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Biocompatible hollow polymeric particles produced by a mild solvent- and template free strategy

Eustolia Rodríguez-Velázquez[a,b,c,d,*], Pablo Taboada[d], Manuel Alatorre-Meda[c,e,**]

[a] Facultad de Odontología, Universidad Autónoma de Baja California, Campus Tijuana, Calzada Universidad 14418, 22390 Tijuana, B. C., Mexico
[b] Departamento de Estomatología, Facultad de Odontología, Universidad de Santiago de Compostela, Campus Norte S/N, E-15782 Santiago de Compostela, Spain
[c] Instituto de Ortopedia y Banco de Tejidos Musculoesqueléticos, Universidad de Santiago de Compostela, Campus Sur S/N, E-15782 Santiago de Compostela, Spain
[d] Grupo de Física de Coloides y Polímeros, Departamento de Física de Materia Condensada, Facultad de Física, Universidad de Santiago de Compostela, Campus Sur S/N, E-15782 Santiago de Compostela, Spain
[e] CONACyT – Instituto Tecnológico de Tijuana, Centro de Graduados e Investigación en Química, Blvd. Alberto Limón Padilla S/N, 22510 Tijuana, B. C., Mexico

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ABSTRACT

Macroscopic hollow polymeric particles are attractive materials for various applications such as surgery, food industry, agriculture, etc. However, protocols reporting their synthesis have hitherto made use of organic solvents and/or sacrificial templates, compromising the encapsulation of different bioactive compounds and the process yield. Here, millimeter-size, hollow polymeric particles were synthesized, for the first time, in a solvent- and template free manner onto superhydrophobic surfaces (SHS). The particles were produced upon assembly and double superficial crosslinking of liquid droplets of DNA and methacrylamide chitosan aqueous solutions (CH:MA), leading to liquid-core particles with a hardened hydrogel shell. The particles displayed appealing physical and biological properties. The millimeter-size hydrogel shell, resulting from the double ionic/covalent crosslinking of CH:MA, endowed the hollow particles with softness to the touch and an outstanding structural stability against manipulation by hand and with forceps. Meanwhile, the liquid DNA core guaranteed a biocompatible cell encapsulation followed by a superior release and proliferation of viable cells, as compared to solid CH:MA particles prepared as a blank. Particles with these characteristics show promise for surgical protocols practiced in Tissue Engineering and Regenerative Medicine, where manipulable and biocompatible synthetic implants are often needed to supply living cells and other sensitive bioactive compounds.

1. Introduction

Hollow polymeric particles, i.e. hybrid particles composed of a hydrogel shell and an inner liquid (or void) space, constitute a special class of carriers that have a tremendous potential for a wide range of biomedical and technological applications such as surgery, drug delivery, food industry, and agriculture; just to cite a few. Their suitability for this range of applications stems from the fact that depending on the nature of the void space and the outer polymeric shell, this kind of particles can encapsulate virtually any kind of cargo [1,2].

Hollow polymeric particles at the macro-, micro-, and nano-scale can be produced in high yields by different well-known methodologies such as template-assisted self-assembly of polymeric chains, *in-situ* polymerization, polymer coacervation, microfluidics, and polymer emulsification followed by solvent extraction [3,4]. However, most of these methodologies, referred to as wet-chemistry routes, suffer the major drawback of requiring the use of organic solvents and toxic surfactants, the synthesis of sacrificial templates that have to be liquefied at post-production stages by treatment with strong acidic solutions, and/or the implementation of extreme working conditions of temperature and pressure [5,6], compromising the encapsulation and release of sensitive bioactive compounds.

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* Corresponding author at: Facultad de Odontología, Universidad Autónoma de Baja California, Campus Tijuana, Calzada Universidad 14418, 22390 Tijuana, B. C., Mexico.
** Corresponding author at: CONACyT—Instituto Tecnológico de Tijuana, Centro de Graduados e Investigación en Química, Blvd. Alberto Limón Padilla S/N, Tijuana, B. C. 22510, Mexico.
E-mail addresses: eustolia.rodriguez@gmail.com (E. Rodríguez-Velázquez), manuel_alatorre@yahoo.com.mx (M. Alatorre-Meda).

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Overcoming the aforementioned pitfalls, we report a mild solvent- and template free strategy to produce biocompatible hollow polymeric particles on top of SHS. Being inspired in the rolling of water droplets over lotus leaves, SHS have been used for some years now as solid substrates for shaping and polymerization of spherical hydrogel particles for diverse applications [7–11]. The distinctive features of the SHS technology are that (i) it entails the simple pipetting and subsequent crosslinking of polymeric solutions which can be loaded with bioactive molecules, (ii) it does not require the use of solvents different than water, (iii) it yields encapsulation efficiencies of ca. 100%, and, very importantly, (iv) it does not compromise the structure/activity of the selected cargoes. Based on these positive characteristics, early reports on this technology demonstrated the synthesis of mono- and multi-compartmentalized solid particles capable of encapsulating cells and proteins [8,9]. Meanwhile, very recent publications have started to describe the synthesis of liquid-core particles with appealing characteristics for applications such as electronics, agriculture, and cosmetics; although entailing the use of sacrificial templates and/or requiring conditioning steps involving either gelling at very low temperatures or exposure of the inner core to salts and acids for liquefaction [12,13], which might compromise the integrity of any given biological cargo even after its release. Improving on this biomimetic technique by eluding the use of templates, the implementation of freezing conditions, and the influx of salts and/or acids to the inner compartment, we herein report, for the first time, on the synthesis of biocompatible hollow polymeric particles from aqueous droplets of polymeric solutions in a solvent- and template free manner. Millimeter-size, liquid-core hydrogel particles were produced onto SHS from droplets of DNA (forming the core) and CH:MA aqueous solutions (forming the shell), which were assembled and hardened by their interface and outer surface by a mild double ionic/covalent gelling process occurring at room temperature. DNA and CH:MA were chosen as the particles' constituents based on their well-known cytocompatibility and the opposite electrostatic charge they present in solution [14–17]. To assess the biocompatibility of the process, human osteoblast cells were encapsulated within the liquid core of the particles and set to release under standard culture conditions. The cytocompatibility and structural stability of the produced samples, as well as the cell release and −proliferation processes, were evaluated by a battery of experimental assays/techniques such as cell staining, fluorescence microscopy, the MTT cell proliferation assay, and organoleptic mechanical tests, among others.

2. Materials and methods

2.1. Materials

Low viscous chitosan from crab shells (CH, viscosity ≤200 mPa·s, 1% in 1% acetic acid, at 20 °C), calcein-AM solution (4 mM in DMSO), and propidium iodide (1 mg/mL in water) were from Fluka. Calf thymus DNA sodium salt with an average molecular weight of 12.5×10^6 g mol^{-1}, methacrylic anhydride (MA, ≥ 92%), 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone (Irgacure 2959 or I2959, 98%), tetraethyl orthosilicate (TEOS, 98%), 1H,1H,2H,2H-perfluorodecyltriethoxysilane (silane, 97%), ammonium hydroxide solution (30−33%), crystal violet, phosphate buffered saline (PBS), and D_2O were from Sigma-Aldrich. Glass microscope slides were from Medline Scientific (Spain). MTT cell proliferation kit was from Roche. Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), MEM Non-Essential Amino Acids solution (NEAA; 100×), sodium pyruvate (100 mM), L-glutamine (L-Glu; 200 mM), penicillin-streptomycin (pen-strep; 100 mg/mL), and trypsin-EDTA (0.25%, 0.913 mM EDTA) solutions were from Invitrogen. Osteoblast (SAOS-2) cell line was from ATCC. Cell culture plates were from Corning Costar. Sterile filtered Milli-Q water was used throughout. Unless otherwise stated, all experiments were carried out under sterile conditions at least in triplicate.

2.2. Preparation and characterization of SHS

SHS were produced as described elsewhere [18]. Briefly, glass microscope slides were held above the flame of a paraffin candle in order to form a soot layer. Afterward, the soot coated substrates were exposed to the chemical vapor deposition (CVD) of TEOS (4 mL) in the presence of ammonia (4 mL) for 24 h in a desiccator, followed by the calcination of the hybrid carbon/silica network at 600 °C for 2 h. Finally, the hydrophilic silica shell was coated with silane (150 μL) by CVD for 3 days. The surfaces were characterized for their topography and water contact angle (WCA) by Scanning Electron Microscopy (SEM, Zeiss FESEM Ultra Plus with EDX operated at 3 kV) and Static WCA measurements from lateral view images of deposited 5 μL droplets (employing an 8-Megapixel iSight digital camera for images' acquisition and the Image J software for images' analysis), respectively. For the SEM characterization, the surfaces were sputter-coated with iridium.

2.3. Synthesis and characterization of CH:MA

CH:MA was synthesized and characterized following the protocols published by Yu et al. [19] Briefly, CH was dissolved to 3 w/v% in 2 wt% acetic acid overnight with constant stirring at room temperature (RT). MA was then added at 0.4 molar equivalents per CH repeat unit and left with constant stirring for 3 h at RT. The obtained blend was afterward dialyzed against distilled water for 3 d, changing the water three times a day. The CH:MA solution was then freeze-dried and stored at −20 °C until use. All steps from the addition of MA on were carried out under dark conditions.

The degree of chitosan methacrylation was evaluated by ^{1}H NMR. To this end, CH:MA was dissolved to 2 w/v% in D_2O and analyzed using an 11.7 T Bruker DRX-500 spectrometer operating at a 500 MHz proton frequency. The obtained spectrum was processed with the MestreNova software (Mestrelab Research Inc., Spain). Chemical shifts are expressed in ppm.

The ζ-potential of the CH:MA polymer was determined with a Zetasizer NanoSeries instrument (Malvern) using folded capillary cells. To this end, CH:MA was dissolved to a concentration of 0.1 w/v% in PBS. The obtained solution was characterized in terms of its electrophoretic mobility from which the ζ-potential was determined using the classical Smoluchowski expression: $\alpha = \varepsilon\zeta / \eta$, where α, ε, ζ, and η correspond to the electrophoretic mobility, permittivity of the medium, ζ-potential of the solute, and viscosity of the medium, respectively. Measurements were carried out at 25 °C. The results are the average of five measurements.

2.4. Covalent crosslinking conditions

DNA-CH:MA and CH:MA particles were gelled upon the UV-light-driven crosslinking of 2 w/v% CH:MA dissolved in PBS containing I2959 0.2 w/v%). I2959 is a cytocompatible photoinitiator [20]. The crosslinking process was carried out for 1 min in both cases employing a UV-lamp (Vilber Lourmat VL-4.LC) working with a power of 120 mW/cm^2 at a wavelength of 365 nm.

2.5. Cryo-SEM characterization of the DNA-CH:MA interface

DNA-CH:MA particles were imaged by cryo-SEM employing a JEOL JSM − 6360LV − GATAN ALTO2100 microscope operating at 15–20 kV. Briefly, the particles were mounted on top of a sample holder and solidified upon quick immersion in liquid nitrogen.

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