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Microfluidic production of perfluorocarbon-alginate core-shell microparticles for ultrasound therapeutic applications

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Abstract
The fabrication of micron size 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 set-up 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 the ultrasound exposure for 15 minutes. 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.

Keywords: microfluidics; ultrasound; microparticles; alginate; perfluorocarbon

Introduction

Nano and microparticles have been described for a large number of drug delivery applications as these systems offer several advantages as controlled release systems and drug carriers, especially they can be administered to the patient by minimally invasive procedures to the target site providing spatial control. Another significant advantage of these systems is the flexibility to select sizes from nano to micro-scale providing 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. In this context, microparticles can be used per se, but also may be used in combination with 3D matrices for delivery of active agents. Furthermore, microparticles allow the development of complex delivery systems such as sequential release of macromolecules from dual release systems providing a time controlled delivery of different agents.

The possibility to develop on-off delivery devices have been largely explored through different stimuli triggered delivery devices. These are particularly attractive to control the delivery of bioactive agents in temporally controlled manner. One way to explore these systems is by the use of ultrasound energy (US). Additionally ultrasound can enhance intracellular delivery of the active compounds promoting tissue uptake. Ultrasound can also be used to promote healing, combined with delivery of bioactive agents, such as cells, signaling molecules, or genes, enhancing the healing processes. The development of ultrasound triggered delivery devices has not been extensively investigated for TERM applications. In a recent work, Fabilli and co-workers reported the development of disperse perfluorocarbon
filled hydrogel microparticles which can be triggered by applying an acoustic field. The microparticles released the growth factors on-demand in a spatio-temporal controlled manner upon stimulation by an acoustic field. US enhances the release of active compounds from a polymeric system due to the response of the system to one of the following physical effects: pressure variation, acoustic fluid streaming, cavitation and/or local hyperthermia. Different carriers such as micelles, liposomes or 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. Micelles and liposomes can be considered as nanocarriers and have a typical average size in the 10-100 nm and 100-200 nm range, respectively. On the other hand, microbubbles are gas-filled particles with 1-10 μm in diameter. Air and nitrogen have been used in the preparation of microbubbles. However, the preparation of these microbubbles presents several challenges as the particles dissolve rapidly when in contact with a liquid phase, thus compromising the stability of the system.

Perfluorocarbons (PFC’s) present several advantages over air and nitrogen. Particularly interesting is the possibility to prepare liquid PFC filled microparticles from volatile PFC’s such as perfluoropentane and perfluorohexane. The choice of the encapsulating material is crucial for the stabilization of the bubbles against coalescence and dissolution. The shell plays a major role in the mechanical stability of microparticles. The more elastic shell material is, the more acoustic energy withstanding longer 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 as they are renewable, produce degradable products, and are biocompatible.
In this work we focus on the application of alginate, a biopolymer derived from sea algae, which has been described for a large number of pharmaceutical and biomedical applications particularly due to its biocompatibility and ease of gelation.\(^2\) Conventional methods that are commonly used for the preparation of microbubble delivery systems include sonication, high-shear emulsification and membrane emulsification.\(^3\) However, these methods present significant disadvantages, namely poor control over 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 size, composition, stability, and uniformity of microparticles.\(^{24,25}\) Microfluidic techniques offer great advantages in fabrication of microparticles over the conventional processes, as 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).
<table>
<thead>
<tr>
<th>#</th>
<th>Microfluidic system</th>
<th>Oil phase</th>
<th>Type of particle</th>
<th>Core fill</th>
<th>Gelation</th>
<th>Particle size (µm)</th>
<th>Application</th>
<th>Ref</th>
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<tr>
<td>1</td>
<td>PDMS microfluidic device</td>
<td>Soybean oil</td>
<td>Beads</td>
<td>-</td>
<td>Internal External</td>
<td>50-110</td>
<td>Cell encapsulation</td>
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<td>Drug delivery</td>
<td>30</td>
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<td>CaCl2 co-flow</td>
<td>60-95</td>
<td>Smart drug delivery system</td>
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<td>-</td>
<td>External</td>
<td>60-105</td>
<td>Drug delivery system</td>
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<td>External</td>
<td>60-230</td>
<td>Cell encapsulation</td>
<td>36</td>
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<tr>
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<td>Glass capillary</td>
<td>Glycerol + tween 20</td>
<td>Beads</td>
<td>-</td>
<td>External</td>
<td>60-230</td>
<td>Drug delivery system</td>
<td>37</td>
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<td>PDMS microfluidic device</td>
<td>undecanol</td>
<td>Beads Microcapsules</td>
<td>Poly-stereene beads dispersed in undecanol phase</td>
<td>External</td>
<td>30-230</td>
<td>-</td>
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<td>12</td>
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<td>Oil</td>
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<td>&lt;10</td>
<td>US and magnetic resonance imaging</td>
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<td>3D microfluidic device prepared by rapid prototyping</td>
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<td>Beads</td>
<td>Air</td>
<td>External</td>
<td>500-800</td>
<td>Cell encapsulation</td>
<td>41</td>
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</tbody>
</table>

Table 1: Literature overview on the production of alginate particles using microfluidic approaches.
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), Amonium persulfate (APS) and N,N,N',N'-tetramethylethlenediamide (TEMED) and were purchased from Sigma-Aldrich. Phosphate buffer without calcium and magnesium ions was obtained from Caroning Cellgo (USA). Perfluorohexane (C6F14) 3M™ Fluorinert™ Electronic Liquid FC-72, was purchased from 3M (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 were collected in a calcium chloride (2M) crosslinking bath. We constructed a new microfluidic device (figure 1) with coaxial injection of multiple fluids.

The new device consisted on 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\textsubscript{6}F\textsubscript{14}). Perfluorohexane has a boiling point above room temperature, 57 \degree C and vapor pressure of 27 kPa. This liquid volatile PFC was selected in order to enhance particle stability and prevent the coalescence of the particles. \textsuperscript{42} The middle aqueous phase was the polymeric solution, constituted by a solution of alginate with 0.25 \% (w/v).

![Image](image.png)

**Figure 1:** (A) Glass capillary microfluidic device designed, (B) Schematic representation of the microfluidic device designed

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

**Table 2: Summary of the experiment performed**

<table>
<thead>
<tr>
<th>Experiment #</th>
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<tr>
<td>10</td>
<td>22.5</td>
<td>70</td>
<td>25</td>
</tr>
</tbody>
</table>
The particles were characterized by image analysis after observation under an inverted microscope Carl Zeiss axivert 200. DaVis 8.1.6 (LaVision, Germany) software was used to image the samples. Image J software was used to measure the mean particle size and distribution of the microspheres. 80-100 particles were analyzed by ImageJ software for each experiment and the mean particle size is represented as the average of the particle size ± standard deviation of three independent experiments.

Statistical analysis of the data was conducted using IBM SPSS Statistics version 20 software. Shapiro-Wilk test was employed to evaluate the normality of the data sets. Once the results obtained do not follow a normal distribution, non-parametric tests, namely Kruskal-Wallis test were used to infer statistical 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 \( \mu l \) water, 150 \( \mu l \) AC solution (30%), 120 \( \mu l \) Bis solution (0.3%), 8 \( \mu l \) APS (10%) and 2 \( \mu l \) TEMED was prepared. After stirring 200 \( \mu l \) of the final solution was dispensed in a 96-well plate. The samples were allowed to polymerize for 24 hours 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 soft lithography-based fabrication of poly(dimethylsiloxane) (PDMS) devices. Glass capillary microfluidics is an advantageous technique to prepare devices for particle production at high rates with controlled particle sizes and 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
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 break-up process. Furthermore, it has been reported in the literature that both polymer molecular weight and polymer concentration in solution affect the break-up dynamics. Solutions with higher extensional viscosity and relaxation time are more effective at retarding break-up. On the other hand, the hydrodynamic resistance on the capillary tubing, in microfluidic systems depends linearly upon the viscosity of the solution, thus, the relevance on the understanding 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. Three viscosity regimes can be identified as a function of the polymer concentration in solution: dilute, semi-dilute unentangled, and semi-dilute entangled. The choice of the alginate concentration was so 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 to this viscosity corresponds a pressure drop of approximately 2.2 bars within the glass capillary tube, for the highest flow rate tested. Air is flown through the outer tube and the air pressure is controlled by a pressure gauge.

Different microfluidic geometries, such as 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 \textsuperscript{31}, soybean oil \textsuperscript{22,29}, n-decanol with 5 wt. % span 80 \textsuperscript{30} or acidic oil solution \textsuperscript{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. Particularly, double or multiple emulsion droplets of water-in-oil-in-water (W/O/W) or oil-in-water-in–oil (O/W/O) double have been described. \textsuperscript{26,51}

To the best of our knowledge microfluidic preparation of alginate microcapsules has been demonstrated by Zhang and coworkers for the first time.\textsuperscript{39} In their work they present as proof of concept the possibility of preparing core-shell particles from a Y shaped microfluidic device prepared by soft-litography method. The preparation of gas core-shell alginate particles has been reported by Park et al who describe a new approach for the development of carbon dioxide filled alginate microbubbles as imaging agents.\textsuperscript{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” which formed droplets in the aqueous polymeric phase that in turn formed spheres when coaxially sprayed with air. The microspheres were precipitated in a crosslinking solution of calcium chloride (2M). 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/minute. The microspheres were collected and kept in CaCl\textsubscript{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\textsubscript{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 2 (A) 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 2 (B) reveal 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 fluorescent intensity provided strong evidence that FITC is contained within the polymeric shell of the particles. The thickness of the shell is $5.5 \pm 1.3 \, \mu m$ based on Image J 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 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.
Figure 2: Optical (A) and Confocal (B, C) microscopy images of the perfluorocarbon-alginate core-shell particles. Particles were prepared at 10 µL/min PFC flow rate; 50 µL/min polymer solution flow rate and 10 Psi air flow. (C) Representative profile of fluorescent intensity for the PFC-alginate core-shell particle presented.

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. \textsuperscript{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 particle size and particle size distribution was studied at different conditions.
Figure 3: Effect of processing conditions (inner fluid flow, middle fluid flow rate and air flow) on the average particle size of microspheres prepared, the different symbols correspond to different air flow ratios (♦) 5 Psi (◊) 10 Psi and (Δ) 15 Psi.

The air flow rate is the parameter most affecting the particle size and size distribution of the alginate spheres (Figure 3). For 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 flow of PFC and alginate solution, respectively are, the parameters that determine whether the system is flowing in the dripping or jetting regime. According to our results, under the conditions tested, changing the inner and middle flow did not produce significant differences in the particle size of the microspheres obtained.

Particles were externally cross-linked in a calcium chloride bath. Ionic crosslinking of alginate is the most common approach to produce hydrogels, even though it may lead to poor stability of the materials in some cases. For this reason, alginate gelation has been object of different studies as the gelation rate is a crucial factor that controls gel strength and homogeneity. Several authors have described the effect of gelation method in the stability of alginate particles prepared by microfluidics. Zhang et al reported the differences observed in alginate particles crosslinked by an internal or external gelation method. While on the external gelation the particles are precipitated in a solution containing the calcium ions, on the internal gelation the continuous phase carries the Ca²⁺, 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 a similar elastic modulus to other alginate beads prepared by conventional methodologies. Capreto and coworkers have studied the effect of three different gelation methods of alginate particles and concluded that the production of
spherical, smooth monodisperse particles were preferentially produced by a partial gelation method which consisted in the addition of barium ions to the alginate solution.\textsuperscript{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 crosslinking solution and height from tip of the microfluidic device to the solution.\textsuperscript{30} We tested the influence of the distance from the tip of the microfluidic device to the crosslinking 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 to 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 10 $\mu$l/min PFC flow rate, 50 $\mu$l/min ALG solution and 10 Psi (air flow) were immersed in 500 $\mu$l of calcium chloride (2M) 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 was evaluated (Figure 4).
Figure 4: (A) Optical images of initial particles. (B) Optical images of 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.
The stability experiments demonstrate that the microspheres are stable 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 bigger droplets in 24h, indicating that the presence of the shell is essential for 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 one day immersed. The poor stability of alginate materials ionically crosslinked with calcium ions in phosphate buffer solutions has been reported by other authors. Ionic crosslinking 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 lead to the preparation of particles with long shelf-life overcoming disadvantages of other technologies previously used for the preparation of this type of systems. The alginate shell further provides a barrier, which is able to prevent PFC diffusion and evaporation and the collapse of the particles up to 21 days.

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

**Table 3: Average particle size (µm) of microspheres in solution**

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<tr>
<th>Time</th>
<th>CaCl$_2$</th>
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</table>


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</tbody>
</table>

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 10 µl/min PFC flow rate, 50 µl/min ALG solution and 10 Psi (air flow) were exposed to ultrasound for 15 minutes (Figure 5).

![Figure 5: Optical microscope images of the alginate microspheres before (A) and after (B) ultrasound exposure](image)

From the images we can observe the break-up of the particles after 15 minutes of ultrasound exposure. In this system, perfluorohexane undergoes a liquid-gas phase transition 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. Acrylamide gels are commonly used as phantom matrices for needle insertion studies as they can mimic different tissue properties, in terms of mechanical and acoustic properties. The mechanical properties of the gels prepared present an elastic modulus $G'$ in the order of 2kPa, according to the results presented by Calvet et al. These are in good agreement with the literature values reported for muscle. 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).
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 and D1) and after (C2 and D2) ultrasound exposure.

Optical and confocal images of the particles injected within the acrylamide 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 green blurry 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 system FITC 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 to load 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 immediately delivered to the site of action. It may also be possible to generate microparticles with sustained release properties using our set-up. The work presented can potentially lead to new 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 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.

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