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Tuneable spheroidal hydrogel particles for cell and drug encapsulation

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Abstract

The need to better mimic native tissues has accompanied the research in tissue engineering and controlled drug delivery. The development of new platforms for cell and drug encapsulation followed the same trend, and studying the influence of the delivery material system's geometry has been gaining momentum. Aiming to investigate how an increase in surface area and varying particle shape could impact drug release and cell viability, a novel method was developed to produce spheroidal hydrogel particles with adjustable circularity, aiming to tune drug delivery. For this purpose, droplets of hydrogel precursor were squeezed between two superamphiphobic surfaces separated with spacers with different height, and then photo-crosslinked to maintain the acquired shape after “de-sandwiching”. Numerical modelling studies were performed to study the polymeric droplet geometry deformation process, which were consistent with experimentally obtained results. The spheroidal particles were produced in mild conditions using methacrylated chitosan, capable of encapsulating proteins or cells. Likely due to their higher surface area to volume-ratio, comparatively to spherical-shaped ones, spheroids presented an improved viability of encapsulated cells due to an enhanced nutrient diffusion to the core, and lead to a significantly faster drug release rate from the polymer network. These results were also assessed numerically, in which the drug release rate was computed for different spheroidal-like geometries. Hence, the described method can be used to manufacture spheroidal particles with tailored geometry that can be broadly applied in the biomedical field, including for drug delivery or as cell encapsulation platforms.

Introduction

Hydrogels are hydrated, crosslinked, highly porous structures that can be applied for microencapsulation of cells and active substances.^{1,2} The fact that hydrogels can be produced using several biomaterials leads to an ample range of samples with varying viscoelasticity, porosity, and interaction with drugs.³

Conflicts of interest

There are no conflicts to declare.

A key aspect for cell survival within a hydrogel matrix is the efficient diffusion of nutrients to the cells and removal of metabolites. Microspheres appear as an option for cell encapsulation, allowing for nutrient diffusion from the particle border to the core in all directions.² Yet, with increasing particle dimensions, diffusion is compromised, resulting in reduced cellular viability within the core.⁴ Moreover, spherical objects present the lowest surface area to volume ratio, which is another impacting factor in diffusion.⁵ The tailoring of geometry allows to produce hydrogels that can be adapted to specific requirements, imposed by the application.^{6,7}

For drug delivery purposes, the effect of particle geometry has been studied as an impacting factor in opsonisation and targeted therapy. For example, particle geometry influences the phagocytosis of micron-sized particles, determined by the initial contact point and consequent orientation.⁸ Particle accumulation in major organs has also been shown to be shape-dependent,⁹ and an increased circulatory half-life has been verified for elliptical disks comparatively to micron and submicron spheres.¹⁰

From the above-mentioned arguments, it is interesting to consider the surface area associated with a given geometry, for the same volume of a microparticle. Geometries other than spherical may be more advantageous in cases where a larger ratio of surface area to volume is beneficial. The variety of geometries found in natural systems highlight the relevance of this parameter in evolution. For example, some bacteria acquire shapes with an increased surface area, enhancing nutrient up-take and disposal of waste products. Even though oblate spheroids present the highest surface area, other factors such as reduced sinking and enhanced swimming, lead these organisms to acquire a prolate spheroidal shape.¹¹ Another example is phytoplankton geometry, where various geometries such as spheroids, cylinders, and disks are preferentially adopted alternatively to spheres.⁵

Since varying microparticle geometry may lead to distinct cellular responses and drug release profiles, studying the impact of their geometry alongside the traditionally used microspheres would be of interest. Superhydrophobic and superamphiphobic surfaces have been widely applied to the production of individual spherical polymer particles using, for example, alginate,¹² chitosan,¹³ dextran,¹⁴ and PLGA.¹⁵ These substrates have been used to manufacture liquid-core capsules¹⁶ and multi-layered particles,¹⁷ under mild processing conditions, as well as hydrogels with different shapes by patterning of wettable superhydrophilic domains.¹⁸

In this work we demonstrate, for the first time, the possibility to deform initially spherical liquid droplets, by the compressive action of another superamphiphobic surface, to produce spheroidal hydrogel particles with controlled deformation. We hypothesised that by varying the distance between the surfaces could deformed the droplets containing a photocrosslinkable polymer which, followed by a UV-light irradiation, would result in spheroidal particles with controlled heights along the *z* axis. Chitosan was selected due to its biocompatibility, and cell-adhesiveness, whereas its methacrylated derivative was chosen for its ability to crosslink using UV-light.¹⁹

We expected that the obtained spheroidal hydrogels could (i) improve cell viability due to an enhanced diffusion of nutrients to cells in the core, comparatively to spheres, and (ii) release the encapsulated drug at a faster rate due to the higher surface area to volume ratio. Spheroidal particles could therefore present advantages for certain applications when compared to conventional spheres. To confirm these hypotheses, particles were characterised experimentally and through numerical modelling studies of droplet deformation, aiming to provide additional insights into the behaviour of the system. Finally, particles were studied numerically and tested experimentally according to their performance for cell encapsulation and kinetics profile of drug delivery.

Experimental

Materials

Tetraethyl orthosilicate (TEOS) was purchased from Merck (Germany); ammonia solution 25% from LabCHEM (Portugal); chitosan 95/20 (degree of deacetylation 92.6%; molecular weight = 40 – 150 kDa) from HMC Chitosan (Germany); and acetic acid from Chem-Lab (Belgium). 1H,1H,2H,2H-perfluorodecyltriethoxysilane, methacrylic anhydride, dialysis tubing cellulose membrane (flat width 33 mm, MWCO 14 kDa), 2-Hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone, albumin from bovine serum (BSA), phosphate buffered saline (PBS), DMEM (Dulbecco's Modified Eagle Medium) low glucose, sodium bicarbonate were purchased from Sigma-Aldrich (USA). Fetal bovine serum (FBS) was purchased from Biochrom AG (Germany); penicillin-streptomycin (100U/100 g.mL⁻¹), trypsin (TrypleExpress), DPBS (PBS without calcium and magnesium), Calcein-AM, propidium iodide (PI), and Micro BCA Protein Assay Kit™ were supplied by ThermoFisher Scientific (USA).

Methods

Superamphiphobic coating of glass microscope slides—The method for the production of the superamphiphobic coating was adapted from Deng *et al.*²⁰ Briefly, a microscope slide was held above the flame of a paraffin candle to form a soot layer. Afterwards, the soot-coated substrates were exposed to chemical vapour deposition (CVD) of TEOS (4 mL) in the presence of ammonia (4 mL) for 24h in a desiccator, followed by calcination of the hybrid carbon/silica network at 550°C for 2h. Finally, the hydrophilic silica shell was coated with 1H,1H,2H,2H-perfluorodecyltriethoxysilane (300 µL) by CVD for 3 days at 50°C. For surface morphology characterisation, samples were sputter-coated with gold-palladium and visualised by scanning electron microscopy (SEM Hitachi S4100), at a working distance of 15 kV.

Synthesis of methacrylated chitosan (M-CHT)—As described by Yu *et al.*,¹⁹ a 3% (w/v) chitosan solution was prepared by dissolving chitosan in 2% (v/v) acetic acid overnight at room temperature (RT) with constant stirring. Subsequently, methacrylic anhydride was added at 1 molar equivalents per chitosan repeat unit and left for 5h at RT. The mixture was dialysed against distilled water for 5 days, changing the water three times a day.

Production of hydrogels—A volume of 4.5 μL of 2, 3, or 4% (w/v) M-CHT in distilled water was used to produce each hydrogel particle. Spacers were produced from polypropylene (PP) films with a thickness of 90 μm , which were stacked to achieve the desired final height. The polymeric solution was placed between two superamphiphobic surfaces with a varying spacer height to control the shape of the polymer droplet. A UV-crosslinking method was employed using a curing system ($\lambda = 320 - 500 \text{ nm}$; Omnicure-S2000 XLA) with a 90 second crosslinking period and 2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone as photoinitiator.

Characterisation of shape—ImageJ software was used to evaluate particle shape and size. The “Hull and Circle” plug-in allowed to calculate the circularity of particles from the images taken using a contact angle measurement equipment. Particle circularity was calculated using Equation 1, varies between 0 and 1, with 1 corresponding to a perfectly spherical particle.

$$\text{Circularity} = \frac{4\pi \times \text{Area}}{\text{Perimeter}^2} \quad (\text{Equation 1})$$

Particle deformation was calculated according to spacer height and an initial average particle diameter of $1.90 \pm 0.05 \text{ mm}$ (Equation 2).

$$\text{Particle deformation}(\%) = \left(1 - \frac{\text{Spacer height}}{\text{Initial particle diameter}}\right) \times 100 \quad (\text{Equation 2})$$

Particle surface area was calculated for oblate spheroids (Equation 3) and spheres ($4\pi a^2$), where a and c represent the semi-axis along the y axis and z axis, respectively. Semi-axis values were measured from experimentally obtained particles, with ImageJ, and used to calculate the surface area for each analysed particle deformation.

$$\text{Surface area (Oblate spheroid)} = \frac{2\pi}{\sqrt{a^2 - c^2}} \left[a^2 \sqrt{a^2 - c^2} + ac^2 \ln \left(\frac{a + \sqrt{a^2 - c^2}}{c} \right) \right] \quad (\text{Equation 3})$$

3)

Modelling of the liquid droplet compression and drug release—The droplet deformation was modelled with the computational library OpenFOAM, using the elasticFoam solver, which can simulate the structural behaviour of linear and non-linear elastic materials. For this specific study, a linear elastic material was considered. To mimic the experimental tests, the initially spherical droplet was deformed by a moving plate, to achieve the same deformation levels imposed experimentally. Additionally, numerical modelling studies were also performed to assess the impact of the geometry of spheroidal particles on the evolution of a specific compound, transported by diffusion. In these studies,

the OpenFOAM solver employed was the laplacianFoam, which was modified to accommodate a Neuman boundary condition at the spheroid surface.

In vitro drug release—10 mg of BSA per millilitre were dissolved in the 2% (w/v) M-CHT solution prior to the production of spheres and spheroids. Four spheres or spheroids loaded with BSA were placed in each well containing 5 mL of PBS solution (pH 7.4) at 37°C, under gentle agitation (60 rpm). Assays were performed in triplicate (n=3). At pre-established time points, 150 µL of supernatant were removed for analysis and replaced with equal volume of fresh PBS. The amount of the drug released was quantified using the Micro BCA Protein Assay Kit™ upon spectrophotometrical quantification at 562 nm against the known calibration curve.

Results were modelled using the Korsemeyer-Peppas equation (Equation 4) for calculation of the diffusional exponent (n) to characterise the transport mechanism, according to the exponential dependence of the amount of drug released (M_t/M_∞) and rate constant (k) over time (t).²¹ GraphPad software was used to perform a two-way analysis of variance (ANOVA) with a Bonferroni's multiple comparison test for a significance levels of 0.1 %, 1%, and 5%.

$$M_t/M_\infty = Kt^n \quad (\text{Equation 4})$$

Cell viability – Live/Dead Assay—L929 cells were cultured with DMEM low glucose supplemented with 3.7 g/L sodium bicarbonate, 10% FBS and 1% penicillin-streptomycin at pH 7.4. Cells were grown in 75 cm² tissue culture flasks and incubated at 37°C in a humidified air atmosphere of 5% CO₂. At 90% of confluence the cells were washed with PBS and detached by a chemical procedure with trypsin solution for 5 min at 37°C in a humidified atmosphere of 5% CO₂. After this period, fresh cell culture medium was added to inactivate the effect of trypsin. The obtained cell suspension was centrifuged at 300 x *g* and 25°C for 5 min. After discarding the supernatant, cells were resuspended in the polymer solution at a cell density of 1 x 10⁶ cells/mL. The resulting suspensions were used to produce spherical and spheroidal particles according to the methods previously described for hydrogel production. After 24h of culture the cell culture medium was removed and 1 mL of PBS containing 2 µL of calcein-AM and 1 µL of PI was added to each well and incubated at 37°C for 10 min protected from the light. After this period, samples were washed several times with PBS to remove all traces of excessive dyes. Samples were visualised by fluorescence microscopy (Axio Imager 2, Zeiss) using appropriate red and green fluorescence filters to detect dead and live cells, respectively.

Results and discussion

Particle characterisation

The obtained superamphiphobic surfaces presented a contact angle of $165.2 \pm 4.2^\circ$, consistent with previous reports.¹⁶ Oblate spheroidal particles ($c < a$) with different thickness were produced by sandwiching liquid M-CHT polymer precursor between

superamphiphobic surfaces with varying spacer height, with subsequent UV-crosslinking, as shown in Figure 1. The particle was thus compressed along the z axis (semi-axis c), whilst the length along the x and y axis increased equally (semi-axis a) (Figure 1 inset).

In Figure 2 it is possible to perceive the effect of decreasing spacer height on the shape of spheroidal particles. Figure 2A shows the droplet geometries predicted by the numerical modelling code, for each prescribed deformation, whilst Figure 2B presents the experimentally observed particles upon deformation. Overlapping of the modelled geometry profile (red dotted lines in figure 2B) on experimentally obtained particles demonstrates that there is a similarity in the disk-like shape for each deformation. Thus, experimental findings were in agreement with the numerical predictions. Figure 2C evidences the spheroidal shape that particles acquire upon removal of the upper superamphiphobic surface, as compared with the disk-like shape they present when “sandwiched” between the surfaces. Green dotted lines represent the theoretical yz plane profiles of spheroids, using the implicit equation of a spheroid $\left(\frac{x^2+y^2}{a^2} + \frac{z^2}{c^2} = 1\right)$, which are in accordance with experimentally obtained particles.

Additionally, the effect of M-CHT concentration on spheroidal particle shape upon removal of the upper superamphiphobic was studied using 2, 3, and 4% (w/v) solutions. As can be observed in figure 3, the recovery of particle shape was identical for all deformations studied, independently of the concentration of the precursor solution.

Figure 4 presents the relationship of particle deformation with circularity and surface area, which were both calculated from the contour of the projected particle. The standard deviation values for circularity of each batch were small (0.04), indicating that the method was quite reproducible. As expected, an increase in particle deformation, was correlated with a decrease in particle circularity. As can also be observed from Figure 3, there was an increase in surface area with increased particle deformation.

BSA release assay

The cumulative release profiles of BSA from M-CHT spheres and spheroids (62% deformation) ($n = 3$) are presented in Figure 5A, as well as the data adjusted according to Korsmeyer-Peppas equation.

The cumulative BSA release from spheroids was faster than from spheres, with a statistical significance of at least 5% was observed for most time-points, including the initial 5 and 30 minutes. All particles presented the same initial volume. Therefore, the distinct results obtained may be attributed to the larger surface area of spheroids comparatively to spheres, thus facilitating diffusion across the particle surface. According to the Korsmeyer-Peppas model, spherical particles presented an n value of 0.0891, and spheroidal particles presented $n=0.0757$. According to Peppas *et al.*,²¹ the diffusional exponent for Fickian release in spheres is $n = 0.432 \pm 0.007$. For values below this threshold the release kinetics can be classified as Quasi-Fickian diffusion, or partial diffusion, where another process in addition to diffusion is responsible for drug transport.^{22,23} Hence, the release mechanism for both spherical and spheroidal particles studied can be classified as Quasi-Fickian.

Additionally, Figure 5B presents the results obtained on the numerical modelling studies comparing the release of a compound from spherical and spheroidal particles with varying compression along the c semi-axis. As shown, the release tends to be more accelerated with increasing compression along the c semi-axis, with the spherical particle (1) presenting the slowest release. This is in accordance with experimentally obtained results, as the release curve for the studied particle with 62% deformation, should be between particles with relative height $\frac{1}{2}$ and $\frac{1}{4}$.

A cumulative release of approximately 100% was observed by Lima *et al.*²⁴ for the encapsulation of low molecular weight model drug dexamethasone within chitosan hydrogel particles, due to the high encapsulation efficiency expected for particles produced on superhydrophobic surfaces. However, this was not the case in the present study, where the maximum cumulative release observed was $50.4 \pm 2.6\%$ for spheroids and $47.4 \pm 3.2\%$ for spheres. Taking into consideration that BSA is negatively charged at physiological pH (pH = 7.4) due to an isoelectric point (pI) between 4.7 and 5.3 (according to product specifications), the formation of complexes between BSA and positively charged chitosan molecules may explain the low cumulative release values.²⁵ This causes the entrapment of a fraction of the encapsulated BSA within the polymer matrix and blocks its capability to diffuse out of the network. The fact that both spheroidal and spherical particles tend towards similar final cumulative release values corroborates the theory of chitosan-entrapped proteins.

The initial hypothesis that spheroids would present a faster release rate comparatively to spheres was confirmed. This may be explained by the higher average distance between the points in the object and its surface, in the case of spherical particles, hence resulting in a larger travel distance for molecule diffusion. Thus, it was possible to significantly alter the release rate of an entrapped molecule by simply changing particle shape from spherical to spheroidal.

Live/Dead assay

Cells were successfully encapsulated within spherical and spheroidal hydrogel particles, showing an even cell distribution within all particles. In Figure 6 it is possible to assess a higher viability degree within L929-laden spheroidal particles, when compared to spherical particles after 24 hours. Spherical particle (0% deformation) presented the highest number of dead cells, followed by 48% deformation spheroid. Spherical particles with 62% and 76% deformation presented virtually no dead cells (> 99% of live cells).

Given the size of the particles studied, it was expected that spherical particles would contain dead cells within the core, seen as oxygen and nutrient diffusion is compromised by the crosslinked polymer network.^{4,16} Interestingly, by increasing the particle deformation and thus increasing particle surface area, it was possible to bypass the size limitation and increase overall cell viability. Since the exchange of nutrients and waste is facilitated in the spheroidal particles, there is likely a promotion of cell growth and proliferation. Our hypothesis that spheroids would improve cell viability was therefore confirmed.

Conclusions

Spheroidal particles with varying thickness were successfully and reproducibly created using photocrosslinkable chitosan and superamphiphobic surfaces. Varying the distance between the superamphiphobic surfaces allowed to control the deformation of hydrogel precursor droplets, resulting in a range of particles after UV-crosslinking. Using chitosan as a model to prove this concept, the application of these particles was further studied as an encapsulation vehicle for either drugs/proteins or cells. For spheroids, a significantly faster cumulative BSA release, and an enhanced cell viability was observed, comparatively to spheres, greatly evidencing the impact of a larger surface area.

Furthermore, the good correlation obtained between the experimental results and the numerical predictions, both for droplet deformation and drug release rates, evidenced that numerical modelling can provide useful support on the design of these systems. The proposed technology could expand the use of superhydrophobic and superamphiphobic platforms, which have been employed to process spherical particles, towards the production of spheroid-like objects with potential to be used in biomedical applications.

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References

1. Seliktar D. *Science* (80-.). 2012; 336:1124–1128.
2. Gasperini L, Mano JF, Reis RL. *J R Soc Interface*. 2014; 11
3. Peppas NA, Hilt JZ, Khademhosseini A, Langer R. *Adv Mater*. 2006; 18:1345–1360.
4. Correia CR, Sher P, Reis RL, Mano JF. *Soft Matter*. 2013; 9:2125–2130.
5. Karp-Boss, L, Boss, E. *Aquatic Microbial Ecology and Biogeochemistry: A Dual Perspective*. Glibert, PM, Kana, TM, editors. Springer International Publishing; Switzerland: 2016. 25–34.
6. Soman P, Chung PH, Zhang AP, Chen S. *Biotechnol Bioeng*. 2013; 110:3038–3047. [PubMed: 23686741]
7. Drury JL, Mooney DJ. *Biomaterials*. 2003; 24:4337–4351. [PubMed: 12922147]
8. Champion JA, Mitragotri S. *Proc Natl Acad Sci U S A*. 2006; 103:4930–4934. [PubMed: 16549762]
9. Decuzzi P, Godin B, Tanaka T, Lee S-Y, Chiappini C, Liu X, Ferrari M. *J Control Release*. 2010; 141:320–327. [PubMed: 19874859]
10. Muro S, Garnacho C, Champion JA, Leferovich J, Gajewski C, Schuchman EH, Mitragotri S, Muzykantov VR. *Mol Ther*. 2008; 16:1450–1458. [PubMed: 18560419]
11. Dusenbery DB. *J Bacteriol*. 1998; 180:5978–5983. [PubMed: 9811657]
12. Song W, Lima AC, Mano JF. *Soft Matter*. 2010; 6:5868–5871.
13. Rodríguez-Velázquez E, Taboada P, Alatorre-Meda M. *Colloids Surfaces B Biointerfaces*. 2017; 160:732–740. [PubMed: 29150150]
14. Lima AC, Song W, Blanco-Fernandez B, Alvarez-Lorenzo C, Mano JF. *Pharm Res*. 2011; 28:1294–1305. [PubMed: 21298327]
15. Takei T, Hamada S, Terazono K, Yoshida M. *Process Biochem*. 2017; 54:28–32.

16. Costa AMS, Mano JF. J Am Chem Soc. 2017; 139:1057–1060. [PubMed: 28071048]
17. Lima AC, Custódio CA, Alvarez-Lorenzo C, Mano JF. Small. 2013; 9:2487–2492. [PubMed: 23296984]
18. Neto AI, Demir K, Popova AA, Oliveira MB, Mano JF, Levkin PA. Adv Mater. 2016; 28:7613–7619. [PubMed: 27332997]
19. Yu LMY, Kazazian K, Shoichet MS. J Biomed Mater Res Part A. 2007; 82A:243–255.
20. Deng X, Mammen L, Butt HJ, Vollmer D. Science (80-.). 2012; 335:67–70.
21. Ritger PL, Peppas NA. J Control Release. 1987; 5:37–42.
22. Peppas, NA, Korsmeyer, RW. Hydrogels in medicine and pharmacy. Vol. III. CRC Press; Boca Raton, Florida: 1987. 109–136.
23. Rehman K, Zulfakar MH. Pharm Res. 2017; 34:36–48. [PubMed: 27620176]
24. Lima AC, Correia CR, Oliveira MB, Mano JF. J Bioact Compat Polym. 2014; 29:50–65.
25. Kang GD, Song S-C. Int J Pharm. 2008; 349:188–195. [PubMed: 17884313]

Figure 1. Schematic representation of oblate spheroidal particle production method. Inset: Axis (x , y , z) and semi-axis (a , c) representation. M-CHT liquid precursor was squeezed between superamphiphobic surfaces separated using a known spacer height, followed by UV-crosslinking. Representative SEM image of topographical features present on superamphiphobic surfaces (scale bar corresponding to $10\ \mu\text{m}$).

Figure 2.

A: Geometry predicted by numerical studies of the shape of the elastic liquid droplet upon deformation. B: Effect of decreasing spacer height on particle format. Dotted red lines correspond to the profiles of predicted particle geometry by numerical studies. C: Spheroidal particle shape acquired upon removal of upper superamphiphobic surface. Dotted green lines correspond to the yz plane representation of spheroids. The particle on the left was used as a spherical control with no deformation and was only in contact with the lower superamphiphobic surface. Increasing deformation was used for the following particles. Scales bars on both sets of images correspond to 1 mm.

Figure 3.

Effect of chitosan concentration on UV-crosslinked spheroidal particles. Particles were produced with concentrations of 2, 3, and 4%, and with equal deformations of 0, 48, 62, and 76%. Scale bars correspond to 1 mm.

Figure 4.

Correlation of particle deformation with circularity (●, $n>8$) and surface area (■, $n>4$). The values of circularity and surface area were obtained from particle images. Second order polynomial fittings are represented only as guidelines for the eye.

Figure 5.

A: Cumulative BSA release from 2% M-CHT spheres (●) and spheroids (62% deformation) (■), up to 216 hours, with a zoom-in for the initial 2 hours (n=3). Values plotted are the mean $\pm$ error for 3 samples. Both experimental and curve fittings using Korsmeyer-Peppas equation are presented in the figure (lines with the same colour of the corresponding symbols). Statistical significance of $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***) are represented for time-points when applicable. B: Numerical modelling studies for the release of a compound from spherical versus spheroidal particles with varying particle height.

Variation in height of spheroidal particles ($\frac{3}{4}$, $\frac{1}{2}$, and $\frac{1}{4}$) is presented relatively to spherical particles, corresponding to 1.

Figure 6.

Live/Dead assay of L929 cells encapsulated within 2% M-CH particles 24 hours after seeding. Spherical (0%) and spheroidal (48%, 62%, and 76%) particles are represented. A: Top views of particles and image magnifications in inset. Scale bars correspond to 750 μm for main images, and 440 μm for inset images. B: Quantification of the relative number of live cells per condition.