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Technical Note

An electro-coalescence chip for effective emulsion breaking in droplet microfluidics

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Droplet-based microfluidics is increasingly used for biological applications, where the recovery of cells or particles after an experiment or assay is desirable. Here, we present an electro-demulsification chip which circumvents the use of harsh chemicals and multiple washing/centrifugation steps and offers a mild way for extracting cells and polymer particles into an aqueous phase from microfluidic water-in-oil emulsions.

The rapid development of droplet-based microfluidics provides new tools for chemical and biological research in picoliter reaction volumes.[1] It has proven to be an invaluable platform for single cell analysis [2] and novel microstructure fabrication.[3] In many of these applications, stable monodisperse water-in-oil emulsions are first generated by droplet microfluidics in the presence of surfactants.[4] However, at a later stage droplet content such as cells or beads [5]often needs to be extracted into a continuous aqueous phase for analytical purposes or for further culturing cells.[6] In most of the examples to date emulsion breaking is realized by the use of chemicals, e.g. perfluorooctanol to disrupt emulsion stability, in combination with several washing/centrifugation steps to completely remove the oil.[4,7] This multi-step procedure is not only time-consuming, but may also be harmful to cells or damage fragile particles. As an alternative, the use of an amphiphilic enzyme for direct transfer of droplet content into the water phase was recently reported.[8] But the re-arrangement of surfactants and proteins appeared to take a long time (24 hrs) and the general application of this method also needs to be further verified. A more robust and general method to manipulate droplets is via the use of electrical fields: droplets can be efficiently sorted and fused at high speeds.[9-13] Here, we report a universal, mild demulsification procedure for the rapid extraction of contents from large volumes of emulsions using a simple electro-coalescence chip. Our method is based on electro-coalescence between water-in-oil emulsion plugs and aqueous spacer plugs. These 'emulsion of emulsions' can be broken in a continuous flow process by applying AC electric fields whereby the droplet content is effectively transferred into the surrounding continuous water plugs in a single step. We demonstrate how this method can be applied to emulsion flow rates from 50 up to 1000 μL hr^{-1} using milli- and microfluidic devices. The viability of extracted cells has also been verified. The additional advantage of electro-demulsification simplifying material functionalization is demonstrated by the one-step fluorescence labelling of poly(acrylamide) hydrogel microbeads.

Materials and Methods

The macro-version of the electro-coalescence chip is made from poly(methyl methacrylate) (PMMA) known for its reusability and good optical transparency. As shown in Figure 1.c, a circular channel of 50 mm in length and 1.6 mm in diameter is drilled using modern micromachining techniques. At 800 μm distance from the circular channels, copper electrodes were mounted on either sides of the channel and can be connected to the high power high voltage amplifier (TREK, cat. no. 623B).

The microfluidic electro-coalescence chip is fabricated by soft lithography as described above. Micro-electrodes are included in the microfluidic device as additional microfluidic channels, which are filled with a metal alloy: the device is heated to 75 °C and a 51In/32.5Bi/16.5Sn low-temperature solder (Indium Corporation) is melted inside the electrode channels and the device is cooled to room temperature to solidify the micro-electrodes. Electrical connections with the solder electrodes were made with short pieces of electrical wire (Novodirect).

The water-in-oil emulsion collected from the microfluidic droplet production chip is directly re-injected into the electro-coalescence chip in the case of the PDMS microfluidic chip or through the use of a polytetrafluoroethylene (PTFE) cross-junction in the case of using the PMMA macro-version chip. The reinjection flow rate was ~ 500 μL h^{-1} for the emulsion phase and ~ 500 μL h^{-1} for the spacing aqueous phase. An Agilent 33220A 20 MHz function generator and a high voltage amplifier (TReK Model 610E COR-A-TOL) was used to apply a signal to the electrodes. An AC field with a sinus waveform of the voltage difference U was imposed at the electrodes around 1-1.9 kV at a frequency of 1-1.5 kHz.

Other experimental details, including microfluidic water-in-oil emulsion production, agarose bead preparation and PAAm microgel preparation can be found in the Supporting Information.

Results and Discussion

Monodisperse water-in-oil emulsion droplets containing cells or hydrogel microbeads were first generated using a flow focusing microfluidic device with a 30-100 µm orifice (Figure 1a). We used a continuous oil phase that consisted of a fluorinated oil (HFE 7500) containing 2 % (w/w) of a biocompatible surfactant (Krytox-jeffamine-Krytox A-B-A triblock copolymer).[7,15] For each individual experiment, ~ 10^6 - 10^7 droplets were collected at the outlet of the microfluidic device for later use. We observed that the random encapsulation of cells in droplets followed a Poisson distribution (Figure 1b). For demulsification purposes, the collected emulsion was re-injected together with a spacing aqueous phase containing e. g. phosphate buffered saline (PBS) at equal flow rates (~ 500 µl h^{-1}) into the demulsification module via a polytetrafluoroethylene (PTFE) cross-junction. This results in the formation of secondary,

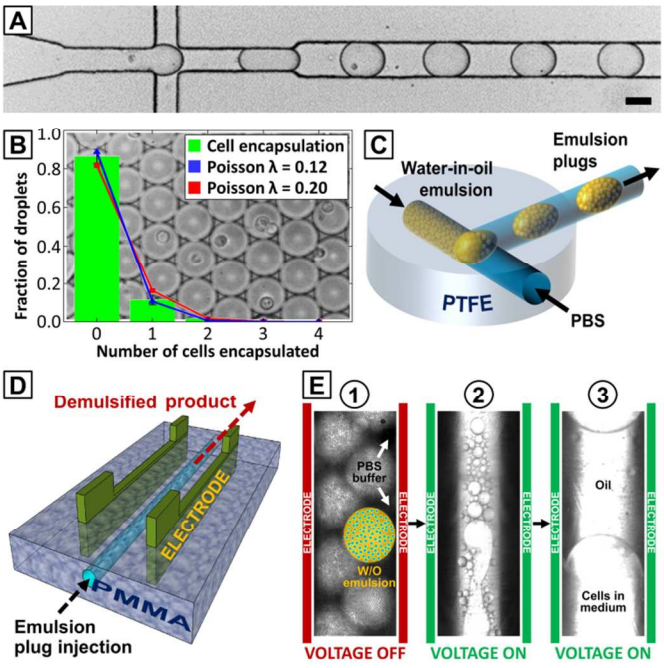

Figure 1 The electro-demulsification chip for the effective recovery of droplet contents such as cells from droplet microfluidic water-in-oil emulsions. (A) Encapsulating cells in water-in-oil emulsion droplets by microfluidic flow focusing devices. Scale bar = 50 µm. (B) The random cell encapsulation in droplets follows a Poisson distribution. (C) Schematic of the formation of a secondary emulsion containing plugs of microfluidic droplets in PBS using a polytetrafluoroethylene (PTFE) cross-junction, whose outlet was connected to the electro-coalescence module. (D) An electro-coalescence chip made from poly(methyl methacrylate) (PMMA) with copper electrodes mounted with a gap of 800 µm on both sides of a 50 mm long circular channel with a diameter of 1.6 mm. A homogeneous electric field will be generated along the channel once a voltage is applied on the electrodes. (E) A working example of emulsion separation by the electro-coalescence chip. When no electric field was applied (voltage off, image 1), the demulsification channel was filled with a secondary emulsion Once a voltage was applied and increased to a certain value between 1 -1.6 kV at a frequency of 1.5 kHz, the droplets started to fuse (image 2). Finally an overall phase separation and transfer of the droplet contents into the aqueous phase were observed (Image 3).

'emulsion of emulsion' plugs at the outlet of the junction, which were directly injected into the electro-coalescence chip (Figure 1c). The schematic of the electro-coalescence demulsification chip is shown in Figure 1d. We made use of AC fields as efficient electrocoalesescence requires the application of AC fields [16,17] and DC fields are more likely to cause electrochemical side effects. An additional advantage of using AC is that an insulating layer can be placed between the electrodes and liquid, preventing short circuits when a voltage is applied.[17]

In the current design, copper electrodes were mounted on both sides of a 50 mm long circular channel with a diameter of 1.6 mm through a poly(methyl methacrylate) (PMMA) block. The spacing between the electrodes and the channel is 800 µm, providing an insulating gap for the generation of a homogeneous electric field along the entire length of the channel. When a high voltage (U) is applied on the electrodes, the strength of the generated electric field (E_0) in the fluidic channel can be calculated from the following equation:[12]

$$\mathbf{E_0} = \frac{\mathbf{U}}{\left(\frac{\mathbf{2d\varepsilon_c}}{\mathbf{\varepsilon_i}} + \mathbf{h}\right)}$$

where d is the distance between the electrode and the channel, h is the distance between the electrodes, ε_c and ε_i are the dielectric constants of the continuous phase and the insulating material (PMMA), respectively. Figure 1e shows how droplet electro-coalescence takes place. When no electric field was applied (voltage off, image 1), the demulsification channel was filled with emulsion plugs separated by plugs of PBS. Once a voltage was applied and increased to a value between 1 -1.6 kV at a frequency of 1.5 kHz, the droplets started to fuse (image 2).

On the mechanism of the demulsification process we believe that the differences in conductivity and permittivity of the dispersed phase (oil) and continuous (water) phase destabilizes the droplet-droplet and droplet-water interface, which eventually leads to droplet/interface deformation. Following the deformation surfactant molecules at all the interfaces will be displaced or re-aligned, eventually leading to electro- coalescence.[18] While the electric field is switched on, droplet fusion and surfactant displacement continues until all droplets in each secondary emulsion plug are fused, first with other droplets and later with the large buffer plug. In the end, complete demulsification and transfer of the droplet contents into the aqueous phase is observed (Image 3). It is important to use a continuous phase that has a low conductivity so that it acts as an insulator between the two electrodes. A similar system is used here and in most of the droplet microfluidic studies and makes our electro-coalescence setup robust for emulsion breaking.

The applied voltage and frequency depends on the liquid emulsion composition, electrode material and the geometrical aspects. The characteristics and geometry of the electrode system influence the performance of the coalescence, and are closely related to the type of the applied electric field and the emulsion used. The optimum voltage and frequency to be applied for the effective demulsification was determined experimentally and summarized in Table 1. At lower applied voltages of up to 0.8 kV, no clear droplet fusion was observed within the channel. With an increasing voltage, the time to complete phase separation (t_{ps}) decreased, until the change in t_{ps} was no longer significant above a voltage of 1.2 kV. It should be mentioned that this method is not material-selective and the same chip can be used for different interfacial coverage conditions using other oil/surfactant combinations with varying surfactant concentrations.[11] However, for satisfactory device performance the optimal parameters (frequency and magnitude) of the electric field should be fine-tuned under the specific interfacial conditions.[19,20]

Table 1 Effect of the applied voltage across the electrodes for complete phase separation of the secondary emulsion inside the electro-coalescence module. E is the field strength and t_{ps} is the time required for complete phase separation. Frequency of the AC field f = 1 kHz, T = 298 K.

***E* (kV)**	0.2	0.5	0.8	1.2	1.6
t_{ps}	no fusion	no fusion	190 ms	160 ms	150 ms

The versatility of the electro-coalescence demulsification chip is demonstrated by the effective recovery of cells, cell- containing gel beads, and one-step functionalization of poly(acrylamide) (PAAM) hydrogel particles (Figure 2). In order to verify the biocompatibility of the electrical demulsification procedure and check for complete oil removal, Jurkat T cells transfected with CD6-GFP was first encapsulated into droplets and subsequently transferred into the aqueous phase using the electro-coalescence chip. After the recovery of cells from the demulsification process, cell viability was tested by staining the dead cells with propidium iodide (PI). As shown in Figure 2a and b, similarly high cell viability of over 90% was observed before encapsulation and after the demulsification processOur approach thus provides a safe, easy and general method for the effective recovery of various cell types, including sensitive ones. The bright green (GFP), and complete lack of PI-derived fluorescence from the cells (Figure 2b) further ensures the effective oil removal and no adverse effects on cells obtained from the demulsification process.

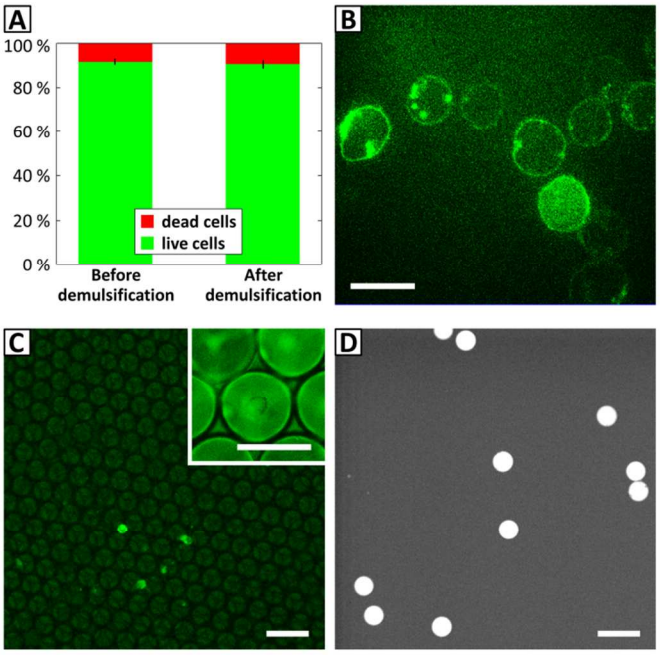

Figure 2 Versatile applications of the electro-demulsification chip in extracting emulsion contents from microfluidic water-in-oil droplets. (A) Similarly high viability of encapsulated Jurkat T cells transfected with CD6-GFP before and after the demulsification process by staining with Propidium Iodide (PI). (B) The bright green and lack of PI-derived fluorescence from the cells provided further evidence for the biocompatibility of the demulsification procedure. (C) PI stained agarose hydrogel microbeads loaded with Jurkat cells collected after demulsification of emulsions containing cells in agarose solutions produced at ~ 37°C and then incubated at 4°C. A high cell viability of > 90% as indicated by the fluorescence emission from the cells demonstrated a complete oil removal from the beads. (D) Direct fluorescent labelling of poly(acrylamide) (PAAM) microparticles when PAAM hydrogel emulsion was demulsified in the presence of a sodium carbonate buffer (pH 9) containing saturated Rhodamine B isothiocyanate (RBITC) as the spacing aqueous phase.

Droplet microfluidics not only enables the encapsulation of cells in a fluidic compartment, but also offers the possibility of putting cells in micron-sized 3D hydrogel environments. Cell-laden hydrogel microbeads are important for biological research in the fields of tissue engineering, studies on cell-cell and cell-matrix interactions.[5] In this context, cells are generally encapsulated together with hydrogel precursors into water-in-oil droplets, which are gelled inside the droplets before the beads are transferred into the aqueous phase for culture and analysis. The electro-coalescence module developed here is particularly useful in purifying the beads as it combines the function of both demulsification and washing steps. To demonstrate the efficient recovery of cell-laden gel beads, agarose solution droplets in DMEM were loaded with Jurkat cells at elevated temperatures (~ 37°C) using droplet microfluidics. The produced emulsion was collected and stored at 4°C for 1 hr. Upon the completion of the gelation process, emulsions containing the cell-laden gel beads were sent to the electro-coalescence module to collect the beads in cell culture medium, and the cell viability was again tested by PI staining. As shown in the fluorescence microscopy image in Figure 2c, monodisperse hydrogel beads were completely recovered in the aqueous phase and cells encapsulated showed high viability (> 90%) even after 3 hours of further culture.

Effective oil removal by the electro-coalescence chip could also simplify material functionalization procedures. Here, we present one example in this direction on the fluorescent labelling of poly(acrylamide) (PAAM) microparticles. Similar to the fabrication of agarose gel beads, emulsions of PAAM beads were produced by incorporating acrylamide, bisacrylamide, N,N,N′,N′-tetramethylethylenediamine and ammonium persulfate into water-in-oil droplets followed by overnight incubation at 65 °C to complete the gelation. The bead emulsion was then sent into the electro-coalescence chip with a sodium carbonate buffer (pH 9) containing saturated Rhodamine B isothiocyanate (RBITC) as the spacing aqueous phase. By applying an electric field, red fluorescence-emitting hydrogel beads were directly collected in the aqueous phase at the outlet of the electro-coalescence module (Figure 2d). In this example, the multi-step procedure of emulsion breaking, washing and subsequent chemical modifications on polymer particles fabricated by droplet microfluidics have beens greatly simplified into one single step.

While the electro-coalescence chip showed above is mainly suitable for relatively large sample volumes (in the sub mL range), the same principle can be applied for the fabrication of microfluidic chips especially suitable for emulsion separation when the sample volume is in the sub-100 µL range. Figure 3 shows an example of a microfluidic chip made from PDMS with integrated electrodes for emulsion separation. As shown in the figure, metal electrodes were melted to follow the entire length of the microfluidic channel. The gap between the electrodes and the microfluidic channel is 20 µm. Emulsions-containing cells or hydrogel microbeads were sent together with an aqueous phase (PBS buffer) into the demulsification device and formed a secondary emulsion (Figure 3b) after the flow junction. Following a short emulsion stabilization section of 100 µm, the secondary emulsion flew through a long coalescence channel of 1 mm in length on which an electric field was applied. Since the dielectric constant of PDMS (2.3-2.8) is similar to that of PMMA (2.6) but the thickness of the insulation layer is lower, the voltage required to fully demulsify the emulsions in the microfluidic electro-coalescence chip is lower. At a total flow rate of 130 µL/h, the residence time for emulsion samples in the electro-coalescence section was 150 ms. This residence time was sufficient to fully separate the oil and aqueous phases. As shown in Figure 3c and d, only two continuous phases are present towards the outlet of the microfluidic chip, demonstrating the completion of the electro-demulsification process.

Conclusions

In conclusion, the design and fabrication of an electro-coalescence chip for efficient emulsion separation has been shown. Contents of

emulsion samples can be extracted into a continuous aqueous phase using either a PMMA macro-version chip or a PDMS microfluidic chip, depending on sample volumes. The electro-coalescence chip avoids the use of harsh chemicals and multiple washing steps, simplifies the recovery of cells and cell-containing hydrogel microbeads encapsulated in microfluidic droplets into an aqueous phase and shows no adverse effect on the viability of the cells. This technique also provides a straightforward way for the functionalization of emulsion contents. Moreover, this electro-coalescence setup is not dependent on the emulsion composition and therefore, can be used for various oil and surfactant combinations, but the optimum frequency and voltage has to be fine-tuned to meet best coalescence conditions as the magnitude of the applied frequency greatly depends on the type of electric field as well as on the arrangement of the electrodes. We believe emulsion separation based on this electro-coalescence setup will find broad applications in droplet microfluidics related to biological, chemical and materials research.

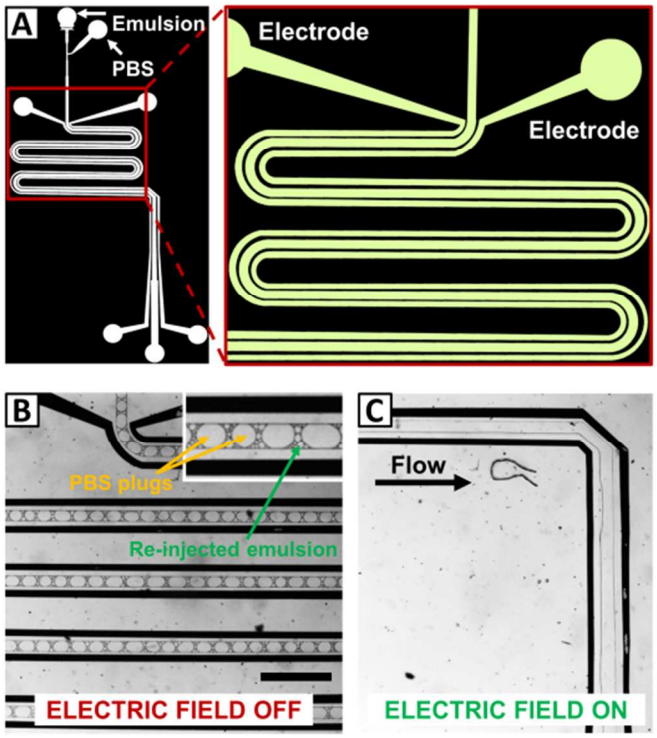

Figure 3 A microfluidic electro-coalescence chip for extracting emulsion contents in case of small sample volumes in the sub-100 µL range. (A) AutoCAD design of the microfluidic electro-coalescence chip with a two-inlet cross-junction for the generation of secondary emulsions containing microfluidic droplets and PBS plugs. Metal electrodes were melted to follow the major part of microfluidic channel after the cross-junction. The gap between the electrodes and the microfluidic channel is 20 µm. Scale bar = 500 µm. (B) The formation a secondary emulsion with emulsion droplets and PBS plugs inside the electro-coalescence microfluidic channel when no electric field was applied. (C) The complete separation of aqueous and oil phases towards the end of the electro-coalescence microfluidic chip when an electric field was applied.

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Notes and references

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