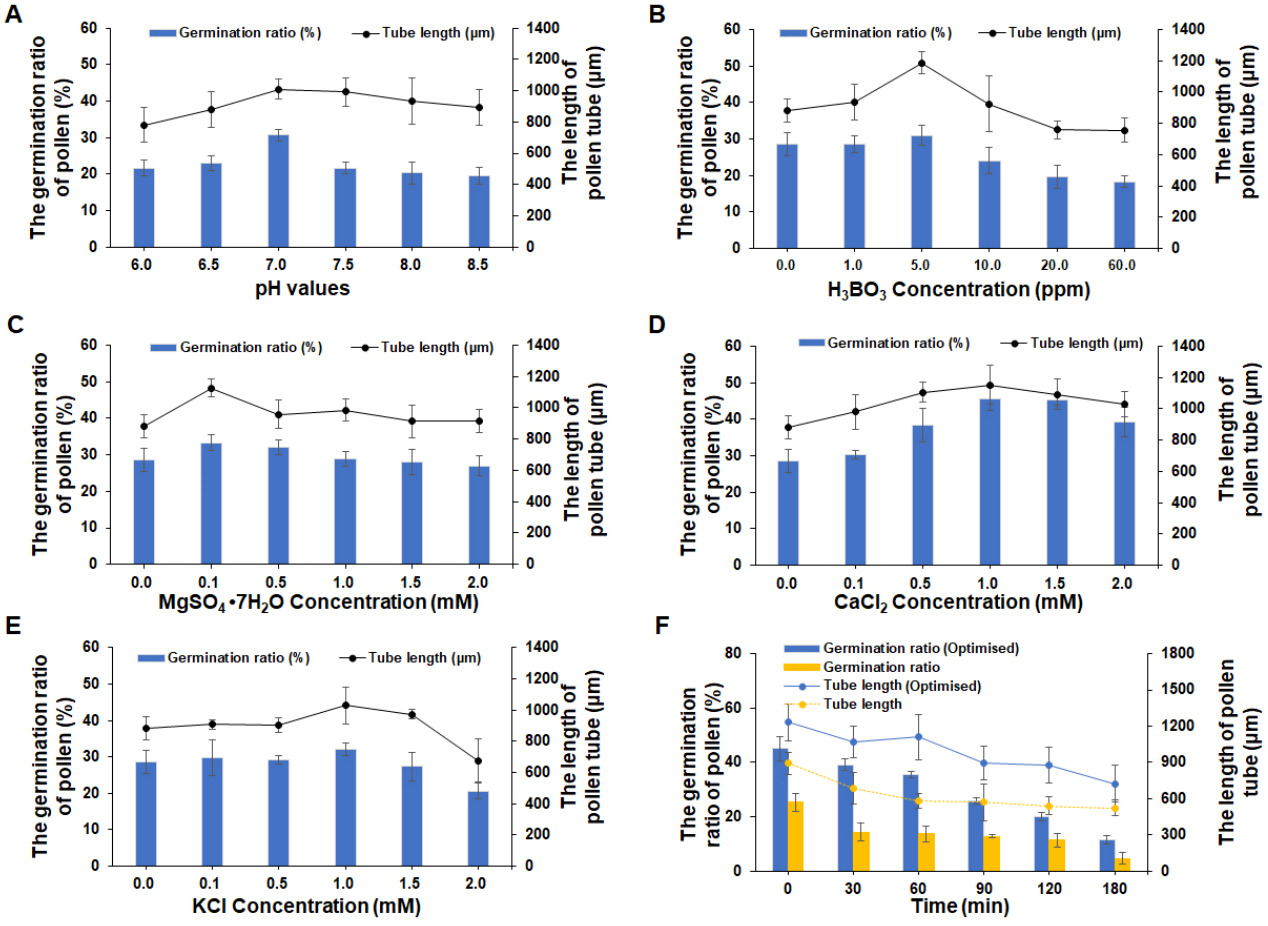

Figure S7. Related to Figure 1. Optimisation of soap bubble solution for the improvement of pollen activity. Effects of (A) the pH value and treatment with (B) H_3BO_3, (C) $MgSO_4 \cdot 7H_2O$, (D) $CaCl_2$ and (E) KCl on the germination ratio and tube length of pollen grains. (F) Pollen activity for 3 h of soap bubble pollination after optimisation of the chemical (H_3BO_3, $MgSO_4 \cdot 7H_2O$, $CaCl_2$ and KCl) treatment in comparison with non-optimised group.

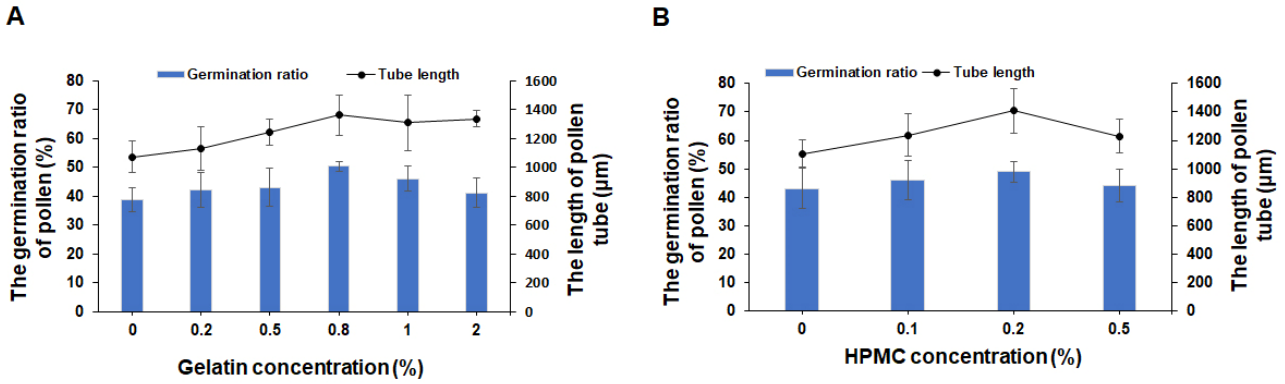

Figure S8. Related to Figure 1. Effects of different concentrations of (A) gelatine (0.0–2.0%) and (B) hydroxypropyl methylcellulose (HPMC) on promoting pollen activity.

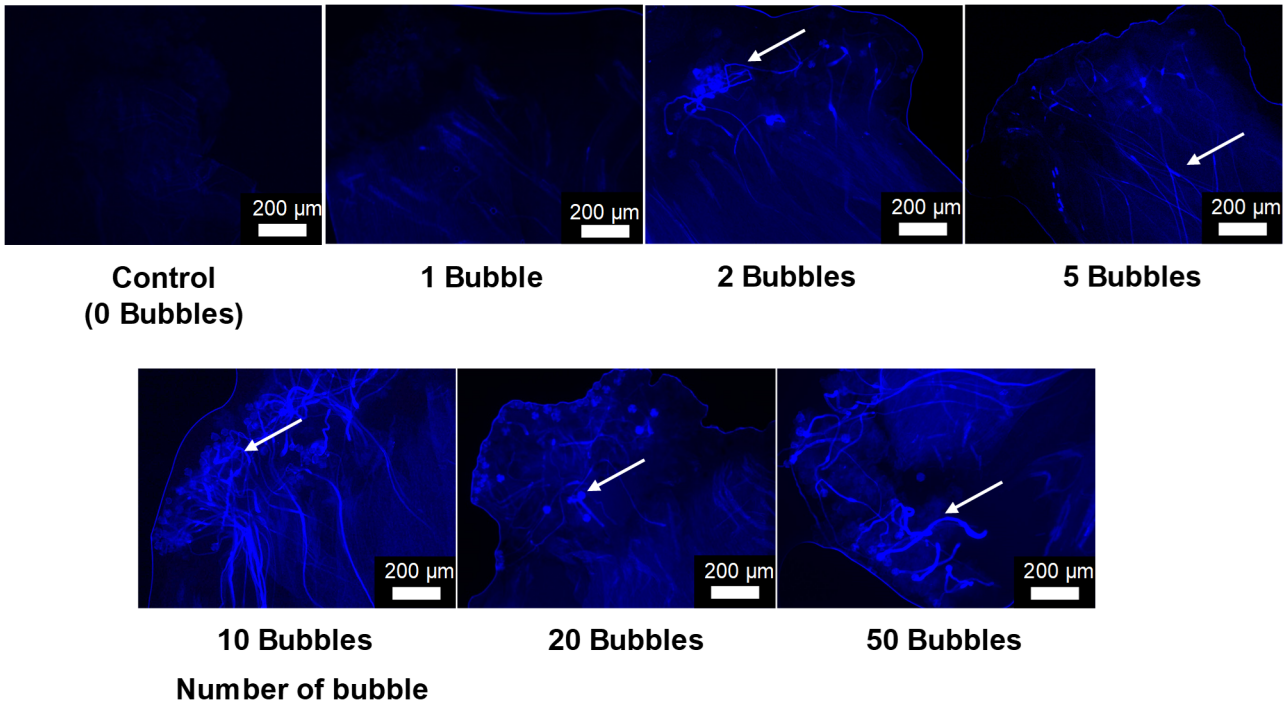

Figure S9. Related to Figure 2. Fluorescence microscopy images of the pistils of *Pyrus pyrifolia* var. *culta* flowers after soap-bubble-mediated pollination using different numbers of soap bubbles containing pollen grains.

Figure S10. Related to Figure 2. Photographs of soap-bubble-mediated pollination on a *P. pyrifolia* var. *culta* flower at a pear orchard.

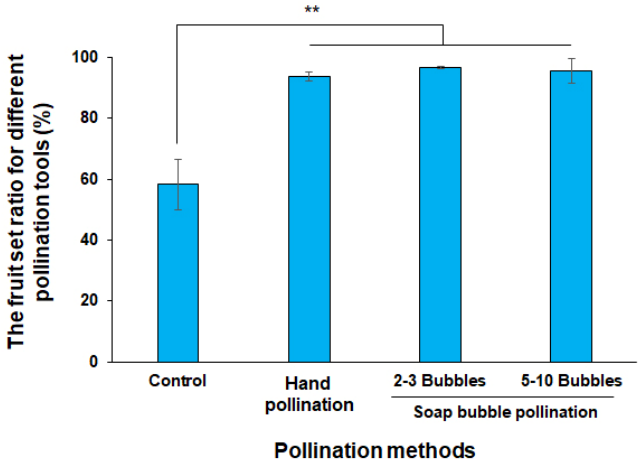

Figure S11. Related to Figure 2. Fruit-bearing rates of various pollination methods. The symbol ** indicates a significant result at $P < 0.01$.

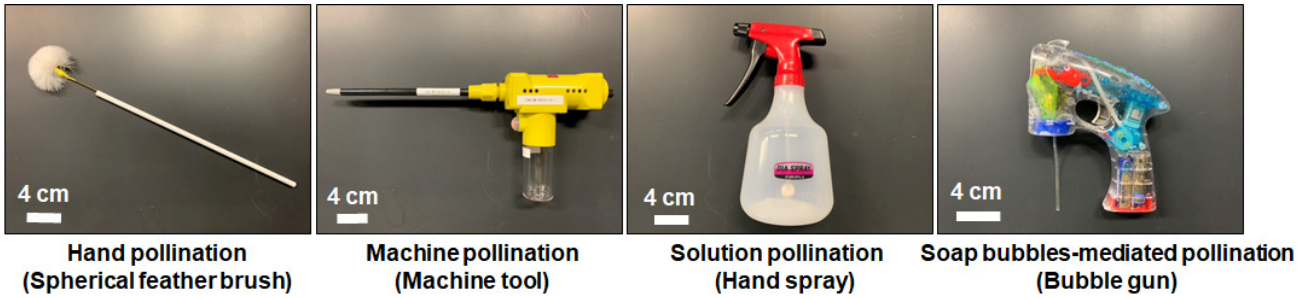

Figure S12. Related to Figure 2. Photographs of various pollination tools.

Table S2. Related to Figure 2. Consumption of pollen grains used in different types of pollination methods with a single trigger.

Methods	Weight of pollen grains (mg)
Soap-bubble-mediated pollination (bubble gun)	0.06 ± 0.00 – 0.08 ± 0.01 (2-3 bubbles) 0.14 ± 0.01 – 0.28 ± 0.02 (5–10 bubbles)
Hand pollination (spherical feather brush)	1,747.29 ± 436.61
Machine pollination (machine tool)	165.31 ± 36.30
Solution pollination (hand spray)	3.24 ± 0.43

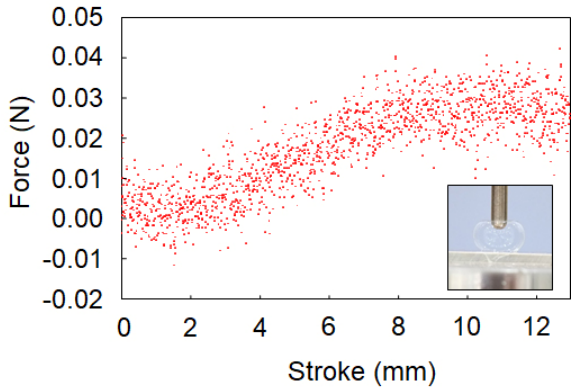

Figure S13. Related to Figure 3. Compression test of a mechanically stabilised soap bubble containing 2% HPMC and 1% A-20AB.

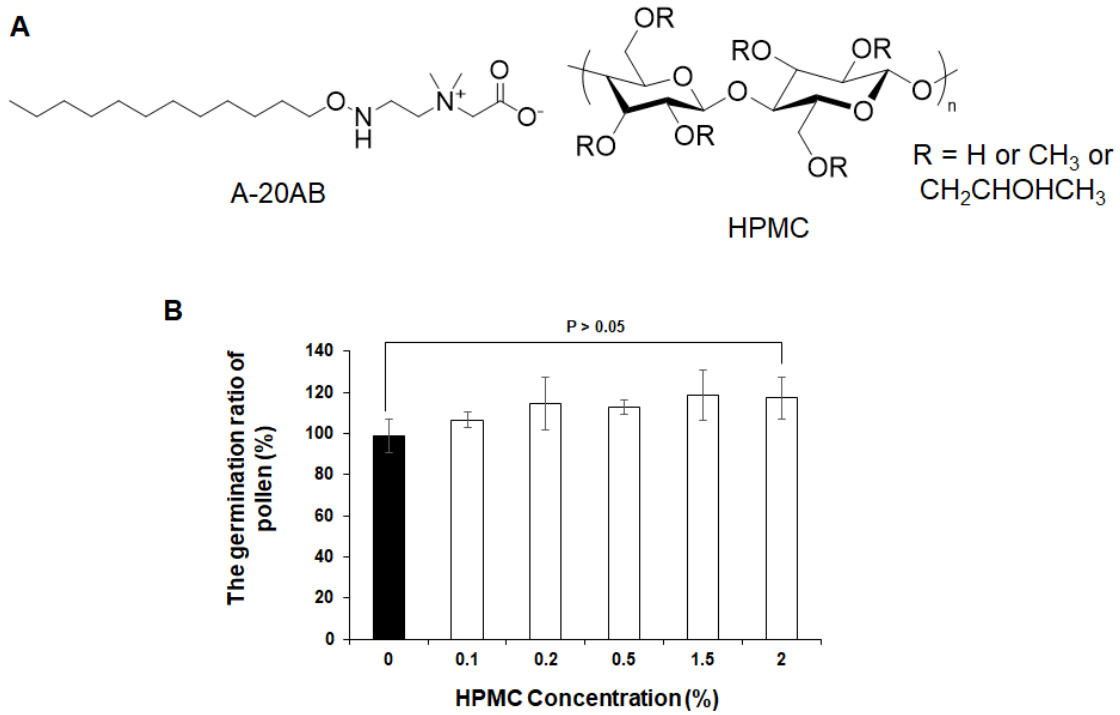

Figure S14. Related to Figure 3. (A) Chemical structures of the ingredients of a mechanically stabilised soap bubble. (B) Effect of HPMC concentration (0–2%) on pollen germination after *in vitro* soap-bubbled-mediated pollination.

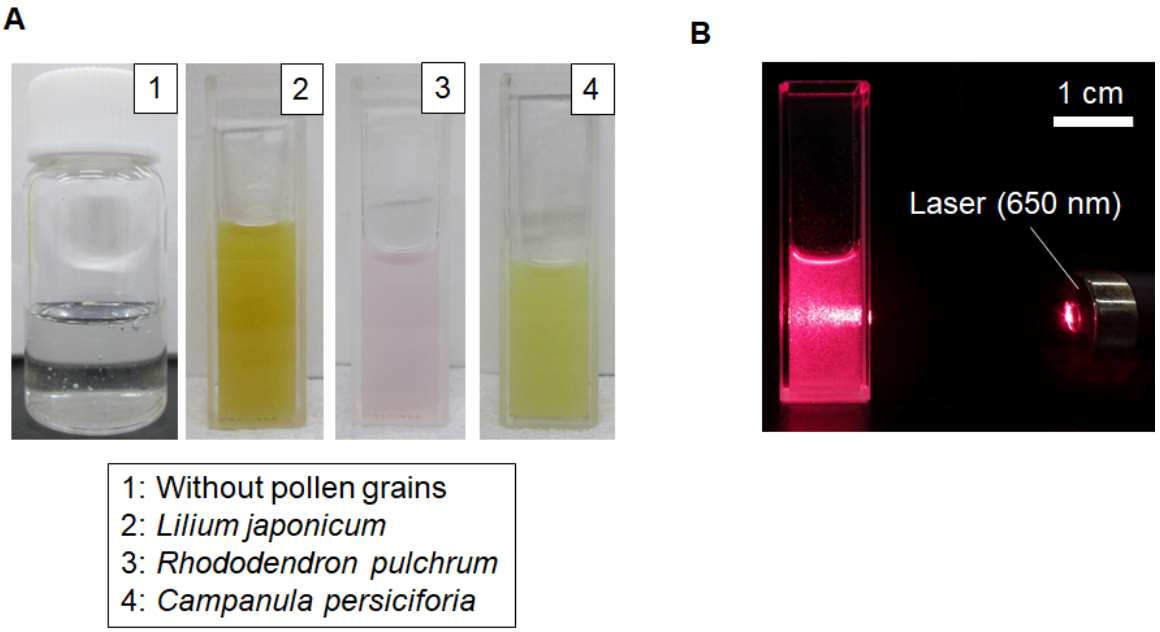

Figure S15. Related to Figure 3. (A) Photographs of a mechanically stabilised soap bubble solution containing the pollen grains of various plants. (B) Tyndall phenomenon of *L. japonicum* pollen grain colloidal particles in a soap bubble solution.

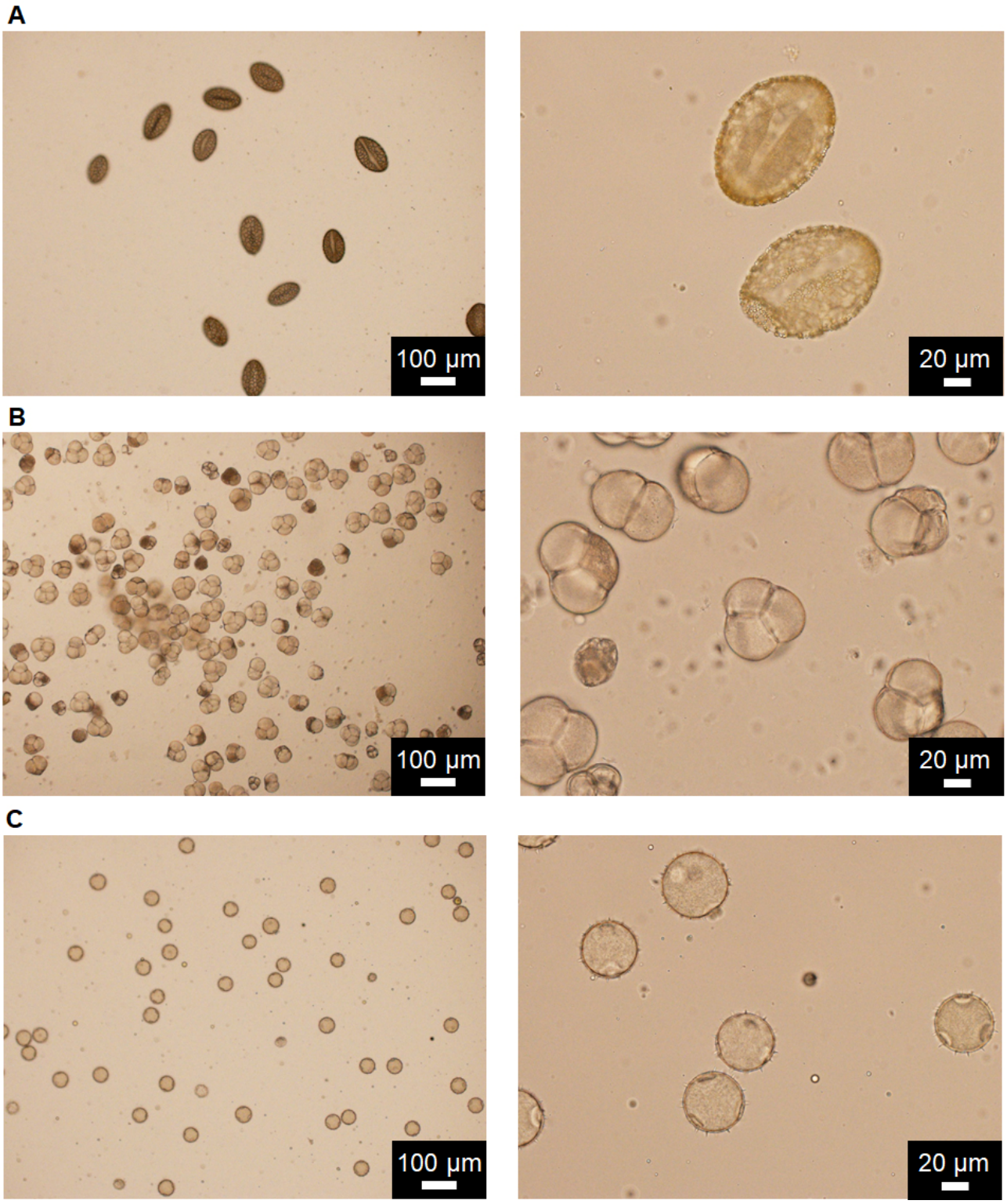

Figure S16. Related to Figure 3. Optical microscopy images of soap bubble dispersions containing (A) *L. japonicum*, (B) *R. pulchrum* and (C) *C. persicifolia* pollen grains.

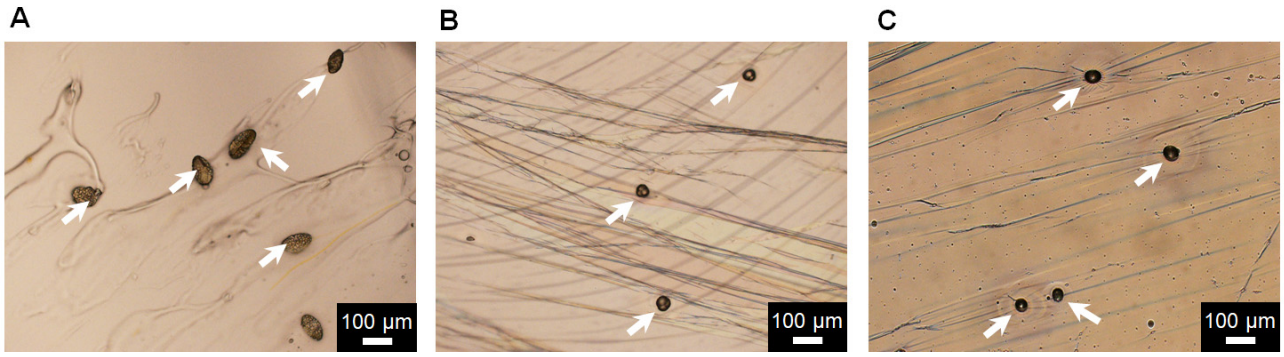

Figure S17. Related to Figure 3. Optical microscopy images of (A) *L. japonicum*, (B) *R. pulchrum* and (C) *C. persicifolia* pollen grains on mechanically stabilised soap bubble films.

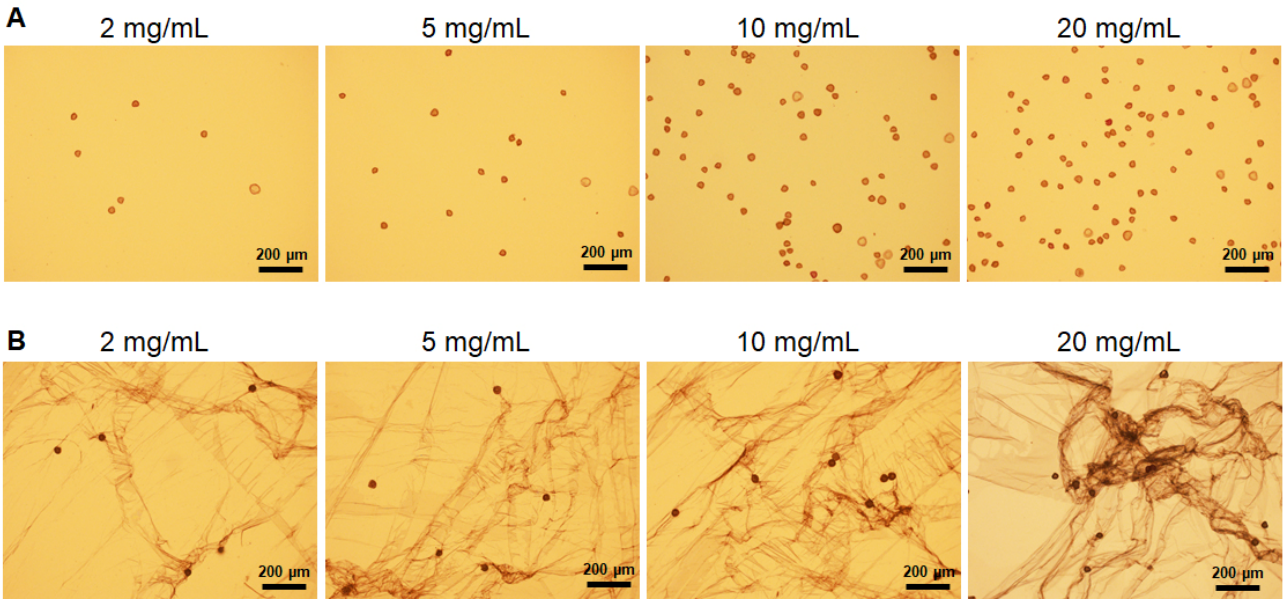

Figure S18. Related to Figure 3. Optical microscopy images of (A) soap bubble dispersions containing spores and (B) spores on mechanically stabilised soap bubble films at different concentrations.

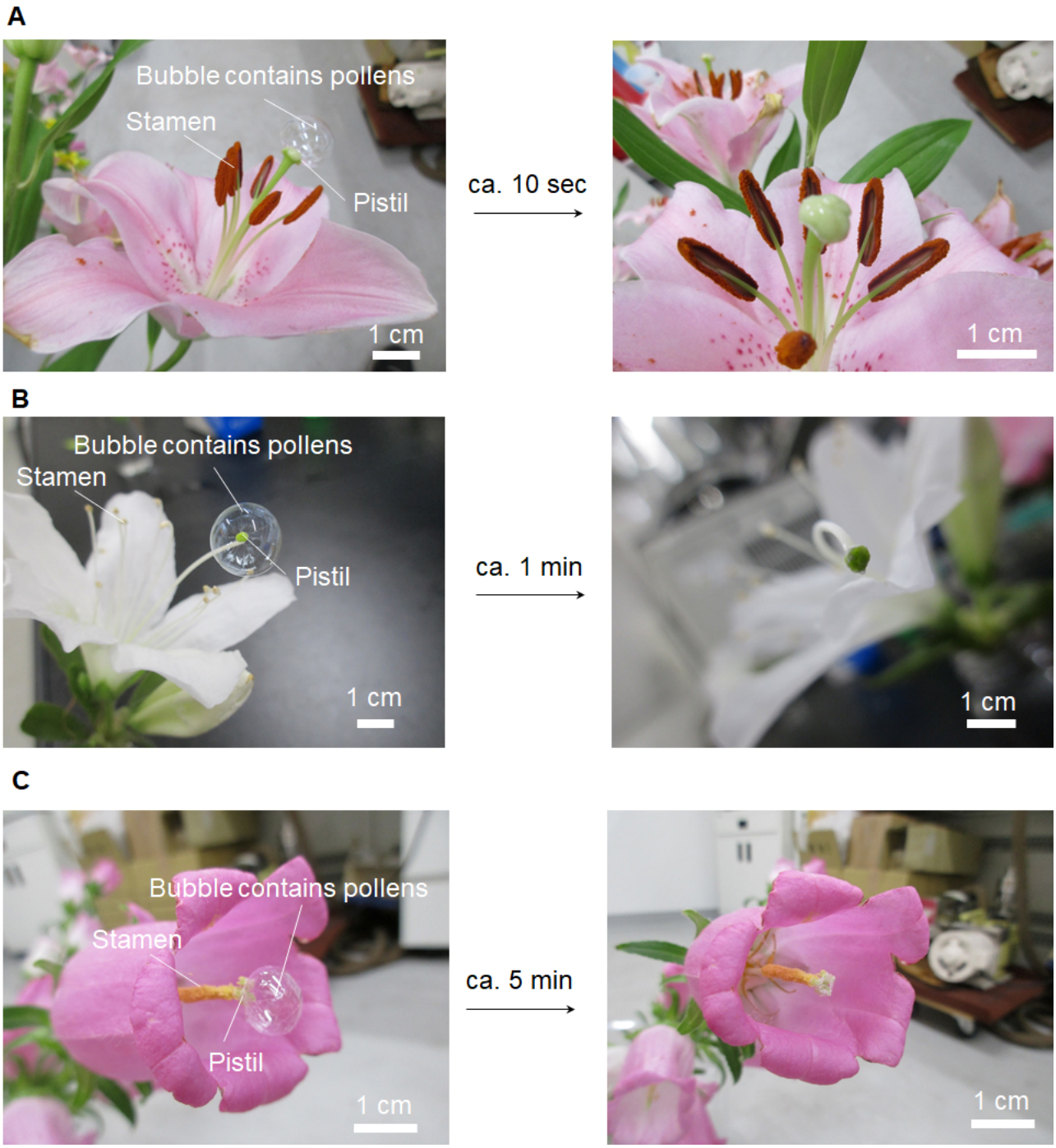

Figure S19. Related to Figure 3. Photographs of (A) *L. japonicum*, (B) *R. pulchrum* and (C) *C. persicifolia* flowers after a single soap bubble attached onto their pistils.

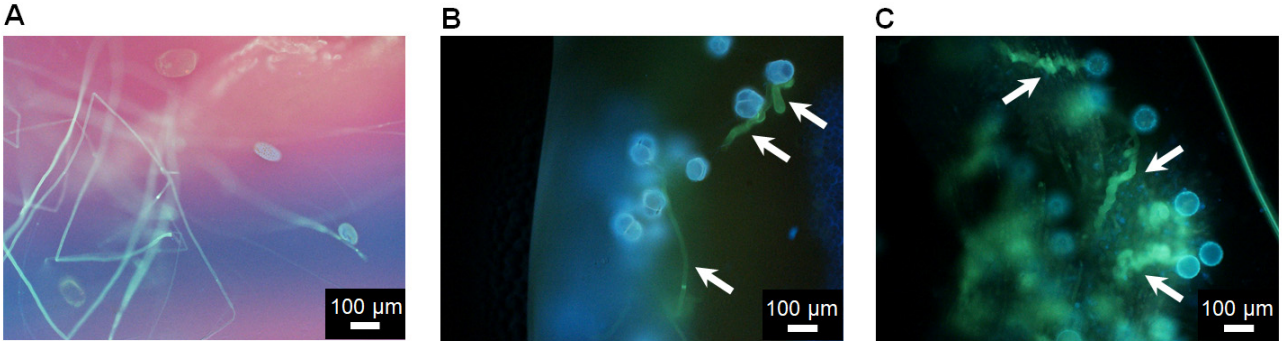

Figure S20. Related to Figure 3. Fluorescence microscopy images of pistils of (A) *L. japonicum*, (B) *R. pulchrum* and (C) *C. persicifolia* flowers after single soap-bubble-mediated pollination.

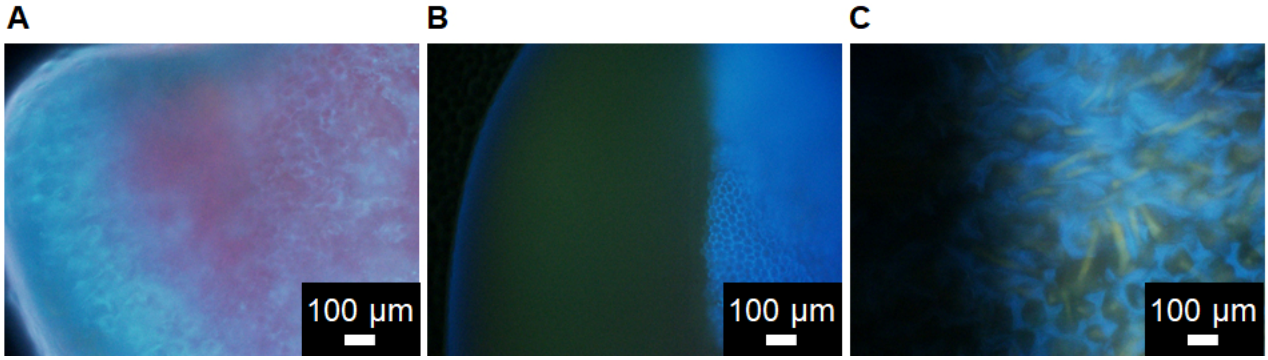

Figure S21. Related to Figure 3. Fluorescence microscopy images of the control samples of (A) *L. japonicum*, (B) *R. pulchrum* and (C) *C. persicifolia* flowers without soap-bubble-mediated pollination.

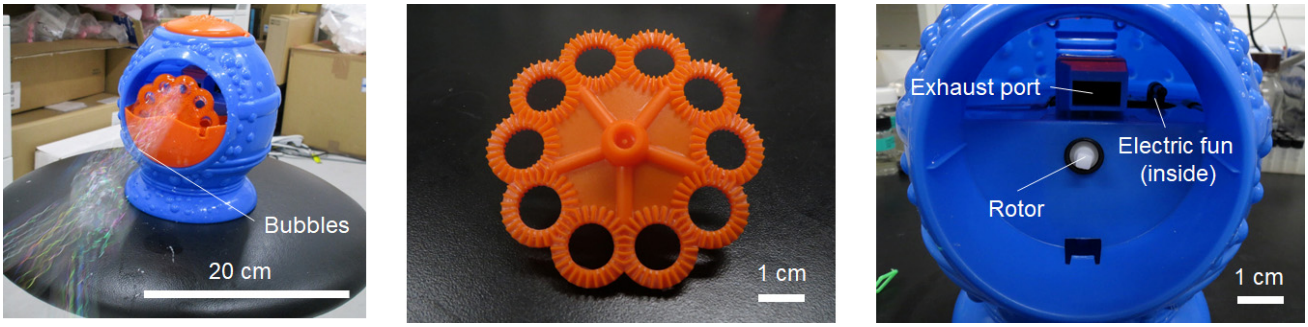

Figure S22. Related to Figure 3. Photographs of the bubble maker for mechanically stabilised soap-bubble-mediated pollination.

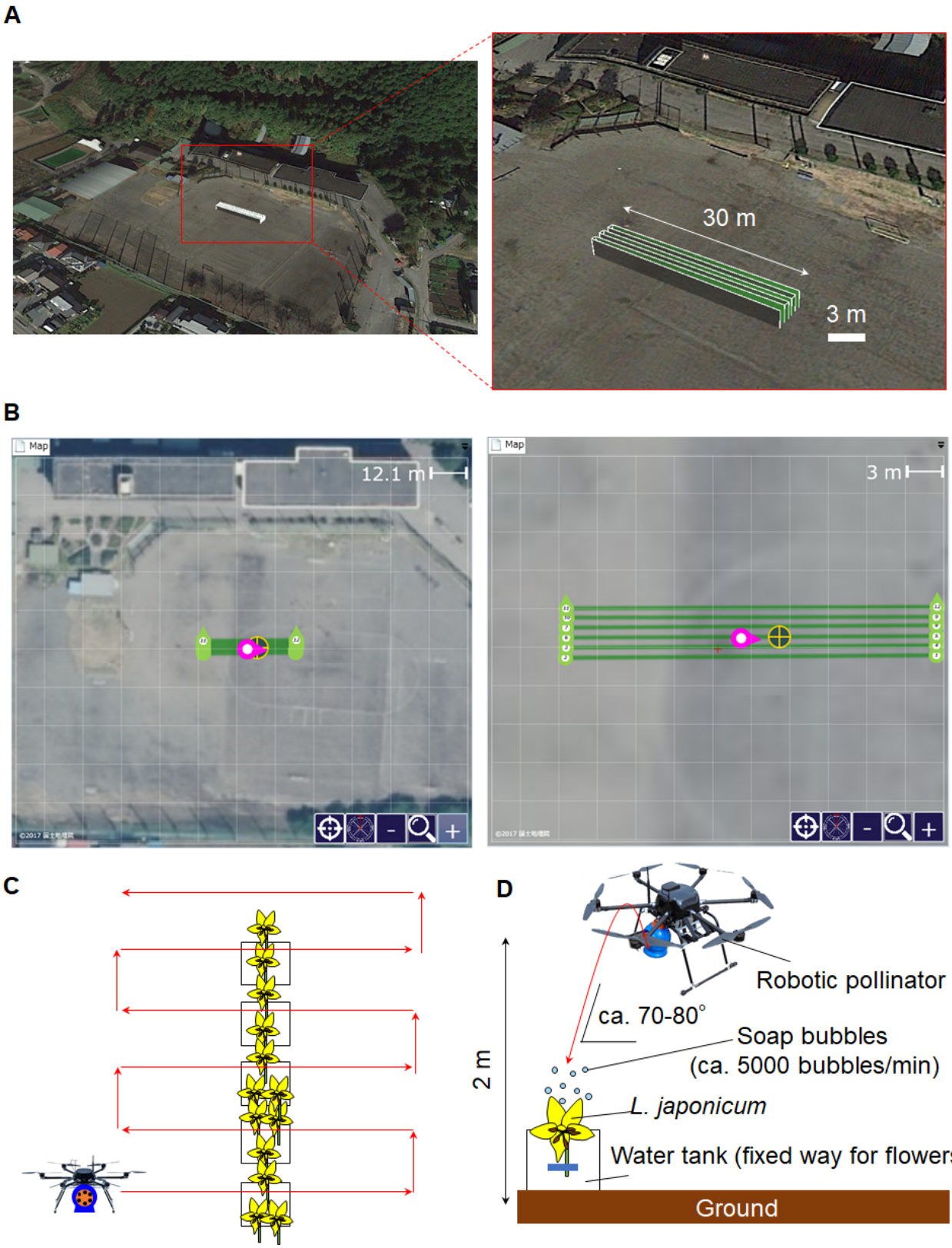

Figure S23. Related to Figure 3. (A, B and C) Route and map of the programmed movements of the robotic pollinator using a fully automatic operation system with a global positioning system and global navigation satellite system. (D) Location and height of an autonomous robotic pollinator for soap-bubble-mediated flower pollination.

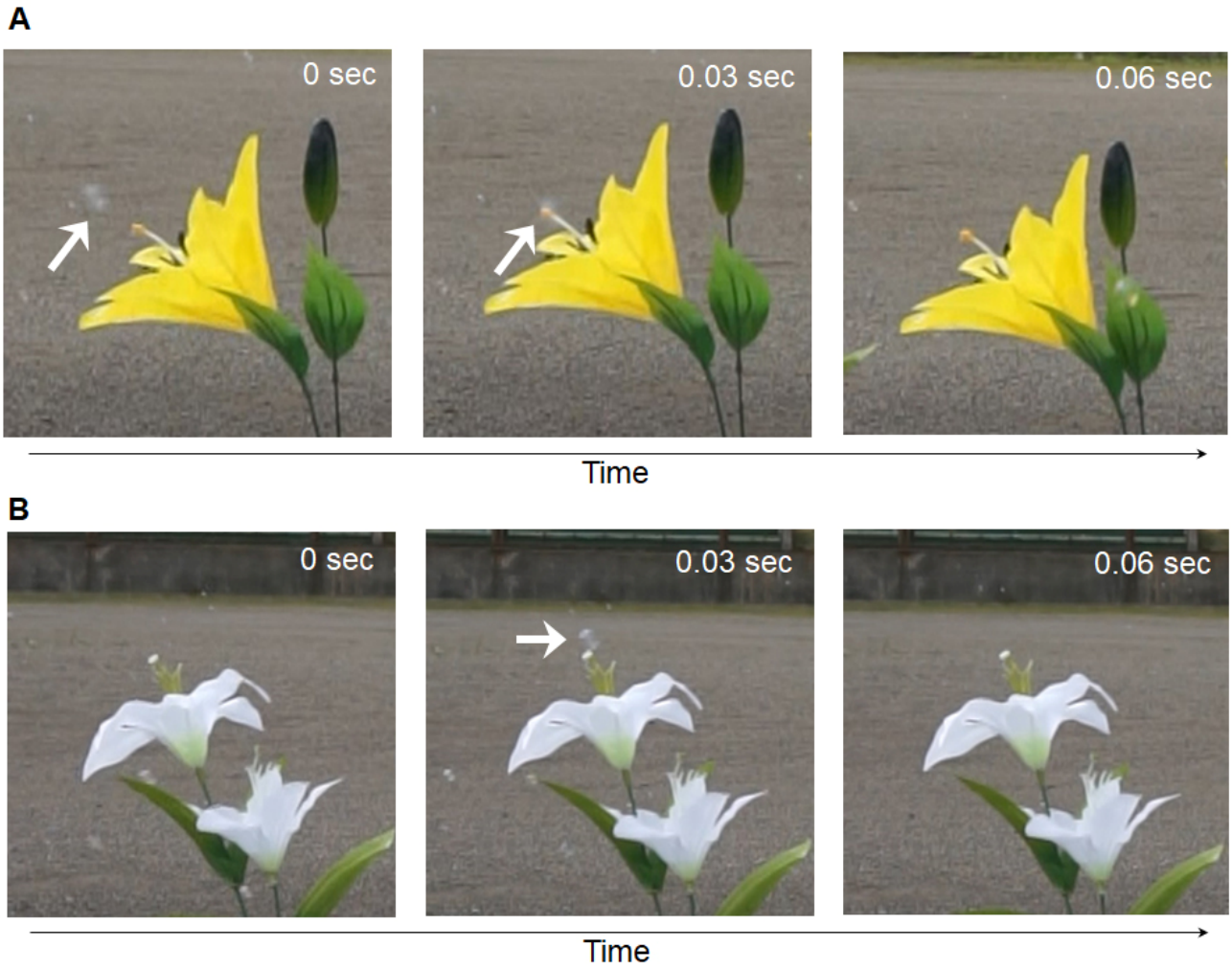

Figure S24. Related to Figure 3. (A and B) Defining moments of shooting soap bubbles onto an *L. japonicum* pistil by soap-bubble-mediated robotic pollination at a speed of 2 m s^{-1}. The white arrows indicate the location of the soap bubble.

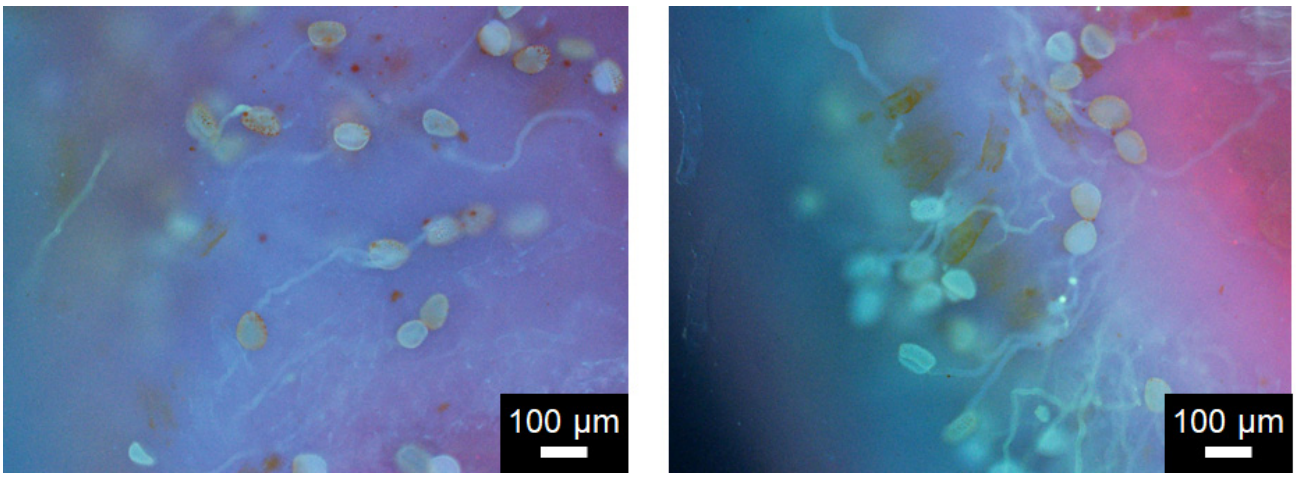

Figure S25. Related to Figure 3. Fluorescence microscopy images of *L. japonicum* pistils after soap-bubble-mediated robotic pollination.

Supplementary References

- Fan, L.-M., Wang, Y.-F., Wang, H., and Wu, W.-H. (2001). *In vitro Arabidopsis* pollen germination and characterization of the inward potassium currents in *Arabidopsis* pollen grain protoplasts. J. Exp. Bot. *52*, 1603–1614.
- Hopping, M. E. and Simpson, L. M. (1982). Supplementary pollination of tree fruit. 3. Suspension media for kiwifruit pollen. N. Z. J. Agri. Res. *25*, 245–250.
- Rodriguez-Enriquez, M.J., Mehdi, S., Dickinson, H. G., and Grant-Downton, R. T. (2013). A novel method for efficient *in vitro* germination and tube growth of *Arabidopsis thaliana* pollen. New Phytol. *197*, 668–679.
- Yano, T., Miyata, N. & Matsumoto, H. (2007). The use of liquid pollen extender thickened with polysaccharides for artificial pollination of kiwifruit. Acta Hortic. *753*, 415–424.