3D printed tablets loaded with polymeric nanocapsules: an innovative approach to produce customized drug delivery systems

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Abstract

The generation of multi-functional drug delivery systems, namely solid dosage forms loaded with nano-sized carriers, remains little explored and is still a challenge for formulators. For the first time, the coupling of two important technologies, 3D printing and nanotechnology, to produce innovative solid dosage forms containing drug-loaded nanocapsules was evaluated here. Drug delivery devices were prepared by fused deposition modelling (FDM) from poly(ε-caprolactone) (PCL) and Eudragit® RL100 (EUD) filaments with or without a channelling agent (mannitol). They were soaked in deflazacort-loaded nanocapsules (particle size: 138 nm) to produce 3D printed tablets loaded with them, as observed by SEM. Drug loading was improved by the presence of the channelling agent and a linear correlation was obtained between the soaking time and the drug loading (r^2 = 0.9739). Moreover, drug release profiles were dependent on the polymeric material of tablets and the presence of the channelling agent. In particular, tablets prepared with a partially hollow core (50% infill) had a higher drug loading (0.27 % w/w) and faster drug release rate. This study represents an original approach to convert nanocapsules suspensions into solid dosage forms as well as an efficient 3D printing method to produce novel drug delivery systems, as personalised nanomedicines.

Keywords: 3D printing, drug delivery, fused deposition modelling, nanocapsules, nanotechnology.

1. Introduction

Three-dimensional (3D) printing is an additive manufacturing technology characterized by a single process to combine different materials to build objects from a 3D model, which can circumvent the use of different unit operations (Frazier, 2014; Jonathan and Karim, 2016). 3D printing comprises a range of different technologies, such as stereolithography (SLA), fused deposition modelling (FDM), pressure-assisted microsyringes (PAM) and selective laser sintering (SLS).

Its application in the development of drug delivery systems has been recently described to produce fast-disintegrating tablets (Yu et al., 2009), time-controlled release tablets (Goyanes et al., 2015a, Pietrzak et al., 2015, Wang et al., 2016), multi-layer caplets (Goyanes et al., 2015b), multi-active solid dosage forms (Khaled et al., 2014), microneedles (Boehm et al., 2014, Kochhar et al., 2012), implants (Water et al., 2015) and topical drug delivery devices (Goyanes et al., 2016).

Among the available techniques, FDM printing has some important advantages, such as low cost, no need for organic solvents, the feasibility of blending of drugs and polymeric materials in a prior hot-melt extrusion step (Jonathan and Karim, 2016), the ability to manufacture of multidrug devices (Goyanes et al., 2015b), the modulation of drug release profiles through shape and density (Goyanes et al., 2014), the preparation of devices from FDA GRAS (Generally Recognized as Safe) raw materials (Pietrzak et al., 2015, Melocchi et al., 2016) as well as the feasibility to produce customizable drug delivery devices for personalised medicine (Choonara et al., 2016, Tucker et al., 2016). Personalised medicine covers the individualisation of the drug therapy, dosing intervals, drug combinations and drug release rate, taking into account an individual's differences in genome, metabolic functions, patient groups, coexisting diseases and the need of multiple drugs (Alomari et al., 2015, Tucker et al., 2016).

On the other hand, nanotechnology has been an important tool for the development of novel drug delivery systems (Bobo et al., 2016) and has great potential to transform medicine, including the realisation of personalised medicines (Herrmann and Rösslein, 2016). Among the reported drug nanocarriers, polymeric nanocapsules have gained special attention due to

their ability to control the drug release profile and their good stability in biological fluids (Pohlmann et al., 2013). Moreover, drug-loaded nanocapsules have been reported to improve the efficacy of drugs, their aqueous solubility and chemical stability, their oral bioavailability as well as to overcome biological barriers (Frank et al., 2015, Friedrich et al., 2015).

Polymeric nanocapsules are composed of an oily core surrounded by a thin polymeric wall stabilized by a surfactant (Pohlmann et al., 2013). They are obtained as liquid suspensions and can be physically stable for months, although they show a high risk of microbiological contamination for long-term storage due to their high water content (Beck et al., 2012). Some efforts have been carried out to produce solid forms containing nanocapsules to overcome drawbacks regarding microbiological contamination, physical stability, storage and shipping, including spray-drying (Beck et al., 2012), freeze-drying (Schaffazick et al., 2003) and wet granulation techniques (Friedrich et al., 2010a). However, there is still one report on the production of tablets containing drug-loaded polymeric nanocapsules (Friedrich et al., 2010b), which were produced by wet granulation reaching a maximum drug loading of 0.083 % (w/w). No report on the formulation of spray-dried or freeze-dried drug-loaded polymeric nanocapsules as tablets is available. In addition, tablets containing Newcastle disease virus loaded in naturally occurring casein micelles, called nanocapsules, were previously formulated by moulding for veterinary use (Wambura, 2011).

To the best of our knowledge, there is no report on the development of 3D printed dosage forms containing drug nanocarriers, although the use of non-polymeric nanoparticles in the production of 3D printed materials by SLA printing has been reported in the literature for purposes other than drug delivery (Zhu et al., 2016, Pawar et al., 2016).

In this scenario, this study was designed to conjugate 3D printing and nanotechnology in the development of tablets containing polymeric nanocapsules, as personalised medicines, to tailor their drug dose and drug release profile. 3D printed tablets loaded with nanocapsules were prepared from Eudragit RL100® or poly(ε-caprolactone) polymers, as aqueous swellable and non-swellable polymers, respectively. The manufacturing process is detailed in Figure 1. Polymeric filaments were prepared by hot met

extrusion (HME), which were then FDM 3D printed to create the delivery devices. Drug-loading was achieved by soaking the printed devices in a nanoparticle liquid suspension. The effect of the type of the main polymeric material of the tablets, the presence of a channelling agent and the infill percentage on their drug loading and drug release profiles were evaluated. Deflazacort was used as a model drug.

Please insert Figure 1 about here.

2. Material and Methods

2.1. Materials

Eudragit RS100® and RL100® were obtained from Evonik Industries (Darmstadt, Germany). Polysorbate 80 was acquired from Sigma-Aldrich (Poole, UK). Miglyol 812 N was kindly donated by Cremer (Witten, Germany). Deflazacort was supplied by Fagron (São Paulo, Brazil). Poly(ε-caprolactone), 50,000 Da MW, as powder (Capa™ 6506), was kindly donated by Perstop (Cheshire, UK). Acetone (Fisher Chemicals) was obtained from Fisher Scientifics (Loughborough, UK). Mannitol (Alfa Aesar®) and PEG6000 were purchased from Fisher Scientifics (Loughborough, UK) and Merck (Darmstadt, Germany), respectively. Microcrystalline cellulose (Avicel PH301®, FMC Biopolymer) was supplied by IMCD (Sutton, UK). Triethyl citrate was acquired from Sigma-Aldrich (St. Louis, USA). HPLC grade acetonitrile was purchased from Fisher Scientifics (Loughborough, UK). All other chemicals and solvents were of analytical grade and were used as received.

2.2. Methods

2.2.1. Preparation and characterization of the filaments

Filaments were prepared by hot-melt extrusion (HME). Four different filaments were produced with a swellable polymer (Eudragit RL100® - ERL) or a non-swellable polymer [Poly(ε-caprolactone) – PCL], as the main polymeric compound, with or without mannitol as a channelling agent. For tablets prepared without the channelling agent, microcrystalline cellulose was used instead of mannitol, due to its low water solubility. Triethyl citrate was used as a plasticizer and PEG 6000 as a hydrophilic lubricant to facilitate the extrusion

process. The composition of the four different filaments produced is detailed in Table 1. The components were weighed and mixed using mortar and pestle for 5 min. Afterwards, the mixture (40 g) was fed in a single-screw hot-melt extruder (Noztek Pro, Noztek, Shoreham, UK). The extrusion was carried out through a nozzle die of 1.5 mm (ERL filaments) or 2.0 mm diameter (PCL filaments). The extruding temperature was set to 110 ± 5 °C and 65 ± 5 °C for ERL and PCL filaments, respectively. The diameter of the filament was measured with a digital Calliper (Fowler High Precision, Auburndale, USA) at different positions along the filament in order to assure a diameter between 1.65 ± 0.05 mm.

Please insert Table 1 about here.

All filaments and their respective physical mixtures (PM-ERL-M, PM-ERL-A, PM-PCL-M and PM-PCL-A) were analysed by Differential Scanning Calorimetry (DSC). Analyses were performed with a Q2000 DSC (TA Instruments LLC, USA) at a heating rate of 10 °C/min. Indium (T_m= 156.6 °C, ΔH_f = 28.71 J/g) was used to calibrate the cell constant and enthalpy. Nitrogen was used as a purge gas at a flow rate of 50 mL/min. Data were collected and analysed using TA Instruments Advantage software in the range between 25 °C and 250 °C. TA aluminium pans and pin-holed hermetic lids (T_{zero}) were used with an average sample mass between 8 and 10 mg. Thermogravimetric analysis (TGA) was performed with a Discovery Series TGA, (TA instruments LLC, USA). Samples were heated at 10 °C/min in open aluminum pans, using Nitrogen as a purge gas (flow rate of 25 mL/min). Data were analyzed using TA Instruments Trios software. The percentage mass loss and/or onset temperature were calculated.

2.2.2. Preparation and characterization of the 3D printed delivery devices

Delivery devices were produced with the four different filaments (ERL-M, ERL-A, PCL-M and PCL-A) using a commercial FDM 3D printer (MakerBot Replicator 2, MakerBot Inc, USA). The printing process was based on a template previously reported by Goyanes and co-workers (2014), comprising a disk-shaped tablet with a mean diameter of 10.0 mm and a height of 3.60 mm. The 3D object was designed with MakerWare Software (v.2.2.2). The

printing process was carried out at an extrusion temperature of 170 °C and 95 °C for the delivery devices prepared from the ERL and PCL filaments, respectively. The following printing parameters were used: speed while extruding (90 mm/s), speed while traveling (150 mm/s), number of shells (2), infill percentage (100%), and layer thickness (0.20 mm). At least 10 devices from each filament were prepared and analysed. In order to evaluate the influence of the infill percentage, devices from the filament ERL-M were also prepared with 50 % of infill percentage.

The mean weight of the delivery devices was evaluated weighing separately 10 devices using a calibrated analytical balance (CPA225D model, Sartorius, Gottingen, Germany). Their mean diameter and mean height were measured using a digital Calliper (Fowler High Precision, Auburndale, USA). The diameter of each device was measured in two different parallels (horizontal and vertical) and their mean values were considered.

2.2.3. Preparation and characterization of deflazacort-loaded polymeric nanocapsules

Nanocapsules were prepared by interfacial deposition of Eudragit RS 100®, (Fontana et al., 2014). An organic phase containing polymer (EUD, 0.5g), Miglyol 812 N (0.83 mL), and deflazacort (25 mg) in acetone (134 mL) was prepared under magnetic stirring at room temperature. Afterwards, this organic phase was injected into an aqueous phase (267 mL) containing polysorbate 80 (0.385 g) under magnetic stirring at room temperature (25 ± 5 °C). This dispersion was left under magnetic stirring for 10 min followed by the evaporation of the acetone and most of the water content under reduced pressure at 50 °C to form a concentrated aqueous dispersion (Rotavapor RC 900, KNF LAB, Balterswil, Switzerland). The final volume was set to 50 mL to reach a deflazacort concentration of 0.5 mg/mL. Three independent batches were prepared and characterized. The batches were kept at room temperature (25 ± 5 °C) and protected from light. This formulation was named as DFZ-ENC. After preparing, the particle size distribution, zeta potential, drug content and drug encapsulation efficiency were evaluated.

Volume-weighted mean diameters ($D_{4,3}$) and polydispersity (Span) (n=3) were analysed by laser diffraction (LD) (Mastersizer 3000, Malvern Instruments Ltd., UK), dropping the sample directly into the compartment disperser

containing ultrapure water (150 mL). Mean particle size and polydispersity index (PDI) (n=3) were also measured using (DLS) (ZetaSizer Nano ZS, Malvern Instruments Ltd., UK) after the dilution of the suspensions (20 µL) in previously filtered (Millex-HA filter, 0.45 µm, Merck Millipore, Darmstadt, Germany) ultrapure water (10 mL). Zeta potential was measured by electrophoretic mobility (ZetaSizer Nano ZS, Malvern Instruments Ltd., UK). Samples were diluted (1:500 v/v) in NaCl solution (10 mM) previously filtered (Millex-HA Filter, 0.45 µm, Merck Millipore, Darmstadt, Germany).

Drug content was measured (n=3) by liquid chromatography (LC), according to a method previously described (Rigo et al., 2016) with some modifications. The chromatographic system consisted of an Eclipse Plus RP-18 column (150 mm x 4.6 mm, 5 µm, Agilent Technologies, Santa Clara, USA) and an Agilent LC instrument (1200 series, G1311A Quaternary Pump, G1329A Autosampler, and G1314B Variable Wavelenght Detector, Agilent Technologies, Santa Clara, USA)**.** The mobile phase (at a flow rate of 0.8 mL/min) was composed of acetonitrile/water (80:20%, v/v), the volume of injection was 100 µl, and DFZ was detected at 244 nm. DFZ-ENC suspension (200 µL) was transferred to a 10 mL volumetric flask. The sample aliquot was completely dissolved adding acetonitrile (8 mL) followed by adjusting the volume with the same solvent. The diluted sample was filtered (0.45 µm, Millipore® filter) and analysed by LC. Encapsulation efficiency was calculated based on the difference between total drug and free drug content in the ultrafiltrate. The ultrafiltrate was obtained by ultrafiltration/centrifugation technique (Ultrafree-MC 10,000 MW, Millipore, Billerica, USA) at 4,120 $\times g$ for 10 min.

In-vitro drug release from DFZ-ENC was evaluated (n=3) using the dialysis bag method (Rigo et al., 2016). Water containing polysorbate 80 at 2 % (w/v) was used as medium at 37 °C in order to maintain the sink conditions. The dialysis bag containing 1 mL of the sample (0.5 mg/mL) was placed in a 250-mL flask filled with release medium (200 mL) under constant stirring (70 ± 10 rpm). Aliquots (1 mL) of the external medium were withdrawn at predetermined time intervals, and replaced by an equal volume of fresh medium. The withdrawn sample was filtered (0.45-µm filter) and DFZ was

assayed by liquid chromatography according to the methodology described above, using 20 µL as injection volume.

2.2.4. 3D printed tablets loaded with polymeric nanocapsules

2.2.4.1. Preparation

3D printed tablets were loaded with polymeric nanocapsules by soaking the 3D printed devices in the DFZ-ENC suspension (2 mL/device) for 24h at room temperature. Afterwards, the 3D printed tablets were removed and dried at 30 °C for 24 h. They were stored at room temperature and protected from light until analysis. At least 6 tablets were prepared for each formulation. The influence of the soaking time (4, 12 and 24 h) was evaluated.

2.2.4.2 Physical Characterization

Physical dimensions (diameter and height) and mean weight of 3D printed tablets were assessed as previously described for the delivery devices.

2.2.4.3 Swelling and Erosion Index

For swelling and erosion experiments, three devices from each formulation were weighed and placed into individual glass flasks. DFZ-ENC suspension (2 mL) was added to each flask. After 24 h of soaking, the tablets were carefully withdrawn with tweezers. The weights of the hydrated tablets were recorded. Afterwards, the tablets were placed in Petri dish glasses at 30 °C for 24 h to remove the water and to reach a constant mass. Tablets were reweighed. The swelling and the erosion index for each formulation was calculated according to Equations 1 and 2 (Groves and Chaw, 2015), respectively:

$$Swelling\ index\ (\%) = \frac{M_t - M_r}{M_r} \times 100 \qquad (1)$$

Where M_t is the mass of the hydrated tablet after the soaking time (g) and M_r is the mass of the swollen tablet after it has been dried (g).

$$Erosion\ index\ (\%) = \frac{M_0 - M_r}{M_0} \times 100 \qquad (2)$$

Where M_0 is the mass of the dry tablet (device) before swelling (g) and M_r is the mass of the swollen tablet after it has been dried (g).

2.2.4.4 Drug content, drug loading and thermal analysis

Each tablet was placed in a sample glass flask containing acetonitrile (10 mL, n = 3). The flasks were sonicated for 5 or 20 minutes (EUD or PCL tablets, respectively). All the content of the sample flask was quantitatively transferred to a 25 mL volumetric flask, which was filled to volume with mobile phase. The sample solution was filtered through 0.45-µm membrane (PTFE filters, 13 mm, Perkin Elmer, China) for the LC analyses, according to the details described earlier (Section 2.2.3). The method was specific, linear (y= 245.31x + 27.58, r=0.9999, n=3) in the range of 2.00-20.00 µg/mL, and precise (SD=0.91% and 2.44% for intra-day and inter-day precision). Accuracy was 100.35 ± 1.68 %. Retention time was 2.4 min for deflazacort. Drug content was calculated as the amount of drug per tablet (as mg/tablet), whereas the drug loading was calculated from the ratio between the amount of drug and the final tablet mass (as %). Thermal analyses were carried out by TGA and DSC, as previously described for the filaments (Section 2.2.1).

2.2.4.5 Confocal Raman microscopy

Confocal Raman microscopy (CRM) measurements were performed with a WITec alpha 300R+ (WITec GmbH, Ulm, Germany). The excitation source was a monochromatic diode laser (excitation wavelength 532 nm) which was adjusted to a power of 20 mW before the objective (Epiplan Neofluar, Zeiss, Germany, 50x magnification, N.A. 0.8).Signals from out-of-focus regions were rejected by a confocal 50-µm pinhole. Raman spectra of the pure compounds were acquired with 10 accumulations and an integration time of 0.5 s. Image scans of the samples were recorded with an integration time of 0.5 s and a step size was 0.5 µm along the x- and y-axis. Spectra were background subtracted, normalized, and finally converted into false color images using the WITec Project Plus software (WITec GmbH, Ulm, Germany). In these images, pixels assigned to mannitol and MCC spectra are depicted in yellow, while ERL and PCL appear in yellow.

2.2.4.6 Scanning Electron Microscopy

SEM analyses were carried out using a Jeol JSM-6360 Scanning Electron Microscope (Jeol, Tokyo, Japan) at different magnifications. Analyses were carried out at the Centro de Microscopia e Microanálise (UFRGS, Porto Alegre, Brazil). Tablet surfaces and their inner compartments were analysed after longitudinal breaking. Samples were analysed after they had been gold sputtered (Jeol Jee 4BSVG-IN, Tokyo, Japan).

2.2.4.7 In-vitro drug release

In-vitro drug release profiles from 3D printed tablets were evaluated (n=3) using the dialysis bag method, as previously described (Section 2.2.3). Each tablet was inserted in the dialysis bag containing the release medium (1 mL water with polysorbate 80 at 2% w/v). In order to evaluate the influence of the polymeric device (PCL or ERS), the presence of the channelling agent and the infill rate, release data (n = 3) were modelled (MicroMath® Scientist® for Windows™) according to a monoexponential equation (Equation 3) (Cruz et al., 2006). Half-life time ($t_{1/2}$) was calculated according to Equation 4. Data corresponding to the drug release curve between 0 and 60% were fitted to the Power Law model (Equation 5) to understand the mechanism of drug release from the tablets (Siepmann and Peppas, 2012). These equations are described below:

$$C = C_0 e^{-kt} \quad (3)$$

where C is the concentration of drug release at time t (h), C_0 is the initial concentration of the drug and k (h^{-1}) is the constant release.

$$t_{1/2} = \frac{0.693}{k} \quad (4)$$

where $t_{1/2}$ is the time (h) corresponding to 50% of drug release and k (h^{-1}) is the constant release calculated for each formulation according to Equation 3.

$$C = at^n \quad (5)$$

where C is the fraction of DFZ released at time t (h), a is the constant incorporating structural and geometric characteristics of the dosage form, and n is the release exponent, which is indicative of the drug release mechanism.

2.2.4.8 In-vitro nanoparticle release

The qualitative release of nanoparticles from the 3D printed tablets containing nanocapsules was evaluated by DLS (ZetaSizer Nano ZS, Malvern

Instruments Ltd., UK). Each 3D printed tablet was placed in a sample flask containing ultrapure water (20 mL), as the release medium. Experiments were carried out for 24 h under magnetic stirring. After 1, 4 and 8 h the whole medium was withdrawn and replaced by a fresh medium. The medium withdrawn at each time as well as the medium after 24 h of experiment were filtered (0.45 µm, Millipore® filter) and analysed by DLS.

2.2.5 Statistical analysis

Statistical analyses were carried out by one-way and two-way ANOVA, followed by Tukey's test, as post-hoc multiple comparisons, at *p*-value ≤ 0.05 (GraphPad Prism software, version 7.0b, GraphPad Software, Inc, USA).

3. Results and Discussion

3.1. PCL and EUD polymeric filaments

All filaments were successfully prepared by hot-melt extrusion with a diameter between 1.60 and 1.70 mm. After preparation filaments were evaluated by DSC. DSC thermograms are shown in Figure 2. Figure 2A shows the thermograms for the main components of the filaments (Eudragit RL100®, PCL, Mannitol, PEG6000 and microcrystalline cellulose). The glass transition temperature of EUD RL100® was found to be 59.7 °C, confirming a previous report by Shinde and co-workers (2012). PCL showed a broader endothermic peak at 61.1 °C, representing its melting point, which corresponds to its known semi-crystalline form (Pohlmann et al., 2013). PEG 6000 and mannitol showed sharp and prominent endothermic peaks at 62.2 °C and 168.5 °C, respectively, representing their melting points (Rowe et al., 2009). For microcrystalline cellulose a broad endothermic event up to about 150 °C was observed, indicating water loss, which corresponded to a mass loss in the TGA analysis of about 4.5%. TGA data showed thermal degradation starting at about 280 °C (data not shown). Taking into account these data, extruding and printing temperatures were chosen to be below the degradation of any of the components. Figures 2B and 2C show the thermograms for the EUD and PCL filaments and their physical mixtures, respectively. EUD filaments containing mannitol (Figure 2B) exhibited the presence of endothermic peaks at 59.9 °C and 167 °C, corresponding to the presence of PEG 6000 and

mannitol, respectively, while the filaments prepared with microcrystalline cellulose showed an endothermic peak at 55.4 °C, corresponding to the presence of PEG 6000. Differences in the thermal behaviour were not found between the EUD filaments and their respective physical mixtures, showing their crystalline state was not changed during HME processing. Similar behaviour was observed for PCL filaments (Figure 2C), whose thermograms showed the presence of a broad and prominent peak about 61 °C, corresponding to the overlapping of the melting points of PCL and PEG 6000 in the filaments due to their semi-crystalline or crystalline state, regardless of the presence of mannitol or microcrystalline cellulose. Samples containing mannitol (FIL-PCL-M and PM-PCL-M) showed an additional sharp and prominent endothermic peak at about 168 °C, representing to its melting point (Rowe, 2009) and confirming its crystalline state to be the stable beta form in the starting material.

Please insert Figure 2 about here.

3.2. 3D printing of the delivery devices

All the filaments could be printed using FDM to produce the 3D printed delivery devices, regardless of the type of the polymer or the presence of the channelling agent. Table 2 shows their properties, such as mean weight, diameter and height. Their physical dimensions were in agreement with the digital template (10 and 3.60 mm for diameter and height, respectively), with less than 10% of variation. Eudragit RL100® tablets were previously reported by Pietrzak and co-workers (2015) for theophylline extended release. They were prepared at a similar extruding temperature, but without any channelling agent. Moreover, to the best of our knowledge, there is no previous report on the preparation of PCL tablets by 3D printing, as drug delivery systems. Goyanes and co-workers (2016) obtained PCL filaments from a higher molecular weight polymer (80,000 Da), from solvent casted films, in the development of anti-acne topical drug delivery systems, which were not able to be printed into 3-dimensional objects.

Please insert Table 2 about here.

In order to evaluate the influence of the infill percentage on the preparation of 3D printed tablets loaded with nanocapsules, delivery devices were also prepared with 50% infill percentage using the filament ERL-M (ERL-M-50%). They showed mean weight, diameter and height of 0.2513 ± 0.0111 g, 9.68 ± 0.15 mm and 3.82 ± 0.06 mm, respectively, similar to that of tablets prepared with 100% of infill percentage, except the mean weight. The lower the infill percentage, the lower the mean weight. From these data, all the 3D printed devices were selected for further studies on their loading with polymeric nanocapsules.

3.3. Deflazacort-loaded nanocapsules

Deflazacort-loaded nanocapsule suspensions, as a model of nanoparticle suspension, had a milky bluish aspect with a Tyndal effect. The particle size distribution in the nanoscale was confirmed by laser diffraction (Figure 3a), with 90% (d_{90}) of the particles smaller than 0.2843 ± 0.0071 µm. In addition, the D(4,3) value was 0.125 ± 0.003 µm (SPAN: 2.9). The absence of any microparticle population confirms the suitable composition of these nanocapsules. The Z-average size was confirmed by DLS (Figure 3b) as 138 ± 1 nm, with a narrow particle size distribution (polydispersity index: 0.10 ± 0.02) (n = 3). The particles showed a cationic surface with a zeta potential of +6.87 ± 0.44 mV, explained by the cationic properties of their polymeric wall (Eudragit RS 100®). The drug content and encapsulation efficiency were 0.458 ± 0.003 mg/mL and 80.8%, respectively. No aggregation or drug degradation occurred during storage at room temperature (1 month). These properties are in agreement with previous reports regarding Eudragit RS100 nanocapsules prepared by the interfacial deposition of preformed polymer (Fontana et al., 2014, Katzer et al., 2014) as well as deflazacort nanoencapsulation (Rigo et al., 2016). Obtaining nanocapsules suspensions with a narrow size distribution and good encapsulation efficiency is fundamental to maintain their properties, as the control of the drug release rate, the overcoming of biological barriers, the increase in the drug solubility and oral bioavailability, as well as the protection of the gastrointestinal mucosa from the adverse effect of some drugs (Pohlmann et al., 2013).

Please insert Figure 3 about here.

A representative in-vitro DFZ release profile from nanocapsules is depicted in Figure 4. DFZ-ENC released about 65% of drug after 24 h. DFZ released from ENC showed a controlled profile compared to an ethanolic drug solution at the same concentration and experimental conditions, which diffusion through the dialysis bag was higher than 90% after 24 h (Rigo et al., 2016). However, the % of drug release from ENC was higher compared to PCL lipid-core nanocapsules, under the same experimental conditions (Rigo et al., 2016). This result may be explained by the difference in the viscosity of the oily core of nanocapsules and their supramolecular structure. According to these properties, DFZ-ENC was considered a suitable formulation model for the preparation of 3D printed tablets loaded with nanocapsules.

Please insert Figure 4 about here.

3.4. 3D printed tablets loaded with nanocapsules

3D printed tablets were prepared using the DFZ-loaded nanocapsules and the different delivery devices prepared from EUD or PCL filaments. Their physicochemical properties are shown in Table 3.

Please insert Table 3 about here.

Their physical properties (dimensions and weight) were similar to the original devices (differences lower than 5%), except the T-ERL-M. T-ERL-M showed a mean weight corresponding to about 75 % of the original device. The highest erosion index of these tablets can explain this decrease, which was close to 30% and in agreement with the percentage of water-soluble components in their composition (20% Mannitol + 10% PEG 6000). Overall, these data showed that soaking the 3D printed devices in the DFZ-ENC suspension does not change their main original physical properties, except the final mass, which may depend on their composition and subsequently their erosion properties.

The swelling index is a property that might have an important influence on the proposed approach, as it may be related to the amount of water (and nanoparticles) absorbed by the 3D printed devices. Swelling indices were dependent on the material of the 3D printed devices ($p < 0.05$, Two-Way ANOVA). Tablets prepared from EUD RL100® devices showed the highest swelling indices, which can be explained by the nature of this polymer. It is a water-insoluble, highly permeable, ammonium methacrylate copolymer with 10% of functional quaternary ammonium group, with known swelling properties (Rowe et al., 2009). The channelling agent (mannitol) had an additional influence on the swelling index of tablets prepared from EUD RL100® devices. Tablets prepared with mannitol showed a higher erosion index compared to those prepared with microcrystalline cellulose ($p \leq 0.05$). On the other hand, tablets prepared from PCL devices showed very low swelling and erosion indices, regardless of the presence of the channelling agent, which can be explained by the low water-solubility of PCL along with its non-swelling property (Rowe et al., 2009; Pohlmann et al., 2013). The low erosion indices might suggest that the amount of the channelling agent was not enough to promote the efficient water intake into inner compartment of PCL tablets. Therefore, 3D printed tablets were produced from PCL filaments containing 30% instead of 20% of mannitol, called as T-PCL-M'. They showed higher erosion and swelling indices (13.20 ± 0.75 % and 18.92 ± 1.26 %, respectively) compared to tablets containing 20 % of the mannitol ($p \leq 0.05$). Their mean weight, diameter and height were 0.2642 ± 0.0067 mg, 9.68 ± 0.02 mm, and 3.54 ± 0.03, respectively.

The erosion index due to the solubilisation of the water-soluble components was confirmed by spatial compound distribution analysis with confocal Raman microscopy. 3D printed EUD tablets prepared with mannitol did not show the presence of mannitol in their structure (blue colour) after the loading of the drug nanocarriers (Figure 5A), due to the release of mannitol from tablets during the soaking process in the nanocapsules suspension. Only the presence of Eudragit (red color) could be detected. On the other hand, when mannitol was replaced by the water insoluble component (MCC), EUD tablets showed the presence of MCC (blue colour) in their structure (Figure 5B) also after soaking. The same behaviour was observed for PCL tablets (data not

shown). Unfortunately, the strong scattering capabilities of the excipients in combination with the huge concentration difference of excipients versus drug-loaded nanocapsules impeded detection of nanocapsules on the tablet surface by confocal Raman microscopy, regardless of the formulation. Consequently, liquid chromatography and scanning electron microscopy analysis were carried out to evaluate the drug content and the presence of the nanoparticles in the tablets structure, respectively.
Please insert Figure 5 about here.

The amount of drug per tablet (drug content) and drug loading (%) were highly dependent on the polymer used (Two-way Anova, $p \leq 0.05$). Tablets prepared from the EUD RL100® devices showed higher drug content and drug loading than tablets prepared from PCL devices, probably due to their higher swelling indices, as previously discussed. On the other hand, the presence of the channelling agent did not show any influence on the drug content, while its influence can be clearly observed on the % of drug loading ($p \leq 0.05$). While the channelling agent in PCL tablets did not affect the drug loading, the increase of mannitol to 30% (T-PCL-M') was able to increase 1.7x the drug loading (0.1040 ± 0.0048 %). This finding corroborates the erosion and swelling indices, as previously discussed.
All drug loadings (%) were higher than expected. It would be expected that the drug loading would be related to the volume of the DFZ-ENC suspension absorbed by the 3D printed device during the soaking process. From this point of view, the expected drug loading values were calculated considering the drug content of the DFZ-ENC, the volume absorbed and the final mass of the tablet. The following expected drug loading (%) were estimated for T-EUD-M, T-EUD-A, T-PCL-M and T-PCL-A, as: 0.1015 ± 0.0057 %, 0.0810 ± 0.0053 %, 0.0047 ± 0.0003 %, and 0.0057 ± 0.0001 %, respectively. However, experimental findings showed higher drug loading (%) than the expected (about 2x for the T-EUD-M and T-EUD-A, 14x for T-PCL-M and 11x for the T-PCL-A). The drug content was not only affected by passive absorption of the DFZ-ENC (volume intake), but the nanoparticles themselves appear to trigger drug movement towards the tablet. This effect was more pronounced for T-PCL, probably due to its lesser swelling properties,

highlighting the important role of the uptake mechanism governed by the nanocapsules. This mechanism should be investigated further in future studies taking into account particles with different surfaces (cationic, anionic and non-ionic surfaces) and loaded with different drugs and/or labelled-markers.

In addition, loading the EUD 3D printed tablets with DFL-ENC using the proposed approach produce tablets with higher drug loading (%) compared to the previous report for tablets containing nanocapsules (Friedrich et al, 2010a; Friedrich et al., 2010b) where the tablets were prepared by wet granulation with a drug loading (%) of 0.082 %. PCL tablets loaded with 30% of mannitol also showed a higher drug loading compared to this previous report. These data are particularly important when it is considered that the PCL delivery devices are composed of biodegradable and biocompatible materials, approved for systemic use, allowing their future design in the shape of subcutaneous or intrauterine implants for drug delivery.

For a deeper evaluation of the effects of the soaking time, 3D printed tablets loaded with nanocapsules and prepared from ERL-M devices were chosen to evaluate the influence of soaking time on their properties due to their higher drug loading. Table 4 shows the results for tablets prepared with different soaking time (4 and 12 h), which shall be compared to those in Table 3 for T-ERL-M, considered as T-ERL-M_{24h} as well as T-ERL-$M_{100\%}$.

Please insert Table 4 about here.

The swelling and erosion indices, drug content and drug loading were dependent on the soaking time (one-way Anova, $p < 0.05$). The longer the soaking time, the higher the drug content and drug loading. This result can be explained by the time necessary for the complete swelling and erosion of the 3D printed device, reaching values close to 205% and 29% after 24 h, respectively, as well as by improving the inflowing of the nanocapsules.

A linear correlation was obtained considering all the evaluated soaking time (4, 8 and 12 h) and the drug content of tablets ($y = 0.0164x + 0.1378$, $r^2 = 0.9211$) or the soaking time (4, 8 and 12 h) and the drug loading ($y = 0.0084 +$

0.0277, r^2 = 0.9739), suggesting that this property can be used to tailor the desired amount of the drug per tablet in a treatment design.

Furthermore, the infill percentage has an important influence on drug loading. 3D printed tablets prepared from 3D printed EUD-M devices with 50% infill percentage (T-ERL-$M_{50\%}$) showed a reduced mean weight (0.2070 ± 0.0055) compared to T-ERL-M, as expected. Their physical dimensions (diameter and height) and drug content (0.4808 ± 0.0137 mg per tablet) were similar to T-ERL-M. Therefore, the reduced infill percentage led to an increase in the drug loading (0.2744 ± 0.0161 %) compared to them ($p < 0.05$). The reduced infill percentage allows the obtaining of 3D printed tablets loaded with nanocapsules using lower amount of excipients, a lighter drug dosage form and higher drug loading. This feature is particularly important taking into account that the control of the infill percentage is not possible in the conventional tableting methods.

DSC analyses were also carried out on the tablets to confirm the hypothesis about the dissolution of the channelling agent during the soaking process. Results are depicted in Figure 6. Thermograms of tablets printed from EUD filaments (T-ERL-M, T-ERL-$M_{50\%}$ and T-ERL-A) show the glass transition temperature of Eudragit of about 56 °C. T-ERL-M shows a less prominent endothermic peak at 167 °C compared to its original filament (Figure 2B), an additional peak at 157 °C and absence of the endotherm peak at about 60 °C. On the other hand, the T-ERL-$M_{50\%}$ does not show any endothermic peak at 171 °C and a very small signal at 57 °C. These data can be explained by the solubilisation of mannitol (mp of 171 °C) as well as the PEG 6000 (mp 63 °C) during the soaking step. In addition, T-ERL-A does not show any significant endothermic peak, except the Eudragit glass transition temperature, suggesting the solubilisation of PEG 6000 during the soaking process. These data are in agreement with the erosion indices previously discussed, which were in the following descending order: T-EUD-$M_{50\%}$ > T-EUD-M > T-EUD-A. However, the presence of the additional endothermic peak around 157 °C in the thermogram of the T-ERL-M was unexpected. This thermal event can be explained by the re-crystallisation of mannitol in another polymorphic form (the metastable delta form) during the heating process (Burger et al., 2000). As this thermal event at 157 °C was observed in the thermogram of T-ERL-M

before the soaking process (data not shown) it means it is formed during extrusion through the printing nozzle, as the printing temperature (170 °C) was close to the melting point of mannitol. Regarding the PCL tablets, an endothermic peak at 63 °C was observed for all tablets, which is the PCL melting point. Formulations prepared with 20% and 30% of mannitol (T-PCL-M and T-PCL-M', respectively) showed the presence of an endothermic peak at 171 °C, confirming the presence of residual mannitol even after the soaking process and explaining the lower aqueous medium intake for EUD tablets.

Please insert Figure 6 about here.

The morphology of the tablets was analysed by SEM. SEM images of 4 formulations (T-ERL-M, T-ERL-A, T-PCL-M', and T-PCL-A) are shown in Figure 7 for their surface and cross-section. The images confirm the presence of nanoparticles with diameters between 100 and 200 nm on the surface as well as in the inner compartment of the 3D printed tablets loaded with nanocapsules, regardless of the polymeric component or the presence of the channelling agent in the original filament.

Please insert Figure 7 about here.

The influence of the main polymeric component of the delivery device (ERL or PCL), the presence of the channelling agent and the infill percentage was evaluated on the drug release profile of the tablets. The following formulations were evaluated: T-ERL-M, T-ERL-$M_{50\%,}$ T-ERL-A, T-PCL-M', T-PCL-A (Fig 8). The data were modelled according to the monoexponential equation (Table 5), showing a good regression coefficient ($r > 0.99$) in order to compare their release rates.

Please insert Figure 8 about here.

Please insert Table 5 about here.

The influence of the main component of the tablets (ERL or PCL) was dependent on the presence of the channelling agent. In the presence of the channelling agent, ERL tablets (T-ERL-M) showed a faster release constant compared to PCL tablets (T-PCL-M). On the other hand, the release rates were similar for those prepared without the channelling agent (T-ERL-A or T-PCL-A). These data may be explained by the higher swelling and erosion indices showed by the tablets prepared with ERL and the channelling agent, as previously discussed (Table 2), facilitating easier access of the release medium to their inner structure and subsequently increasing the drug release rate. For ERL tablets, a faster release profile was observed for the tablets containing the channelling agent (T-ERL-M) compared to that without it (T-ERL-A). Although both formulations are composed of a swellable polymer, their swelling indices in aqueous medium are significantly different (about 200% and 150% for T-ERL-M and T-ERL-A, respectively), as previously shown (Table 2). Moreover, the infill percentage has an additional influence of the drug release from T-ERL-M. Tablets prepared from the delivery devices containing 50% of infill percentage showed the fastest drug release. This result can be explained by the easier and faster access of the dissolution medium to the whole structure of the 3D printed tablets, in agreement with a previous report on the modulation of the release of a non-encapsulated substance (fluorescein) by changing the infill percentage of 3D printed PVA tablets (Goyanes et al., 2014).

Conversely, 3D printed PCL tablets did not show any influence of the channelling agent on their drug release profiles (Figure 6B) and release constants (Table 6), which can by explained by the low swelling and erosion properties of these tablets. Moreover, the half-life time for the different formulations, that means the time for the release of 50% of the drug, was calculated as 20.67 ± 0.99 min, 12.93 ± 0.60 min, 10.79 ± 0.16 min, 23.75 ± 0.63 min, and 22.10 ± 1.47 min for T-ERL-A, T-ERL-M, T-ERL-$M_{50\%,}$ T-PCL-A, and T-PCL-M. These data confirm the faster release rate by the lower infill percentage, followed by the ERL tablets containing the channelling agent (T-ERL-M). The ERL tablets and PCL tablets without the channelling agent showed the most prolonged drug release.

From the release data discussed above, it is evident that the release behaviour is not only influenced by the drug release from the nanocarrier, but also by the tablet composition and its properties. In this proposed approach, the drug is nanoencapsulated and the release behaviour may be a combination of different mechanisms: the release of the drug from the nanocapsules followed by diffusion of the drug and/or the release of the nanocapsules from the tablets followed by the drug release from the nanocarriers. The release data were fitted to the Power Law ($r > 0.99$) to understand the mechanisms underlying the drug release from the 3D printed tablets. Their polymeric content, infill percentage and the presence of the channelling agent did not influence the mechanism of drug release, which could be described as anomalous transport (Supplementary Information - S1), considering their cylindrical geometry (Siepmann and Peppas, 2012). The drug release from the 3D printed tablets may be governed by the superposition of the Fickian diffusion and the case-II transport, where the relaxation of the polymer chains by the water imbibition into the systems would be the rate-controlling step.

It was also important to determine whether the nanocapsules themselves were released during dissolution testing. The experiment was carried out based on the particle size profile showed by the release medium after 1, 4, 8 and 24 h (Figure 9). Ultrapure water without any additives was used as the medium in order to avoid any interference. Results were analysed considering the distribution analysis, since the samples were too polydisperse for cumulant analysis (Mastersizer v. 3.0, Malvern Instruments Ltd., UK).

Please insert Figure 9 about here.

Particle size release was dependent on the main polymeric component of the tablets as well as the presence of the channelling agent. ERL tablets containing the channelling agent showed the highest similarity of the particle size distribution compared to the original suspension. This similarity increases with time, if the data after 1h, 4h and 8h are compared. This result is in agreement with their faster drug release, supporting the importance of the improved water intake by tablets. Regarding the PCL, a closer similarity to the

original suspension was also observed in presence of the channelling agent as a function of time. The intensity (%) of the size distribution was lower for PCL tablets compared to ERL tablets, which may be related to the lower amount of particles in the PCL tablets, in agreement with the drug content data (showed in Table 4).

Taking all the data discussed above into account, nanocapsules embedded in 3D printed materials may be a strategy to improve the oral or implantable systemic delivery of drugs. The conjugating of nanotechnology and 3D printing technique may bring their advantages together, using a personalised method of manufacture to tailor the drug release and the dose as well as using a nanocarrier to control the drug release rate, to increase drug solubility and bioavailability, its chemical stability, or preventing its immediate contact with the gastrointestinal mucosa or tissue.

4. Conclusion

The present study widens the approaches to producing 3D printed tablets, as drug delivery systems. It represents the first report on conjugating nanotechnology and a 3D printing to produce innovative nanomedicines. Polymeric nanocapsules were successfully loaded in FDM printed devices prepared from novel printable polymeric filaments. The type of the polymer, the presence of a channelling agent and the infill percentage all had an important influence on the drug loading and drug release profiles from the tablets. Moreover, it is an original strategy to convert nanocapsules liquid suspensions into solid drug dosage forms with improved drug loading. As a proof of concept, this study proposed a new platform for the development of oral dosage forms, or even biodegradable implants, with tailored dose and drug release profiles, as personalised medicines.

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Figure Captions

Figure 1. Schematic representation of the proposed process to prepare 3D printed tablets loaded with polymeric nanocapsules. HME: Hot melt extrusion. FDM: Fused deposition modelling.

Figure 2. DSC thermograms for (A) raw materials: Eudragit RS100® (EUDRS100), Poly(ε -caprolactone) (PCL), Poly(ethylene)glycol 6000 (PEG 6000), mannitol, and microcrystalline cellulose (MCC); (B) EUD filaments (FIL) and their physical mixtures (PM): FIL-EUD-M, PM-EUD-M, FIL-EUD-A, and PM-EUD-A; (C) PCL filaments (FIL) and their physical mixtures (PM): FIL-PCL-M, PM-PCL-M, FIL-PCL-M', PM-PCL-M', FIL-PCL-A, PM-PCL-A.

Figure 3. Particle size distribution of formulation DFZ-ENC by (A) Laser Diffraction and (B) Dynamic Light Scattering. Results represent three independent batches.

Figure 4. In vitro deflazacort release profile from Eudragit RS100® nanocapsules (DFZ-ENC) (n = 3). Results are expressed as mean ± standard deviation.

Figure 5. False-color Raman images of 3D printed tablets: (A) T-ERL-M and (B) T-ERL-A. Eudragit RL (ERL) is depicted in red, while mannitol (M, figure A) or microcrystalline cellulose (A, figure B) are depicted in blue in the respective image.

Figure 6. DSC thermograms for 3D printed tablets loaded with nanocapsules prepared with EUD (T-EUD-M, T-EUD-M50% and T-EUD-A) and PCL (T-PCL-M, T-PCL-M' and T-PCL-A). ERL: Eudragit RL100; PCL: Poly(e-caprolactone); M: Mannitol A: Avicel PH 301, MCC.

Figure 7. SEM images of surface (left side) and cross sections (right side) of the following tablets loaded with nanocapsules: (A) T-ERL-M, (B) T-ERL-A, (C) T-PCL-M', and (D) T-PCL-A (at 50,000X magnification, scale bar: 1 μm, EHT 10 kV). Arrows indicate nanoparticles. ERL: Eudragit RL100; PCL: Poly(e-caprolactone); M: Mannitol A: Avicel PH 301, MCC.

Figure 8. DFZ release profile (n = 3) from (A) 3D printed tablets prepared from EUD devices and (B) 3D printed tablets prepared from PCL devices. Results are expressed as mean ± standard deviation. ERL: Eudragit RL100; PCL: Poly(e-caprolactone); M: Mannitol A: Avicel PH 301, MCC.

Figure 9. Particle size distribution by DLS of 3D printed tablets loaded with nanocapsules after (A) 1h, (B) 4h, (C) 8h, and (D) 24h of immersion in ultrapure water. DFZ-ENC represents the original particle size distribution of the liquid suspension. ERL: Eudragit RL100; PCL: Poly(e-caprolactone); M: Mannitol A: Avicel PH 301, MCC.