Formation of liquid-core capsules having a thin hydrogel membrane: liquid pearls

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We report an experimental investigation on the formation of liquid-core capsules having a thin hydrogel elastic membrane, named 'liquid pearls'. These fish-egg-like structures are initially made of a millimetric liquid drop, aqueous or not, coated with an aqueous liquid film containing sodium alginate that gels once the double drop enters a calcium chloride bath. The creation of such pearls with a micrometer-thick membrane requires the suppression of mixing until gelling takes place. Here, we show that superimposing a two-dimensional surfactant precipitation at the interface confers a transient rigidity that can damp the shear-induced instability at impact. Based on this, pearls containing almost any type of liquid can be created. This opens the possibility to use such structures as a new tool for screening microorganism survival and growth in various three-dimensional environments.

1 Introduction

The term 'liquid pearls' refers to liquid drops, on the millimeter scale or below, enclosed within an elastic spherical skin. The skin must be thin while also remaining sufficiently robust to efficiently preserve the integrity of the drop, similar to the well-known fish-egg. Such structures may find a myriad of applications depending on the nature of the encapsulated fluid and the properties of the skin. They can obviously become caviar-like structures for various types of uses, as well as being unique mini-dose containers able to preserve the contents from bacteria contamination. Moreover, because the skin can have adjustable permeability, such structures would also find applications in biotechnologies,[1] as for example to create large three-dimensional libraries of independent chemostats,[2] a unique way to select microorganism mutants resistant to a given selective pressure imposed within the bulk. Up to now, the formation of such biomimetic fish-egg-like structures has always required the encapsulation of the polymers used for building up the shell, formed by diffusion of the gelling agent toward the core of the drop[3–5] or *vice versa*.[6] The development of microfluidic technology has offered the possibility to form such structures by a multiple emulsification procedure, but this approach leads to a rather thick membrane.[7]

Here, we describe the formation of liquid pearls with millimeter-scale diameter having a hydrogel alginate membrane a few micrometers thick. These pearls can contain almost any type of liquid from pure water to oil. Their formation is based on a two-step procedure: the breakup of a compound pendant drop followed by the solidification of the coating layer once the drop enters a gelling calcium bath. Making the skin thin but still able to turn into an elastic sheet is shown to be possible by controlling the interfacial properties with surfactants. Here we show how to suppress the most important instabilities inherent to that two-step procedure. Indeed, the main instability arises from the mixing of the thin alginate solution layer with the surrounding miscible liquids. This is suppressed by inducing a two-dimensional surfactant solidification at the drop interface, which surprisingly transforms this interface into a transient elastic membrane. This allows the persistence of the surrounding layer until gelling takes place, allowing formation of a membrane a few micrometers thick. For hydrophobic liquid cores, the stability against dewetting of the outer layer has of course to be considered. We reveal that the pinch-off of the compound drop can trigger the dewetting and therefore sets the minimum achievable thickness of the shell, a phenomenon that should be generic during the fragmentation of compound non-miscible liquids.

2 Experimental

2.1 Compound drop formation

A schematic of the protocol used for creating liquid pearls is reported in Fig. 1. The compound drop is formed from two coaxial tubes in a dual dripping regime. The outer diameter D of the outer needle, that sets the drop diameter, is 3 mm. The flow of both liquid phases are driven by syringe pumps (PHD 2000, Harvard Apparatus). The average thickness h of the shell is estimated from the ratio between the inner and the outer flow rates, $r_q = q_i/q_o$, and the drop radius R, *i.e.* $h = R((1 - r_q/(1 + r_q))^{1/3})$. Depending on the outer solution surface tension and the core density, the compound drop radius R varies between 1.8 mm and 2.2 mm and the flow rate ratio r_q is in the range 1 to 1000, leading to an average thickness h that varies from 0.6 μm to 150 μm. We build up a triple coaxial tube system made of glass capillaries[8] when a culture medium is used for growing cells, since the presence of divalent ions in the culture medium ultimately alters the formation of the capsules. In that case, the outer diameter of the outer glass tube is 2 mm. The formation of the compound drop as well as its impact into the gelling bath are visualized with the help of a high-speed camera (Fastcam-x 1024, Photron) through a macro lens.

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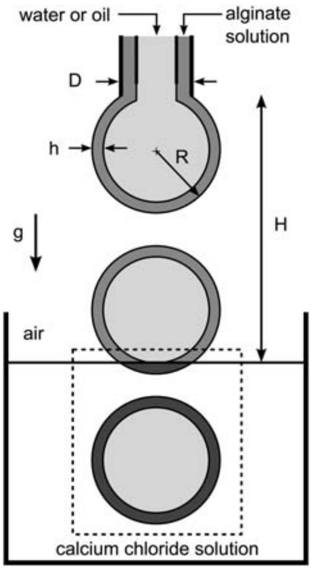

Fig. 1 Schematic of the protocol used for creating liquid pearls: a liquid drop coated by an alginate solution is formed in a dual dripping regime in air and then falls into a calcium chloride solution, leading to the sol–gel transition of the outer layer. The dotted frame indicates the location of the observation area during the high-speed recording of the impact of the drop into the gelling bath.

2.2 Materials

The outer fluid is an aqueous solution containing 20 g of sodium alginate (VWR, Prolabo), for 1000 g of milli-Q water. The use of alginate, a polysaccharide produced by brown algae, makes such capsules suitable for a wide encapsulation application from flavors to cells, as it is edible and biocompatible.[9] The solution is centrifuged and then filtered at 1.2 μm in order to remove impurities. The alginate solution exhibits a shear thinning behavior with a viscosity of 5 500 mPa s at a zero frequency. The interfacial properties are modified by adding to the alginate solution an anionic surfactant, sodium dodecyl sulfate (SDS, Fluka), that has a critical micellar concentration (cmc) close to 8 mM. The pure alginate solution is characterized by a surface tension γ_o of 72 mN/m that drops down to 35 mM/m when 10 mM of SDS is added. Milli-Q water is used as aqueous core. For hydrophobic cores, we choose silicone oils (200 fluid, Dow Corning) of various viscosities that span from 1 mPa s to 1000 mPa s. The surface tension γ_i of the oils is around 21 mN/m, and the interfacial tension γ_{io} between the oil and a pure alginate solution is 40 mN/m, dropping to 14 mM/m when 10 mM of SDS is added to the solution.

The encapsulated microorganisms are yeast cells, *Saccharomyces cerevisiae*, that grow in YPD broth medium (Amresco). A glucose (VWR, Prolabo) solution of 280 mM is used as the second liquid layer in the triple coaxial tube configuration to prevent direct contact of the culture medium and the alginate layer before it gels.

The compound drop is then gelled once it enters a water bath containing 15% in weight of calcium chloride ($CaCl_2$, Fluka) by diffusion of the calcium ions through the alginate solution.[10] The $CaCl_2$ concentration leads to a density of the solution of 1130 kg/m^3.

3 Results and discussion

3.1 Water core

The encapsulation of an aqueous phase with a thin hydrogel membrane by the dual dripping/gelling procedure is not as straightforward because the alginate layer can indeed mix with the core or the bath before it solidifies. Fig. 2 shows a catastrophic entry of a water drop coated by a 2% alginate solution layer of 30 μm thickness into the calcium bath. As expected, the core shell structure bursts, although partial solidification of the membrane is detectable on the bottom part of the drop. This instability can be obviously suppressed by increasing the ratio of inner volume to outer volume to be close to unity, and therefore leading to a very thick shell, which is not our purpose here. Let us instead focus on the conditions that could lead to a very thin membrane as aimed in the experiment reported in Fig. 2. Surfactants are known to modify the properties of liquid interfaces and can, for example, stabilize emulsions or foams against coalescence or being responsible for surface visco-elasticity.[11,12] We thus investigate the potential role of surfactants in suppressing the instability depicted in Fig. 2.

In Fig. 3(a), we show the mixing scenario of a pure water drop entering a water bath ($CaCl_2$ is still present in order to preserve refractive index mismatch). During the impact, the drop is highly deformed along an air cavity whose signature in the snapshots is a conical dark area. As expected, the drop is then immediately mixed through a cascade of vortex rings.[13] If a surfactant is added into the water drop at about its cmc and if this surfactant is chosen to be non-soluble in the gelling bath, we discover that the scenario becomes totally different, as shown in Fig. 3(b). We further find that adding traces of surfactant into the bath (about a few ppm) allows the triple line to move further inside the air cavity and therefore leads to a deeper entry of the drop, as shown in Fig. 3(c). The early dynamic of the water drop impact (with surfactants) is similar to the one observed for an oil drop impact (Fig. 3(d)), but the shape relaxation is different. The oil drop smoothly relaxes to its final lens shape. On the other hand, for an aqueous drop containing surfactants, the drop is left with

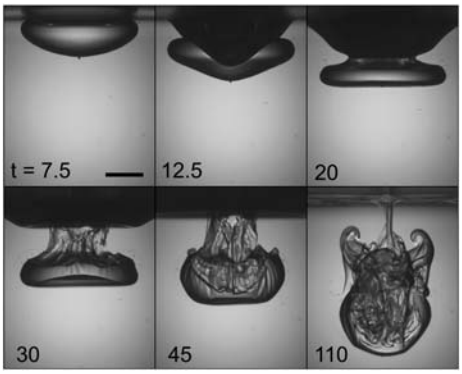

Fig. 2 Image sequence showing the entry of a water drop coated by a pure sodium alginate solution ($H = 26$ mm, $h = 30$ μm) into a calcium chloride solution. The outer alginate layer is disrupted before it completely gels. Time in milliseconds from the initial contact between the drop and the liquid bath is indicated on each image. The scale bar is 2 mm. Note the dark conical area, which is the signature of air cavity formation during the impact.

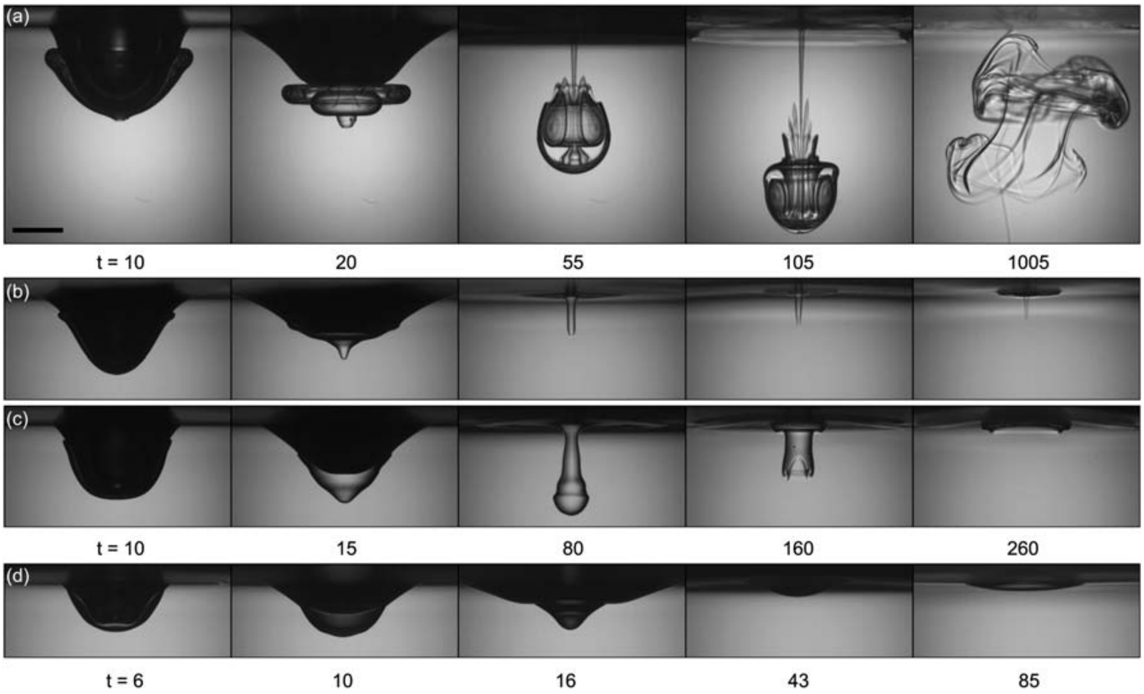

Fig. 3 Image sequences showing the impact of different liquid drops into a $CaCl_2$ solution as seen beneath the free surface. (a) Mixing of a water drop through a cascade of vortex rings after the impact as revealed by refractive index mismatch. (b) Non-mixing of a water drop containing 10 mM of SDS. (c) Non-mixing of a water drop containing 10 mM of SDS that impacts a $CaCl_2$ solution with surfactant. (d) Impact of an oil (dodecane) drop into a $CaCl_2$ solution. Time from the initial contact is indicated in milliseconds; the scale bar is 2 mm.

a cylinder shape, corrugated or not, having a solid skin that moves up and buckles because of buoyancy. During the impact, the mixing is initiated by the velocity contrast between the bath and the drop,[14] which is maximum at the equator where vortices are generated (Fig. 3(a)) as a result of a Kelvin–Helmholtz instability.[15] Thus, the insolubility nature of SDS confers to the interface a rigidity that, in turn, damps the shear instability.

Let us go back to the situation of interest: a water drop coated by an alginate solution. As shown in Fig. 4, the presence of SDS at the cmc in the alginate solution layer allows the formation of pearls that can have a membrane 30 μm thick or less. As previously anticipated from the experiments reported in Fig. 3(c), the presence of traces of surfactant in the gelling bath is required for achieving a complete wetting of the compound drop. During the impact, the compound drop is deformed and thus experiences a surface area increase. Enough surfactant should be present in the alginate layer in order to precipitate as fast as the interface is being stretched. Therefore, this healing process suppresses the convective mixing with the bath but provides enough time for calcium ions to diffuse into the alginate layer that gels. As a consequence, the capsule formation is more and more robust when the concentration reaches the cmc, as shown in Fig. 5(a). For a given SDS concentration, the critical shell thickness h^* below which the capsule formation is unstable is an increasing function of the height fall H and thus of the impact velocity that sets the magnitude of the shear at the drop surface during the impact (Fig. 5(b)). Moreover, the internal flow induced by the impact is still a source of mixing of the shell with the core, whose efficiency may be related to the impact velocity. For an aqueous core, the minimum achievable average thickness of the membrane is then around 8 μm. Decreasing even further the height fall does not allow one to form capsule having a thinner membrane. Indeed, for H smaller than about 20 mm, the air film between the drop and the gelling bath does not initially rupture at the impact. Instead, the compound drop rebounds onto the surface and then stays on the surface for a while until the air film becomes unstable and breaks.[16] This scenario does not lead to a complete engulfing of the drop into the gelling solution, which is required for obtaining efficient capsule formation.

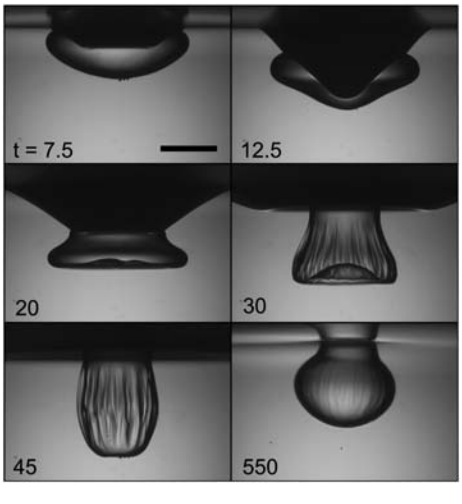

Fig. 4 Formation of a water-core pearl when 10 mM of SDS is added to the shell ($H = 26$ mm, $h = 30$ μm). Time in milliseconds from the initial contact is indicated on each image; the scale bar is 2 mm.

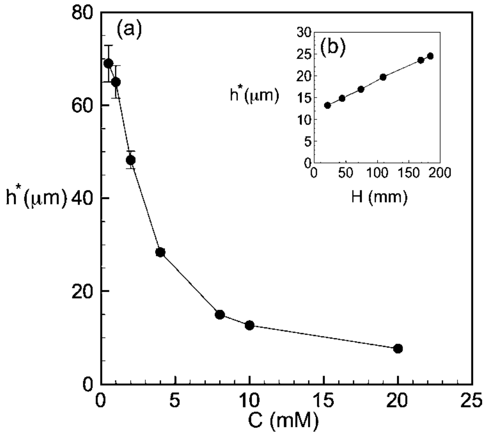

Fig. 5 Critical shell thickness h^* below which a water drop coated by an alginate layer bursts before being gelled as a function of the concentration of SDS in the alginate layer. The height fall H is set to 45 mm. (b) Critical shell thickness h^* as a function of the height fall H for a concentration of SDS equal to 8 mM.

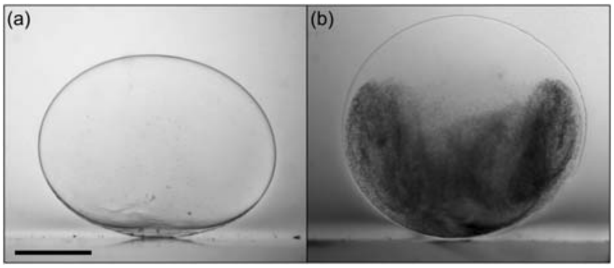

Fig. 6 (a) Snapshot of a capsule containing a few yeast cells just after its formation ($h = 40$ μm). (b) Snapshot of a capsule that initially contained a few yeast cells and that has been suspended in a test tube filled with the culture medium and incubated at 30 °C with constant agitation for 17 h. Note the vertical heterogeneity of the cell concentration due to sedimentation. The scale bar is 1 mm.

Finally, we investigate the potential use of such structures as a new tool for cell culture. First of all, it appears that common culture media contain calcium ions and other divalent ions. The presence of those ions therefore alters the formation of the capsules. Indeed, the alginate layer starts to gel at the exit of the coaxial needles and only rather thick membranes are possible. We have thus added another liquid layer, a glucose solution, that protects the outer alginate layer from the divalent ions contained in the core composed of the culture medium and the cells. The compound drop is then formed by dripping three aqueous solutions from three coaxial tubes. In order to validate the use of such capsules as a reservoir for cell cultures, we encapsulate and incubate yeast cells as a model of eukaryotic cells. An example of a millimetric capsule that contains a few cells just after its formation is shown in Fig. 6(a). The capsules are then suspended in a test-tube filled with the culture medium and incubated at 30 °C under constant agitation for one night. Fig. 6(b) shows a capsule after 17 h of incubation, where we clearly see the growth of a large population of yeast cells.

3.2 Oil core

For oil pearls, in addition, we must of course consider the wetting properties of both liquids forming the drop, since capsule formation is not possible once the coating film dewets the pendant oil drop. In the situation for which the aqueous film coating the oil drop is intrinsically metastable (*i.e.*, where the spreading coefficient, $S = \gamma_i - (\gamma_o + \gamma_{io})$, is negative[17]), surfactant at the cmc is necessary to stabilize the thin film.[18] However, we found that dewetting can be strongly activated by the recoiling of a thin thread after pinch-off, as shown in Fig. 7(a,b). This phenomenon actually sets the minimum achievable thickness for core viscosity η_i larger than 5 mPa s. For this particular nucleation process, the critical shell thickness h^* is an increasing function of the substrate viscosity η_i onto which the thread impacts (Fig. 8(a)). For an intermediate viscosity, $5 < \eta_i < 50$ mPa s, a micro-thread is observed, whereas for a higher

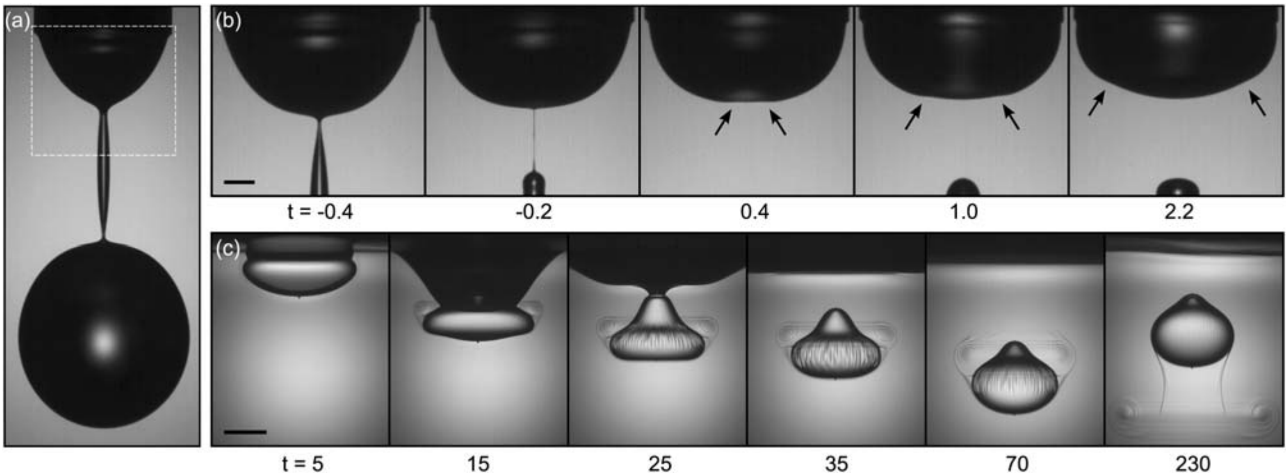

Fig. 7 (a) Snapshot of a compound pending drop prior to pinching. (b) Dewetting of an oil-in-water pendant drop triggered by the recoiling of a micro-thread after the pinch-off that sets the origin of time ($\eta_i = 10$ mPa s, $h = 1$ μm). The arrows indicate the rim of the dewetting hole. (c) Gelling of an oil-in-water drop when it enters a $CaCl_2$ solution ($\eta_i = 50$ mPa s, $H = 26$ mm, $h = 30$ μm). Time in milliseconds is indicated on each image sequence; the scale bars are 0.5 mm and 2 mm, respectively. Note the vortex ring shedding revealed by adding a small amount of non-salted water at the free surface of the bath.

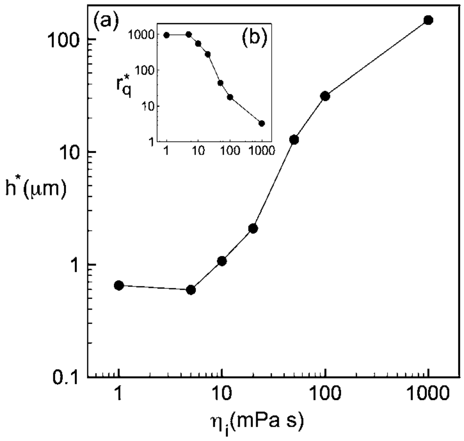

Fig. 8 (a) Critical film thickness h^* below which the dual dripping of the silicone oil in the alginate solution becomes unstable, *i.e.* the outer liquid dewets the inner one, as a function of the inner liquid viscosity η_i. The alginate solution contains 10 mM of SDS. This critical film thickness sets the minimum achievable thickness of the hydrogel membrane. (b) Corresponding critical flow rate ratio r_q^*.

viscosity we note that the radius as well as the length of the recoiling filament increases, a feature that may be related to the complexity of compound thread break-up, which depends on the viscosity ratio as well as on the relative outer layer thickness.[19,20] As far as we know, this kind of dewetting nucleation has never been reported, and a detailed description of the mechanism that may explain the general observed trend, *i.e.* that the critical thickness is an increasing function of the substrate viscosity, is lacking. We also note that, for the smallest viscosities, 1 mPa s and 5 mPa s, the destabilization of the alginate solution film results from the heterogeneous nucleation of holes taking place preferentially at the bottom part of the pendant drop. This type of nucleation sets a minimum achievable thickness that is close to 0.6 μm.

The formation of an oil core pearl, with a viscosity η_i of 50 mPa s, is reported in Fig. 7(c). As compared to the formation of a liquid pearl with a pure-water core falling from the same height (Fig. 4), we note a deeper penetration into the gelling bath and a smaller deformation of the compound drop. We also note that by reducing the core viscosity to 1 mPa s, we recover the same penetration depth and deformation as for a water-core pearl. We then conclude that this trend is a direct consequence of the degree of the deformation at impact, which is linked to the liquid-core viscosity.[21] We finally note that, in contrast to an aqueous-core pearl, the gelling of a membrane around an oil core with a thickness of the order of 1 μm does not fail at impact, since the core and the shell are not miscible.

4 Conclusion

The principal finding of this work concerns the consequence of the two-dimensional precipitation of surfactant during the impact of a water drop into an electrolyte solution. Indeed, though the two phases are intrinsically miscible, this phase transition confers a transient elasticity of the interface which allows it to suppress shear-induced mixing. As shown in this work, this effect has permitted the creation of new structures similar to the well-known fish-egg. Moreover, both the size of these liquid pearls as well as the permeability of their membrane can be in principle varied. This opens the possibility to use such structures in various fields of application, and particularly as a new tool for screening microorganism survival and growth in various three-dimensional environments.

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