MULTICOMPONENT NANOSCALE SYSTEMS AS METASTATIC MELANOMA THERAPEUTIC CANCER VACCINES

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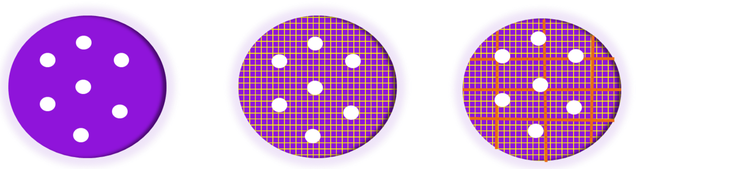
INTRODUCTION

Metastatic melanoma is one of the leading causes of morbidity and mortality worldwide. At present, there are no therapeutic vaccines approved for the prevention or treatment of metastatic melanoma, however different strategies have been devised to modulate immune cells to eradicate tumor cells. Different materials will be explored for the preparation of biocompatible and biodegradable nanoscale systems to overcome the delivery, efficacy and safety issues associated to immunomodulators, including antigens, oligonucleotides and carbohydrates.

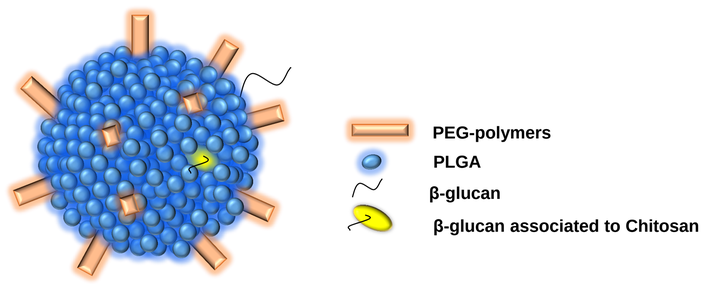
AIM

Design of multivalente nanoscale systems for the delivery of melanoma antigens and immune modulators to dendritic cells (DCs) to improve their cross-talk with T cells.

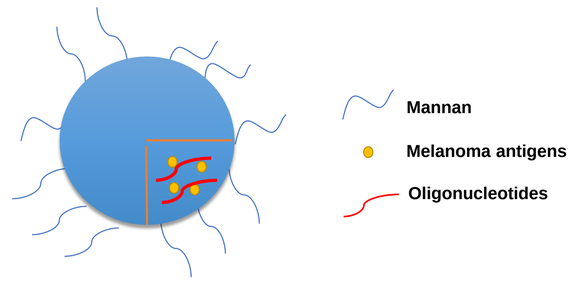
Hybrid lipid-polymeric nanoparticles



Glucan-associated polymeric nanoparticles



Mannan-surface modified P(HEMA-co -MAA) nanogel



MATERIAL AND METHODS

Hybrid lipid-polymeric and glucan-associated polymeric nanoparticles were obtained by the double emulsion-solvent evaporation method. OVA Alexa Fluor® 647 conjugate was entrapped as a model antigen and two different lipids, 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphorylcholine (POPC) and 1,2-Dimyristoyl-sn-glycero-3-phosphorylglycerol (DMPG), were used to modify nanoparticle matrix. Beta-glucan carbohydrate was entrapped in PLGA-based NPs, in the presence or absence of glycol chitosan. The physicochemical properties of nanosystems were addressed following the methods described on Table 1.

Tabela 1 . Nanoplatform caracterization

DYNAMIC LIGHT SCATTERING	ATOMIC FORCE MICROSCOPY (AFM)	LASER DOPPLER ELECROPHORESIS	ALAMAR BLUE ASSAY	FLOW CYTOMETRY	FLUORESCENCE
Size (Z-Ave) Polidispersity index (PdI)	Size Morphology	Zeta Potential (ZP)	Cell Viability	Cellular uptake	Entrapment efficiency (EE) and loading capacity (LC)

RESULTS

Table 2. PLGA-lipid hybrid nanoparticle physicochemical properties

Formulations	Z-Ave (nm)	PdI	ZP (mV)	EE (w/v) (%)	LC (µg/ml)
PLGA	198.6 ± 11.00	0.100 ± 0.029	-3.89 ± 1.72	0.0 ± 0.0	0.0 ± 0.0
OVA-PLGA	190.7 ± 3.66	0.063 ± 0.005	-3.80 ± 1.11	69.9 ± 4.6	3.5 ± 0.2
PLGA/POPC	167.3 ± 4.21	0.085 ± 0.010	-3.12 ± 0.34	0.0 ± 0.0	0.0 ± 0.0
OVA-PLGA/POPC	175.2 ± 12.94	0.100 ± 0.059	-2.42 ± 0.29	60.6 ± 4.9	3.0 ± 0.2
PLGA/POPC/DMPG	143.9 ± 15.29	0.088 ± 0.009	-7.83 ± 1.04	0.0 ± 0.0	0.0 ± 0.0
OVA-PLGA/POPC/DMPG	136.6 ± 0.59	0.078 ± 0.015	-5.26 ± 0.48	84.1 ± 1.4	4.2 ± 0.1

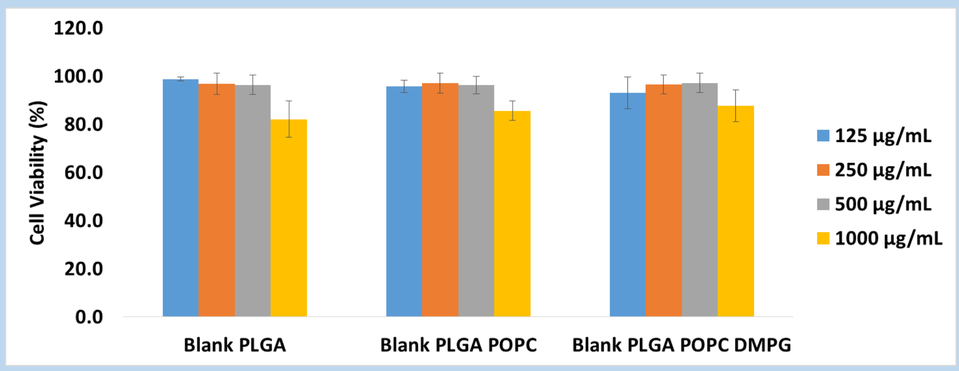


Figure 1. Cell viability of JAWSII cells determined by Alamar Blue® assay (N=3, n=6), 24 h

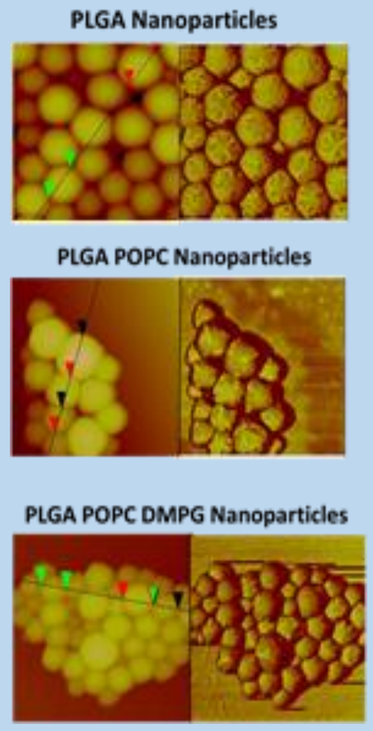


Figure 2. Surface morphology of nanoparticles by AFM

Table 3. Carbohydrate associated nanoparticle physicochemical properties

Formulations	Immunomodulators	Z-Ave (nm)	PdI	ZP (mV)
PLGA 90 % + PLGA-PEG 10 %	no	163.40 ± 12.04	0.04 ± 0.02	-2.46 ± 0.15
	Beta-glucan 10 %	208.50 ± 2.45	0.18 ± 0.08	-2.04 ± 0.08
	Glycol Chitosan 0.1 %	219.28 ± 6.78	0.18 ± 0.04	-1.04 ± 0.06
	Beta-glucan 10 % + Glycol Chitosan 0.1 %	197.67 ± 5.63	0.15 ± 0.03	-0.47 ± 0.05
PLGA 90 % + PCL-PEG 10 %	no	178.67 ± 4.16	0.06 ± 0.01	-2.13 ± 0.07
	Beta-glucan 10 %	231.76 ± 16.23	0.20 ± 0.07	-3.55 ± 1.78
	Glycol Chitosan 0.1 %	206.64 ± 2.44	0.15 ± 0.02	-0.41 ± 0.02
	Beta-glucan 10 % + Glycol Chitosan 0.1 %	215.74 ± 16.36	0.17 ± 0.04	-1.59 ± 0.99
PLGA 70 % + PLGA-PEG 90 % +PCL-PEG 10 %	no	178.58 ± 1.11	0.05 ± 0.01	-2.34 ± 1.10
	Beta-glucan 10 %	182.90 ± 4.15	0.09 ± 0.02	-2.65 ± 0.78
	Glycol Chitosan 0.1 %	233.55 ± 6.47	0.14 ± 0.05	-1.38 ± 0.44
	Beta-glucan 10 % + Glycol Chitosan 0.1 %	195.65 ± 13.14	0.19 ± 0.04	-0.39 ± 0.02
PLGA 70 % +PCL-PEG 30 %	no	166.90 ± 2.58	0.09 ± 0.01	-0.01 ± 0.08
	Beta-glucan 10 %	163.76 ± 3.61	0.08 ± 0.03	-3.82 ± 0,98
	Glycol Chitosan 0.1 %	219.90 ± 10.98	0.13 ± 0.05	-1.64 ± 1.12
	Beta-glucan 10 % + Glycol Chitosan 0.1 %	196.42 ± 8.75	0.16 ± 0.09	-1.57 ± 0.45

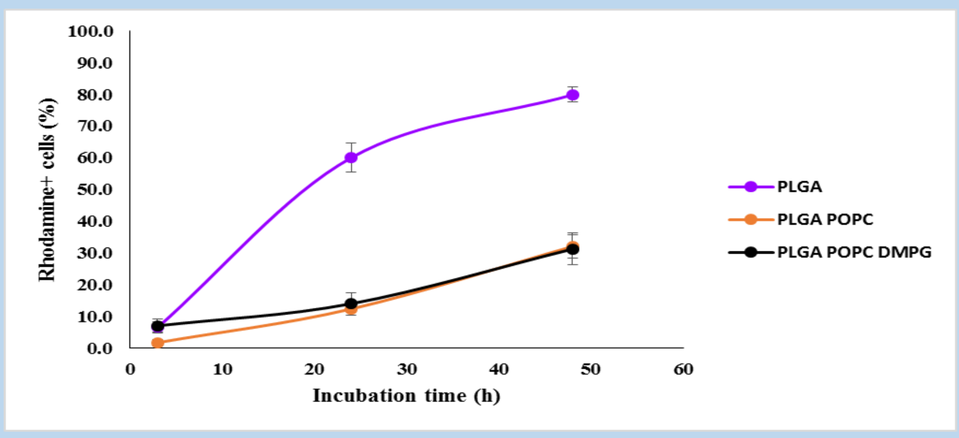


Figure 3. Internalization profile of nanoparticles by JAW SII cells after 3, 24 and 48 h of incubation (N = 3, n = 3)

- **Modification of polymeric nanoparticle matrix by lipids lead to lower mean diameters and smoother surfaces.**
- **Hybrid lipid-polymeric formulations presented a monodispersed size population, with ZP close to neutrality and relevant EE and LC values for immunization purposes.**
- **Nanoparticle did not affect DCs viability.**
- **Nanoparticle internalization levels by DCs were time-dependent.**
- **Successful co-entrapment of the immune-modulators glycol chitosan and beta-glucan**

CONCLUSIONS

Hybrid lipid-polymeric nanoplatforms showed favourable properties for antigen delivery and DC activation and maturation towards an effective immune response against tumor cells. Preliminary results of carbohydrate associated nanoparticles showed a potential nanoplatform, using carbohydrates (beta-glucan), for the targeted delivery of antigens to DCs. Mannan-modified nanogels will be developed for oral vaccination. Additional studies focused on the characterization of nanoparticle-cell interaction and intracellular trafficking may clarify the potential of these different nanoparticles for immune modulation.

ACKNOWLEDGEMENTS

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