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A Novel Microfluidic Assembly of a Biodegradable Nanostructures Designed for Site Specific Delivery of an Anticancer Peptide

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SUMMARY

Ran is a small RAS-related GTPase and is overexpressed in breast carcinoma to induce malignant transformation and metastatic growth. A novel series of antiRan-GTPase peptide (CK-10), which inhibits Ran hydrolysis and activation, have suboptimal activity *in vitro* due to low bioavailability and poor delivery. To overcome these disadvantages, we delivered the CK-10 peptide by encapsulating it in PLGA-based nanoparticles (NP). The successful delivery of CK-10 can prevent Ran activation by blocking a regulator of chromosome condensation 1 (RCC1) following peptide release directly in the cytoplasm after endocytosis of the novel NP(s). A novel hydrodynamic flow technique is designed to avoid the drawbacks with a double emulsion solvent evaporation technique. Loading efficiency and *in vitro* release were measured by modified Lowry assay, size was characterized by dynamic light scattering, tuneable pore resistive sensing and laser obscuration time, zeta potential was measured by laser anemometry, morphology was scanned by electron microscopes and laser obscuration time. Water absorption and its associated changes in the physicochemical properties were measured by various color indicator and potentiometric titration techniques to understand the fundamental biodegradation process. PLGA/β-cyclodextrin nanoparticles showed the highest peptide loading (53.92%m/m) for the novel microfluidic technique with the highest cumulative release of 91.38%.

INTRODUCTION

Nanocarriers can escape the reticuloendothelial system (RES), which attacks any foreign material by opsonisation, tracked by macrophage phagocytosis. Particles with hydrophilic surfaces undertake less opsonisation and clearance by RES. This prolongs the circulation time of NPs in the blood and lets the NPs target to sites of action other than the RES (Buske et al., 2012). The increased circulation time occurs because the hydrophilic polymers reduce the protein interactions on the surface by preventing the opsonins binding (Bertrand et al., 2014). Generally, delivery of NP for anticancer drugs to cancerous/tumor tissues can be accomplished by either passive or active targeting. Passive targeting depends on the advantage of the inherent NP's size and the unique pathophysiological abnormalities of cancerous/tumor vasculature, such as the enhanced permeability and retention (EPR) effect.

MATERIALS AND METHODS

All the chemicals were purchased from Sigma Aldrich UK and the novel CK-10 peptide was purchased from a GL Biochem in China. Nanoparticles were prepared using the double emulsion/solvent evaporation (DE/SE) technique and novel modified hydrodynamic flow technique using the benchtop NanoAssemblr™. The peptide loading efficiency and *in vitro* were measured by modified Lowry assay method. The particle size was measured by dynamic light scattering (DLS), tunable pore resistive sensing technique (TPRS) and laser obscuration time (LOT) techniques while zeta potential was measured by laser

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anemometry technique (LAT). The NP morphology was examined using scanning electron microscopy (SEM), transmission electron microscopy (TEM) and (LOT). Water absorption was analysed by Karl Fischer titration (KF).

RESULTS AND DISCUSSION

The use of the NanoAssemblr™. attained greater loading efficiency, smaller size with narrower polydispersivity index (PDI) and higher cumulative *in vitro* release than the DE/SE for the whole nanoparticles comprising the PLGA and the PLGA blends (Fig.1). PLGA/β-Cyclodextrin (CD) demonstrated the uppermost loading efficiency (53.92%) with the cumulative amount of the *in vitro* release in 4 weeks by 91.38 % by means of the NanoAssemblr™. Under rapid microfluidic mixing of the NanoAssemblr™, solvent exchange is complete even before the polymers start aggregation. Therefore, the PLGA nanoparticles formation occurs in solvent conditions that more closely equal the final solvent, i.e., water with a small portion of acetonitrile may also help for enhancement of the CK-10 entrapment within the PLGA and PLGA blends. Augmentation of the loading efficiency and the greater surface area of the microfluidic NP(s) linked to the smaller sizes can boost the CK-10 release in a direct dependent way (Bertrand et al., 2014 and Buske et al., 2012).

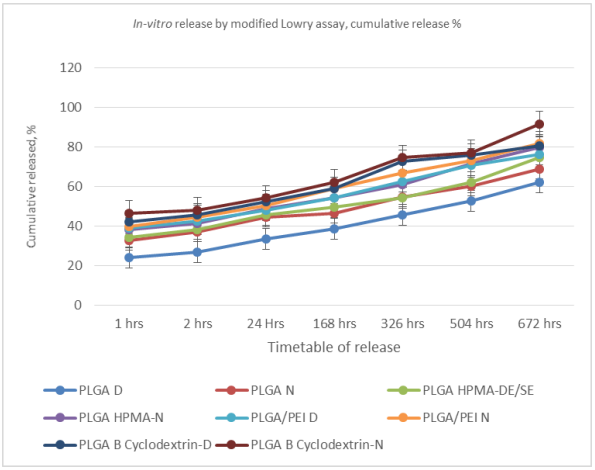

Fig. 1. *Cumulative release of PLGA NP(s) over 1 month.*

Table 1. *Characterization of PLGA nanoparticles.*

Properties	PLGA. D	PLGA. N	PLGA/ β-CD. D	PLGA/ β-CD. N
z-average, nm (LOT)	264.3 ± 5.5	249. ± 13.15	265.83 ± 2.93	253.57 ± 9.21
z-average, nm (DLS)	259.3± 2.68	245.4 ±10.1	261.2 ±3.21	247.67 ±2.6
PDI (DLS)	0.26 ± 0.02	0.18 ± 0.06	0.34 ± 0.03	0.27 ± 0.05
Zeta potential, mV (LAT)	-60.67 ± -7.85	- 56.77 ± -7.27	-40.07 ± 3.21	-39.07 ± -4.61
Loading Efficiency %	25.8 ± 3.52	37.14 ± 5.14	43.88 ± 2.4	53.92 ± 7.95
Water content % (KF)	2.34 ± 0.24	2.71 ± 0.13	3.77 ± 0.38	4.13 ± 0.51

± s.d n= 3 in each case. D: double emulsion /solvent evaporation technique. N: novel microfluidic technique.

Table 2. *Characterization of PLGA nanoparticles.*

Properties	PLGA/ HPMA. D	PLGA/ HPMA. N	PLGA/ PEI. D	PLGA/ PEI.N
z-average, nm (LOT)	270.10 ± 8.80	257.83 ± 4.15	273.3 ± 11.7	265.3 ± 11.7
z-average, nm (DLS)	267 ± 5	251.4 ± 8.3	268.4 ± 6.1	262.7 ± 6.2
PDI (DLS)	0.41 ± 0.04	0.28 ± 0.06	0.33 ± 0.02	0.29 ± 0.02
Zeta potential, mV (LAT)	-48.61 ± -5.21	-42.90 ± -4.71	-35.8 ± -5.85	-33.6 ± -1.48
Loading Efficiency %	34.86 ± 4.46	40.19 ± 3.64	34.89 ± 1.85	45.29 ± 2.19
Water content % (KF)	3.29 ± 0.16	3.53 ± 0.47	3.44 ± 0.11	3.72 ± 0.11

± s.d n= 3 in each case. D: double emulsion /solvent evaporation technique. N: novel microfluidic technique.

CONCLUSIONS

PLGA/β- cyclodextrin nanoparticles have the best physicochemical properties comprising the highest peptide loading efficiency, highest in vitro release, and the smallest size rates than the other types of amphiphilic PLGA nanoparticles.

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