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Microfluidic Production of Perfluorocarbon-Alginate Core–Shell Microparticles for Ultrasound Therapeutic Applications

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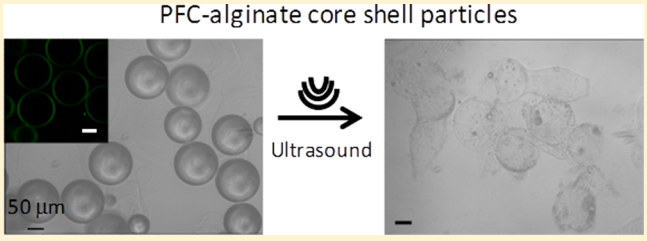
ABSTRACT: The fabrication of micrometer-sized core–shell particles for ultrasound-triggered delivery offers a variety of applications in medical research. In this work, we report the design and development of a glass capillary microfluidic system containing three concentric glass capillary tubes for the development of core–shell particles. The setup enables the preparation of perfluorocarbon-alginate core–shell microspheres in a single process, avoiding the requirement for further extensive purification steps. Core–shell microspheres in the range of 110–130 μm are prepared and are demonstrated to be stable up to 21 days upon immersion in calcium chloride solution or water. The mechanical stability of the particles is tested by injecting them through a 23 gauge needle into a polyacrylamide gel to mimic the tissue matrix. The integrity of the particles is maintained after the injection process and is disrupted after ultrasound exposure for 15 min. The results suggest that the perfluorcarbon-alginate microparticles could be a promising system for the delivery of compounds, such as proteins, peptides, and small-molecule drugs in ultrasound-based therapies.



INTRODUCTION

Nanoparticles and microparticles have been described for a large number of drug-delivery applications because these systems offer several advantages as controlled release systems and drug carriers. In particular, they can be administered to the patient by minimally invasive procedures to the target site, providing spatial control.[1] Another significant advantage of these systems is the flexibility to select sizes from the nano to the microscale, providing an easily adjustable surface area per volume, drug loading, and bioavailability. Extensive reviews on the application of microparticles in tissue engineering and regenerative medicine (TERM) have been published in the literature.[2−6] In this context, microparticles can be used per se but also may be used in combination with 3D matrices for the delivery of active agents. Furthermore, microparticles allow the development of complex delivery systems such as the sequential release of macromolecules from dual-release systems, providing a time-controlled delivery of different agents.[7−10]

The possibility to develop on–off delivery devices has been largely explored through different stimuli-triggered delivery devices.[11] These are particularly attractive for controlling the delivery of bioactive agents in a temporally controlled manner.[12−14] One way to explore these systems is by the use of ultrasound energy (US).[11,15] Additionally ultrasound can enhance intracellular delivery of the active compounds promoting tissue uptake. Ultrasound can also be used to promote healing, combined with the delivery of bioactive agents, such as cells, signaling molecules, or genes, enhancing the healing processes.[16] The development of ultrasound-triggered delivery devices has not been extensively investigated for TERM applications. In a recent work, Fabilli and coworkers reported the development of disperse perfluorocarbon-filled hydrogel microparticles that can be triggered by applying an acoustic field. The microparticles released the growth factors on demand in a spatiotemporally controlled manner upon stimulation by an acoustic field.[17] US enhances the release of active compounds from a polymeric system because of the response of the system to one of the following physical effects: pressure variation, acoustic fluid streaming, cavitation, and/or local hyperthermia.[15]

Different carriers such as micelles, liposomes, and microbubbles have been described in the literature as systems able to respond to external ultrasound stimuli. Such microparticles can be cavitated by ultrasound energy, acting as mediators through which the energy of pressure waves is concentrated, producing forces able to disrupt the particles.[18] Micelles and liposomes can be considered to be nanocarriers and have a typical average size in the 10–100 and 100–200 nm ranges, respectively. However, microbubbles are gas-filled particles that are 1–10 μm in diameter. Air and nitrogen have been used in the

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Table 1. Literature Overview of the Production of Alginate Particles Using Microfluidic Approaches

no.	microfluidic system	oil phase	type of particle	core fill	gelation	particle size (μm)	application	ref
1	PDMS microfluidic device	soybean oil	beads		internal external	50−110	cell encapsulation	24
2	PDMS microfluidic device	soybean oil	beads		external	50−60	immobilization of antibodies	29
3	glass capilary (cylindrical + square)	decanol	beads		external	100−200	drug delivery	30
4	microfluidic chip "snake mixer slide"	sunflower oil	beads		internal partial external	225−320	cell encapsulation	31
5	PDMS microfluidic device	soybean oil	beads		internal	20−80		32
6	PDMS microfluidic device	*n*-hexadecane	beads		CaCl2 coflow	60−95	cell encapsulation	33, 34
7	PDMS microfluidic device	oil	beads		external		smart drug delivery system	35
8	PDMS microfluidic device	sunflower seed oil	beads		external	60−105	drug delivery system	36
9	glass capilary	glycerol + tween 20	beads		external	60−230	cel encapsulation	37
10	PDMS microfluidic device	Novec 7500 fluorocarbon oil	beads		internal	25	cel encapsulation	38
11	PDMS microfluidic device	undecanol	beads microcapsules	polysterene beads dispersed in undecanol phase	external	30−230		39
12	glass capilary	oil	core−shell particles	oil	CaCl2 coflow	250−340	drug delivery system	40
13	PDMS microfluidic device		microbubbles	CO_2	internal	<10	US and magnetic ressonance imaging	25
14	3D microfluidic device prepared by rapid prototyping		beads	air	external	500-800	cell encapsulation	41

preparation of microbubbles. However, the preparation of these microbubbles presents several challenges because the particles dissolve rapidly when in contact with a liquid phase, thus compromising the stability of the system.

Perfluorocarbons (PFCs) present several advantages over air and nitrogen. The possibility to prepare liquid-PFC-filled microparticles from volatile PFCs such as perfluoropentane and perfluorohexane is particularly interesting.[19] The choice of the encapsulating material is crucial to the stabilization of the bubbles against coalescence and dissolution. The shell plays a major role in the mechanical stability of microparticles. The more elastic the shell material, the more acoustic energy that can be withstood for a longer time before bursting or breaking up. Various types of shell materials can be used, including proteins, carbohydrates, phospholipids, and biodegradable polymers. Polymers that are obtained from natural sources, such as polysaccharides and proteins, have been reported in numerous applications. These materials have been widely used in biomedical applications because they are renewable, produce degradable products, and are biocompatible.[20,21]

In this work, we focus on the application of alginate, a biopolymer derived from sea algae, that has been described for a large number of pharmaceutical and biomedical applications because of its biocompatibility and ease of gelation.[22] Conventional methods that are commonly used for the preparation of microbubble delivery systems include sonication, high-shear emulsification, and membrane emulsification.[23] However, these methods present significant disadvantages, namely, poor control over the particle size and distribution. To date, engineering core−shell microparticles remains a challenging task. Thus, there is a demand for new techniques that can enable control over the size, composition, stability, and uniformity of microparticles.[24,25] Microfluidic techniques offer great advantages in the fabrication of microparticles over the conventional processes because they require mild and inert processing conditions. Furthermore, particles produced from microfluidic systems present a narrow size distribution.[26−28] Research groups have reported the production of alginate particles using different microfluidic devices; however, only few report the development of capsules or core−shell particles, particularly from a glass capillary microfluidic device (Table 1).

Herein, we report a new fabrication technique for the development of "infusion-like" systems for local delivery triggered by ultrasound, which can potentially be used in various therapeutic applications. More specifically, we present the design and implementation of a new glass capillary microfluidic device to fabricate and engineer stable perfluorocarbon-biopolymer core−shell microspheres.

MATERIALS AND METHODS

Materials. Alginic acid sodium salt from brown algae was purchased from Fluka (Switzerland). Calcium chloride dihydrate powder was obtained from Mallinrock (Japan). Fluorescein 5(6) isothiocyanate (FITC), acrylamide (AC), *N*,*N*′-methylenebis-(acrylamide) (Bis), ammonium persulfate (APS) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamide (TEMED) and were purchased from Sigma-Aldrich. Phosphate buffer without calcium and magnesium ions was obtained from Caroning Cellgo (USA). Perfluorohexane (C6F14) 3 M Fluorinert Electronic Liquid FC-72 was purchased from 3 M (Germany). All chemical were used as received without further purification.

Methods. A new robust microfluidic device was designed for the fabrication of perfluorocarbon microspheres. Perfluorohexane and sodium alginate solutions (0.25 wt %) were injected using two independent syringe pumps at a constant flow rate. The microparticles

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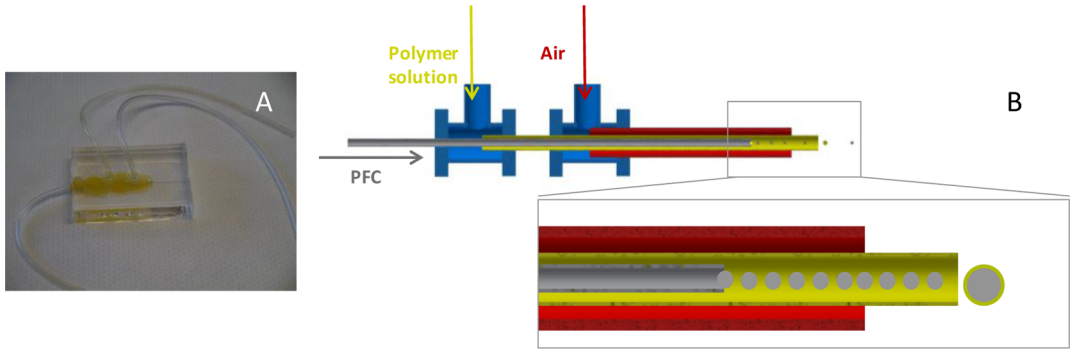

Figure 1. (A) Glass capillary microfluidic device designed. (B) Schematic representation of the microfluidic device designed.

were collected in a calcium chloride (2 M) cross-linking bath. We constructed a new microfluidic device (Figure 1) with the coaxial injection of multiple fluids.

The new device consisted of three concentric glass capillaries as shown in Figure 1. The inner capillary had an inner diameter (ID) of 50 μm and an outer diameter (OD) of 80 μm, the middle capillary had an ID of 150 μm and an OD of 250 μm, and the outer glass capillary tube had an ID of 800 μm. The device has been designed to allow the coaxial flow of three different solutions in each capillary, and the flow rates of each solution can be controlled by independent syringe pumps (Harvard Apparatus PHD 2000 infusion). The inner fluid was a perfluorocarbon, namely, perfluorohexane (C_6F_{14}). Perfluorohexane has a boiling point above room temperature, 57 °C, and a vapor pressure of 27 kPa. This liquid volatile PFC was selected in order to enhance particle stability and prevent the coalescence of the particles.[42] The middle aqueous phase was the polymeric solution, constituted of a solution of 0.25% w/v alginate.

A summary of the experiments performed is listed in Table 2.

Table 2. Summary of the Experiment Performed

		flow rate (μL/min)	
experiment no.	air (psi)	alginate	PFC
1	5	50	10
2	10	50	10
3	15	50	10
4	20	50	10
5	22.5	50	25
6	22.5	50	20
7	22.5	50	15
8	22.5	50	10
9	22.5	60	25
10	22.5	70	25

The particles were characterized by image analysis after observation under a Carl Zeiss Axivert 200 inverted microscope. Da.Vis 8.1.6 (LaVision, Germany) software was used to image the samples. ImageJ software was used to measure the mean particle size and distribution of the microspheres. Particles (80−100) were analyzed by ImageJ software for each experiment, and the mean particle size is represented as the average of the particle size ± the standard deviation of three independent experiments.

A statistical analysis of the data was conducted using IBM SPSS Statistics version 20 software. The Shapiro-Wilk test was employed to evaluate the normality of the data sets. Once the results obtained do not follow a normal distribution, a nonparametric test, namely, the Kruskal-Wallis test, was used to infer statistically significant differences. Differences between the groups with $p < 0.05$ were considered to be statistically significant.

A Zeiss LSM710 confocal microscope (Carl Zeiss Microscopy, Thornwood, NY) was used to image the stability of microparticles that containing FITC over a period of 21 days.

Acrylamide gels were prepared according to standard protocols described in the literature.[43] Briefly, a solution containing 522 μL of water, 150 μL of AC solution (30%), 120 μL of Bis solution (0.3%), 8 μL of APS (10%), and 2 μL of TEMED was prepared. After stirring, 200 μL of the final solution was dispensed in a 96-well plate. The samples were allowed to polymerize for 24 h before they were used in further experiments.

RESULTS AND DISCUSSION

Microfluidic devices can be prepared from glass capillary tubes or by microfabrication techniques such as the soft lithography-based fabrication of poly(dimethylsiloxane) (PDMS) devices. Glass capillary microfluidics is an advantageous technique for preparing devices for particle production at high rates with controlled particle sizes and a narrow size distribution. Different designs have been reported in the literature.[29,44,45] Most of the glass capillary microfluidics systems described are based on a circular glass capillary inserted in a square capillary. The major constraint of these devices is their limited ability to inject only two different fluids at the same time. In this work, we develop a new robust microfluidic device designed to promote the injection of multiple fluids coaxially as described in the Methods section.

The rheological properties of the alginate solution, including the intrinsic viscosity of the polymeric solution, are an important aspect to consider. Cooper and coworkers report that for a very low viscosity polymeric solution the elasticity of the polymeric solution will affect droplet formation in a drop or capillary breakup process.[27,46] Furthermore, it has been reported in the literature that both the polymer molecular weight and polymer concentration in solution affect the breakup dynamics. Solutions with a higher extensional viscosity and relaxation time are more effective at retarding breakup.[47,48] However, the hydrodynamic resistance on the capillary tubing in microfluidic systems depends linearly upon the viscosity of the solution, thus the relevance to the understanding of the viscoelastic properties of the polymeric solution. In particular, alginate solutions present non-Newtonian behavior and are considered to be complex fluids. Small amounts of alginate in water lead to a drastic increase in the viscosity of the solutions.[49] Three viscosity regimes can be identified as a function of the polymer concentration in solution: dilute, semidilute unentangled, and semidilute entangled.[50] The choice of the alginate concentration was such that the solution presents a diluted regime, i.e., there are no interactions or overlapping of the polymeric chains. At 0.25% w/v, the viscosity of the solution was calculated to be 5.31 mPa·s, and this viscosity corresponds to a pressure drop of approximately 2.2 bar within the glass capillary tube for the highest flow rate

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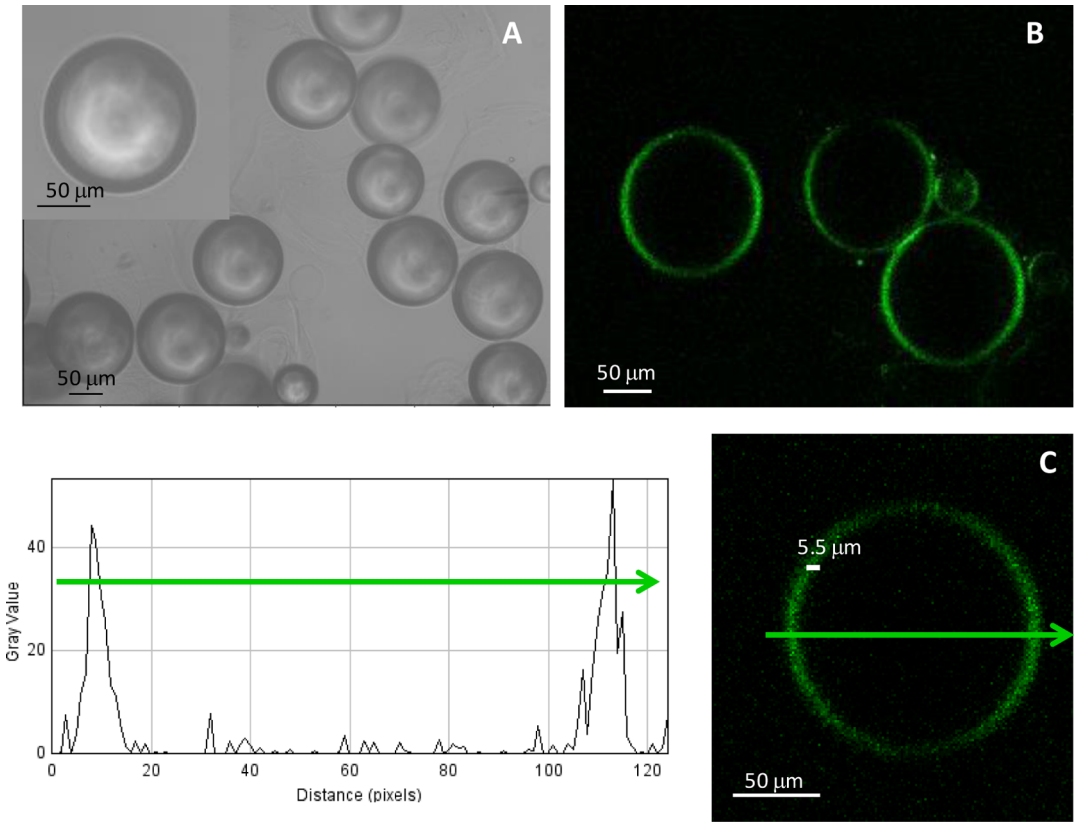

Figure 2. (A) Optical and (B, C) confocal microscopy images of the perfluorocarbon-alginate core−shell particles. Particles were prepared at a 10 μL/min PFC flow rate, a 50 μL/min polymer solution flow rate, and 10 psi of air flow. (C) Representative profile of fluorescence intensity for the PFC-alginate core−shell particle presented.

tested. Air is allowed to flow through the outer tube, and the air pressure is controlled by a pressure gauge.

Different microfluidic geometries, such as a T-junction or cross-junction, were tested for the preparation of various alginate systems for different applications. The papers reported in the literature refer to the preparation of alginate microspheres from multiple emulsions using different oil phases (Table 1), including sunflower oil,[31] soybean oil,[22,29] *n*-decanol with 5 wt % Span 80,[30] and an acidic oil solution.[38,51] However, these systems refer mostly to the preparation of beads and not to capsules or core−shell alginate microparticles. Other microfluidic approaches have been reported in the literature for the preparation of liquid core−shell particles. In particular, double or multiple emulsion droplets of water in oil in water (W/O/W) or oil in water in oil (O/W/O) have been described.[26,51]

To the best of our knowledge, the microfluidic preparation of alginate microcapsules has been demonstrated by Zhang and coworkers for the first time.[39] In their work, they present as a proof of concept the possibility of preparing core−shell particles from a Y-shaped microfluidic device prepared by a soft lithography method. The preparation of gas core−shell alginate particles has been reported by Park et al., who describe a new approach to the development of carbon dioxide-filled alginate microbubbles as imaging agents.[25] In this work, we propose the preparation of perfluorocarbon-alginate core−shell microspheres using a simple glass capillary microfluidic device and a single oil-in-water (O/W) emulsion where no additional stabilizing agents and/or surfactants are required. The system studied consisted of an inner oil phase that formed droplets in the aqueous polymeric phase that in turn formed spheres when coaxially sprayed with air. The microspheres were precipitated in a cross-linking solution of calcium chloride (2 M). The methodology developed provides a simple technique for the preparation of liquid-core particles and eliminates the need for any subsequent washing or purification steps to remove the oil phase, with a production rate of approximately 200 particles/min. The microspheres were collected and kept in $CaCl_2$ solution. PFC particles were processed as a control, following the same procedure but using water as the middle fluid instead of the polymeric solution. As a result, small droplets of PFC were dispersed in $CaCl_2$ solution. After some time, the particles start to coalesce and larger particles of PFC are observed, indicating that PFC droplets per se are not stable in an aqueous solution.

Figure 2A presents an optical microscope image of the microspheres prepared. After stable spheres were produced, the shell-like structure was visualized by dispersing FITC within the polymeric shell. Confocal microscopy (Figure 2B) reveals the alginate shell covering the inner liquid core of PFC. The green signal of FITC, present in the aqueous polymeric phase, is observed in a thin concentric circle. The profile of fluorescence intensity provided strong evidence that FITC is contained within the polymeric shell of the particles. The thickness of the shell is 5.5 ± 1.3 μm on the basis of ImageJ analysis of the confocal images (Figure 2C). The images demonstrate that the particle is composed of two different phases in addition to proving the successful encapsulation of the perfluorcarbon in the alginate shell. These images show the feasibility of preparing core−shell particles from the newly designed

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capillary glass microfluidic device. Although the particles consist of a hydrophobic core and an aqueous shell, it is also possible to fabricate particles having a inner hydrophilic core and a hydrophobic shell using the same device.

In a liquid−liquid flow, capillary instabilities produce segmented flows with uniform droplet size and depend on the superficial velocities, inlet geometry, and wetting properties of the microfluidic channel.[52,53] Particles from microfluidic devices are generated in either dripping or jetting regimes, depending on the balance between the applied forces and the surface tension forces. The effect of the flow rate of the inner and middle flows and air flow on the particle size and particle size distributions was studied under different conditions.

The air flow rate is the parameter most affecting the particle size and size distribution of the alginate spheres (Figure 3). For

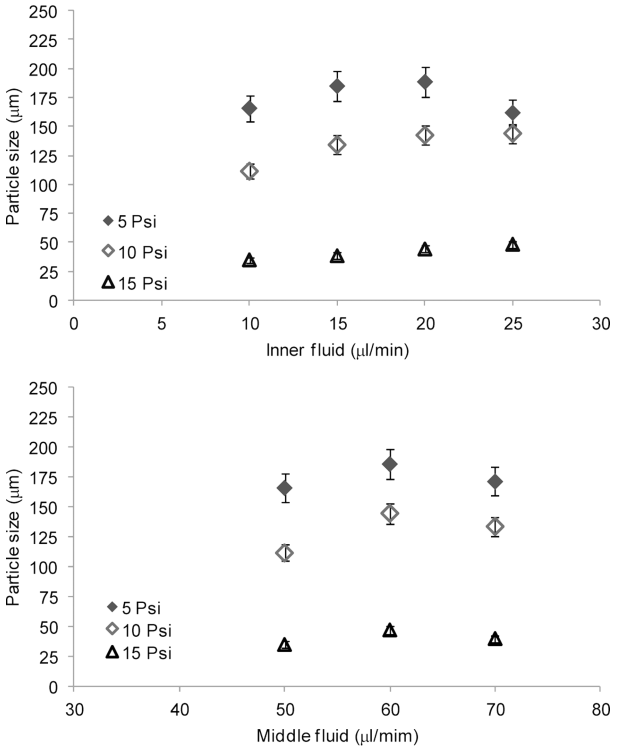

Figure 3. Effect of processing conditions (inner fluid flow, middle fluid flow rate, and air flow) on the average particle size of microspheres prepared, with the different symbols corresponding to different air flow ratios: (♦) 5, (◊) 10, and (△) 15 psi.

all conditions tested, the average particle size decreases with increasing air flow. The particle size distribution is broader for samples prepared at 15 psi, and the samples are not as homogeneous as the samples prepared at 5 or 10 psi. The inner and middle fluid flows of PFC and alginate solution are the parameters that determine whether the system is flowing in the dripping or jetting regime, respectively.[54,55] According to our results, under the conditions tested, changing the inner and middle flows did not produce significant differences in the particle size of the microspheres obtained.

Particles were externally cross-linked in a calcium chloride bath. Ionic cross-linking of alginate is the most common approach to producing hydrogels, even though it may lead to poor stability of the materials in some cases.[22] For this reason, alginate gelation has been the object of different studies because the gelation rate is a crucial factor that controls gel strength and homogeneity. Several authors have described the effect of the gelation method in the stability of alginate particles prepared by microfluidics. Zhang et al. reported the differences observed in alginate particles cross-linked by an internal or external gelation method.[22] Although upon external gelation the particles are precipitated in a solution containing calcium ions, upon internal gelation the continuous phase carries Ca^{2+} in the form of calcium carbonate and the cross-linking will be triggered by a change in the pH of the solution. Their findings suggest that alginate particles cross-linked by an external gelation method would be more stable than the ones prepared by an internal gelation method with an elastic modulus similar to those of other alginate beads prepared by conventional methodologies. Capreto and coworkers have studied the effect of three different gelation methods of alginate particles and have concluded that spherical, smooth monodisperse particles were preferentially produced by a partial gelation method that consisted of the addition of barium ions to the alginate solution.[4] In their work, when an external gelation method was tested, the particles presented a tail-like structure, but this was not observed in the case of our experiments. Another work by Hu et al. reports the differences in particle morphology depending on the cross-linking solution and height from the tip of the microfluidic device to the solution.[30] We tested the influence of the distance from the tip of the microfluidic device to the cross-linking solution bath on the geometry and morphology of the particles prepared and did not observe significant differences. Furthermore, the particle size of the core−shell particles produced was not affected by this parameter. The height between the tip of the microfluidic device and the solution was hereafter adjusted to 3 cm in all experiments.

The poor stability of alginate materials in solution has been reported to be mostly due to the exchange of ions from the matrix to the solution. It is therefore important to determine the stability of the microspheres produced. The stability of the microspheres was evaluated during 21 days in different solutions. Particles prepared at a 10 μL/min PFC flow rate, a 50 μL/min ALG solution, and 10 psi (air flow) were immersed in 500 μL of calcium chloride (2 M) solution, phosphate buffer (PBS), and water. At days 0, 7, 14, and 21 the samples were analyzed by optical and confocal microscopy and the particle size and particle size distribution were evaluated (Figure 4).

The stability experiments demonstrate that the microspheres are stable for up to 21 days in calcium chloride or water. As a control, PFC particles in the absence of polymer were sprayed into $CaCl_2$ solution and observed. PFC particles coalesced into larger droplets in 24 h, indicating that the presence of the shell is essential to the preservation of particle size. The presence of the alginate shell was further confirmed by confocal microscopy. In PBS, however, the particles are not stable and degrade after 1 day of immersion. The poor stability of alginate materials ionically cross-linked with calcium ions in phosphate buffer solutions has been reported by other authors. Ionic cross-linking of alginate molecules results from the chelation of two alginate molecules by a calcium ion. In the presence of monovalent ions, there will be a competition between these and Ca^{2+}, i.e., there will be an exchange of Ca^{2+} by the monovalent ions and consequently the loss of mechanical properties of the materials and eventually disintegration of the structures.[22,56]

The results demonstrate, nonetheless, that the methodology proposed for the development of perfluorocarbon-filled alginate microspheres leads to the preparation of particles with a long shelf life, overcoming disadvantages of other technologies

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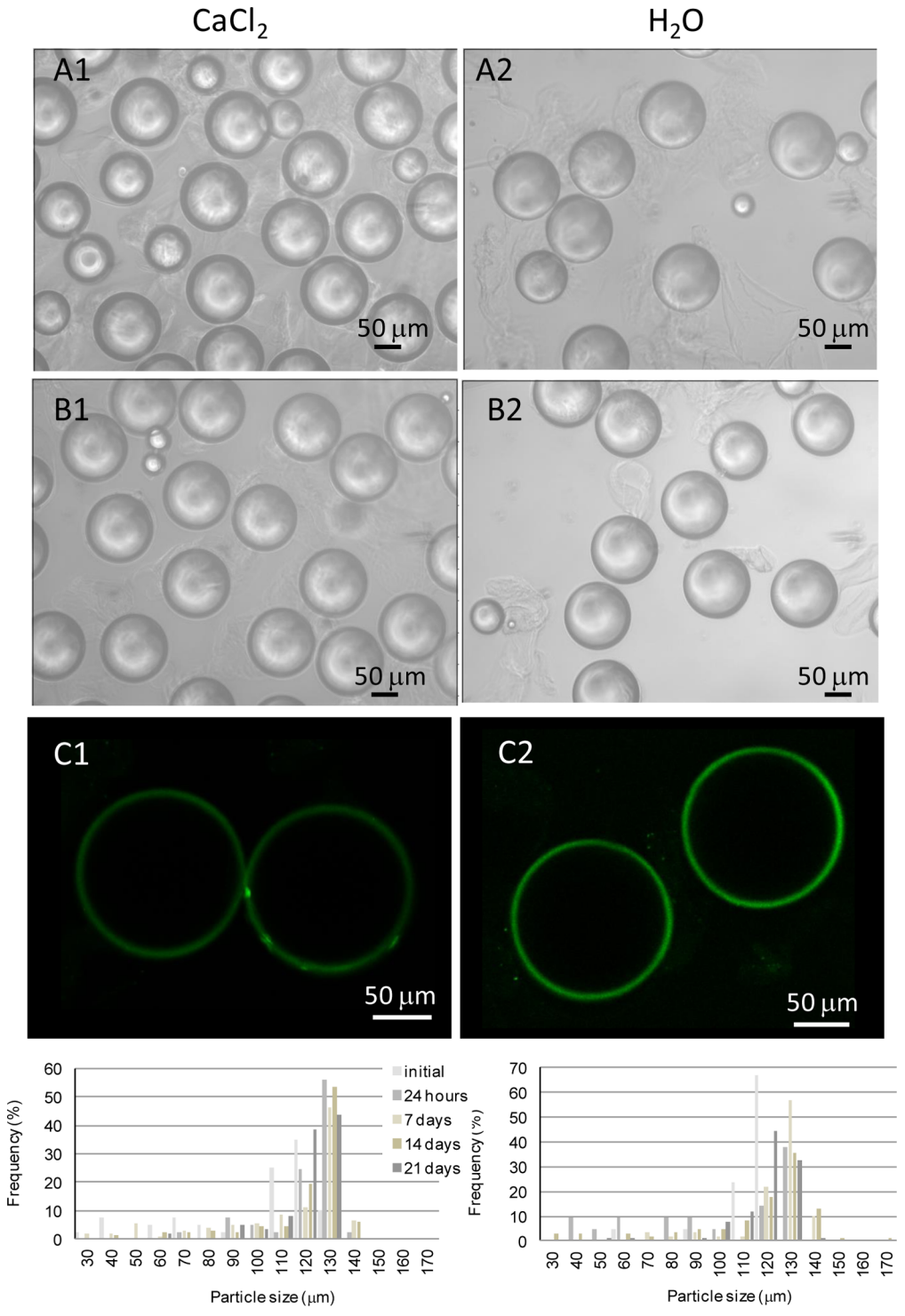

Figure 4. (A) Optical images of initial particles. (B) Optical images after 14 days of immersion (C) Confocal microscope images of particles immersed after 12 days and particle size distribution of particles immersed in calcium chloride and water as a function of immersion time.

previously used for the preparation of this type of system. The alginate shell further provides a barrier that is able to prevent PFC diffusion and evaporation and the collapse of the particles for up to 21 days.

Statistical analysis performed on the data revealed that no significant differences were observed for the average particle size of the microspheres for up to 21 days of immersion (Table 3). The particle size distribution (Figure 4C) was also not affected throughout the length of this study.

Perfluorocarbon-filled particles are interesting for ultrasound-triggered delivery systems. To demonstrate the ability to disrupt the PFC-loaded microspheres by ultrasound, the particles prepared at a 10 μL/min PFC flow rate, 50 μL/min ALG solution, and 10 psi (air flow) were exposed to ultrasound for 15 min (Figure 5).

From the images, we can observe the breakup of the particles after 15 min of ultrasound exposure. In this system, perfluorohexane undergoes a liquid–gas phase transition

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Table 3. Average Particle Size (μm) of Microspheres in Solution

	$CaCl_2$		water	
time (days)	average	SD	average	SD
0	119.0	4.9	124.5	3.3
0.1	122.3	0.2	116.7	11.8
0.2	124.6	0.1	122.8	0.2
1	126.4	0.3	106.8	23.7
2	121.3	0.2	119.7	3.3
7	117.2	1.8	118.5	9.2
14	118.7	9.5	119.2	1.0
21	123.5	1.4	123.5	0.1

promoted by an increase in local temperature and acoustic energy provided locally after ultrasound application, leading to the disruption of the particles.

To demonstrate the mechanical strength of the particles and simulate the intramuscular injection without disruption, acrylamide gels were prepared following a protocol described by Park.[43] Acrylamide gels are commonly used as phantom matrices for needle insertion studies because they can mimic different tissue properties in terms of mechanical and acoustic properties.[57] The mechanical properties of the gels prepared present an elastic modulus G' on the order of 2 kPa, according to the results presented by Calvet et al.[58] These are in good agreement with the literature values reported for muscle.[59] Microspheres loaded with FITC were injected using a 23 gauge needle in the gel and were observed under optical and confocal microscope before and after US exposure (Figure 6).

Optical and confocal images of the particles injected within the acryalmide gel demonstrate that the perfluorocarbon-alginate microspheres have enough resistance to be used as an injectable system. The particles were able to resist manipulation and present intact morphology within the gel just like they were in the solution presented previously in Figure 2. The optical and confocal microscopy images provided complementary information in the case of the particles after US exposure. The optical images indicated the presence of smaller PFC droplets within the gel, proving the disruption of the alginate spheres. By confocal microscopy, the round alginate particles are no longer observed, and instead a blurry green image was observed.

The homogeneous FITC dispersion within the alginate shell, observed by confocal microscopy, provided strong evidence that the incorporation of another molecule in this FITC system

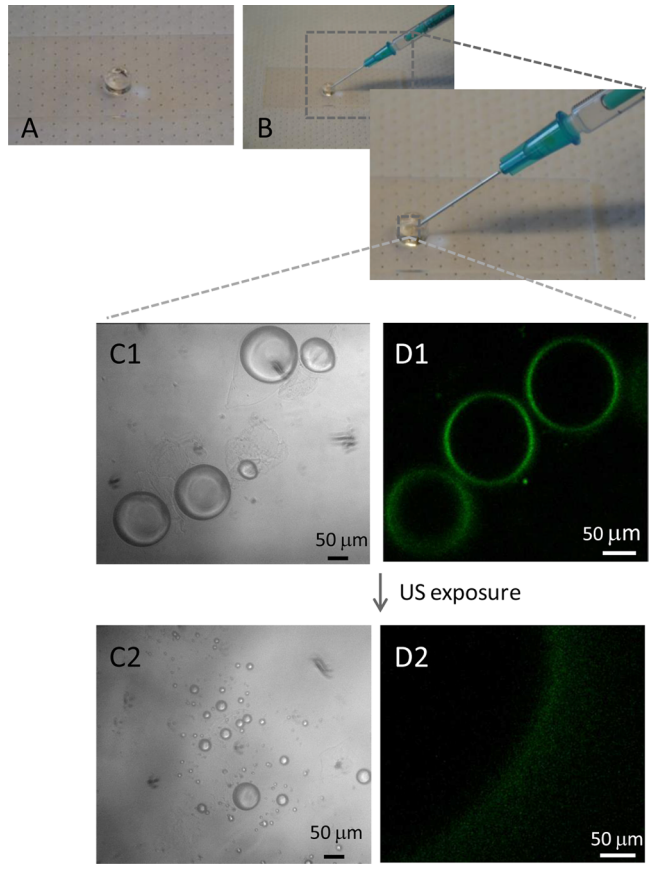

Figure 6. (A) Acrylamide gel. (B) Injection of perfluorocarbon-alginate particles within a gel. (C) Optical and (D) confocal microscopy images of the encapsulated particles before (C1, D1) and after (C2, D2) ultrasound exposure.

did not affect the preparation of the microspheres, suggesting that more complex systems can be prepared using the microfluidic device designed and presented in this work. As future perspectives, we envisage the possibility of loading proteins, growth factors, or other hydrophilic molecules dispersed in the shell and the encapsulation of hydrophobic molecules in the liquid core. Upon exposure to US, the particles will be disrupted and the active compounds will be immediately delivered to the site of action. It may also be possible to generate microparticles with sustained release properties using our setup. The work presented can potentially lead to new

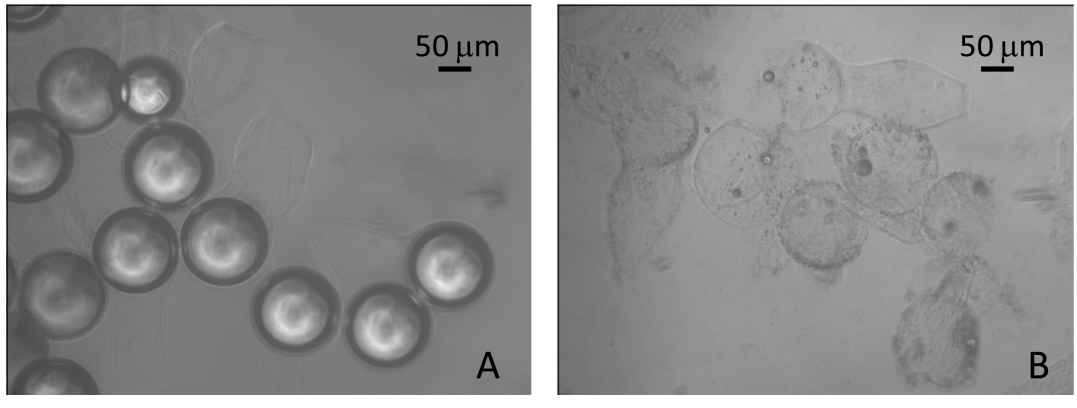

Figure 5. Optical microscope images of the alginate microspheres (A) before and (B) after ultrasound exposure.

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approaches in therapies by integrating local and targeted delivery of the active agents encapsulated within the microparticles with low-pulsatile ultrasound.

CONCLUSIONS

A new microfluidic device based on three concentric glass capillary tubes was designed and implemented for the preparation of perfluorocarbon-filled alginate microspheres. The results demonstrate that the core−shell microspheres prepared have an average particle-size diameter of 120 μm. The presence of the outer shell was proven by confocal microscopy. The particles prepared following the proposed methodology are intact for up to 21 days when immersed in calcium chloride solution or water. The disruption of the particles can be triggered by ultrasound exposure as the perfluorohexane undergoes a liquid−gas phase transition offering potential advantages in regenerative therapies. Furthermore, we have proven that the particles maintained their integrity upon injection in a hydrogel matrix, mimicking intramuscular injection, and that the injected microspheres can be disrupted after ultrasound exposure. The work presented herein may open new possibilities in ultrasound regeneration therapies, providing systems for the simultaneous delivery of hydrophilic and hydrophobic active compounds such as proteins, growth factors, cells, and anti-inflammatory agents.

AUTHOR INFORMATION

Notes

The authors declare no competing financial interest.

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