Two-Phase Emulgels for Direct Ink Writing of Skin-Bearing Architectures

Siqi Huan, Bruno D. Mattos, Rubina Ajdary, Wenchao Xiang, Long Bai,* and Orlando J. Rojas*

Direct ink writing (DIW) provides programmable and customizable platforms to engineer hierarchically organized constructs. However, one-step, facile synthesis of such architectures via DIW has been challenging. This study introduces inks based on two-phase emulgels for direct printing and in situ formation of protecting layers enveloping multicomponent cores, mimicking skin-bearing biological systems. The emulgel consists of a Pickering emulsion with an organic, internal phase containing poly(lactic acid) stabilized by chitin/cellulose nanofibers and a continuous, cross-linkable hydrogel containing cellulose nanofibers and any of the given solid particles. The shear during ink extrusion through nozzles of low surface energy facilitates the generation of the enveloped structures via fast and spontaneous phase separation of the emulgel. The skin-bearing architectures enable control of mass transport as a novel configuration for cargo release. As a demonstration, a hydrophilic molecule is loaded in the hydrogel, which is released through the core and skin, enabling regulation of diffusion and permeation phenomena. This 3D-printed functional material allows independent control of strength owing to the hierarchical construction. The new method of fabrication is proposed as a simple way to achieve protection, regulation, and sensation, taking the example of the functions of skins and cuticles, which are ubiquitous in nature.

1. Introduction

The skin in mammals, as one of the most important organs, and the cuticle in plants, designed by nature for protection, are critical for survival, adaptation, and function. They also regulate the interactions with the surrounding environment, e.g., by channeling connections with the inner microstructure. Such spatial confinement preserves morphology and structural organizations. This usually implies multiple stage-wise processes, since single-step, simultaneous fabrication of outer/inner structures, which are relevant to a wide range of man-made constructs used in biomedicine, separation, and others. Materials that exhibit hierarchically layered structures have been shown to surpass their counterparts, for instance, in self-adaptation and property adjustment. Bioinspired layered stiff plate/soft matrix composite materials have been reported, but more universal, complex systems that use layered structures have been studied very scarcely, mainly owing to the challenges posed by their synthesis.

3D printing allows a high level of topological complexity while ensuring robust custom-shaped structures. Among various 3D printing techniques, extrusion-based direct ink writing (DIW) enables programmable, precise layer-by-layer assemblies in 3D. The inks suitable for DIW must exhibit shear-thinning to enable efficient flow through the nozzles but demand fast recovery into objects with a sufficiently high yield stress and storage modulus, to ensure shape retention and distortion-free geometries. Unlike lithography or laser-based polymerization, DIW materializes sophisticated microfabrication for a wide range of materials, such as hydrogel, emulsions, and foams. To this end, DIW can be used to integrate the functions of materials across multiple length-scales, a prerequisite for the construction of hierarchical organizations. This usually implies multiple stage-wise processes, since single-step, simultaneous fabrication of outer/inner construct via DIW remains intractable, given the requirement for the inks to self-separate upon printing. Moreover, the formation of spatially resolved and gradient structures often requires multiple inks that are printed either simultaneously or sequentially, namely, multimaterial printing. The latter process usually alters the fidelity and increases the operational complexity. The development of all-in-one ink is therefore paramount to facilitate the deployment of hierarchically layered constructs.

To tackle the current challenges, we propose multicomponent materials consisting of immiscible but metastable phases given the possibility of spontaneous phase-separation. For
example, we have recently reported on multiphase emulsions to generate complex structures via 3D printing using DIW.\textsuperscript{[17]} While our initial aim was to gain control on the properties of the emulsion ink by changing the formulation (e.g., emulsion morphology), the results hinted to the possibility of achieving hierarchical architectures.\textsuperscript{[18]} However, this depends on the ability to engineer phase-separation to achieve high fidelity without compromising the required balance between ink composition and printability.\textsuperscript{[19]} In line with this effort, we noted that the absence of a solid support in the aqueous phase of the emulsion prevented phase separation;\textsuperscript{[20]} otherwise, such phenomenon could be enhanced by the incorporation of a hydrophilic component that is miscible with the aqueous phase to solidify the emulsions prior to printing.\textsuperscript{[21]}

Hence, here we propose a two-phase emulgel ink comprising an oil-in-water Pickering emulsion. More specifically, the dispersed phase contains poly(lactic acid) (PLA), while the continuous phase is a physical cross-linkable hydrogel loaded with silica particles. An alkaline solution (ammonia) is titrated to solidify the hydrogel phase and generate the printable emulgels (Figure 1a). Such emulgels are easily transformed into layered materials via spontaneous phase-separation upon deposition.
during DIW printing, representing a one-step process to create struts or filaments with a core covered by a coating or skin layer.

2. Results and Discussion

2.1. Structure and Rheological Properties of Two-Phase Emulgels

The effect of the emulgel composition was studied by varying the emulsion-to-hydrogel mass ratio, more precisely, the emulsion-to-silica particle ratio (hereafter referred to as ESR#, where # is a number between 0 and 4, as shown in Table 1). The choice of Pickering emulsion stems from the fact that colloids can adsorb irreversibly at the oil/water interface, generating an interfacial barrier that limits droplet coalescence and endows emulsions with superior stability compared to those produced with surfactants.[22] We have previously shown that a mixture of anionic cellulose nanofibrils (CNF) and cationic chitin nanofibers (ChNF) is highly efficient in stabilizing oil-in-water emulsions (e.g., PLA/CHCl₃-in-water Pickering emulsions).[17,23] In such systems, cooperative adsorption and network formation at the oil/water interfaces are the main reasons for achieving high stability. Here, PLA/CHCl₃ droplets with sizes of ≈8 μm were well dispersed in the aqueous phase, which contained CNF and ChNF at low concentration (0.5 wt%), ensuring a homogeneous emulsion for emulgel preparation (Figure 1b; Figure S2, Supporting Information). The high stability of PLA droplets results from their high surface charge (=+60 mV, Figure S1c, Supporting Information), as well as the strong steric hindrance originated by the fibrillar network formed around the droplets (Figure S2, Supporting Information). No free CNF or ChNF are present in the continuous, aqueous phase, as confirmed by fluorescence imaging (staining CNF/ChNF by Calcofluor white, Figure S2, middle image, Supporting Information). Moreover, the calculated surface coverage of the stabilizer on PLA droplets is much less than that corresponding to full coverage (see discussion in the Supporting Information). Thus, the aqueous phase of the emulsion took no part in the cross-linking that was used to obtain post-cross-linked emulgels.

The adoption of silica-loaded hydrogel was inspired from our previous report wherein mechanically robust materials were produced after drying suspensions containing silica microparticles and CNF.[24] Herein, the hydrogel was formed by the addition of poly(acrylic acid) (PAA) that is cross-linked in the presence of ammonia solution.[23] Subsequently, Pickering emulsions and hydrogels were mixed to generate emulgel precursors, which could be solidified upon PAA cross-linking (an illustration of the system is included in Figure 1a). Before titrating ammonia solution, PLA droplets in the emulsion were homogeneously mixed with silica particles within the hydrogel (Figure 1c, top; Figure S3a, Supporting information). After cross-linking, however, silica particles strongly interacted with CNF via PAA-induced interparticle hydrogen bonding, making PLA droplets to diffuse out and to envelope the solid particles (Figure 1c, bottom; Figure S3b, Supporting Information), as also illustrated in Figure 1a. The weak interaction between PLA droplets and silica particles arises from the fact that CNF is not available on the surface of the droplets, which otherwise would produce CNF–silica–PAA networks. In fact, most of CNF on the droplets interacts electrostatically with ChNF to enable higher stabilization ability. Such segregated structure within the emulgel, which is formed upon cross-linking, facilitates separation of the ink into two phases during printing; however, macroscopically, phase separation is not apparent in the cross-linked emulgel (Figure 1e, inset).

The printability of the emulgels is determined by their rheological properties: see Figure 1d and Figure S4 (Supporting Information) for the rheological behavior of the emulgels at different emulsion-to-silica particle ratios. All the emulgels underwent pronounced shear thinning and displayed similar flow profiles; the apparent viscosity decreased, by several orders of magnitude by increasing the shear rate from 10¹² to 10⁵ s⁻¹ (values typically applied in DIW to ensure flow through the deposition nozzle) (Figure S4, Supporting Information). Oscillatory rheological measurements at low strain indicated that the storage modulus (G’⁰) of the all the emulgel inks dominated at lower shear stresses, whereas the loss modulus (G″⁰) became more relevant at high shear stresses after crossing the yield stress point (τ_y, G’ = G″) of the respective emulgel (Figure 1d). This behavior is attributed to the significant post-cross-linking effect between CNF, PAA, and silica particles, even at the lowest silica loading (ESR4). On the other hand, Figure 1d indicates that the G’ and τ_y decreased with increasing loading of the emulsions (i.e. decreasing the amount of silica particles, ESR1 to ESR4). This can be attributed to the less solid-like nature of the emulgels at high emulsion content. It should be noted that the G’ and τ_y of the silica particle-based hydrogel (no emulsion, ESR0) were slightly lower than those of ESR1. Thus, although both samples contained identical amounts of silica particles (Table 1), there is an indication that the addition of a small amount of emulsion promoted a stronger gelation.

We note that even if the rheological properties are appropriate for DIW, a high τ_y and apparent viscosity of the emulgel may generate a high printing pressure, for example, if the maximum yield stress (τ_max) generated within the nozzle is not sufficient to overcome the τ_y of the emulgels. In such case, plug flow occurs and prevents continuous printing. To determine if

| Sample | Emulsion [wt%] | Silica [wt%] | PAA/CNF [wt%] | Dry solid content [wt%] |
|--------|----------------|-------------|---------------|-------------------------|
| ESR1   | 19             | 32          | 49            | 33.2                    |
| ESR2   | 29             | 22          | 49            | 23.5                    |
| ESR3   | 39             | 12          | 49            | 14.6                    |
| ESR4   | 45             | 6           | 49            | 7.8                     |
| ESR0²  | 0              | 32          | 68            | 33.1                    |
| ESR0¹  | 0              | 12          | 88            | 13.5                    |
| ESR0²  | 0              | 6           | 94            | 7.6                     |

²The ratio for PAA to CNF was 2:1 in all samples; ¹Dry solid content corresponds to the mass of the printed structures after drying; ²ESR0 indicates that no emulsion was added to the ink.
this is the case of the present inks, we calculated the radial $\tau$ within the deposition nozzle during printing, Equation (1)\(^{13a}\):

$$\tau = \frac{\Delta P}{2L} r$$  \hspace{1cm} (1)

where $\Delta P$ is the maximum pressure applied at the nozzle, $r$ is the radial position from the center of the nozzle, and $L$ is the nozzle length. Using $\Delta P = 7 \times 10^5$ Pa, $r = 6.3 \times 10^{-3}$ m, and $L = 1.27 \times 10^{-2}$ m, the calculated $\tau_{\text{max}}$ for the nozzle is $1.7 \times 10^4$ Pa. Comparing the $\tau$ of the emulgels and $\tau_{\text{max}}$ within the printer nozzle (dotted line in Figure 1e), it was determined that $\tau < \tau_{\text{max}}$ and therefore no plug flow was expected for any of the two-phase emulgels used as DIW inks. Overall, the rheological results highlight the ability of the emulgel inks to maintain their shape upon printing.

2.2. Printing 3D Structures from Two-Phase Emulgels

2.2.1. Skin-Bearing Structures Printed from Emulgels

As will be shown, the emulgels used in DIW allow the design of hierarchically layered architectures with functions that can be independently controlled at the micrometer and millimeter scales. By using a 0.63 mm diameter nozzle, layered cubic grids were easily created. As an illustration, ESR2 emulgel was extruded through the nozzle and underwent rapid solidification after ceasing the shear, enabling continuous DIW printing (Video S1, Supporting Information). Upon freeze-drying, the shape and size of the original designs were fully retained, with no apparent collapse, deformation, or shrinkage. Moreover, the boundary between the stacked layers was visible and indicated 3D objects with a fine resolution at the micrometer scale (Figure S5b, Supporting Information). As a control, a grid composed of pure silica-filled hydrogel (with no emulsion, ESR0) was printed. No observable, macroscopic difference existed in comparison with the grid produced from the emulgel of similar rheology, e.g., ESR3 (Figure S5a, Supporting Information).

However, at the microscopic level, the grids printed from different emulgels exhibited remarkable differences (Figure 2). For a neat hydrogel ink (ESR0), a homogeneous arrangement of particles and fibrils was observed across the structure of the printed filaments or struts (Figure 2a1). This is owing to the ability of CNF/PAA to bind strongly with silica particles after cross-linking (Figure 2a3). The cross-section of ESR0 indicates filaments comprising well-packed networks of silica particles (Figure S6a, Supporting Information). In the presence of the emulsion phase and with its increased content, the emulgels formed filaments that were enveloped by a skin, or outer layer, even at low emulsion loading (ESR1, Figure 2b1). The skin coverage on the filaments increased with increasing the emulsion fraction in the emulgel, and full coverage took place at the highest emulsion content (ESR4, Figure 2e1). The surface morphology of the skin transformed from large pores or cracks, uneven mesopores, to dense surface-containing nanopores (Figure 2b1–e1). The results indicate that the formation and morphology of the skin correlate with the emulsion fraction in the emulgels (Table 1). Interestingly, the skin formed separately from the core of the filaments (Figure 2b2,c2). Such phase separation upon solidification was confirmed by the cross-section images of filaments ESR2 and ESR4 obtained using scanning electron microscopy (SEM) (see high magnification image of ESR4, Figure S6b–d, Supporting Information). The cross-section also indicates the possibility of adjusting the thickness of the skin, depending on the emulsion loading. Furthermore, we note that neither the emulsion droplet size nor PLA loading level had an influence on the formation of the skin (see Figures S7 and S8 in the Supporting Information and discussion). Overall, the results demonstrate two-phase emulgels for a one-step fabrication of structures via DIW printing producing architectures with a core protected by a skin.

Additional emulgels were formulated with hydrogels containing solid particles different than silica, namely, nanoclays, fumed silica, iron (II, III) oxide, and hydroxyapatite. All these particles are commercially available, and reported to have distinctive physicochemical properties (see Table S1 in the Supporting Information for the refractive index and zeta potential). Such systems were successfully printed into structures similar to those discussed before (Figure S9, Supporting Information). The versatility of the proposed method owes to the fact that the composition of each phase depends only on their individual components, that is, a broad range of solid particles, regardless of their size, shape, hydrophilicity, and surface properties, could be used to prepare suitable hydrogels. It should be emphasized that the surface charge (type and value) of the particles showed

Figure 2. Scanning electron microscopy (SEM) images of the printed structures (crossing in the grids are shown) prepared from two-phase emulgels: a1,a2) ESR0, b1,b2) ESR1, c1,c2) ESR2, d1,d2) ESR3, and e1,e2) ESR4. The needle size was 0.63 mm for all the samples. All the grids were freeze-dried at least 2 days before imaging. The scale bar is 200 µm for (a1) to (e1), and 100 µm for (a2) to (e2).
no significant effect on the formation of the skin although the mixture of CNF/PAA was negatively charged (Table S1, Supporting Information). We also replaced the PLA encapsulated in the emulsion by polystyrene. As was the case of the hydrogel, the ink was successfully prepared provided an emulsion was formed. In the above experiments, we used a fixed emulsion-to-particle ratio (45:6, as in ESR4) and although the properties of supporting particles and polymer were different, the printed grids reflected the intended design (Figure S9a1–e1, Supporting Information). Moreover, SEM images of the grids confirmed skin structures fully covering the surface of the filaments (Figure S9a2–e2, Supporting Information, similar to the case of ESR4, Figure 2e). Importantly, the microstructural details did depend on the particles used (Figure S9a3–e3, Supporting Information), which expands the property space for the emulgels as versatile inks toward hierarchical architectures and for given applications.

2.2.2. Formation Mechanism of Skin-Bearing Structures upon Printing

We thoroughly investigated the mechanism underlying skin formation (Figure 3). In the emulgel, the PLA was dissolved in

![Figure 3](https://www.afm-journal.de/)

_Figure 3._ a) Schematic illustration (not to scale) of the skin formation mechanism upon printing. Surface morphology of printed structures including b) silica particle and c) nanoclay after removing PLA by immersing in chloroform. The ratio of emulsion-to-supporting material is 45:6 for both samples. The dashed circles in (c) indicate PLA spheres trapped in the nanoclay layers. d) Microstructure of the grid printed through a metal needle (ESR4). The scale bar is 200 µm for (b) to (d). Microstructure of the filaments printed from ESR4 after drying at e) room temperature and f) 85 °C oven. The scale bar is 200 µm for (e) and (f). The insets in (b) to (f) are high magnification images, and the scale bar is 20 µm.
induced phase separation, but this may not occur spontaneously, depending on the affinity between the phases. On the other hand, it has been shown that in multiphase systems segregation of a hydrophobic phase or component toward hydrophobic surfaces occurs readily. Given that a hydrophobic needle (polypropylene) was used for printing, it is reasonable to assume that the skin is principally composed of the hydrophobic component of the emulsion, wherein the shear upon printing and segregation promoted phase separation and subsequent surface minimization (Figure 3a). Additionally, as the PLA was emulsified by the mixture of CNF and ChNF, outward transport of PLA-based droplets was facilitated by the preseparated internal structure of the emulgel once cross-linking (Figure 1c). The hydrophobic N-acetyl groups in ChNF facilitated interactions with PLA droplets and the inner surface of the needle. On the other hand, the evaporation of chloroform within PLA droplets upon printing might create a convective flow along with the hydrophobic inner surface of the needle, which may further increase the wettability of PLA droplets on the inner surface, thereby rendering a more effective phase separation. We selectively dissolved PLA from the grids using chloroform to confirm the composition of the skin. SEM images of the structure showed no residual skin on the filament surface; however, bound particles were still evident, demonstrating that the skin contained the nonpolar phase inside the emulsion, in this case PLA (Figure 3b,c). Particularly, after washing, some PLA spheres were trapped in the nanoclay layers, as shown in the inset of Figure 3c, which may be caused by the tight stacking of nanoclay layers. This result clearly demonstrates that the phase separation of PLA droplets occurred during printing, leading to skin formation.

To confirm the role of hydrophobic interactions in phase separation at the needle surface, a hydrophilic needle (0.63 mm, made from passivated stainless steel) was used for printing (ESR4, Figure 3a). SEM images of the grid printed through a hydrophilic metal needle was remarkably different compared to those produced from hydrophobic needle (Figure 3d). Particularly, no continuous skin was formed, and the dimension of PLA-based patches was quite wide, implying that the phase separation within the emulgel was either weak or partial. Additional evidence supporting poor phase separation within the later system is the observation of fragmented PLA films trapped in the inner regions of the filaments (Figure 3d, inset). The surface wettability of both needle types by the emulsions was evaluated by measuring the contact angle. A poor surface wettability was noted for the hydrophilic metal needle (Figure S10, Supporting Information), which highlights the role in phase separation of the interactions between the emulsion phase and the inner surface of the needle. It is worth noticing that the filaments printed from metal needles fused in the grid structure (no boundary was presented). As a result, the skin structures prevented the merging or fusion of the different layers upon printing and drying. Notably, the mechanical strength of the printed grids increased with the skin coverage (Figure S12, Supporting Information). In order to show the high fidelity of objects bearing skin at the millimeter scale, we used emulgel ink ESR4 to print grids with needles of different diameters (Figure 4a–d; Video S2, Supporting Information). All the grids maintained the original dimensions and clear grids, which were not possible by DIW printing of low solid inks. Moreover, the skin was formed regardless of the needle diameter (Figure 4e,f), demonstrating the universality of skin structure under different printing conditions. Therefore, the presence of a spatially segregated skin enveloping the filaments has a protective effect, maintaining the morphological integrity and even enhancing the mechanical strength of the grids, both at the micrometer and millimeter scales. In this context, these effects are taken as mimics to those found in skins and cuticles in nature.

The macroscopic shape of 3D printed objects is also critical for any application. A two-phase emulgel was used to produce complex architectures at the millimeter scale by printing a human dental replica using hydroxyapatite as solid filler (Figure 4g), which clearly retained details as designed. Letters were also printed to demonstrate the ability to produce high curvatures and round structures (Figure 4h). Moreover, the macroscopic layers produced during printing were clearly identified from the enlarged images, indicating the ability of emulgels to
create objects showing high levels of geometrical complexity, at the millimeter scale (Figure S13, Supporting Information). Overall, objects bearing skin structures printed from emulgels exhibit high fidelity at relatively low solid content.

2.3.2. Controlled Release of Hydrophilic Cargo

An outer layer in the 3D printed structures is expected to contribute to enhanced protection and mechanical performance. It also facilitates control on the interaction with the surrounding medium, which is an important function for skin mimicry. A point in case with relevance to drug delivery is the effect of the skin in channeling cargos between the core and the surrounding medium (Figure 5). We assessed this feature by testing the release of a hydrophilic compound (sodium salicylate, SAL) that was encapsulated in the hydrogel. Owing to its hydrophilicity, SAL is expected to be retained in the hydrogel phase during printing. Here, we utilized the hydrophobic skin to tune the permeability and diffusivity of SAL to the surrounding medium. The drug payload was fixed at 5 wt% of the final dry mass of the grids (Table 1); no alterations in grid microstructure were observed (Figure S14, Supporting Information). In order to verify the effect of the skin, we determined the drug release profiles for various grids, e.g., those prepared from ESR0 (no skin) to ESR4 (fully covering, thick skin). All the release profiles were similar in shape but presented distinctive release rates, with an initial burst release followed by a slow one (Figure 5a). Within the burst release time range, a remarkable control was enabled by the skin: an
increased skin coverage gradually slowed down the release rates (Figure 5a₂).

The mechanism for drug release was elucidated by using kinetic models that describe diffusion either through pores or by polymer relaxation, both of which fitted reasonably well the drug release profiles but indicated anomalous cargo release (Table S2, Supporting Information). Thus, the experimental data and the kinetics describing the mechanism of drug release indicate the synergistic effects that arise from the combination of a pore matrix and a polymeric shell or skin. The Peppas–Sahlin model was selected to describe the individual contribution of each mechanism (diffusion through pores and relaxation). By plotting the release coefficients for diffusion ($k_1$) and relaxation ($k_2$) as a function of the skin, a reverse scaling of the coefficients was observed (Figure 5b). With the increased skin coverage, $k_2$ played a more prominent role, which is attributed to more hydrophobic and less porous PLA layer that acted as a barrier hindering the spontaneous release from the grid. As observed in the Figure 5b, $k_2$ is an order of magnitude lower than $k_1$, which results in slower drug diffusion through the relaxed polymer when compared to the porous, inner filaments. Particularly, a fine tuning of the diffusion and permeability kinetics can be achieved by changing the composition of the inner particles or the skin, as demonstrated for different silica particle loadings in the hydrogel (Figure S15, Supporting Information). From these observations, the mechanism underlying the controlled drug release is illustrated in Figure 5c. The single circular cross-section used in this visualization is meant to represent the release rate of a single component of the printed objects that were in fact built from more complex architectures (Figures 2 and 4; Figure S6, Supporting Information). Here, the diffusion along the length is negligible compared to that in the radial direction. In summary, the drug release tests for skin-bearing grids demonstrate the capability of the skin to controllably regulate the channeling of the inner component to the outer medium, which offers a great potential for applications in advanced functional devices.

3. Conclusions

We propose two-phase emulgel systems for one-step fabrication of 3D-printed, skin-bearing architectures. DIW printing of the emulgels enabled precise phase-separated structures, which cannot be achieved through any other monocomponent ink. Moreover, the versatility of the emulgel concept was demonstrated for diverse types of components, allowing the tailoring of hierarchical microstructures that are suited to different demands. The generation of skin structures around the filaments occurred from spontaneous phase separation of the emulgel, which was enhanced by shearing upon printing and depended on the surface energy of the surface of the printing nozzle. The extrusion of emulgels by DIW allowed for the independent control of robustness and functionality of the
skin-bearing objects at the micrometer and millimeter scales. The skin on the filaments was used not only as a protection to keep the integrity of the hierarchical structure and to improve the mechanical strength, but also to tune the diffusion and permeability of cargos, enabling the regulation of transport and channeling to the outer environment. It is anticipated that the proposed emulgel strategy for DIW printing will open the possibility to engineer complex hierarchical constructs. On the ground of biomimicry from animal skins and plant cuticles, protection, regulation, and sensation are some of the possibilities for this new generation 3D-printed architectures.

4. Experimental Section

Materials: CNF were obtained from never-dried, bleached, and fines-free sulfite hardwood fibers via passing through a microfluidizer (M110P, Microfluidics Int. Co., Newton, MA), as described previously. ChNF were extracted from crabs following a series steps of washing, decolorizing, deacetylation, and ultrasonication, according to the procedures described previously. PLA 6060D (Mn = 191 000) was kindly provided by Nature Works, United States. Chloroform (CHCl3), PAA (Mw = 4 000 000), polystyrene, silica gel particles (40–75 µm), fumed silica (0.2–0.3 µm), nanoclay (≤ 25 µm, hydrophilic bentonite), iron (II, III) oxide (< 5 µm, 95%), and hydroxyapatite (2.5 µm, ≥ 100 m2 g−1) were purchased from Sigma-Aldrich (Helsinki, Finland) and used as received.

Preparation of All-in-One Two-Phase Emulgel as Ink for DIW: Pickering emulsions were prepared similarly as previous studies. Suspensions of CNF and ChNF were both diluted to 0.5 wt% and then mixed thoroughly to obtain homogeneous suspension (Figure S1a, Supporting Information). 10 mL of PLA/CHCl3 (4 wt%) was added into 10 mL of CNF/ChNF suspension and pre-emulsified by hand-shaking, and then fine emulsions were obtained via sonicating for 60 s under 40% power level with alternating on-off cycles (3–2 s, respectively) (Titanium tip sonicator, Sonifier 450, Branson Ultrasonics Co., Danbury, CT, USA).

The hydrogel was prepared by mixing silica particles, CNF (1 wt%), and PAA (2 wt%) at designed mass combinations wherein the PAA to CNF ratio was 2:1. For emulgel preparation, emulsions were added into the hydrogel under vigorous blending. The emulsion/hydrogel precursor was placed at room temperature for 30 min prior to cross-linking. Several drops of ammonia solution were injected into the precursor with thorough mixing to generate emulgel. The emulgel was placed at room temperature for at least 2 h to enable sufficient cross-linking.

To test the universality of emulgel system, nanoclay, fumed silica, iron, and hydroxyapatite were used to replace silica particles under the same approach for preparing the hydrogel. Polystyrene was used to replace PLA with the same procedure to produce Pickering emulsion.

Morphology of Emulgel: The morphology of emulsion/hydrogel precursors and post-cross-linked emulgel was characterized by confocal laser scanning microscope with a 40x oil immersion objective lens (Leica DMRXE, Leica, Germany) at transmittance and fluorescent modes. The oil phase of the emulsion was dyed with Nile red solution (1 mg mL−1 ethanol) prior to emulsion preparation. The sample was placed on a microscope slide and covered with a glass coverslip before observation. The excitation and emission spectrum for Nile red were 488 and 539 nm, respectively.

Rheological Measurement: Rheological behavior of the emulgel was measured with a rheometer (MCR 302, Anton Paar, Germany) using parallel plates (PP25) with a gap fixed at 0.5 mm. All samples were presheared at 10 s−1. The apparent shear viscosity was monitored by increasing the shear rate from 102 to 103 s−1. Oscillatory measurements were carried out at a constant frequency of 1 Hz and increasing stress from 10−2 to 103 Pa. All measurements were performed at 25 °C.

3D Printing of Emulgel Inks: 3D bioprinter (BIO X, CELLINK, Sweden) with pneumatic functioning printing head was used to print the emulgels. The device utilized 3 mL pneumatic syringe provided by CELLINK and sterile blunt needles (plastic or metal, Drifton, Denmark). The nozzle size of the needle was 0.25, 0.41, 0.63, and 0.84 mm. Cubic grids (20 mm × 20 mm × 5 mm) and specially designed shapes (dental and letters) were printed on the plastic Petri dish, for which the grids had rectilinear infill pattern and 30% infill density. Based on primary optimization, the moving speed of the printhead was 8 mm s−1, the extrusion speed was 0.012 mm s−1, and the extrusion pressure was controlled in the range of 20–200 kPa. After printing, the samples were freeze-dried at least 2 days before characterization. For comparing different drying methods, the printed samples were dried by placing at room temperature or 85 °C oven.

Characterization of the Grids: The microstructure of the filaments in the grids was observed by SEM (Zeiss Sigma VP, Germany) operated under vacuum and at an accelerated voltage of 2 kV. The structure of cross-section was revealed from clean knife cuts. The samples were sputter-coated with platinum before imaging.

Drug Release Test: The drug was incorporated in the grids prior to printing. For this, 5 wt% of sodium salicylate related to the dry mass of the filaments was dissolved in the hydrogel phase. A calibration curve in the range of 0.01–0.1 mg mL−1 for the determination of the drug concentration in water was built using the linear correlation between absorption at 296 nm and concentration (Figure S15a,b, Supporting Information). The release study was carried out at 25 °C, using Milli-Q water containing 0.5 w/v% NaCl as releasing medium (pH = 7). The grids (known mass) were individually placed in the bottom of the separated flasks, which directly contacted the releasing medium (known volume). Aliquots of the medium were withdrawn at given times for UV spectra acquisition. Before sampling the flask was agitated for homogenization of the dissolved drug. The shape of the all the grids loaded with drugs was kept unchanged during test. The release profiles were acquired with triplicates.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

3D printing, controlled delivery, DIW, Pickering emulsions, two-phase emulgel
