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Impact of electrostatic potential on microcapsule-formation and physicochemical analysis of surface structure: Implications for therapeutic cell-microencapsulation

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Abstract

Cell-encapsulation is used for preventing therapeutic cells from being rejected by the host. The technology to encapsulate cells in immunoprotective biomaterials, such as alginate, commonly involves application of an electrostatic droplet generator for reproducible manufacturing droplets of similar size and with similar surface properties. As many factors influencing droplet formation are still unknown, we investigated the impact of several parameters and fitted them to equations to make procedures more reproducible and allow optimal control of capsule size and properties. We demonstrate that droplet size is dependent on an interplay between the critical electric potential (U_c), the needle size, and the distance between the needle and the gelation bath, and that it can be predicted with the equations proposed. The droplet formation was meticulously studied and followed by a high-speed camera. The X-ray photoelectron analysis demonstrated optimal gelation and substitution of sodium with calcium on alginate surfaces while the atomic force microscopy analysis demonstrated a low but considerable variation in surface roughness and low surface stiffness. Our study shows the importance of documenting critical parameters to guarantee reproducible manufacturing of beads with constant and adequate size and preventing batch-to-batch variations.

Keywords

Therapeutic cell encapsulation, droplet formation, electrostatic generator, modeling, high-speed camera

Introduction

Since the introduction of the so-called electrostatic droplet generator,^{1,2} electrostatic potential has been widely used to regulate the diameter of microcapsules for cell encapsulation. The field of cell encapsulation is expanding fast as it allows minute-to-minute regulation of hormones and metabolites, which cannot be achieved with a pharmaceutical intervention.³ This technology is used for treating diseases such as anemia,⁴ dwarfism,⁵ hemophilia B,⁶ kidney⁷ and liver⁸ failure, pituitary⁹ and central nervous system insufficiency,¹⁰ and diabetes mellitus.¹¹ In recent years, microencapsulation has also been considered an essential technology to allow immunisation of stem cell-derived therapeutic cells that protect insulin-producing cells obtained from replenishable progenitor cells.³

Despite its broad application, the process by which microcapsules are formed in electrostatic droplet

generators is not well understood. Although it is broadly accepted that the capsules should be small to allow rapid exchange of glucose and insulin and to allow diffusion of nutrients into the capsules,¹² not much is

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known about the factors influencing the capsule diameter or formation process. To date, capsule size has been mainly controlled by changing the electrostatic potential, but many more factors influence capsule properties in the process. Items such as viscosity of the polymer solution, needle diameter, and their impact on droplet size are essential for capsule formation^{2,13} but have not been studied in great detail and are limited mostly to experimental studies without mathematical verification.

The study of microcapsules manufactured from biodegradable polymers has received special attention due to its low-cost applicability and the versatile properties of these materials. Among this class of polymers, one of the most commonly applied for cell encapsulation is alginate^{14,15} which is preferred as core material by many groups because of its biocompatibility, heat stable nature, non-antigenicity and chelating ability.^{16,17} To make capsule formation possible, the solidification of the alginate is necessary. Solidification may include gelation in contact with a divalent cation, such as calcium, or cooling of a melt solution, such as liquid wax in cool air. Capsules should be produced at a relatively high speed to avoid long exposure of cells to divalent cations. The speed at which droplets form depends on the flow rate of the solution through the needle in the droplet generator and the force acting on the droplets. Under low flow rates, droplets are formed on the nozzle until their mass surpasses the surface tension force between the droplet and the tip of the nozzle¹⁸

$$mg = \frac{\pi d^3}{6} \rho g = \pi \gamma d_{ne} \quad (1)$$

$$d = \sqrt[3]{\frac{6m}{\pi \rho}} = \sqrt[3]{\frac{6\gamma d_{ne}}{\rho g}} \quad (2)$$

where m is the mass of the droplet (kg), g the gravitational constant (m/s^2), γ the surface tension (N/m), d_{ne} the external diameter of the needle (m), d the droplet diameter, and ρ the solution density.

If the flow rate is enhanced, this process of droplet formation is disrupted and the alginate solution is ejected as a jet, which requires a different technology to break down the solution into droplets. Jet breakage for droplet formation is generally performed by vibration or by a cutting device, producing homogeneous droplets (200 μm to 2 mm) with a higher flow rate (up to several liters for large droplets).

Large quantities of capsules are not required for biomedical applications, as the production of several milliliters per hour usually suffices. For example, one

million capsules of insulin-producing cells are needed for treating human diabetes, and this quantity can easily be produced in an hour. Using capsules of 500 μm , the volume of 10^6 capsules is only 60 mL.¹⁹ This is one of the reasons why relatively slow producing processes but precise, fine-tuning technologies such as electrostatic droplet generators are widely used in the cell-encapsulation field.^{20,21}

Fine-tuning droplet size with electrostatic potential is mainly accomplished by changing the electric charge on the surface of the droplets, leading to some repulsion effects counteracting the surface tension. The surface tension is related to the electrostatic potential, as proposed by Poncelet et al.^{22,23}

$$\gamma = \gamma_0 \left(1 - \frac{U^2}{U_c^2} \right) \quad (3)$$

where γ_0 is the surface tension without electric potential, U is the electrostatic potential applied on the needle, and U_c is the critical electrostatic potential. The breakage process is then determined by

$$mg = \frac{\pi d^3}{6} \rho g = \pi \gamma_0 d_{ne} \left(1 - \frac{U^2}{U_c^2} \right) \quad (4)$$

The electro-dripping theory was developed by measuring the size of alginate beads. However, droplets shrink during gelation due to the binding of opposing guluronic acid backbones to alginate molecules with divalent cations, according to the so-called egg-box model.²⁴ Shrinking may range from 30 to 70%, depending on the alginate type and the type and concentration of the divalent cation applied, and it may lead to issues such as protrusion of cells.²⁵ Here we studied droplet formation because a better understanding of processes related to capsule formation is essential to prevent capsule issues such as protrusion, the formation of tails, or other irregularities on the surface that might cause immune responses *in vivo*.

A previous study has shown that using a high-speed camera may document and define the optimum conditions to realize adequate dripping techniques for capsule formation.²⁶ In the present study, we used such high-speed camera to study a more complex dripping process using an electrostatic droplet generator. To this end, we studied droplet formation with a novel high-speed camera (up to 5000 Hz) during alginate droplet formation at different electrostatic forces and determined the optimal equations to predict droplet formation. Furthermore, as surface properties are essential for the final capsule product and its behavior after implantation, we performed X-ray photoelectron

spectrometry analysis and atomic force microscopy on the surface of the final capsules.

Materials and methods

Chemicals

Sodium alginate powder Algogel 3001 was kindly provided by Algaia, France. Algogel 3001 has a high guluronic acid content and a M/G ratio of 0.8. Calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) was purchased from Sigma-Aldrich. All solutions were prepared with deionized water.

Solutions

Alginate solution was prepared by dissolving 15 g alginate in 1 L deionized water under magnetic stirring. The alginate was allowed to dissolve for 60 min under stirring. It was stored at 4 °C but used at room temperature. Calcium solution was prepared by dissolving 7.35 g of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ in 1 L deionized water under magnetic stirring (50 mM).

Viscosity and surface tension measurements

The viscosities of alginate solutions were determined by using a viscometer (Haake VT 550, USA) and Rheowin Job Manager Software. To this end, 15 mL of alginate was placed into the chamber and subjected to an increasing shear rate from 180 to 1000 s^{-1} followed by a decreasing shear rate from 1000 to 180 s^{-1} , over 60 s.

The surface tensions of the solutions were determined by using a tensiometer (KRÜSS K-12, France) according to the du Nouy ring method and LabDesk™ software. For the surface tension measurement, 70 mL of alginate solution was used.²⁷ The temperature during measurements was kept at 25 °C and three replicates of the experiments were performed.

Equipment

The alginate solution was extruded using a syringe pump (Fisher Scientific, Illkirch, France) through a needle connected to an electrostatic energy generator (Bertan, USA, positive connector). This generator was placed above a beaker containing the calcium solution and connected to the negative but grounded connector of the electrostatic generator. The needle was purchased from Eleco Produits (Gennevilliers, France) and fixed on a 3 D printed build block through luer lock connectors (Fisher Scientific, Illkirch, France) (Figure 1).

Study of droplet formation using a high-speed camera

We measured the shape and size of droplets as they develop over time and studied the volumes and velocities of the jets and the droplets using a high-speed

Figure 1. Set-up for the electrostatic dripping experiments and droplet formation studies.

camera (HighSpeedStar 6 from Lavision) with a Precise Eye lens. The image capture was performed at 5000 fps, shutter speed of 10^{-8} s, and image resolution of 1024x1024 Pixels.² The images were recorded and analyzed using ImageJ software (National Institutes of Health, USA). The mean size and size dispersion were quantified by measuring 50 droplets.

X-ray photoelectron spectroscopy (XPS)

To quantitatively study the atomic composition, samples of fresh capsules were washed three times with ultrapure water and placed on gold plates. Excess fluid was carefully aspirated and slowly dried to keep the capsules in their original round shape. The sample holder was inserted into the chamber of an X-ray photoelectron spectrometer (Surface Science Instruments, S-probe, Mountain View, CA). An aluminum anode was used for the generation of X-rays (10 kV, 22 mA) at a spot size of $250 \times 1000 \mu\text{m}$. During the measurements, the pressure in the spectrometer was approximately 5×10^{-9} mbar. The scans were performed over the binding energy range of 1–1100 eV at low resolution (150 eV pass energy). Next, C1s, N1s, and O1s peaks over a 20 eV binding energy range were recorded at high resolution (50 eV pass energy). The polymer contents of the capsule surface were expressed as a percentage of the total C, N, and O content of the membrane.

Surface roughness of capsules

Single capsules were carefully selected using a dissection microscope (Leica MZ75 microsystems), washed, and placed on microscopic slides. The surface roughness (R_a) and Young's modulus (YM) of different microcapsules were determined by a Bruker Catalyst atomic force microscope (AFM) with a Bruker DNP silicon nitride cantilever. Topographic imaging of the

capsules was performed at room temperature using the contact mode. Ra was calculated by using the NanoScope Analysis 1.8 software. The YM was calculated from 25 force curves on three different capsules with the same software.

Results

Alginate solution properties

As surface tension is considered an essential factor in droplet formation, we quantified the viscosity and surface tension for 15, 17, 19, 22, and 25 g/L alginate solutions. As shown in Table 1 and Figure 2(a), the viscosity increased with a gradual increase in the concentration of the alginate while the surface tension decreased. For all solutions, the viscosity was constant over a large range of shear rate (180 to 1000 s^{-1}) and the relation between shear stress and shear rate was proportional (Figure 2(b)). The solution was, therefore, considered to be Newtonian.

Droplet formation and release

As 17 g/L still allows for reasonable production speeds for cell-containing capsules, this concentration was

Table 1. Impact of the alginate concentration on the viscosity and surface tension of the solutions. Values are expressed as mean $\pm$ standard deviation.

Alginate (g/L)	Surface tension (mN/m)	Viscosity (mPa.s) (from 180 to 1000 s^{-1})
15	52.30 ± 0.30	25.00 ± 5.01
17	52.10 ± 0.30	35.00 ± 5.00
19	65.30 ± 0.80	36.00 ± 6.02
22	65.30 ± 0.80	53.00 ± 7.00
25	65.30 ± 0.90	84.00 ± 9.00

chosen for the follow-up study on droplet formation and impact of the gradual increase in the electrostatic potential. Figure 3 illustrates the formation and the release of the droplet with a gradual increase in the electrostatic potential. Without voltage, the droplet gained a relatively spherical form on the tip of the needle. When its mass became too high, the droplet deformed creating a throttle until the droplet detached. With moderate voltage ($<7\text{ kV}$), the deformation occurred faster, and smaller droplets detached from the tip. For higher voltages ($>10\text{ kV}$), the liquid formed a neck or even a jet. The droplet detached from the end of the neck/jet and not from the needle itself. The transition between the two processes was progressive. The size dispersion of droplets (standard deviation divided by the mean size) was in general lower than 3%. However, for the higher electrostatic potential, some satellite droplets were observed resulting from breakage of the liquid filament connecting the droplet to the neck just before detachment.

Droplet formation according to theoretical models

Figure 4(a) shows a typical evolution of the mean droplet size with a gradual increase in the electrostatic potential. Without electrostatic potential, the size of the droplet corresponded mainly to equation (1). As the electrostatic potential increased, the size was fitted to equation (2). However, for electrostatic potential higher than a critical value, the size remained relatively constant. In the absence of electrostatic voltage, there was a well-fitted correlation between experimental and theoretical diameter (equation (1)). For higher voltages, i.e. 10 kV , the diameter was constant and equal to approximately twice the internal diameter of the nozzle (vide infra).

Figure 2. (a) Variation in the surface tension (mN/m) and viscosity (mPa.s) for 15, 17, 19, 22, and 25 g/L alginate solution. (b) Relationship between shear rate ($1/\text{s}$) and shear stress (Pa) for alginate 17 g/L.

Figure 3. Impact of the electrostatic potential on the droplet formation and release of droplets (alginate concentration: 17 g/L, needle: G25, flow rate: 1 mL/min).

Figure 4. Impact of the electrostatic potential on droplet size (alginate concentration: 17 g/L, internal/external needle diameters: 0.61 mm/0.92 mm, flow rate: 1 mL/min, height: 20 cm). Data correspond to the mean value of 10 batches with a batch-to-batch dispersion variation between 2 and 5%.

The critical voltage (U_c) was then determined by fitting the experimental data to equation (2), assuming a minimum diameter for the droplets at high voltage. The correlation coefficient between the experimental data was optimized by fixing the initial droplet size without applying the electrostatic voltage (d_{om}) and the final droplet size at 10 kV (d_{fm}), and by adjusting the critical electrostatic voltage (Figure 5). In all calculations, the correlation between the experimental and theoretical droplet diameter was higher than 0.99 (Figure 4(b)). From Figure 5, we also deduced that the critical electrostatic voltage may be determined

with a precision of ± 200 V (coefficient correlation higher than 0.99).

Influence of the needle internal and external diameters

It is insufficiently studied in the field of cell encapsulation how the needle internal and external diameters might impact droplet formation. Figure 6 and Table 2 present experimental data and fitted models for different needle sizes. Up to the critical electrostatic potential, the data fitted well to equation (2)

($R^2 > 0.99$). At the highest tested voltage, i.e. 10 kV, the minimum size of droplets varied between 1.6 and 2.2 times the internal diameter. The extruded liquid formed a jet, which broke down into droplets. The min-

imum jet velocity, $u_{j,min}$, given by Lindblad and Scheider et al.,²⁸ was as follows

$$u_{j,min} = 2\sqrt{\frac{\gamma}{\rho d_j}} \quad (5)$$

where d_j is the jet diameter, generally assimilated to the internal nozzle diameter, and ρ is the liquid density. The surface tension decreased with higher electrostatic potential applied to the needle, initiating the transition from drop-by-drop to jet process. In the jet regime, the droplet formation followed the Rayleigh theory,²⁹ which predicts that jet primarily breaks in cylinders of an optimum length or wavelength, λ , equal to

$$\lambda_{opt} = \pi\sqrt{2}d_j \quad (6)$$

Taking into account that each jet cylinder will be converted to a spherical droplet of the same volume

$$\frac{\pi}{4}d_j^2\lambda = \frac{\pi}{6}d^3 \quad (7)$$

Combining equations (5) and (6), after rearrangement, the mean droplet diameter was then given by

$$d = 1.88d_j \quad (8)$$

Moreover, the optimum wavelength may vary as a function of the liquid properties and the flow rate.³⁰ The experimental data for the minimum droplet size were similar to but not exactly the same as the predicted value.

The critical electrostatic voltage, U_c , increased with increasing needle size. Combining equations (3) and (5), assuming that, for a defined needle, the minimum jet velocity is constant, one would expect the critical electrostatic potential to increase proportionally by the root square of jet diameter or internal nozzle diameter. This critical electrostatic potential was determined

Figure 5. Determination of the critical electrostatic voltage by optimization of the correlation coefficient (alginate concentration: 17 g/L, internal/external needle diameters: 0.33 mm/0.65 mm, flow rate: 1 mL/min, height: 20 cm).

Figure 6. Impact of the needle diameter on the droplet size (E: experimental data, M: model, alginate concentration: 17 g/L, internal/external needle diameters: 0.61/0.92 mm, flow rate: 1 mL/min).

Table 2. Impact of the needle diameter on the droplet size (alginate concentration: 17 g/L; flow rate: 1 mL/min; height: 20 cm).

Needle gage	Symbol	G27	G25	G23	G20
Needle external diameter (mm)	d_e	0.44	0.45	0.65	0.92
Needle internal diameter (mm)	d_i	0.20	0.254	0.33	0.61
Initial droplet size (equation (1)) (mm)	d_o	2.41	2.43	2.74	3.08
Initial droplet size (experimental) (mm)	d_{om}	2.37	2.30	2.60	3.00
Final droplet size (equation (4)) (mm)	d_f	0.38	0.48	0.62	1.15
Final droplet size (experimental) (mm)	d_{fm}	0.32	0.52	0.78	0.98
Critical electrostatic voltage (experimental) (V)	U_c	5800	6100	7800	8400
Correlation coefficient (R^2)		>0.99	>0.99	>0.99	>0.99

by fitting to the experimental data but the sensitivity to this parameter was not very high.

Influence of distance between needle tip and gelation bath

The impact of the distance between the needle and the gelation bath was tested as well. The distance between the gelation bath and the needle was changed (i.e. 5, 10, and 20 cm) while keeping the needle diameter and the alginate concentration constant. As shown in Figure 7, the 5- and 10-cm distance did not influence the diameter of the capsules, but the capsules were larger at a higher voltage when a 20-cm distance was applied.

Physicochemical surface properties of alginate microcapsules produced by electrostatic forces

To gain insight into the elemental composition of the capsule surface, we performed X-ray photoelectron spectrometry of the first layers of the capsule surface (Table 3). The absence of Na indicated that most of the carboxyl groups of alginate were complexed with calcium. Predominantly, C1s (47.14%) was present at the surface corresponding to C-C, C-O-C, and O-C=O groups. In addition, we performed atomic force microscopy (Figure 8), which can be used to determine the surface roughness (Ra) and the surface stiffness (Young's modulus). The resulting mean values and the standard deviation of the Ra and YM are

Figure 7. Influence of the distance between the nozzle and gelation bath on the droplet size (see Table I for needle size, E: experimental data, M: model, alginate concentration: 17 g/L, internal/external needle diameters: 0.61/0.92 mm, flow rate: 1 mL/min).

summarized in Table 4. The Ra value was 39 ± 36 nm and the large standard deviation was caused by a relatively large heterogeneity of surface roughness of the capsules. Young's modulus was 45 ± 11 kPa, indicating a rather homogenous surface stiffness, which was relatively low as expected for hydrogels.

Discussion

Cell microencapsulation is a technology involving immobilization of therapeutic cells using polymer scaffolds or semi-permeable hydrogel capsules to provide cells with a protective environment that shields the cells from harmful effects of the host immune system.^{19,31} Microcapsules made from biodegradable polymers such as applied here have been used for biomedical applications such as for controlled delivery of low molecular weight drugs as well as for delivery of bioactive proteins.^{32–36} These natural polymers are commonly applied as they are biodegradable and have favorable biocompatibility properties which makes them suitable for implantation in the body.³⁷ One of the challenges of cellular microencapsulation involves controlling capsule size and stability in a reproducible way and avoiding tissue responses against encapsulated cells.^{25,38} Therefore, the droplet formation for cell encapsulation should ensure the production of beads with adequate size distribution parameters and stability in a reproducible way.³⁹ Insight into manufacturing processes, as tested here, contributes to a better understanding of capsule formation and therewith to reproducibility and side-by-side comparison of capsule characteristics.⁴⁰

Previous studies have demonstrated that the size of beads gradually decrease with increasing electrostatic potential.^{2,13,41,42} However, these experiments were based on quantification of capsules sizes and not of droplet sizes. This is an important difference as capsules are smaller due to gelification and shrinking and variable in size as the extend of shrinkage is alginate and polymer concentration dependent.^{41,43} Here we did direct measurements of the droplet sizes after production, which allows fitting in mathematical equations and prediction of sizes independent of shrinkage dependent parameters. We demonstrate that droplet size is influenced not only by the electrostatic voltage applied to alginate solutions but also by the needle diameter,

Table 3. Study of the elemental surface composition of capsules by X-ray photoelectron spectroscopy (XPS).

C, %	Cl, %	C2, %	C3, %	C4, %	O, %	N, %	Na, %	Ca, %
59.44	47.14	35.66	15.12	2.07	32.37	0.48	1.49	3.29

Figure 8. Representative AFM topographical images of three different microcapsules.

Table 4. Average surface roughness (Ra) and Young's modulus (YM) of alginate microcapsules.

Ra (nm)	Ra max (nm)	Young's modulus (kPa)
39 ± 36	263 ± 186	45 ± 11

which potentially provides options for even more precise regulation of the droplet size. We observed that in the absence of electric potential, the droplets detach from the needle with a size of 2 to 3 mm, depending on the external needle diameter. When the electric potential is gradually increased, the droplet size decreases until a critical value at which transition from drop-by-drop to a jet process occurs. For the higher electric potential (i.e. 10 kV), the size of the droplet is constant and equals approximately twice the internal diameter of the needle and the process is accompanied by some undesired satellite droplets. The formation of many satellite droplets could trigger issues in the process since they are associated with protrusions of cells³⁹ and inflammatory responses.⁴³ If this type of setting needs to be applied to cell encapsulation, it is advisable to use alginates containing higher amounts of guluronic acid (G)-chains at 2% concentrations as these are associated with lesser formation of these satellites.²⁵ Therefore, the possibility of manufacturing smaller and defect-free capsules (average diameter around 0.44 mm, size dispersion lower than 3%) could be achieved by working in the jet flow regime and by a pre-defined choice for alginates concentrations.

In the present study, the alginate concentrations ranged from 15 g/L to 25 g/L which corresponds to a viscosity range of 25 ± 5 to $84 \pm$ mPa.s. Chan et al.⁴⁴ reported that an alginate solution should have a viscosity above 60 mPa.s to produce beads with adequate mechanical properties and desired spherical shape. Lower viscosities lead to weaker capsules but also

high viscosities (>500 mPa.s) should be avoided as it causes shape deformations of beads and production issues due to low production rates.⁴⁵

Our study also demonstrates that the droplet formation process is dependent on an interplay between the electric potential (U_c), the needle size, and the distance between the needle and the gelation bath. The critical electric potential, i.e. when the surface tension becomes zero, increases with the needle size and the distance between the needle and the gelation bath. The distance between the needle and the gelation bath only had a diameter-increasing effect at a higher distance and a higher voltage. This should be explained by a lower and slower electric (ions) charge accumulation on the surface of the droplet at a higher distance. Consequently, at the same alginate flow through the needle, it will take longer to build a surface tension around the droplets to divert them from the needle tip. As a consequence, the droplets will be larger.

The surface properties of capsules determine the biocompatibility after implantation *in vivo*.⁴⁰ As shown here, the applied process results in a complete absence of sodium on the surface, which implies that the gelation process was performed correctly, because sodium was substituted by calcium,⁴⁶ subsequently connecting the constitutive blocks of G-G polymers in alginate.⁴⁷ Also, surface roughness is an important factor determining tissue responses. Although the value at which responses occur likely depends on the system applied, it is generally accepted that it should stay as low as possible to prevent innate immune cells, such as circulating monocytes, to adhere to the surface. Our study shows that the roughness was low, but it varied considerably between capsules and also per capsule, even though capsule size distribution was rather monodisperse. What is causing this variation is unknown, but it might be a characteristic of electrostatic droplet

generation as the process requires upbuilding of electrostatic charge around the capsule and disconnection of the needle tip, which always implies a heterogeneity of roughness of the surface between the part of the capsule connected to the needle tip and the other parts of the droplet. The surface stiffness was also determined, and the values of the alginate beads (45 kPa) were similar to previously reported literature values⁴⁷ and considered to be conventional for a hydrogel.

The electrostatic droplet generator provides adequate control over the droplet formation process, making it possible to manufacture microcapsules with regular shapes, uniform sizes, and smooth surfaces. Moreover, this technology can be performed under less stressful conditions without the use of organic solvents that reduce cell viability.⁴⁸ Other technologies that has generated research interest that also allows a precise control over size, morphology, and properties of the droplets formed are microfluidic devices.⁴⁹ Although droplet-based microfluidic devices offer size-controlled particles, monodispersed capsules are not always obtained due to the need for subsequent solvent evaporation and application of a droplet solidification step.⁵⁰ In addition, due the complexity of handling and the optimization of the fluidic circuit, broad application of the process is not yet possible.⁵⁰

Conclusion

Our study shows that the critical electric potential (Uc), the internal and external diameters of the needle, and the distance between the needle and the gelation bath influence the microcapsule formation in an electrostatic droplet generator used for therapeutic cell-encapsulation. Although this technology has been applied for cell-encapsulation for more than 20 years, we demonstrate that many factors influencing droplet generation still need to be identified. We also highlight the importance of documenting critical parameters to guarantee reproducible manufacturing of beads with constant and adequate size.

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All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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References

- Hallé JP, Leblond FA, Pariseau JF, et al. Studies on small (< 300 microns) microcapsules: II—parameters governing the production of alginate beads by high voltage electrostatic pulses. *Cell Transplant* 1994; 3: 365–372.
- Bugarski B, Amsden B, Goosen MFA, et al. Effect of electrode geometry and charge on the production of polymer microbeads by electrostatics. *Can J Chem Eng* 1994; 72: 517–521.
- Hu S and de Vos P. Polymeric approaches to reduce tissue responses against devices applied for islet-cell encapsulation. *Front Bioeng Biotechnol* 2019; 7: 134.
- Koo J and Chang TSM. Secretion of erythropoietin from microencapsulated rat kidney cells. *Int J Artif Organs* 1993; 16: 557–560.
- Chang PL, Shen N and Westcott AJ. Delivery of recombinant gene products with microencapsulated cells in vivo. *Hum Gene Ther* 1993; 4: 433–440.
- Liu HW, Ofosu FA and Chang PL. Expression of human factor IX by micro-encapsulated recombinant fibroblasts. *Hum Gene Ther* 1993; 4: 291–301.
- Cieslinski DA and Humes HD. Tissue engineering of a bioartificial kidney. *Bio-technol Bioeng* 1994; 42: 678–681.
- Wong H and Chang TM. Bioartificial liver: implanted artificial cells micro-encapsulated living hepatocytes increases survival of liver failure rats. *Int J Artif Organs* 1986; 9: 335–336.
- Aebischer P, Russell PC, Christenson L, et al. A bioartificial parathyroid. *ASAIO Trans* 1986; 32: 134–137.
- Aebischer P, Goddard M, Signore AP, et al. Functional recovery in hemiparkinsonian primates transplanted with polymer-encapsulated PC12 cells. *Exp Neurol* 1994; 126: 151–158.
- Lim F and Sun AM. Microencapsulated islets as bioartificial endocrine pancreas. *Science* 1980; 210: 908–910.
- De Vos P, Andersson A, Tam SK, et al. Advances and barriers in mammalian cell encapsulation for treatment of diabetes. *Immunol Endocr Metab Agents Med Chem* 2006; 6: 139.
- Klokk TI and Melvik JE. Controlling the size of alginate gel beads by use of a high electrostatic potential. *J Microencapsul* 2002; 19: 415–424.
- Wang J-P, Zhang X-X and Wang X-C. Preparation, characterization and permeation kinetics description of calcium alginate macro-capsules containing shape-stabilize phase change materials. *Renew Energy* 2011; 36: 2984–2991.
- Cañibano-Hernández A, Saenz Del Burgo L, Espona-Noguera A, et al. Hyaluronic acid enhances cell survival of encapsulated insulin-producing cells in alginate-based microcapsules. *Int J Pharm* 2019; 557: 192–198.
- Bhujbal SV, de Haan B, Niclou S, et al. A novel multi-layer immunoisolating encapsulation system overcoming protrusion of cells. *Sci Rep* 2014; 4: 6856.

17. Sun J and Tan H. Alginate-based biomaterials for regenerative medicine applications. *Materials* 2013; 6: 1285–1309.
18. Harkins WD and Brown FE. The determination of surface tension and the weight of falling drops. *J Am Chem Soc* 1919; 41: 499–452.
19. De Vos P, Bucko M, Gemeiner P, et al. Multiscale requirements for bioencapsulation in medicine and biotechnology. *Biomaterials* 2009; 30: 2559–2570.
20. Goosen MFA, Mahmoud ESE, Al-Ghafri AS, et al. Immobilization of cells using electrostatic droplet generation. In: Bickerstaff G (ed.) *Methods in molecular biology: Immobilization enzymes and cells*. Totow, NJ: Human Press, 1996.
21. Goosen MFA, Al-Ghafri AS, El-Mardi O, et al. Electrostatic droplet generation for encapsulation of somatic tissue: assessment of high voltage power supply. *Biotechnology* 1997; 13: 497–502.
22. Poncelet D, Neufeld R, Goosen M, et al. Formation of microgel beads by electric dispersion of polymer solutions. *AIChE J* 1999; 45: 2018–2023.
23. Poncelet D, Babak VG, Neufeld RJ, et al. Theory of electrostatic dispersion of polymer solution in the production of microgel beads containing biocatalyst. *Adv Colloid Interf Sci* 1999; 79: 213–228.
24. Sikorski P, Mo F, Skjåk-Braek G, et al. Evidence for egg-box-compatible interactions in calcium-alginate gels from fiber X-ray diffraction. *Biomacromolecules* 2007; 8: 2098–2103.
25. Bhujbal SV, Paredes-Juarez GA, Niclou SP, et al. Factors influencing the mechanical stability of alginate beads applicable for immunoisolation of mammalian cells. *J Mech Behav Biomed Mater* 2014a; 37: 196–208.
26. Davarci F, Turan D, Ozcelik B, et al. The influence of solution viscosities and surface tension on calcium alginate microbead formation using dripping technique. *Food Hydrocolloids* 2017; 62: 119–127.
27. Lee B, Ravindra P and Chan E. A critical review: surface and interfacial tension measurement by the drop weight method. *Chem Eng Commun* 2008; 195: 889–924.
28. Lindblad NR and Schneider JM. Production of uniform-size liquid droplets. *J Sci Instrum* 1965; 42: 635–638.
29. Rayleigh JWS. On the instability of jet. *Proc Lond Math Soc* 1878; s1–10: 4–8.
30. Weber C. Zum zerfall eines flussikeitstahles. *Z Angew Math Mech* 1931; 11: 136–155.
31. Opara EC. Applications of cell microencapsulation. *Methods Mol Biol* 2017; 1479: 23–39.
32. Pekarek KJ, Jacob JS and Mathiowitz E. Double-walled polymer microspheres for controlled drug release. *Nature* 1994; 367: 258–260.
33. Jeong B, Bae YH, Lee DS, et al. Biodegradable block copolymers as injectable drug delivery systems. *Nature* 1997; 388: 860–862.
34. Ulbrich K, Pechar M, Strohalm J, et al. Synthesis of biodegradable polymers for controlled drug release. *Ann N Y Acad Sci* 1997; 831: 47–56.
35. Hejazi R and Amiji M. Chitosan-based gastrointestinal delivery systems. *J Control Release* 2003; 89: 151–165.
36. Zhao Z, Wang J, Mao HQ, et al. Polyphosphoesters in drug and gene delivery. *Adv Drug Deliv Rev* 2003; 55: 483–499.
37. Park J, Ye M and Park K. Biodegradable polymers for microencapsulation of drugs. *Molecules* 2005; 10: 146–161.
38. De Vos P, Van Straaten JF, Nieuwenhuizen AG, et al. Why do microencapsulated islet grafts fail in the absence of fibrotic overgrowth? *Diabetes* 1999; 48: 1381–1388.
39. Lewinska D, Rosinski S and Werynski A. Influence of process conditions during impulsive electrostatic droplet formation on size distribution of hydrogel beads. *Artif Cells Blood Subst Immobil Biotechnol* 2004; 32: 41–53.
40. Orive G, Emerich D, Khademhosseini A, et al. Engineering a clinically translatable bioartificial pancreas to treat type I diabetes. *Trends Biotechnol* 2018; 36: 445–456.
41. Shi P, He P, Teh TKH, et al. Parametric analysis of shape changes of alginate beads. *Powder Technol* 2011; 210: 60–66.
42. Gryshkov O, Pogozhykh D, Zernetsch H, et al. Process engineering of high voltage alginate encapsulation of mesenchymal stem cells. *Mater Sci Eng C Mater Biol Appl* 2014; 36: 77–83.
43. De Vos P, De Haan B, Pater J, et al. R. Association between capsule diameter, adequacy of encapsulation, and survival of microencapsulated rat islet allografts. *Transplantation* 1996; 62: 893–899.
44. Chan ES, Lee BB, Ravindra P, et al. Prediction models for shape and size of calcium-alginate macrobeads produced through extrusion–dripping method. *J Colloid Interface Sci* 2009; 338: 63–72.
45. Moghadam H, Samimi M, Samimi A, et al. Electro-spray of high viscous liquids for producing mono-sized spherical alginate beads. *Particuology* 2008; 6: 271–275.
46. De Vos P, De Haan B, Wolters GH, et al. Factors influencing the adequacy of microencapsulation of rat pancreatic islets. *Transplantation* 1996; 62: 888–893.
47. Ceccaldi C, Fullana SG, Alfarano C, et al. Alginate scaffolds for mesenchymal stem cell cardiac therapy: influence of alginate composition. *Cell Transplant* 2012; 21: 1969–1984.
48. Yao R, Zhang R, Luan J, et al. Alginate and alginate/gelatin microspheres for human adipose-derived stem cell encapsulation and differentiation. *Biofabrication* 2012; 4: 025007.
49. Leong JY, Lam WH, Ho KW, et al. Advances in fabricating spherical alginate hydrogels with controlled particle designs by ionotropic gelation as encapsulation systems. *Particuology* 2016; 24: 44–60.
50. Damiati S, Kompella UB, Damiati SA, et al. Microfluidic devices for drug delivery systems and drug screening. *Genes* 2018; 9: 103.