Sustained release and pharmacologic effects of human glucagon-like peptide-1 and liraglutide from polymeric microparticles.

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Running title: *Sustained release of GLP-1 and liraglutide*

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ABSTRACT

The GLP-1 class of peptide agonists has been shown to exert regulatory key roles in both diabetes, obesity and related complications. Given the short half-life of GLP-1 its use has been historically discouraged. We developed polymeric microparticles loaded with either human GLP-1 (7-37) or liraglutide peptides by double emulsion and solvent evaporation approach. The size distribution of all formulations was of about 30-50 µm. The *in vitro* kinetic release assays showed a sustained release of the peptides extending up to 30 to 40 days with varying profiles. Morphologic analysis demonstrated a more regular particle surface for those comprising polymers PLA, PLA-PEG and PLGA. *In vivo* evaluation in Swiss male mice demonstrated a similar extension of effect of decreasing in body weight gain for up to 25 days after a single subcutaneous administration of either hGLP-1 or liraglutide peptide-loaded microparticles (200 µg peptide / kg body weight) compared to controls. These demonstrate the effectiveness of hGLP-1 as a therapeutic agent in long-term, continuous release from peptide-load microparticles, and thus its plausibility as an unmodified therapeutic agent.

Keywords: glucagon-like peptide-1; GLP-1; liraglutide; microparticles; long-acting release

1. Introduction

The glucagon-like peptide-1 (GLP-1) is a natural peptide secreted in response to meal that stimulates glucose-dependent insulin release and suppresses postprandial glucagon secretion. Also this hormone delays gastric emptying, helping to increase satiety (Zander et al., 2002). The GLP-1 is rapidly metabolized by dypeptidil-peptidide IV (DPP4) enzyme, resulting in a half-life of about 10 min (Holst, 2007).

The physiological and pharmacological features of GLP-1 is highly attractive, in particular for additional cardiovascular outcomes and favorable risk/benefit balance, having motivated the development of several agonists and prospection of analogues (Bethel et al., 2018; Kim and Kim, 2017; Marso et al., 2016). Currently two main products are available commercially for therapeutic purposes, known as liraglutide and exenatide (exendin-4). Liraglutide is a GLP-1 analogue to which the amino acid substitution of lysine with arginine at position 34 and attachment of a C16 acyl chain via a glutamoyl spacer to lysine at position 26, resulting in a compound with increased half-life (Agersø and Vicini, 2003), decreased susceptibility to digestion by DPP4 (Ahrén and Schmitz, 2004; Brunton, 2014), and is currently available as injectable solution and allowing a s.i.d. therapeutic scheme (Gough, 2012; Ponzani, 2013; Sjöholm, 2010) either alone (Victoza®) or in combination with long-acting insulin analogue degludec (Tresiba).

Exending is a GLP-1 peptide analogue discovered from the salive of guila monster, which display GLP-1 agonism pharmacologic effect, and currently marketed as a pos-prandial injection or a once-a-week or once-a-month formulation in polymeric microparticles (Liu et al., 2010; Minze et al., 2013; Pinelli and Hurren, 2011). An adverse reaction observed with exendin therapy is pancreatite (Aroda and DeYoung, 2011; Ryan et al., 2013a), and without the cardiovascular benefits observed with liraglutide.

Biodegradable polymers are commonly used to design and to synthesize drug delivery systems (DDS). Among them, the most widely used are polyethylene glycol (PEG), poly(lactic acid) family and its copolymers with glycolic acid (Chen et al., 2003; Reddy, K. R., 2000). Conjugation of PEG to polymeric systems or drugs prevents recognition by various defense systems of organisms because of its potential for biomasking (Fontana et al., 2001; Huang et al., 2005; Perry et al., 2012). Furthermore they are biodegradable and biocompatible polymers which are approved by the US FDA for use in biomedical applications (Kamaly et al., 2016; Kapoor et al., 2015; Klose et al., 2008; Makadia and Siegel, 2011). In consequence some resent experimental researches in diabetes field are directed to the design of different types of DDS based on these co-polymers as a useful alternative for diabetes treatment (Cai et al., 2013; Guerreiro et al., 2012a, 2013). Although a long-acting release (LAR) system based on polymeric particle has been used for exenatide (Aroda and DeYoung, 2011; Liu et al., 2010; Pinelli and Hurren, 2011; Ryan et al., 2013b), to date there is no known sustained release formulation of either liraglutide or GLP-1 itself (Cai et al., 2013).

In this work, we explored the development of biocompatible polymeric microparticles loaded with either human GLP-1 (hGLP-1) and liraglutide and the comparative evaluation of *in vitro* kinetic release and *in vivo* pharmacologic evaluation of these products.

2. Material and Methods

2.1 Materials.

Polyvinyl alcohol Mw 89 kDa-98kDa, >99% hydrolyzed (PVA; lote # MKBD2262V, Cat: 9002-89-5) was obtained from Sigma-Aldrich. Liraglutide (Victoza®, Lots # FS60K24 and FS6W992) was purchased at local drug store, and glucagon-like peptide-1 (GLP-1, 7-37, sequence "HAEGTFTSDVSSYLEGQAAKEFIAWLVKGRG", > 95 % purit, lot #98664; Certificate of Analysis in **Supporting Material**) was purchased from Genemed Synthesis Inc, USA). Fluorescamine was obtained from Sigma-Aldrich (Cat #F9015). The branched PLGA-glucose (lots 0811001762 and Cat #5004-A) (Bodmer et al., 1992) and the linear PLGA 50:50 Lactide/Glicolide (lot 14007) were purchased from Purasorb. Linear PLGA 85:15 (Cat. #430471), 75:25 (Cat. #P1941) and 65:35 (Cat. #P2066) Lactide/Glicolide (lots #MK861113V, #089K134V and #050M1668V, respectively) were purchased from Sigma-Aldrich. PLA, PLA-PEG and PLA-PEG-F (fluorescein) were synthesized and characterized as described elsewhere (Icart et al., 2016). All other reagents were from analytical grade. All reactants were used as received.

2.2 Preparation of GLP-1 or Liraglutide-loaded polymeric microparticles by double-emulsion and solvent-evaporation procedure.

Peptide loaded-polymeric microparticles were prepared using all the polymeric materials (PLA, PLA-PEG, PLA-PEG-F and PLGA) by using the double emulsion-solvent evaporation methodology (Allahyari and Mohit, 2015) with some modifications. In this methodology, we used 140 mg of the polymer solubilized in 1.00 mL of dichloromethane (DCM) (organic phase) and 1.2 mg or 3.6 mg of peptide solubilized in 200 µL of water (internal aqueous phase), targeting respectively a theoretical maximum load of 0.86 % and 2.6 % (peptide/polymer). First emulsion was formed mixing both immiscible phases under stirring at 20,000 rpm during 5 min, producing a w/o emulsion. Them, the resulting emulsion was transferred into a beaker containing 40 mL of PVA water solution (0.1 wt %) (external water phase). This system was kept under stirring at 20,000 rpm for 5 min, producing a w/o/w emulsion. Them the system was kept under mechanical stirring for 2 h at room temperature (25°C) to harden the microparticles by solvent evaporation. The peptide loaded-polymeric microparticles were then collected by centrifugation at 5,000 rpm and washed 3 times with water. Finally, the collected microparticles were freeze-drier by liofilization and stored at 12°C until use.

2.3 Characterization of polymeric microparticles

2.3.1 Scanning electron microscopy: The morphologic analysis of the polymeric microparticles were carried out with a FEI-Quanta 259 Tungsten scanning electron microscope (at the analytical platform of CENABIO-UFRJ), using acceleration voltages of 12.5 kV. Samples were coated with gold and the materials were sampled by taking several images of various magnifications to ensure that the analysis was based on a representative region of the sample.

2.3.2 Particle size distribution. The polymeric microparticles size distribution was accessed with the material dispersed in water and evaluated in a Mastersizer 2000 laser (Malvern instrument. Ltd. UK; at the analytical platform of EngePol, UFRJ) at room temperature.

2.3.3 Peptide quantification: The quantification of the peptides was performed by derivatization with fluorescamine and fluorimetric analysis (Guerreiro et al., 2012b; Udenfriend et al., 1972). Briefly, an analytical curve (between 0.4 and 0.0065 µg/µL) was performed by using the respective peptide (either liraglutide or hGLP-1) as standards. Samples

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were assessed in serial dilution in 96-well microplates (Corning A010993), in 50 mM sodium phosphate buffer pH 7.0 (final volume = 150 μL), added of 75 μL fluorescamine (500 μg/mL DMSO, for final concentration = 250 μg/mL) and read in a Spectramax M5 (Molecular Devices) after 5 min incubation at room temperature, using excitation at 390nm and emission at 480nm.

2.4 Encapsulation yield and encapsulation efficiency.

Encapsulation yield was determined as the weight of the microparticles recovered (MPt) with respect to sum of all the starting material, peptide (PEPo) and polymer (POLo). The encapsulation yield of prepared microparticles was determined using Equation (1):

Eq(1)   Encapsulation yield = (MPt / (PEPo + POLo) × 100

Encapsulation efficiency of peptides encapsulation was performed by quantification of the peptide by using the fluorescamine method (previously described) in the supernatant (PEPsn) after the microencapsulation process. This value was subtracted from the initial amount of peptides (PEPo) at the beginning of the process in order to calculate the amount of peptides incorporated to the microparticles. The percentage encapsulation efficiency of peptides was calculated with Equation 2:

Eq(2)   Encapsulation efficiency = (PEPo – PEPsn / PEPo)*100

2.5 *In vitro* kinetic release of hGLP-1 or liraglutide-loaded microparticles.

Peptide loaded-microparticles (containing 1 mg of peptide) were dispersed in 2 mL of phosphate buffer pH 7.2 and 100 mM of NaN_3). Then 200 μl of the same microparticles suspension were placed in eppendorf tubes. The tubes were sealed and left under orbital agitation (150 rpm) at 37 °C. At the indicated time interval (0, 1h, 24h, 48h, 4d, 8d, 15d, 20d, 25d, 30d and 45d) the tubes were removed from the shaker and they were centrifuged at 5,000 rpm at 4^0C for 15 min, the supernatant was transferred to another eppendorf tube, flash frozen and kept at -20 °C until use. Total peptide release was inferred by quantification by the fluorescamine method as described above.

2.6 Pharmacologic assays.
The pharmacologic assays were approved by the Institutional Bioethics Committee on Animal Care and Experimentation at UFRJ (CCS, UFRJ, Protocol #057/17).

2.6.1 Animals.
Swiss male mice aged 7-8 weeks (25-28 g) were housed at a constant room temperature (22 ± 3°C) in a 12 hours light/dark cycle and fed *ad libitum* with standard diet purchased from Nuvilab (DSPS803 Cat # 92-33634).

2.6.2 Single dose of hGLP-1 or liraglutide-loaded polymeric microparticles.
The mice were randomly distributed into three groups (n=5/groups) and left 7days of housing and adaptation before subjected to experimentation. The experimental design took

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into account the idealization of a hypothetical linear, constant flux of peptide release from the particles resulting in an approximate dose of 200 μg peptide/body weight/day, during 40 days (based on the estimate extension of the kinetic *in vitro* release). Based on this estimation we achieved a dose of about 5 μg peptide/animal (of approximately 25 g body weight).

The suspension (in 0.9 % NaCl saline) contained 40 mg microparticles/100 μL, either dummy (formulated without peptide) or loaded with peptide (either liraglutide or hGLP-1, at 5.6 μg peptide / mg particles), corresponding to a dose of about 224 μg peptide / 25 g body weight (or 8.96 mg peptide / kg body weight). Each animal received 100 μL suspension/25 g body weight. The formulation was administer by subcutaneous injection (over the shoulders, into the loose skin over the neck) using a standard 29 gauge needle (BD™).

2.6.3 Measurement of blood glucose and weight.

Mice were weighing regularly and had they glycemia measured weekly (at about 2 pm); with a portable glucometer ACCU-CHEK, Cat # TYP: 05680456003), both in baseline and after microparticle administration.

2.6.4 Statistical analysis

Data are the Means ± Standard Media Error (SME). Weight change, glucose change, food and water intake and feces and urine production were compared across groups using two-way ANOVA, with Bonferroni multiple comparison subtests. Graphs and statistical anaylsis were obtained using GraphPadPrism 5.0 (GraphPad Software Inc.).

3. Results

3.1. Preparation of hGLP-1- or liraglutide-loaded polymeric microparticles.

The preparation of polymeric microparticles by emulsion and solvent evaporation is a satisfactory procedure for the entrapment of small compounds aimed their chemical of physical stabilization and sustained release ("Emulsion Solvent Evaporation Microencapsulation Review | Emulsion | Polyethylene Glycol," n.d.; R and V, 2015; Yüksel and Baykara, 1997). A large set of variables can be explored in their preparation according to the compound characteristics and aiming to achieve the desired morphology, size, polidispersity, release profile, yield and entrapment efficiency (Tu and Lee, 2012). We choose using the double emulsion and solvent evaporation approach in the preparation of hGLP-1 and liraglutide loaded microparticles since this procedure is one of the most useful methods for entrapping water-soluble substance, such as proteins and peptide. The high hydrophilicity of these molecules favors their quantitative introduction into the internal aqueous phase which results in an increased encapsulation efficiency in comparison with some others emulsifications procedures such as single-emulsion and solvent evaporation. In addition, the use of proteins induces a stabilizing effect of the emulsions formed, which contributes to the success of the double emulsion process and loading.

We used a set of 8 polymers in this study, covering the classes of PLA, PLA-PEG and PLGA, which are the most commonly used biocompatible polymers for therapeutic purpose (Han et al., 2016; Kamaly et al., 2016; Makadia and Siegel, 2011). The characteristics of the polymers used here and the resulting microparticles are detailed in **Table 1**. The double emulsion and solvent evaporation process prepared with a ratio of 0.86 % peptide/polymer yielded microparticles with satisfactory narrow polydispersity of size distribution (Pd = 0.2-0.8). The microparticles had a mean diameter of 44.1± 10.8 and 47.8 ±14.7 µm for hGLP-1 and liraglutide respectively, well below the safety cutoff of 100 µm (due to overall risks of embolization) (Lee and Henthorn, 2012). The microparticles was in the range of 57-76% for hGLP-1 and 82-95% for liraglutide. Peptide encapsulation efficiency ranged from 47-68% to hGLP-1 and 54-74% for liraglutide.

The particle size of the microparticles obtained in this work were mainly influence by the average molecular weight of the polymer. Microparticles which were prepared with PLA or PLA-PEG showed an smaller particle sized than microparticles prepared with the co-polymers of PLGA , (**Table 1**). The morphological characterization of prepared microparticles containing hGLP-1 (**Fig. 1**) and liraglutide (**Fig. 2**) by SEM revealed the influence of the polymer composition on the morphological surface of microparticles. Microparticles which were prepared using PLA, showed a smoother surface with a few randomly distributed small pores. In turn, microparticles prepared with co-polymer of PLA-PEG showed a rough surface with a few randomly distributed small pores. This might be attributed to the known effect of PEG in the induction of small pores in the surfaces of polymeric microparticles. During the precipitation step, the PEG branches of the diblock copolymer are known to be orientated toward the internal and surrounding aqueous phase forming a sponge-like structure. This phenomenon was also reported for microparticles based on blends of PLGA/PLA and PEG and also applies to microparticles composed of diblock polymer (Essa et al., 2010; Lochmann et al., 2010). The amount of glicolide into the composition of the PLGA was also likely to influence the morphological characteristics of the prepared microparticles.

Microparticles prepared using PLGA-A and PLGA-E with high glicolide composition higher that those based on PLGA-B, PLGA-C and PLGA-D showed a more irregular surface and several apparent large pores. Instead, the microparticles based on PLGA with lower glicolide content were more regular and showed smoother surface with fewer pores.

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Microparticles prepared with a 3 times higher load (2.6 % liraglutide/polymer) resulted in microparticles similar in morphology (**Fig. S1**) and particle size, encapsulation efficiency and encapsulation (**Table S1**) to the microparticles preparations formulated with 0.86 % load (**Table 1; Fig. 2**). These results are in agreement with those reported for different types of peptides microencapsulation by double emulsion and solvent evaporation (Parikh et al., 2003; Ravi et al., 2016; Xu et al., 2006; Yang and Owusu-Ababio, 2000).

3.2. *in vitro* kinetic release profile of hGLP-1 and liraglutide from microparticles.

We next evaluated the *in vitro* kinetic release profile of hGLP-1 and liraglutide peptides from the polymeric microparticles. The **Figure 3** shows the results of the kinetic release assay performed with hGLP-1 (**Fig. 3a** and **Fig. 3b**) and liraglutide (**Fig. 3c** and **Fig. 3d**) for 45 days in phosphate buffer pH 7.4 at 37^0C. Within the first days an initial 'burst' of peptide release took place for hGLP-1 ($\sim$30% of total peptide) and liraglutide ($\sim$10% of total peptide). A hyperbolic profile comprised by a burst phase followed by a slower kinetic release between approximately day 5 up to the end of the study is observed for the hGLP-1-loaded microparticles, with either PLGA (**Fig. 3c**) or PLA (**Fig. 3d**), Instead, the *in vitro* kinetic release profile liraglutide from the microparticles showed a sigmoidal pattern after the small burst phase, with a plateau up to about day 20, followed by an exponential phase for the rest of the assay, for both PLGA (**Fig. 3a**) and PLA (**Fig. 3b**). This dissimilar kinetic release behavior between microparticles loaded with hGLP-1 or the palmitoylated analogue liraglutide suggests a deterministic effect of the fatty acid moiety of the conjugate on the resulting microparticles.

3.3. Pharmacologic evaluation of hGLP-1- and liraglutide-loaded microparticles

We have further evaluated the *in vivo* effect of these formulations of hGLP-1 and liraglutide polymeric microparticles. The microparticles produced with PLA resulted in a faster kinetic release profile compared to the extended release obtained with the microparticles formulated with the PLGA polymers. The polymers PLGA-A and PLGA-E have both the more equivalente amount of lactide:glicolide, while PLGA-B and PLGA-C were higher in lactide – such as the PLA only. However, the particles obtained with PLGA-A and PLGA-E were irregular in morphology and were hollow. Instead, the PLGA-D showed uniformity in particle surface when used in preparation of microparticles with both peptides. Based on these features, we choose the PLGA-D as a polymer for the pharmacologic assay of the peptide-loaded microparticles (hGLP-1 and liraglutide), along with control with polymer-only microparticles.

Swiss male mice aged 7-8 weeks (25-28 g) were randomly distributed in three groups (n=5/group) and they received each subcutaneously a single dose of unloaded, hGLP-1-loaded or liraglutide-loaded polymeric microparticles. The group receiving microparticles containing either hGLP-1 or liraglutide showed a decline in the rate of body weight gain after at 9 days period with no significative effect being observed. From day 10 up to day 25 after dosing, a significative difference was observed, both for body weight (**Fig. 4a**) and reduction in glycemia (**Fig. 4b**). Collectively, these results demonstrate the similar long-term effectiveness of both unmodified hGLP-1 and the analogue liraglutide in the present *in vivo* pharmacological assays.

4. Discussion

In this work we have shown that the well-established pharmaceutical system of long-acting release based on microparticles developed with biocompatible polymers could be used for the formulation of the regular human GLP-1 and its analogue liraglutide. The effect of reducing body weight and glycemia in mice for over 3 weeks provided evidence for the *in vivo* effect of these formulations, which upon further optimization could be considered as an alternative approach in the therapeutic portfolio for diabetes, obesity and comorbidities.

The very short serum half-life of hGLP-1 of about 2 min has been hampering its direct therapeutic use, and motivated the development of other GLP-1 receptor agonists such as its analogue liraglutide. However, upon formulation into polymeric microparticles with same PLGA polymer, these products resulted in close *in vivo* effect of both hGLP-1 and liraglutide (**Fig. 4**), suggesting similar potency, overall release and absorption flux, although further other pharmacokinetic, pharmacodynamics and toxicological investigation would be desirable to establish a better foundations for the understanding of those systems.

From our kinetic release *in vitro* we could observe a multi-pattern profile, mainly in three phases:

i) an initial fast release (defined as "burst release"), starting within the first hours and can last for a few days, and is most commonly attributed to drug adhered to the external wall of the microparticles,

ii) an slow release phase, which depends mainly on the degradation kinetics of the polymeric matrix used to prepare the microparticles, and

iii) the last accelerated release, which depends on the microsphere diameter and drug diffusion or solubility (Kamaly et al., 2016).

In this study we observed a deterministic effect of both the polymer (PLGA versus PLA) and the nature of the entrapped compound (hGLP-1 *versus* its palmitoylated analogue liraglutide) in the peptide release profile from the microparticles. Hence microparticles based on PLGA showed a faster hGLP-1 and liraglutide release compared to PLA, most likely due to the increase in the ductility of the PLGA material (Song et al., 2011). In the case of the PLGA microparticles, no large effect of the proportion of lactide and glicolide was observed on the peptides release profiles.

In vitro polymer degradation is generally considered as heterogeneous process. First degradation probably occurs on the surface due to the polymer water intake, however the higher concentration of carbonyl groups in the center of the particle helps the self-catalyzing degradation. This behavior is common during the degradation of aliphatic polyesters (Ruan and Feng, 2003). Furthermore it was observed a faster release of hGLP-1 compared to liraglutide, which may be attributed to the enhanced aqueous solutiliby of hGLP-1 compared to the fatty acid acylated liraglutide. Finally, liraglutide loaded-microparticles prepared using the triple load of peptide (2.5 % instead of 0.86 %) showed both similar kinetic release (**Supporting Material**).

The relationships between GLP-1 or GLP-1-analogous with appetite and weight maintenance was previously reported by several authors (Aroda and DeYoung, 2011; DeFronzo et al., 2005, p. 1; Larsen et al., 2001; Madsbad, 2009; Madsbad et al., 2004). Some evidence demonstrates that GLP-1 reduces body weight when administered by intracerebroventricular route in animals (Ronveaux et al., 2015; Tang-Christensen et al., 1996; Turton et al., 1996) and by subcutaneous route in humans (Abbott et al., 2005; Rüttimann et al., 2009; Talsania et al., 2005; Williams et al., 2009). It´s known that they have action on the gastrointestinal tracts as well as the direct regulation of appetite which provoke inhibition of gastric secretion and motility. Some recent research suggests that long-acting analogs of the GLP-1 could be a useful alternative to obesity treatment (Cai et al., 2013). In consequence the GLP-1 receptor agonist

was recently approved by the US Food and Drug Administration as an obesity treatment option, showing interesting results in human reducing and sustaining body weight loss ("FDA Approves Liraglutide (Saxenda) for Weight Loss," n.d.; Isaacs et al., 2016). In addition, **Figure 3b** also confirms the effects of the released GLP-1 or Liraglutide from microparticles on the blood glucose levels of the treated groups. In which an important decline of blood glucose levels was observed with statistical significance difference as compared with control group (p value = 0.0003). No statistical significance difference was observed between both treated groups. This effect was sustained during at least fourth week of study. From this moment and to the end of the experiment a tendency to increase of this parameter was observed. Currently several pre-clinical and clinical studies demonstrated the role of GLP-1 on the blood glucose homeostasis due to its capacity to slow gastric emptying, to enhance pancreatic insulin secretion, and to suppress pancreatic glucagon secretion (Farilla et al., 2003; Gough, 2012, p. 1; Madsbad, 2009, p. 1; Madsbad et al., 2004, p. 1; Minze et al., 2013, p. 1; Ronveaux et al., 2015).

In summary, the present study demonstrated the effective ability of trapping hGLP-1 and its analogue liraglutide into biocompatible polymeric microparticles, resulting in an extended release profile and overcoming the limitations of the very short half-life of hGLP-1.

5. Conclusions

The emulsion and solvent evaporation procedure allowed obtaining hGLP-1 or liraglutide loaded-microparticles with satisfactory peptide encapsulation yield and microparticles recovery. The peptide-loaded microparticles showed an extended kinetic release profile spanning over 5 weeks *in vitro* with pharmacological effect sustained for over 3 weeks, even for the hGLP-1 known for its short half-life. These results pave a possibility for new long-acting release formulation based either on the unmodified hGLP-1 or the well-established analogue liraglutide.

Acknowledgments:

Conflict of interest:

The authors have no financial conflicts of interest with the contents of this article. LMTRL is a participant in patent applications by the UFRJ on controlled release of peptides unrelated to the present work.

Author Contributions:
Conceived and designed the experiments: LPI, FGSJ, LMTRL
Performed the experiments: LPI, FGSJ, LMTRL
Analyzed the data: LPI, FGSJ, LMTRL
Contributed reagents/materials/analysis tools: LPI, FGSJ, LMTRL
Wrote the manuscript: LPI, FGSJ, LMTRL

Table 1: Characteristic of the polymer and resulting microparticles formulated with hGLP1 or liraglutide (0.86 % load, peptide/polymer).

Materials			hGLP1-loaded microparticles				Liraglutide-loaded microparticles			
Polymer	L/G*	Mw (kda)	Size (µm)	Pd	Yield (%)	E.E** (%)	Size (µm)	Pd	Yield (%)	E.E (%)
PLGA (A)	50:50	60-80	48.2	0.3	75.1	52.0	53.0	0.3	95.5	62.2
PLGA (B)	85:15	50-75	56.2	0.4	60.5	54.5	57.2	0.4	82.3	74.6
PLGA (C)	75:25	66-77	61.1	0.4	57.9	59.7	68.5	0.2	84.2	61.9
PLGA (D)	65:35	40-75	43.3	0.3	64.8	56.9	40.9	0.3	86.6	64.2
PLGA (E)	55:45	50-90	48.4	0.3	73.5	57.3	66.9	0.4	82.7	54.2
PLA	n/a	10-20	30.7	0.6	76.8	53.9	32.3	0.4	91.2	61.4
PLA-PEG	n/a	10-20	28.1	0.3	59.6	47.5	27.1	0.4	87.7	66.5
PLA-PEG-F	n/a	10-20	37.5	0.8	75.3	43.2	37.2	0.6	92.8	73.8

*L/G: Lactide/glicolide composition.
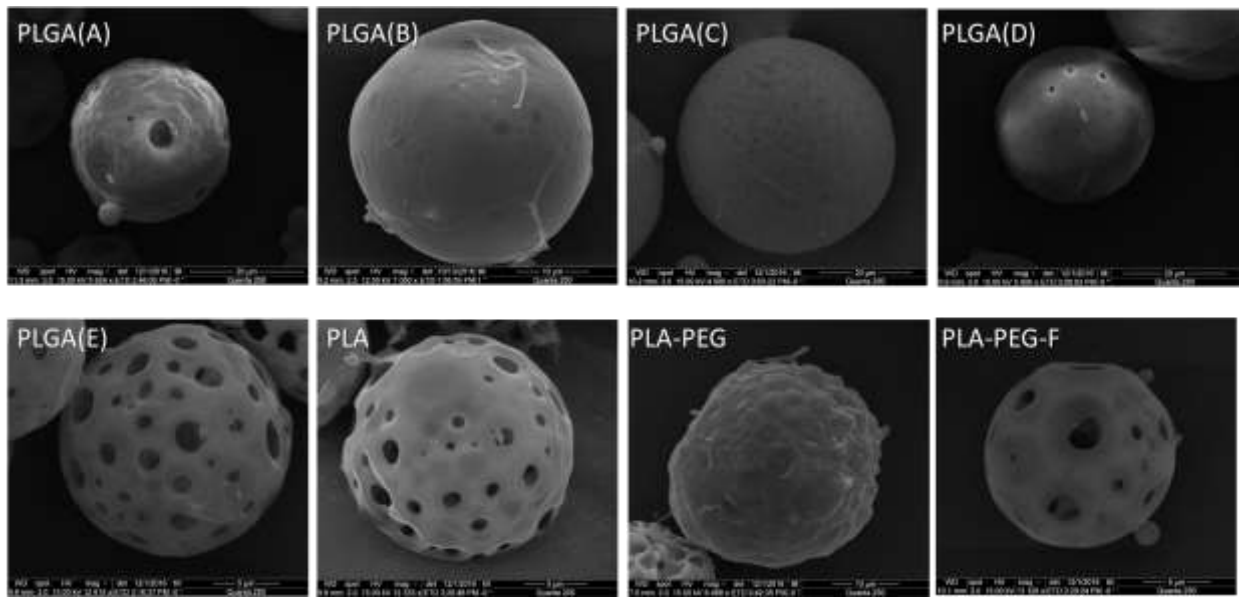
**Encapsulation efficiency.

Figure 1. Morphologic characterization of the h-GLP-1 loaded microparticles by scanning electron microscopy (SEM). The hGLP-1-loaded microparticles were formulated used the different PLGA commercial co-polymers (A, B, C, D and D) and the synthetic polymers (PLA, PLA-PEG and PLA-PEG-F) as depicted in the respective panels. The detailed information about the methods and the polymers cam be found in the Material and Methods section and in **Table 1**.
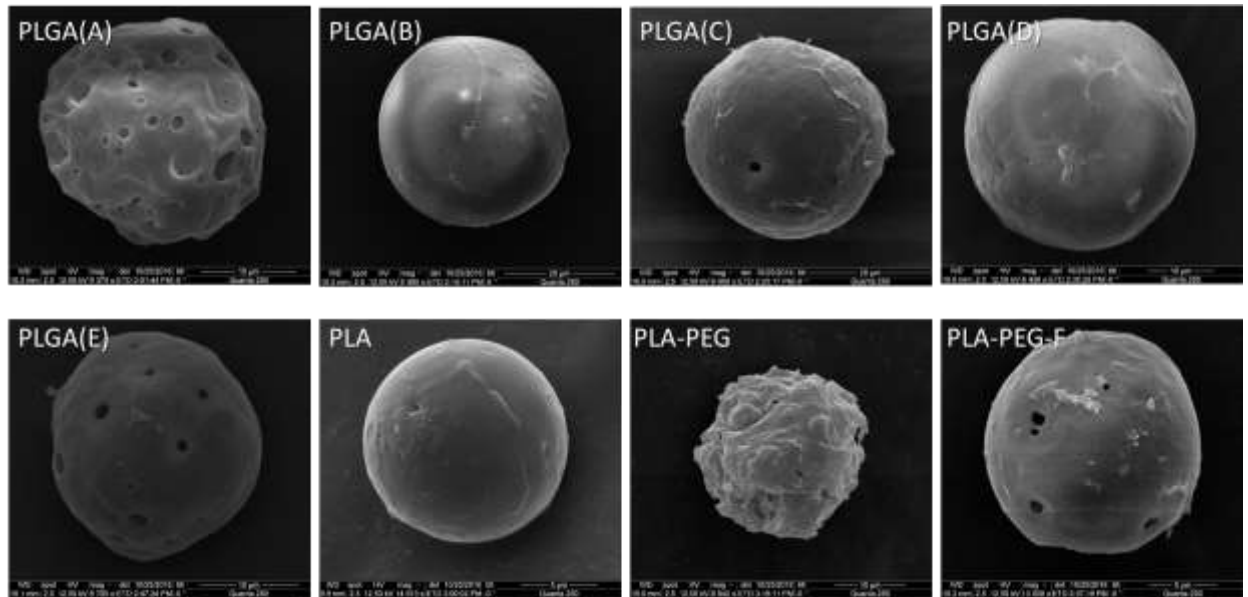
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Figure 2. Morphologic characterization of the liraglutide loaded microparticles by scanning electron microscopy (SEM). The liraglutide-loaded microparticles were formulated used the different PLGA commercial co-polymers (A, B, C, D and D) and the synthetic polymers (PLA, PLA-PEG and PLA-PEG-F) as depicted in the respective panels. The detailed information about the methods and the polymers cam be found in the Material and Methods section and in **Table 1**.
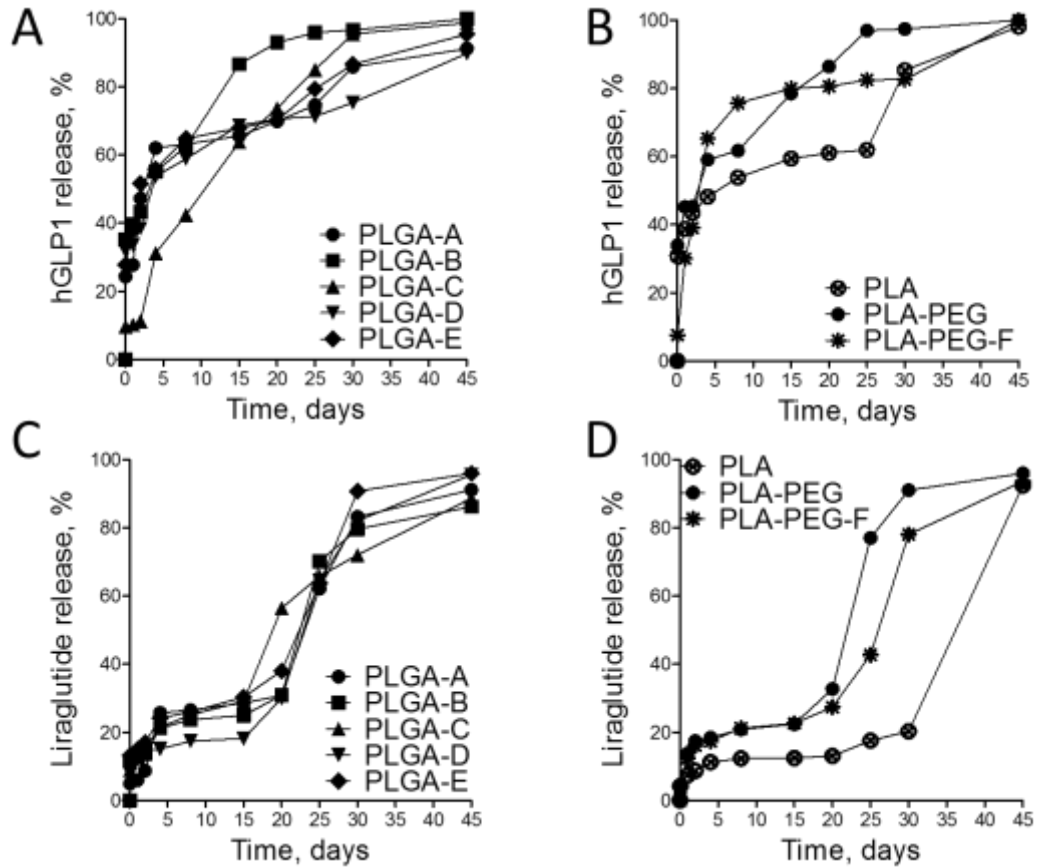
Figure 3. *In vitro* kinetic release profile of hGLP-1 and liraglutide from peptide-loaded microparticles. The microparticles loaded with hGLP-1 (**A** and **B**) or liraglutide (**C** and **D**) were prepared using the PLGA (**A** and **C**) and PLA (**B** and **D**) depicted in **Table 1**. The release was performed in phosphate solution pH 7.4, 37^0C, under orbital agitation set at 75 rpm. The peptide (hGLP-1 or liraglutide) released from the polymeric microparticles at each time interval was quantified from supernatant harvested after centrifugation (4^0C, 15 min, 5,000rpm) of aliquots and them quantified by the fluorescamine procedure. Details in the *Material and Methods* section.

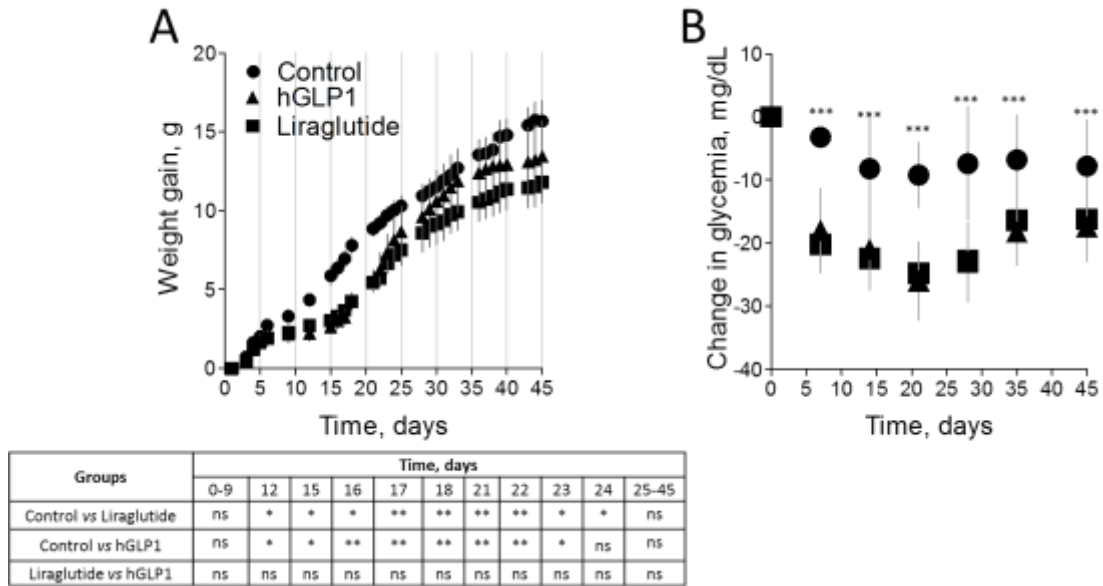| Groups	Time, days										
	0-9	12	15	16	17	18	21	22	23	24	25-45
Control vs Liraglutide	ns	*	*	*	**	**	**	**	*	*	ns
Control vs hGLP1	ns	*	*	**	**	**	**	**	*	ns	ns
Liraglutide vs hGLP1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Figure 4: Pharmacologic evaluation of hGLP-1-loaded or liraglutide-loaded microparticles. Swiss male mice aged 7-8 weeks (n=5/groups) received a single dose of microparticles formulation (either with no peptide, loaded with hGLP-1 or liraglutide) and were monitored for 45 days for change in body weight (A) and glycemia (B). $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

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