Liquid marble-based digital microfluidics – fundamentals and applications

Chin Hong Ooi, Raja Vadivelu, Jing Jin, Kamalalayam Rajan Sreejith, Pradip Singha, Nhat-Khuong Nguyen and Nam-Trung Nguyen

Liquid marbles are droplets with volume typically on the order of microliters coated with hydrophobic powder. Their versatility, ease of use and low cost make liquid marbles an attractive platform for digital microfluidics. This paper provides the state of the art of discoveries in the physics of liquid marbles and their practical applications. The paper first discusses the fundamental properties of liquid marbles, followed by the summary of different techniques for the synthesis of liquid marbles. Next, manipulation techniques for handling liquid marbles are discussed. Applications of liquid marbles are categorised according to their use as chemical and biological reactors. The paper concludes with perspectives on the future development of liquid marble-based digital microfluidics.

Introduction

A liquid marble is a droplet coated with tiny particles. These particles form a flexible and porous shell that prevents the core liquid from wetting the surrounding surfaces. Liquid marbles are also alternatively called particle-laden droplets or armoured droplets. With its behaviour like a flexible and stretchable solid, a liquid marble represents the digital subfield of the recently established field called micro elastofluidics. A large variety of liquids and shell materials have been reported to form stable liquid marbles. Both hydrophobic and hydrophilic particles can form liquid marbles, provided that the particles do not dissolve into the core liquid. As a liquid marble is perfectly non-wetting, it can easily roll on a solid surface, float on a liquid surface, and sink in a carrier liquid with a lower density. Although a liquid marble consists mainly of liquid, it behaves like a soft solid. Liquid marbles can be squeezed, stacked on top of each other, merged, or even split into two smaller liquid marbles. Research on liquid marbles started off as a curious topic two decades ago owing to their interesting properties. Since then, continuing research efforts positioned liquid marbles as a practical and attractive platform to manipulate discrete droplets, e.g. digital microfluidics. Digital microfluidics handles discrete microdroplets instead of a continuous fluid flow. Most conventional digital microfluidic systems are closed devices that cover the droplets between two plates. Liquid marbles on the other hand can be used in an open environment, because the coating prevents the working liquid from wetting the carrier substrate.

This review provides a comprehensive discussion on the state of the art of liquid marbles as a digital microfluidic platform, providing insights into their further development and current theoretical and practical challenges. First, the paper discusses the fundamental properties of a liquid marble. The knowledge of liquid marble properties lays the foundation for the subsequent sections focusing on its production and manipulation schemes, Fig. 1(a-c). Subsequently, the paper discusses how these manipulation schemes can be used for various applications, Fig. 1(d and e). The paper is concluded by the perspectives and challenges of liquid marble-based digital microfluidics.

Fig. 1 Liquid marble-based digital microfluidics: (a) fundamental properties of a liquid marble. (b) Effect of the shell on liquid marble properties. (c) Liquid marble synthesis. (d) Manipulation of a liquid marble. (e) Liquid marbles as chemical and biological microreactors.
For practical applications, a liquid marble serves as a digital microfluidics platform with great advantages. First, a liquid marble can serve as a highly effective micromixer due to its relatively small size. Mechanical or electrical schemes can generate convective and advective flows within a liquid marble, providing excellent mixing capabilities. Second, fluid flow with solid particles may affect the function of conventional continuous-flow microfluidic devices as they clog the microchannels. A liquid marble is insensitive to the clogging problem as its length scale is generally much larger than that of the solid particles it contains. Third, a liquid marble is a low wastage platform. Conventional microfluidic devices require a relatively large amount of liquid to prime the device, whereas a liquid marble can operate as a standalone batch microreactor. Fourth, liquid marbles are highly scalable and cost effective. The amount of materials used is diminutive compared to most microfluidic devices. For the proof of concept, a liquid marble can be easily produced only requiring a powder bed and a micropipette. This simplicity is in stark contrast to typical microfluidic devices that involve complex microfabrication techniques such as soft lithography, mask alignment, dry etching and wet etching. Thus, the liquid marble represents a promising engineering solution for some of the current challenges in digital microfluidics.

As the volume of a liquid marble spans across the sub-millilitre to sub-microlitre range, this platform encompasses fluid physics from the microscopic to the macroscopic regime. A liquid marble can be simultaneously subject to significant capillary and gravity forces, thus pushing the physics behind its behaviour into the mesoscale. Since a liquid marble displays fluid-like properties, fundamental research topics revolve around its effective surface tension. One of the key challenges is understanding this effective surface tension as it is fundamental for macroscopic properties of liquid marbles. Furthermore, the effective surface tension is affected by the liquid marble shell, which consists of random agglomerates. Investigations of the liquid marble shell revealed the self-assembly capability of the particles during the formation process. The pseudo-solid shell also displays interfacial jamming effects, similar to floating particle rafts. The present review discusses in detail the effective surface tension and the macroscopic properties arising from it.

Liquid marble production invariably involves coating a droplet with particles. This coating process is achieved by various means and complexities. A liquid marble can be generated simply by dispensing a droplet on a powder bed and rolling it vigorously. Rolling ensures that the droplet surface is completely covered with particles. Alternatively, a difference in electric potentials between the droplet and particles allows for electrostatic coating of the liquid droplet. Li et al. used a more complex method to generate the particle from the liquid droplet via precipitation. This method can form a monolayer shell with nanometre thickness with interesting properties. However, the main bottlenecks of liquid marble production remain the reproducibility and the scalability. To date, no generation method can yet produce a large quantity of liquid marbles with decent uniformity and monodispersity. The reasons behind low uniformity in terms of size, shell thickness, shell porosity, robustness have yet to be systematically investigated. A section of this paper later provides a critical review on the various means of liquid marble synthesis.

As mentioned above, a liquid marble can be considered as a self-contained digital microfluidics or digital micro elastofluidics platform. A liquid marble is often used in an open environment surrounded by air, unlike most continuous-flow microfluidic devices based on microchannels. Consequently, individual liquid marbles can be actuated or transported in the same way as handling solid particles. Liquid marbles can easily roll or slide on surfaces to deliver a small amount of liquid. On the other hand, liquid marbles can release their liquid content via well controlled actuation. With these capabilities, a liquid marble can perform basic sample handling tasks such as encapsulation, transport, mixing, coalescence, and splitting. The components of a liquid marble can be active or passive. Passive components include most inert materials used to form the coating that are meant to protect the liquid core. Active components are directly involved in the reaction and manipulation. A liquid marble can be manipulated using a variety of schemes including electromagnetism, mechanical forces, thermal gradients, and chemical gradients. The present review systematically discusses the state-of-the-art manipulation schemes of the liquid marble as a versatile digital microfluidics platform.

As a liquid marble attracts significant interest from the research community, a range of applications have been explored. Most notably, liquid marbles can serve as micro chemical- or bioreactors. A liquid marble shell could accommodate multi-phase reactions as it represents a solid–air matrix partially embedded on a liquid surface. Liquid-phase reactions can take place within the core. Solid–liquid phase reactions can happen at the interface between the shell and the core liquid. Liquid–gas phase reactions can occur at the pores on the surface of the core liquid. Solid–gas phase reactions can arise within the shell. The multilayered microscopic particles provide a large surface area that improves the reaction rate. Engineered shell materials provide liquid marbles with highly specific functions and improve their robustness. Therefore, a liquid marble has significant potential as a chemical microreactor. As a micro bioreactor, a liquid marble contains sufficient culture medium required for cell growth. The porous liquid marble shell enables gas exchange, thus allowing aerobic activities within the core liquid. The shell also serves as a shield to prevent contamination and to reduce evaporation of the core liquid. Interestingly, a liquid marble as a cell culture platform produces three-dimensional cell tissues known as spheroids. Actuating this scaffold-free, three-dimensional cell culture platform induces controlled mixing that promotes the assembly of cells into a spheroid. This review discusses the
various chemical and biological reactions that are highly relevant to the liquid marble platform.

Fundamental properties of a liquid marble

Compared to a liquid droplet, a liquid marble possesses unique properties such as robustness, relatively low evaporation, high elasticity, and low friction, which enable it to serve as a potential digital microfluidics platform. Both the core liquid and the shell of a liquid marble determine its fundamental properties. The growing interest in liquid marbles for microfluidic applications requires insights into their fundamental properties. This section discusses the role of the core liquid and the shell in the formation mechanism and properties of a liquid marble.

Formation mechanism of a liquid marble

A liquid marble consists of a liquid core and a shell of micro- or nanoparticles.\(^4\) The conventional manual preparation of a liquid marble relies on rolling a dispensed droplet over a bed of hydrophobic particles, Fig. 2(a). The coating mechanism of the droplet can be explained by the concept of surface energy minimisation. When a particle attaches to the free surface of a dispersed droplet, a portion of the solid–air interface is replaced by the solid–liquid interface, Fig. 2(b). The surface free energy \(G\) of the attached particle is calculated as: \(^{4,10}\)

\[
G = \gamma_{la}(A_{la} - \pi R_1^2) + \gamma_{sl}A_{sl} + \gamma_{sa}A_{sa}
\]

(1)

where \(R_1 = R_p(1 - \cos \theta)/2\), \(A_i\) and \(\gamma_{ij}\) are respectively the interfacial area and the interfacial tension at the i-j interface, i and j represent the phases: solid (s), liquid (l) or air (a), \(R_p\) is the radius of the particle and \(\theta\) is the three-phase contact angle.

If this particle is fully immersed into the droplet or released fully from the droplet into the air, the surface free energy changes to \(G_1\) or \(G_2\) respectively:

\[
G_1 = \gamma_{la}A_{la} + \gamma_{sl}(A_{sl} + A_{sa})
\]

(2)

\[
G_2 = \gamma_{la}A_{la} + \gamma_{sa}(A_{sl} + A_{sa})
\]

(3)

Therefore, the energy required for the particle to detach from the droplet is: \(^{4,31}\)

\[
\Delta G_{1,2} = \pi R_p^2\gamma_{la}(1 - \cos \theta)^2
\]

(4)

The energy \(\Delta G_{1,2}\) is usually higher than the energy released during the attachment of the particle at the droplet surface. Therefore, the particle does not detach after it is adsorbed onto the droplet surface without an external effort. This mechanism applies to all particles adsorbed onto the droplet surface. Hence, after rolling over a bed of hydrophobic particles the dispersed droplet produces the stable system of a liquid marble.

The stabilisation mechanism of a liquid marble can also be explained with the interfacial energy.\(^{4,31}\) The attachment of a particle onto the droplet surface during the rolling process changes the interfacial area. For a particle with a contact angle of \(90^\circ\), the solid–air interfacial area \((A_{sa})\) and the solid–liquid interfacial area \((A_{sl})\) are the same. The proportion of \(A_{sa}\) and \(A_{sl}\) changes with the particle properties, Fig. 2(b). For a hydrophobic particle with a contact angle of more than \(90^\circ\), \(A_{sa}\) is larger than \(A_{sl}\), whereas \(A_{sa}\) is smaller than \(A_{sl}\) for a hydrophilic particle with a contact angle of less than \(90^\circ\). The change of the interfacial energy \(\Delta f\) due to the attachment of the particle is calculated as:

\[
\Delta f = A_{sa}(\gamma_{sa} - \gamma_{sl}) - A_{la}\gamma_{la}
\]

(5)

According to Young’s law:

\[
\cos \theta = \frac{\gamma_{sa} - \gamma_{sl}}{\gamma_{la}},
\]

(6)

the interfacial tension between i and j phases, \(\theta\) is the contact angle of the droplet with respect to the solid. Combining eqn (5) and (6) results in:

\[
\Delta f = -A_{sa}\gamma_{la}\left(\cos \theta + \frac{A_{la}}{A_{sa}}\right)
\]

(7)

In eqn (7), \(A_{la}/A_{sa}\) is either zero or greater than zero. Therefore, the surface energy is always reduced after particle attachment, representing a favourable condition of a stabilized system.\(^32\) The explanation is always applicable except for the special case of a contact angle of \(180^\circ\).

A dispersed droplet with a significant impact on the bed of hydrophobic powder eliminates the need for rolling for preparing a liquid marble.\(^33\) The droplet collects a larger number of particles during the spreading stage of the impact. During the receding stage of the impact, these particles are compressed over the droplet surface and form a liquid marble. The kinetic energy of the impact determines the surface coverage. McEleny et al. showed that larger encapsulating particles may not be able to climb up the droplet surface. Hence, a portion of the liquid marble could remain open at the top.\(^34\) Quantitative analysis showed that the droplet diameter
A liquid marble obtains either a spherical or a puddle shape, depending on the capillary length \( l_c \) of the core liquid and the radius \( R \) of the liquid marble.\(^{36}\) Capillary length is a scaling variable that reflects the balance between the surface tension force and the gravitational force. The capillary length correlates the former two factors as, \( l_c = \sqrt{\gamma/g} \). A liquid marble is spherical when \( R < l_c \), and has a puddle shape when \( R \gg l_c \).\(^{36}\)

**Effect of the shell on the liquid marble properties**

The shell of a liquid marble consists of encapsulating particles and pores. The size, properties, and distribution of the encapsulating particles determine the macroscopic properties of a liquid marble such as robustness,\(^{37–40}\) elasticity,\(^{31,42}\) effective surface tension,\(^{27,43–47}\) and shell thickness. Smaller encapsulating particles form a strong inner network among themselves, which opposes the deformation upon external application of force. Therefore, the robustness of a liquid marble increases with smaller encapsulating particles.\(^{38,49}\) Moreover, the close packing of these particles reduces the pores at the shell and reduces defects that are responsible for the evaporation of a liquid marble. Cengiz *et al.* determined the effective surface tension and the lifetime of a floating liquid marble as a function of particle size.\(^{46}\) The team found that the effective surface tension of the liquid marble decreased with increasing size of encapsulating particles. Therefore, the repulsion between encapsulating particles increases, allowing the underlying liquid to flow through the channels formed between the larger particles. So, the lifetime of a liquid marble decreases with increasing particle size. Later, Wang *et al.* investigated the lifetime and stability of a floating liquid marble by controlling the concentration of the surfactant Triton X-100 in the core liquid.\(^{50}\) Triton X-100 molecules adsorbed at the surface of encapsulating silica particles in the innermost layer of the shell. The adsorption enhanced the hydrophilicity of the silica particles. As a result, the level of the core liquid around the silica particles increases and reduces the air pockets in the shell. An increase in Triton X-100 concentration causes a portion of the innermost layer of the shell to sink into the core liquid and enlarges the liquid clearings at the shell, destabilising the liquid marble. Recently, Singha *et al.* demonstrated the effect of core liquid surface tension on the shell thickness of a liquid marble.\(^{51}\) A decrease in surface tension of the core liquid enhances the penetration of the encapsulating particles into it. Hence, the shell thickness decreases with decreasing surface tension of the core liquid. Further reduction of the surface tension beyond a certain threshold causes interfacial jamming of the encapsulating particles, which increases the shell thickness.

**Effective surface tension of a liquid marble**

The surface tension of a droplet is caused by the unbalanced force that enables the molecules at the liquid-air interface to experience an inward pulling force. In the case of a liquid marble, the droplet is penetrated by the encapsulating particles. Therefore, a portion of the liquid-air interface is replaced by the solid-liquid interface.\(^{52}\) Fig. 2b. The penetration of a particle largely relies on the core liquid surface tension, properties of the encapsulating particles, and capillary interactions between the encapsulating particles. These parameters are likely to influence the force balance at the core liquid surface and change the surface tension of the liquid marble. The modified surface tension is widely termed as the ‘effective surface tension’ of a liquid marble.\(^{43,44}\) The effective surface tension \( \gamma_{\text{eff}} \) is formulated as:\(^{4,53}\)

\[
\gamma_{\text{eff}} = \gamma_{\text{la}} + \gamma_{\text{int}}
\]

where \( \gamma_{\text{la}} \) is the surface tension at the liquid–air interface, and \( \gamma_{\text{int}} \) is the contribution due to capillary and electrostatic interactions.\(^{43,44}\) Depending on the nature of interactions, the contribution \( \gamma_{\text{int}} \) may be either positive or negative, which differs the effective surface tension of a liquid marble from the surface tension of a bare droplet. Positive and negative values represent the net attractive and repulsive forces, respectively.\(^{53}\)

The effective surface tension of a liquid marble opposes its deformation against externally applied force. Liu *et al.* determined the robustness of a liquid marble from its effective surface tension.\(^{39}\) A liquid marble compressed between two parallel flat plates obtains a quasi-spherical shape. In this situation, the Laplace pressure of the liquid marble opposes the compression. The Young–Laplace equation determines this Laplace pressure \( (P_L) \) from the effective surface tension:\(^{39}\)

\[
P_L = \gamma_{\text{eff}} \left( \frac{1}{R_{\text{max}}} + \frac{2}{h} \right)
\]

where \( \gamma_{\text{eff}} \) is the effective surface tension of the liquid marble, \( R_{\text{max}} \) is the maximum radius of the liquid marble before rupture and \( h \) is the height of the liquid marble under compression. Eqn (9) implies that the robustness of a liquid marble depends on the effective surface tension. The effective surface tension also determines the lifetime of a liquid marble.\(^{46}\) Therefore, measurement of the effective surface tension is necessary to understand its behaviour.

Several methods exist for the measurement of the effective surface tension of a liquid marble, e.g. the Wilhelmy plate method,\(^{43}\) capillary rise method,\(^{43}\) pendant drop method,\(^{54,55}\) sessile drop method,\(^{9,56}\) maximum height method,\(^{4,44,55,57}\) vibrational analysis of liquid marbles,\(^{44,45,58}\) and shape analysis.\(^{27,45}\) Table 1 lists the typical effective surface tension values of water liquid marbles obtained with these methods.

A thorough comparison between these values indicates that the reported effective surface tensions of liquid marbles are not consistent. The values vary with the measurement methods, particle type, particle size, homogeneity of the particles, and degree of their agglomeration. For example, the Wilhelmy plate method relies on the size of the particles.
interparticle forces. This journal is © The Royal Society of Chemistry 2021

This manual method is simple and has been used in the formation of a liquid marble, coating a liquid droplet with hydrophobic microparticles by rolling it over a powder bed.4 This method does not consider the effect of multiple layers. On the other hand, the effective surface tension measured with the pendant drop method varies with the number of shell layers. The pendant drop method relies on the accuracy of surface fitting by the image analysis software. A multi-layered liquid marble has a fluffy surface, bringing uncertainties into the fitting procedure. Conversely, a monolayer nanoparticle-covered liquid marble has a smoother surface which minimises the uncertainties.28,59 Present understanding of the effective surface tension provides only a macroscopic insight that relies on the measurement methods of these values. Recently, Li et al. attempted to address the inconsistencies of the effective surface tension with the concept of equivalence quality.27 The team proposed to consider several factors such as particle size, shape, and interfacial jamming for the measurement of effective surface tension. Nevertheless, a systematic approach to address the inconsistencies is required, which should also consider the effect of interparticle forces.

### Synthesis of liquid marbles

A relatively large number of works have been reported on the fundamental physics, engineering, and applications of liquid marbles over the last two decades.5–7,60,61 Liquid marble-based digital microfluidic platforms have been demonstrated for applications in biology, medicine, biochemistry, forensics, and disease diagnosis.62–68 However, the commercialisation of liquid marble-based digital microfluidics for these applications remains challenging. The major bottleneck lies in the very fundamental process – the synthesis of liquid marbles. Fig. 2b shows the most common method for the formation of a liquid marble, coating a liquid droplet with hydrophobic microparticles by rolling it over a powder bed.4 The majority of reported works on liquid marbles and their applications. However, the commercialisation of liquid marble-based digital microfluidics would require the synthesis of liquid marbles to be fully automatic with high throughput, reliability, accuracy, precision, and more importantly independence from a specific application. This section discusses the different techniques for the formation of liquid marbles reported in the literature over the last two decades.

Following the seminal papers by Aussillous and Quéré4 on liquid marbles demonstrating a simple manual method for the formation of a liquid marble, Binks and Murakami introduced another method of liquid marble synthesis.69 The team demonstrated the technique of phase inversion of particle-stabilised air-water systems, from air-in-water foams to water-in-air powders and vice versa either by increasing the inherent hydrophobicity of the stabilizing particles at a constant air/water ratio or by increasing the air/water ratio at fixed particle hydrophobicity. The team has successfully demonstrated the synthesis of liquid marbles using dichlorodimethyl-silane coated silica particles. Although the method was unique, it has many disadvantages. First, this method still involves a number of manual interventions at the various stages of the synthesis. Second, the method is highly complex and time-consuming. Third, there is no control over the size of the liquid marble generated.

Bhosale and Panchagnula demonstrated another method to synthesize liquid marbles70 called dropwise condensation on hydrophobic nanoparticulate materials. A glass vial containing the working liquid covered with hydrophobic nanoparticles on the liquid surface was heated from the bottom, Fig. 3(a). The liquid vapor generated at the liquid–air interface was condensed at the hydrophilic sites of the powder. The condensed droplets are enveloped by the particles through a self-assembly process, forming a parent liquid marble. The parent liquid marbles are collected and heated again. Subsequently, small daughter liquid marbles

<table>
<thead>
<tr>
<th>Encapsulating particles</th>
<th>Method</th>
<th>Effective surface tension value (mN m⁻¹)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyvinylidene fluoride (130 nm diameter)</td>
<td>Maximum height</td>
<td>70 ± 5</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Pendant drop</td>
<td>70 ± 5</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Shape analysis</td>
<td>70 ± 5</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Vibrational analysis</td>
<td>70 ± 5</td>
<td>44</td>
</tr>
<tr>
<td>Polytetrafluoroethylene (100–200 nm diameter)</td>
<td>Maximum height</td>
<td>60 ± 5</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Pendant drop</td>
<td>70 ± 5</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Maximum height</td>
<td>60 ± 5</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Shape analysis</td>
<td>53 ± 5</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Vibrational analysis</td>
<td>53 ± 5</td>
<td>44, 45</td>
</tr>
<tr>
<td>Lycopodium (30 μm diameter)</td>
<td>Maximum height</td>
<td>51</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Maximum height</td>
<td>50 ± 5</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Pendant drop</td>
<td>54 ± 47</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>Shape analysis</td>
<td>62 ± 5</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Vibrational analysis</td>
<td>43 ± 3</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Vibrational analysis</td>
<td>56 ± 5</td>
<td>58</td>
</tr>
<tr>
<td>Polytetrafluoroethylene (1 μm diameter)</td>
<td>Capillary rise</td>
<td>71</td>
<td>43</td>
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<tr>
<td></td>
<td>Wilhelmy rise</td>
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<td></td>
<td>Puddle height</td>
<td>68</td>
<td>12</td>
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<tr>
<td></td>
<td>Pendant drop</td>
<td>70 ± 5</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>Maximum height</td>
<td>60 ± 6</td>
<td>44</td>
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<td></td>
<td>Shape analysis</td>
<td>53 ± 5</td>
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</tr>
<tr>
<td></td>
<td>Vibrational analysis</td>
<td>53 ± 3</td>
<td>44, 45</td>
</tr>
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<td></td>
<td>Maximum height</td>
<td>70 ± 7</td>
<td>44</td>
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<td>Pendant drop</td>
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<td>Shape analysis</td>
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<tr>
<td></td>
<td>Vibrational analysis</td>
<td>75 ± 3</td>
<td>44</td>
</tr>
</tbody>
</table>
were formed at the air interface of the parent liquid marble. Fig. 3(b) depicts various phases of dropwise condensation on hydrophobic particles. The team was able to demonstrate control over the size of liquid marbles by adjusting the temperature and time of heating. Liquid marbles with a diameter ranging from 3–1000 μm were synthesised using this method. This method has the potential of large-scale manufacturing, because the number of parent and daughter liquid marbles can be easily controlled by changing the diameter of the glass vial. Manual intervention still exists. However, the prospect of automation is very good since the process is not too complicated. The major drawback of this method is heating the working liquid. This process may not apply to samples that are sensitive to temperature change.

Liu et al. established a millifluidic method that could generate 200 liquid marbles per minute. The team utilized an assembly automation machine called a vibration bowl feeder to continuously generate liquid marbles, Fig. 3(b).71

Ireland et al. reported an electrostatic method for the generation of liquid marbles.72 A vertical capillary with a droplet was kept over a hydrophobic powder bed and an electric field was applied between the pendant droplet and the hydrophobic powder bed, Fig. 3(c). The hydrophobic particles were electrically charged and migrated to the droplet due to the attractive electrostatic force. The migrating hydrophobic particles assemble into a shell to form a pendant liquid marble. This pendant liquid marble can be subsequently placed on any surface accordingly. This method lacks the capability of high throughput and control over the size of the liquid marble. The size greatly depends on the diameter of the capillary. This method was not fully automated and not tested for suitability for liquid samples containing biological samples as the high electric field (applied voltage is around 2.5 kV) can adversely affect the samples.

Our research group demonstrated a fully automated liquid marble generator based on electrohydrodynamic pulling, Fig. 3(d).73 The operation was based on electrohydrodynamic pulling. A pendant droplet formed at the tip of a metal capillary is pulled down by the applied electric field. The volume of the formed droplet can be reduced by increasing the applied electric field. The control system and the corresponding software were developed for the automatic operation. The user can enter the volume of the liquid
marble. The electric field required for a specific droplet volume was automatically setup between the metal capillary and a spatula containing the hydrophobic powder. The piston of a syringe containing the working liquid was subsequently actuated by a motorized stage and generated a pendant drop at the tip of the metal capillary. The liquid droplet was pulled to the hydrophobic powder bed when the required volume was reached. The hydrophobic powder bed containing the deposited droplet was oscillated so that the droplet can roll over to form the liquid marble. This automatic system successfully synthesised liquid marbles with volumes ranging from 5 μL to 700 μL with an average relative error of 8.6%. This system was the first completely automated on-demand liquid marble generator with good accuracy. However, the throughput was relatively low as the system can only form one liquid marble at a time. Higher throughput can be achieved by modifying the hydrophobic powder bed assembly. Another drawback of this system is the high voltage on the order of kV. This relatively high voltage may adversely affect biological samples in the core liquid. Further works must be conducted to adapt this system to handling biological samples.

Synthesis of composite liquid marble and core–shell bead

A composite liquid marble consists of two liquid phases forming a concentric spherical geometry with the exterior liquid phase covered with hydrophobic/oleophobic powder coating. The major issue of conventional liquid marbles for practical applications is the evaporation of the liquid sample through the porous coating. Biochemical reactions such as DNA amplification through polymerase chain reaction (PCR) or loop-mediated isothermal amplification (LAMP) occur at elevated temperature. Rapid evaporation of the liquid at elevated temperature prevents the meaningful use of conventional liquid marbles for DNA amplification. The composite liquid marble technology can be effectively used to synthesise core–shell beads – an alternative for conventional liquid marbles – to solve the evaporation problem.

A core–shell bead consists of a core liquid droplet and a nonporous solid shell. However, the synthesis of core–shell beads in air is complex. Takei et al. recently demonstrated a simple method to synthesize core–shell beads using the composite liquid marble technology. Trimethylolpropane trimethacrylate (TRIM) monomers with photo-polymerization initiators were dropped and rolled over an oleophobic marshmallow gel-like powder to form TRIM liquid marbles. Water was subsequently injected into the TRIM liquid marble. The resulting composite liquid marble consists of the liquid core surrounded by the TRIM monomer that is coated with oleophobic powder. The composite liquid marble was then exposed to blue light and simultaneously rotated using a rotary shaker. The light initiated the photopolymerisation of the shell liquid resulting in a hard nonporous solid shell. The centrifugal force imparted by the rotation maintained the position of the water core in the centre of the bead. The super amphiphobic powder coating was later washed with water to obtain a transparent core–shell bead, Fig. 3(e). Our group has employed a similar technique to make core–shell beads and successfully demonstrated polymerase chain reaction in core–shell beads. Compared to conventional plastic PCR vials, the core–shell beads represent an 85% reduction in plastic waste.

Manipulation of liquid marbles

P. Aussillous and D. Quéré first proposed in their pioneering work the concept of liquid marbles and introduced their control through gravitational, electric, and magnetic forces. Following this work, a considerable number of studies have been conducted to manipulate liquid marbles on a solid or liquid surface. Reliable actuation concepts for liquid marbles, especially controllable schemes using external fields, are of great interest and have been extensively reported in recent years, owing to advances in coating materials and the promising versatile biochemical applications of liquid marbles, Fig. 4. 

Electric scheme

A direct way of handling small liquid marbles is using an electric field. Electric actuation can effectively handle microlitre-volume liquid marbles with a simple experimental setup, which avoids loss of mass thanks to the lack of physical contact. This scheme allows for contactless controllable manipulation of non-magnetic liquid marbles without the need for functionalising the shell or the core liquid. However, this scheme also has drawbacks such as the high voltage and unavoidable charging of the coating particles. By tuning the field strength of an electric field, in either alternate-current (AC) or direct-current (DC) mode, the motion of individual or multiple liquid marbles can be initiated on solid and liquid surfaces. The electric field can induce deformation, gripping, rolling, sliding, oscillation, mixing and coalescence of liquid marbles.

Electrowetting-on-dielectric (EWOD) has been utilised to induce the movement of bare droplets on a hydrophobic surface. This manipulation method can be deployed to transport non-wetting liquid marbles with a static contact angle close to 180°, Fig. 4(a). McHale et al. reported the completely reversible electrowetting phenomenon of liquid marbles. Liquid marbles return to their initial condition after activation with both AC and DC electrowetting. This work presents the proof-of-principle demonstration of controlling the rolling motion of a liquid marble using a finger electrode. Furthermore, the team reported the modal shapes of the oscillating liquid marbles in an alternating electric field. Both up and down motion and oscillation in multiple vibration modes were observed for lycopodium-coated liquid marbles containing potassium chloride using EWOD with a frequency ranging from 1 Hz to 250 Hz. Later,
Fu et al. systematically studied the controlled actuation of sessile liquid marbles on a dielectric using an DC electric field. The insight into electric manipulation allows for sustained and on-demand directional actuation of individual liquid marbles such as rolling and coalescence.

Bormashenko et al. conducted extensive research on the electrostatic deformation of sessile liquid marbles. The team reported that if the electric field passes a threshold, a uniform electric field may counter the gravity and keep a liquid marble in its undeformed spherical shape. Furthermore, the team investigated the dynamic responses of composite liquid marbles and Janus liquid marbles in an electric field. In terms of composite liquid marbles, the team focused on the actuation with a uniform DC electric field for a composite liquid marble consisting of di-iodomethane and water droplets, which are encapsulated by a common shell of hydrophobic particles. Apart from the deformation, the water droplet can climb onto the di-iodomethane droplet as well. Janus liquid marbles are water droplets wrapped with two particle types including carbon black as a semiconductor and PTFE as a dielectric. The team demonstrated stable physical and chemical properties of the Janus liquid marble and its rotation in an electric field.

Another study worth mentioning was reported by Ooi et al. The team recently proposed a simple but practical manipulation method for picking and placing sessile liquid marbles using dielectrophoresis (DEP) force. Liquid marbles of different volumes (2–25 μL) were picked up using an electrode with a high DC voltage bias, then transported to a different location, and gently released after turning off the voltage. This method provides reliable handling of liquid marbles without the need to modify their coatings. Therefore, this actuation scheme could enable the future automation of liquid marble handling. Subsequently, Jin et al. applied this method to induce the vertical collision of two identical liquid marbles for coalescence. A liquid marble was picked up using the DEP force from an inhomogeneous electric field and then dropped vertically towards another sessile liquid marble resting on a hydrophobic powder bed. Liu et al. reported that two or more contacting liquid marbles can be forced to coalesce if they are charged by a sufficiently high voltage, Fig. 4(b). By evaluating the electric stress and surface tension effect, the authors attributed such coalescence to the formation and growth of the connecting liquid bridge driven by the electric stress. Liu et al. further reported that liquid marbles could serve as an effective reactor for miniaturised chemical reactions. By applying a voltage to the liquid marble, the induced electro-mixing inside the liquid marble-based microreactor promotes the reaction rate and the product yield.

Unlike the rolling motion of sessile liquid marbles on a solid substrate, liquid marbles floating on a free liquid surface tend to slide smoothly with less friction. Our group has recently conducted a series of experimental and theoretical studies on manipulating floating liquid marbles using the DEP force induced by a non-uniform DC electric field. Ooi et al. utilised DEP to handle liquid marbles floating on water, Fig. 4(c). In this study, a relatively simple setup can move liquid marbles of various volumes (2.5–30 μL) across the water surface at a relatively high speed up to 30 mm s⁻¹. The team also presented a one-dimensional analytical model to describe and accurately predict the motion of a floating liquid marble. Furthermore, Jin et al. extended this electric manipulation scheme to trap floating liquid marbles from a stationary position and with a given initial launch velocity. Apart from trapping, positioning of floating liquid marbles is also essential for efficient sample handling. Jin et al. reported the concept of accurately positioning a floating liquid marble using dielectrophoresis with a two-electrode configuration. In this work, high voltages applied to each electrode generated a non-uniform electric field, which attracted the floating liquid marble.
towards the corresponding electrode. The combined DEP forces of the electrode pair effectively trapped the floating liquid marble at the desired position between the two electrodes. By controlling the voltages of two individual electrodes, the position of the floating liquid marble can be precisely controlled and monitored. The precise and real-time monitoring of the position of a liquid marble was also reported by Ooi et al. to trace the motion of a self-propelled liquid marble containing ethanol. In general, tracking a liquid marble is similar to tracking a particle, albeit with a larger size and field of view. Therefore, well-established particle tracking methods should be adequate.

**Magnetic scheme**

Magnetic actuation is another effective scheme for controllable liquid marble manipulation, Fig. 4(d). Recently, an increasing number of magnetic manipulation methods were presented for both sessile and floating liquid marbles. The magnetic scheme provides an excellent means to remotely control the motion of a liquid marble without an external power supply. However, magnetic components either in the liquid core or the solid shell are essential for this actuation concept. Besides actuating a liquid marble, the magnetic scheme could be used to reversibly open and close the magnetic shell of a sessile liquid marble, Fig. 4(e). For this purpose, a permanent magnet is simply placed in the vicinity of the target liquid marble and then removed. This unique feature enables magnetic liquid marbles to function as microreactors, where reagents can be added, and products removed on demand.

Lin et al. investigated liquid marbles coated with hydrophobic magnetite particles. Zhao et al. reported the planar and spatial magnetic actuation of a water marble coated with highly hydrophobic Fe₃O₄ nanoparticles. The team found that these magnetic liquid marbles have the remarkable ability to be opened and closed reversibly with a magnetic field. Furthermore, Xue et al. utilized silane-doped Fe₃O₄ nanoparticles to prepare magnetic liquid marbles of both aqueous solution and organic liquids with a surface tension as low as 20.1 mN m⁻¹. The team subsequently demonstrated the feasibility of using the above magnetic liquid marble as a universal miniature reactor for various chemical reactions. Zhao et al. later demonstrated the manipulation of a magnetic liquid marble under an external magnetic field, considering the maximum friction force, the boundary actuation force, the effective surface tension, and the threshold marble volume for shape transition. Taking advantage of reversible opening and closing, Lin et al. proved that the liquid inside a magnetic marble can be probed for “on-line” quantitative electrochemical measurement or optical detection.

There has been great interest in magnetically handling liquid marbles due to the wide range of applications and simplicity of implementation. Vialetto et al. described an additive-free way to magnetically control the motion of discrete liquid entities, especially liquid marbles. The method uses a paramagnetic liquid as a deformable surface to direct the motion of floating liquid marbles, aided by a small permanent magnet. A variety of liquids, including diamagnetic (water, oil), could be efficiently transported using a moderate magnetic field (ca. 50 mT). This paramagnetofluidic method eliminates the need for complex equipment or electric supply, in-line with the development of robust and low-cost analytical and diagnostic microfluidic devices.

**Optical scheme**

Optical actuation allows for the remote control of a liquid marble without the restriction of the working path. Light not only drives the liquid marbles on demand, but also triggers the mixing process and the reactions inside them, Fig. 4(f). However, excessive heat load due to extended exposure to illumination needs to be considered in practical applications. Tang et al. demonstrated the photochemical actuation of WO₃ coated galinstan liquid metal marbles. These marbles were placed in a H₂O₂ solution and illuminated with a moving UV light source. The semiconducting WO₃ coating behaves as a photocatalyst to trigger a photochemical reaction, generating oxygen bubbles that propel the liquid marble.

Kavokine et al. reported the light-driven transport of floating liquid marbles on water with photosensitive surfactants. Irradiation of the solution generates photo-reversible Marangoni flows that transport the liquid marbles toward UV light and away from blue light when the thickness of the liquid layer is large enough (Marangoni regime). Below a critical thickness, the liquid marbles move against the surface flow at a speed that increases with decreasing liquid thickness (anti-Marangoni effect).

**Acoustic scheme**

Acoustically induced pressure could be utilised to lift sessile liquid marbles effectively without physical contact. Furthermore, the shell of most liquid marbles can be opened and closed reversibly under acoustic actuation. However, the strong acoustic field may cause the liquid marble to rupture. Zang et al. recently reported the dynamic behaviour of liquid marbles actuated by an acoustic field. The team successfully levitated liquid marbles using an acoustic field and can manipulate the particle shell in a controlled manner, Fig. 4(g). Upon increasing the sound intensity, the stable levitated liquid marble changes from a quasi-spherical shape to a flattened ellipsoid, and the particles migrate from the polar region of the droplet surface to the equatorial region, leading to the formation of a cavity on the liquid marble surface. Moreover, the opened cavity can close completely upon decreasing the field intensity, allowing these liquid marbles to serve as a microreactor. The driving force for particle migration is the acoustic radiation pressure exerted on the part of the particle surface protruding into air. Zang
et al. utilized acoustic levitation to induce the coalescence of multiple liquid marbles, and thus trigger chemical reactions.\textsuperscript{24} Driven by the sound pressure field, the liquid marbles rested on a copper sieve moved toward each other, collided, and eventually coalesced into a larger liquid marble.

Gravitational scheme

Gravitational potential energy is a traditional yet efficient means to initiate the movement of sessile liquid marbles, Fig. 4(h). This actuation scheme does not require supporting equipment as the liquid marble simply rolls along an inclined plane\textsuperscript{23,101,102} or falls from a height.\textsuperscript{23,103} This scheme has several inherent drawbacks such as a fixed working path and uncontrollable acceleration. Aussillous and Quéré systematically investigated the actuation and physical properties of liquid marbles under the effect of gravity in their pioneering works.\textsuperscript{4,53} Following these studies, more research was conducted on handling liquid marbles with gravity. Planchette et al. studied the coalescence of two liquid marbles with different sizes through direct vertical collisions.\textsuperscript{23} The team observed two different collision regimes and proposed a geometric criterion depending on the size of coating particles and the diameter of the droplet to describe this transition. Jiang et al. focused on the dynamic behaviour of a sessile liquid marble moving on an inclined plane and coated with a thin oil film.\textsuperscript{102} Draper et al. also explored the possibility of using liquid marbles with a relatively low mass and momentum under gravity as a mechanical flip-flop actuator for microfluidic logic systems.\textsuperscript{105} Due to the rocking motion of the switch, sequential liquid marbles are distributed along different channels, allowing the liquid marbles to traverse parallel paths.

Other schemes

Apart from the above-mentioned schemes, there are meaningful attempts of manipulating sessile and floating liquid marbles using other methods. Bormashenko et al. conducted a few experiments on the self-propulsion of floating liquid marbles on the water surface.\textsuperscript{106,107} The liquid marbles were filled with solutions such as alcohol and sulfuric acid, and moved randomly and rapidly by the Marangoni capillary flow. Furthermore, the change of environmental conditions such as pH, temperature, humidity, and even surfactant concentration\textsuperscript{104,108–111} can be used to induce various motions of liquid marbles.

Chemical applications

In the past two decades, liquid marbles have been explored for chemical applications. The unique characteristics of a liquid marble make it a perfect candidate for a chemical sensor or microreactor. This section focuses on the application of liquid marbles as chemical sensors and microreactors.

**Microsensor**

Liquid marble-based sensors make use of the selective response of the coating particles or the core liquid to a particular analyte. Liquid marbles provide a facile, cost-effective, and portable sensing method with distinct advantages including the small amount of required reagent and the ease of monitoring the sensor.

Floating liquid marbles are effective platforms for detecting contaminants on a water surface based on the decrease of surface tension with the introduction of organic compounds. The coating particles tend to adhere to the low surface tension of the carrier liquid surface rather than to the core liquid, thus causing the liquid marbles to collapse, Fig. 5(a). Bormashenko et al. first demonstrated the feasibility of using floating liquid marbles for detecting organic compounds.\textsuperscript{110} By floating and observing the collapse of liquid marbles on the water surface, the team could detect pollutants such as oils, petroleum, and its derivatives. Using polyvinylidene fluoride (PVDF) coating particles, the detection range of this method is below 61–62 mN m\textsuperscript{-1}. Zhao et al. employed the same method using colourful liquid marbles fabricated from organic salts that were easily distinguishable to detect various organic compounds including ethanol, acetone, petroleum, and ether.\textsuperscript{112}

Utilizing the pH-responsiveness of coating particles, it is feasible to detect the change in pH of the external liquid environment with liquid marbles.\textsuperscript{113,114} The change in pH of the interior liquid or external carrier liquid induces a change in wettability of the pH-responsive coating particles, thus leading to the collapse of the liquid marble. Fujii et al. used poly(2-vinylpyridine) particles to quantitatively determine the pH of the water surface.\textsuperscript{111} The authors observed that liquid marbles coated with the aforementioned particles immediately burst when placed on solutions with a pH lower than 2.9. Inoue et al. used a similar method to detect solutions with pH higher than 9 using poly(6-(acrylamido) hexanoic acid)-grafted silica particles.\textsuperscript{115} Ohno et al. prepared coating particles with a carboxyl group that is reactive with alkalis such as NaOH, effectively destroying the floating liquid marbles when alkaline solutions were introduced onto the water surface.\textsuperscript{116}

The relative gas permeability of the coating layer enables the use of liquid marble for gas sensing applications. Acidic or basic gases can be absorbed across the porous coating layer into the liquid core and change its pH, thus increasing the wetting of the particles and disintegrating the liquid marbles. Many studies reported the use of liquid marbles coated with pH-responsive particles to detect the presence of gaseous HCl and CO\textsubscript{2}.\textsuperscript{117,118} Generally, this method can be applied to any solutions or gases that can change the pH of the core liquids.

When an indicator is added into the core liquid, liquid marbles can be used as colourimetric gas sensors, Fig. 5(b). Bormashenko et al. first used liquid marbles containing Hantzsch reaction reagents to detect formaldehyde vapour.\textsuperscript{119}
Tian et al. introduced different indicators into liquid marbles to sense ammonia and hydrochloric acid based on colourimetric and fluorescent detection. Li et al. coated phenolphthalein solution with a superhydrophobic sol–gel film to form rod-shaped gas sensors that are able to indicate gas distribution along their length. Nguyen et al. recently reported the use of liquid marbles with a transparent coating for digital colourimetry. This enables the reaction process to be accurately characterised in real-time. A calibration curve was built to conveniently determine the instantaneous reactant concentration.

Liquid marble-based sensors also find application in heavy metal ion sensing which is of great significance in chemical industry. Sivan et al. utilised metal liquid marbles coated with nanoparticles of semiconducting WO$_3$ for sensing heavy metal ions and reported a remarkable enhancement in the detection sensitivity of Pb$^{2+}$ and Cd$^{2+}$ ions. Floating liquid marbles are effective platforms not only for detecting but also absorbing heavy metal ions. By encapsulating lignin–gold nanoparticle composites in liquid marbles and floating on Pb$^{2+}$ solution under near-infrared (NIR) irradiation, the authors could release the lignin–AuNP composites, eventually absorbing Pb$^{2+}$ and forming red-colour Pb$^{2+}$–lignin complex precipitates.

Microreactor

Liquid marble-based microreactors can reduce the amount of reagents used and the associated waste. The coating layer acts as a shield to protect liquid marbles from evaporation and contamination. Furthermore, liquid marble-based microreactors minimize the hazards of corrosive or toxic reagents as the core liquid is isolated from the environment.

These distinct advantages of liquid marbles make them a promising universal miniaturised reactor platform. Liquid marbles can conveniently accommodate a broad range of liquid-phase reactions. The simplest method is encapsulating reagents into a single liquid marble and then triggering the reaction. Magnetic particles are usually employed to perform the triggering reaction and mixing in standalone liquid marbles by manually moving them using permanent magnets. Nonetheless, multi-liquid marble reaction systems that can be triggered by coalescence are gaining research interest as they enable on-demand and non-contact initiation. The coalescence of liquid marbles containing reagents can be induced by various strategies including electromagnetic, mechanical or chemical schemes.

The feasibility of liquid marble-based microreactors involving gas-phase reactants has been demonstrated. The gas-permeable coating layers allow gases to be absorbed into the interior liquids and the large area to volume ratio of liquid marbles facilitates diffusion. Sato et al. reported the use of liquid marbles containing 2-hydroxyethyl sorbate (HES) to absorb oxygen for the synthesis of polyperoxide of HES, obtaining higher selectivity and reaction yield compared to conventional reactors. Liquid marbles containing polyperoxide of HES can then be used as pressure-sensitive adhesives. Gas-phase reactions also occur on the coating layer interface as it provides a site for condensation.

Reactions with solid-phase reactants tend to face difficulties such as clogging in microfluidic reactors. Liquid marbles can easily overcome this limitation. Generally, solid particles are dispersed within liquid marbles and subjected to external stimulations to trigger the reactions. Liquid marbles also play an important role in tuning the
characteristics of the products. Chu et al. synthesised graphene/Ag nanocomposites by coating the dispersion of graphite oxide and silver nitrate with Fe$_2$O$_3$/C and performed the reduction reaction on the dispersion at elevated temperature.\textsuperscript{137} Gu et al. studied the synthesis of colour pigments by exposing liquid marbles containing colloidal crystal solutions of silica nanoparticles to UV light to polymerise the interior liquid.\textsuperscript{138} Recently, Rong et al. successfully demonstrated the use of liquid marbles to synthesise solid–liquid hybrid superparticles to capture CO$_2$ by co-assembling mesoporous silica particles with tetraethylenepentamine using slow evaporation of water in the core liquid.\textsuperscript{139} Reactions can also take place on the interface between the coating particles and core liquid. Sheng et al. fabricated Janus particles by depositing polydopamine on the surface of coating particles submerged in the liquid marbles.\textsuperscript{140} In another study, Sheng et al. investigated hydrophobic silica as a coating layer for the silver mirror reaction in liquid marbles. This coating layer provides a reactive surface for free silver ions that can bind to and to convert into elemental silver atoms. These silver atoms then act as nucleating sites for the formation of the silver mirror.\textsuperscript{141}

Coating particles can be endowed with specific properties to enhance the functions of liquid marble-based microreactors. For instance, hydrophobic catalysts can be used as coating particles to fabricate catalytic microreactors. Carter et al. utilised Ru/Al$_2$O$_3$ as coating particles to encapsulate maleic acid solution and catalyse hydrogenation reactions.\textsuperscript{142} Significantly higher conversion in comparison to a bulk mixture of maleic acid and coating particles was obtained owing to the large catalyst surface area. In another work, hydrophobic silver nanowires treated with perfluorodecanethiol were used to fabricate catalytic liquid marbles, reducing methylene blue with high catalytic efficiency that is stable after 5 cycles of reactions, Fig. 5(d).\textsuperscript{143} Silver nanoparticles can also be dispersed and immobilised onto polymer particles synthesised from cyclomatrix polyphosphazene (PZAF particles), creating coating particles that have high catalytic activity and averting the agglomeration of catalyst particles.\textsuperscript{144} Wang et al. demonstrated photocatalytic liquid marbles with magnetic-responsive and upconversion Yb$^{3+}$/Er$^{3+}$/Gd$^{3+}$-doped NaYF$_4$ nanoparticles as coating materials.\textsuperscript{94} Liquid marbles can convert protoporphyrin IX (PpIX) into reactive oxygen species (ROS) which find application in photodynamic therapy of cancer cells. Plasmonic liquid marbles enable surface-enhanced Raman scattering spectroscopy (SERS) for real-time and \textit{in situ} reaction monitoring.\textsuperscript{145,146} Han et al. demonstrated SERS in a multi-liquid marble system to monitor the multi-step synthesis of azo-dye.\textsuperscript{147} The SERS detection limit of the plasmonic liquid marble for bisphenol A is 50,000 times lower than the safety limit of this compound. Another interesting function of coating particles is photothermal heating enhancement. Using graphene as the coating layer leads to a high surface temperature when the liquid marble is subjected to laser irradiation.\textsuperscript{148} Substantially higher reaction kinetics was achieved for the degradation of methylene blue under these photothermal heating conditions.

**Biological applications**

In recent years, micro bioreactors have progressed to a versatile tool that provides potentially effective solutions for industrial biotechnology and development of biotherapeutics. These low-cost and easy-to-use bioreactors demonstrated convincing benefits through improved and reduced fluidic processing steps as compared to traditional bioreactors. Digital microfluidics bioreactors are increasingly adapted for biomedical applications. This technology is affordable, easy to handle, capable of high throughput and suitable for a wide range of biological processes. This section discusses the applications of liquid marbles as micro bioreactors, Fig. 6(a).

The absence of contact with the surrounding allows liquid marbles to roll on a solid surface or even propel themselves on a liquid surface.\textsuperscript{106} The motion of a liquid marble generates an internal flow that greatly enhanced mixing of solutes or cells without an external liquid handling system. The stability of liquid marbles is extremely suitable for applications with physical perturbations during handling. Mechanically, liquid marbles are robust and soft but exhibit the properties of an elastic solid. The elasticity of the liquid marble is critical to resist deformation due to impact during handling. These features are essential for dispensing liquid marbles; for instance, by pressure fitting the marble into a cut pipette tip.\textsuperscript{149} In addition, the robustness of a liquid marble allows reversible opening and closing of the particle shell.\textsuperscript{100} For instance, a micro-syringe can be used to inject and remove materials into and from the liquid marble or even removing and refilling the core liquid. The coating particles also allow for optical access to monitor the content of a liquid marble.\textsuperscript{150} The properties and the manipulation of liquid marbles mentioned in the previous sections meet the criteria of a practical micro bioreactor.

**Microbioreactors for cell culture**

Three-dimensional (3D) cell spheroids are appropriate pre-clinical models for biological experiments. A 3D tissue model complements conventional two-dimensional (2D) cell culture systems and animal models. Cell spheroids closely mimic the spatial architecture of native tissue and model the complex cell interactions. Particularly, the 3D model provides more accurate cell–cell and cell–matrix interactions.\textsuperscript{151} Furthermore, organoid aggregates consisting of self-organised, mixed cell types derived from stem cells are capable of mimicking \textit{in vivo} physiology.\textsuperscript{152} Both spheroids and organoids represent the basic unit for clinical translation, disease modelling, drug screening, and developing innovative therapeutics.
The unique and beneficial characteristics of liquid marbles allow for their use in culturing cells in three dimensions. The porous nature of the particle coating at liquid–gas interfaces permits gas exchange, making liquid marbles suitable for culturing cell tissues ranging from spheroids to organoids. This capability is also well suited for growing microorganisms and performing analysis of biochemical reactions. Arbatan et al. first demonstrated the use of liquid marbles to grow tumour spheroids. This technique was later utilised for growing various types of cells including fibroblasts, cells of the nervous system, embryoid bodies, and stem cells, in vitro maturation of sheep oocytes and organoid development.

Cells are commonly grown on non-adhesive substrates or in a hanging drop to form cell spheroids. In the liquid marble method, a small culture medium droplet containing cells is dispensed on the surface of a hydrophobic powder bed. The rolling motion causes the powder particles to envelope the droplet, creating a robust and elastic hydrophobic shell. This system of a liquid core and particle shell serves as a compartmentalised and miniaturised cell culture system, which is suitable for studying cellular behaviour in a discrete microliter scale volume. A liquid marble creates a niche similar to the native tissue microenvironment and plays a key role in orchestrating rapid self-assembly of cells to form a multicellular tissue. The coating material acts as a non-adhesive space, which prevents cells from attaching to the internal surface. Instead, the coating material promotes suspension and aids cells to freely interact with neighbouring cells. Subsequently, cells undergo self-assembly to form cellular spheroids. In a liquid marble, the assembled cells result in multiple spheroids, which are uniform in geometry and size. In contrast, other conventional techniques lead to the formation of larger aggregates.

The lifetime of a liquid marble is critical for maintaining cell growth for an extended duration and satisfactory drug treatment regime. Compared to droplets, the particle coating of a liquid marble acts as a barrier to reduce evaporation. Nevertheless, liquid marbles still suffer from undesirable evaporation. Eventually the liquid marble loses the liquid core and flattens. This problem was addressed by floating the liquid marble on a free liquid surface, Fig. 6(b). The liquid surface increases the humidity surrounding the liquid marble and slows down evaporation. As a result, a floating liquid marble is able to culture cells for more than 3 days. Additionally, the spherical shape of a liquid marble can be maintained. This strategy of floating a liquid marble on a carrier liquid to extend its lifetime does not require the liquid marble to be permanently encapsulated in a solid shell, thus making the process more convenient and reversible. A liquid marble could be completely submerged if it is substantially denser than the carrier liquid. This effectively forms a Pickering emulsion where the liquid marble interior is separated from the carrier liquid by the coating layer. A submerged liquid marble that is stable can last much longer than those exposed to air as the complete encapsulation of the coating surface drastically reduces the evaporation rate.

Another advantage of using a floating liquid marble is that the motion of the liquid marble seems to prevent cells from sedimentation and improves movements of cells which is critical for inducing cell–cell interactions and attachments. Vadivelu et al. demonstrated that the floating liquid marble prevents cell aggregation and is useful to generate co-cultures of olfactory ensheathing cells (OECs) with Schwann cells or astrocytes cells. Interestingly, the OECs exhibit cellular response resembling the native physiological behaviour by enwrapping other cells. In another study, 3D cell interaction was carried out by merging liquid marbles with different cellular entities. The major challenge of a floating liquid marble is stability and dispensability. The floating condition is prone to external motion and vibration. Hence, floating liquid marbles are fragile...
and can easily break. In this regard, as a step forward in the enhancement of its lifetime, a slow-evaporating liquid marble was developed by filling the liquid marble with a spherical hydrogel, Fig. 6(c). Our team observed that the presence of hydrogel reduced the evaporation process and maintains the integrity of the liquid marble for over 3 days. In addition, the hydrogel can be loaded with growth factors and chemotactic function to boost cell migration. The migrated cells assemble at the bottom surface, around the hydrogel sphere in a ring shape and subsequently forms a toroidal tissue.\textsuperscript{157} Besides the application in evaporation reduction, this platform can be adopted to further improve the method proposed by Serrano \textit{et al.} for cryoprotectant-free cryopreservation.\textsuperscript{160} Cryoprotectants such as dimethyl sulfoxide and glycerol are toxic. A liquid marble with embedded hydrogel containing serum can replace the harmful cryoprotectant. The release of serum during the thawing process facilitates the cell recovery.\textsuperscript{65}

**Microbioreactors for diagnostics and therapeutics**

Arbatan \textit{et al.} were the first to demonstrate the use of liquid marbles for blood typing.\textsuperscript{66} Blood was dropped on the hydrophobic powder of precipitated calcium carbonate to generate a blood liquid marble. Next, antibody solution (anti-A, anti-B and anti-D) was injected into the marbles. The reaction with the corresponding antigen and the agglutination process occurred immediately showing the separation of light and dark-red colours. The absence of separation indicates that there was no reaction. This method is simple, low-cost and does not require special tools. The encapsulation of blood samples prevents biohazard contamination and can be disposed of easily. In another study, Zhao \textit{et al.} used a magnet to move, close, and open a magnetic liquid marble, Fig. 6(d).\textsuperscript{92} This method allows automated measurement by moving the marble to different detection sites.\textsuperscript{92} The marble was encapsulated with human serum, upon opening the dopamine level was quantified using electrochemical detection and optical absorbance was used to conduct the glucose colorimetric assay. Injection of needles through the liquid marble shell did not disrupt the structure. Taking advantage of this feature, Oliveira \textit{et al.} scaled the liquid marble for high-throughput drug screening by detecting cell viability using AlamarBlue.\textsuperscript{68}

Droplet-based digital polymerase chain reaction (PCR) was recently possible with microfluidic technologies. These advanced PCR systems compartmentalise genetic materials in individual microdroplets. The amplification results of each individual droplet can then be analysed, hence the “digital” nomenclature. Digital PCR systems typically rely on large quantities of microdroplets (in the order of $10^4$ to $10^6$) to approximate the theoretical Poisson distribution. These droplets are suspended in a surfactant and transported in surface-treated microchannels to prevent droplet coalescence and wetting of the channel walls. As such, the efficacy of the PCR process depends on the microdroplets which in turn are highly sensitive to the surfactant and the protecting oil. Consequently, the surfactants are closely guarded as trade secrets in commercial platforms and are being sold at high prices that hinder the widespread use of this technology. To address these issues, Sreejith \textit{et al.} developed a liquid marble-based digital PCR platform.\textsuperscript{62} A humidified chamber was designed to maintain the liquid marble volume during thermal cycling. Unlike its droplet counterpart, liquid marbles are stable and more resistant to coalescence. To further enhance the stability of a liquid marble, the team demonstrated that composite liquid marbles containing photopolymers can be converted into core–shell beads. As the solid beads are non-porous, the product from the PCR can be stored for extended duration without the risk of cross-contamination. The PCR concept with core–shell beads generated from liquid marbles was successfully demonstrated by Gorgannezhad \textit{et al.} for the detection of different \textit{E. coli} strains.\textsuperscript{161}

**Perspectives and conclusions**

A relatively large body of works have been dedicated to understanding the fundamentals of liquid marbles. Tuning the properties of both the core liquid and the shell material can control their macroscopic properties. Further investigations are required to increase the lifetime of a liquid marble and to find a method to measure the effective surface tension with consistency. Knowledge from the fundamental study of liquid marbles would enable their practical applications as a digital microfluidics platform.

Replacing the porous shell with a solid shell would make a liquid marble more robust. This platform represents a core–shell bead with a liquid core. The use of core–shell beads as microreactors could offer a material saving solution for sample storage and significantly address the problem of plastic waste generated by chemical and biological laboratories. Although the generation of core–shell beads using composite liquid marble technology is well demonstrated in the laboratory, the lack of a commercially available, high-throughput, completely automated, compact, and reliable system for their synthesis is the limiting factor for their broad applications. Core–shell beads with a liquid core are potentially another novel digital microfluidics platform that requires more research effort in the near future.

In terms of manipulation of liquid marbles, external energy sources such as electric, magnetic, optical, acoustic and gravitational fields can be implemented in tools and automated systems that handle liquid marbles. The active manipulation schemes allow for precise control of the movement of liquid marbles and have great potential for automation. For large scale applications, emerging manipulation techniques utilising the surrounding conditions such as the temperature, pH value and surfactant concentration would allow for self-propulsion and random dispersion of liquid marbles.
In conclusion, liquid marbles have shown their promise as a digital microfluidics platform for microsensors and microreactors. Liquid marbles are relatively simple to synthesise and to handle, thus providing a cost-effective solution for many applications that previously relied on conventional digital microfluidics. The volume of a liquid marble can be adjusted from a few hundred nanolitres to a few microlitres. Therefore, a liquid marble could serve a versatile microreactor that utilises a minimum amount of reagents. The large variety of coating particles allows for the synthesis of liquid marbles with unique properties such as magnetic, fluorescent and stimuli-responsive, promising novel yet to be explored applications.

Author contributions

CHO wrote the introduction and compiled the initial contributions of the other authors. RV wrote the section on biological applications. JJ wrote the section on manipulation of liquid marbles. KRS wrote the section on fundamentals of liquid marbles. NKN wrote the section on chemical applications. NTN developed the overall paper structure and contributions of the other authors. RV wrote the section on magnetic, fluorescent and stimuli-responsive, promising liquid marbles. N.-T. Nguyen, Z. Li and Y. Gu, Adv. Sci., 2019, 6, 1802033.

Conflicts of interest

There are no conflicts to declare.

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