PEGylated polymeric nanoparticles targeting the CD44v6 receptor in colon cancer cells

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BACKGROUND

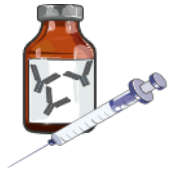
Metastatic colorectal cancer (mCRC)

1. Accumulation of both genetic and epigenetic modifications
2. Normal epithelial evolves to adenomatous lesions: carcinoma
3. Metastatic disseminations

Treatment - Bevacizumab (Avastin®)

- First angiogenesis inhibitor to be approved by FDA in 2004 for the treatment of CRC
- First- or second-line treatment for mCRC

Tumor tissue penetration
Drug distribution
Multiple administrations



POLYMERIC NANOPARTICLES

- Overcome limitations of antibody-based delivery
- Poly(ethylene glycol) (PEG) coating: higher penetration into the site of action
- Active targeting: conjugation with ligands to produce a targeted nanosystem

Specific binding to overexpressed molecules on the tumor cell surface or tumor vasculature

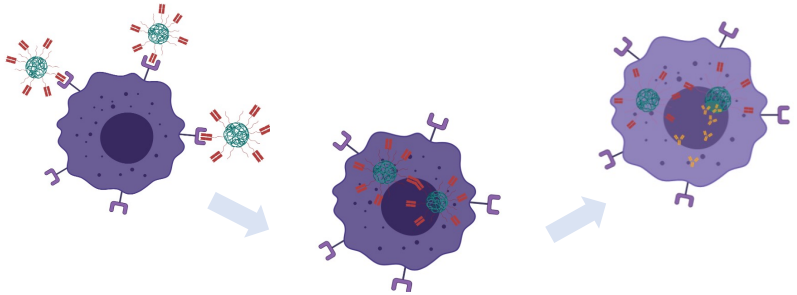
CD44v6
Overexpressed in 50% of CRCs
Major role in CRC metastatic behavior

AIM

1. PEGylated polymeric nanoparticles loaded with anti-VEGF bevacizumab

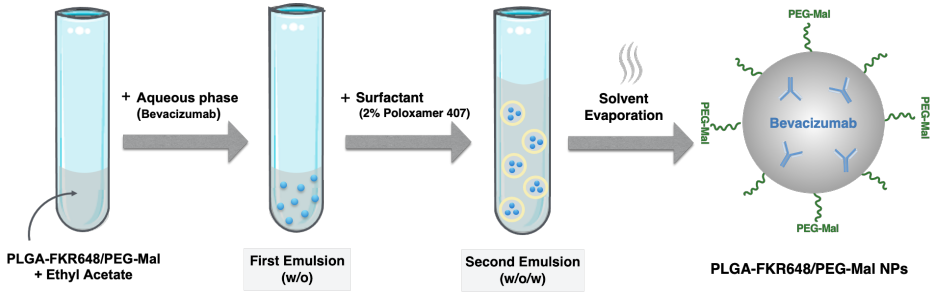
2. Functionalization with a human antibody fragment specific to human CD44v6 (v6 Fab, AbD15179)

Intracellular delivery of bevacizumab through interactions of NPs with the CD44v6 receptor in colon cancer cells

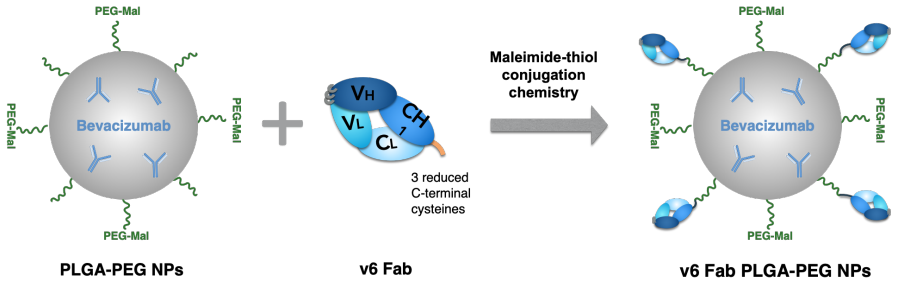


METHODS

1. Production of PLGA PEG NPs



2. Functionalization of NPs with v6 Fab



3. Characterization of NPs

- Physicochemical characterization (ELS and DLS)
- Fab conjugation efficiency (ELISA) and bevacizumab content of NPs (HPLC)
- *In vitro* cytotoxicity
- Binding of NPs to the surface of cells expressing CD44v6 and *in vitro* cellular uptake studies
- Intracellular staining of bevacizumab and VEGF

Gastro-intestinal cell model with overexpression of CD44v6:
MKN74-CD44v6+

RESULTS AND DISCUSSION

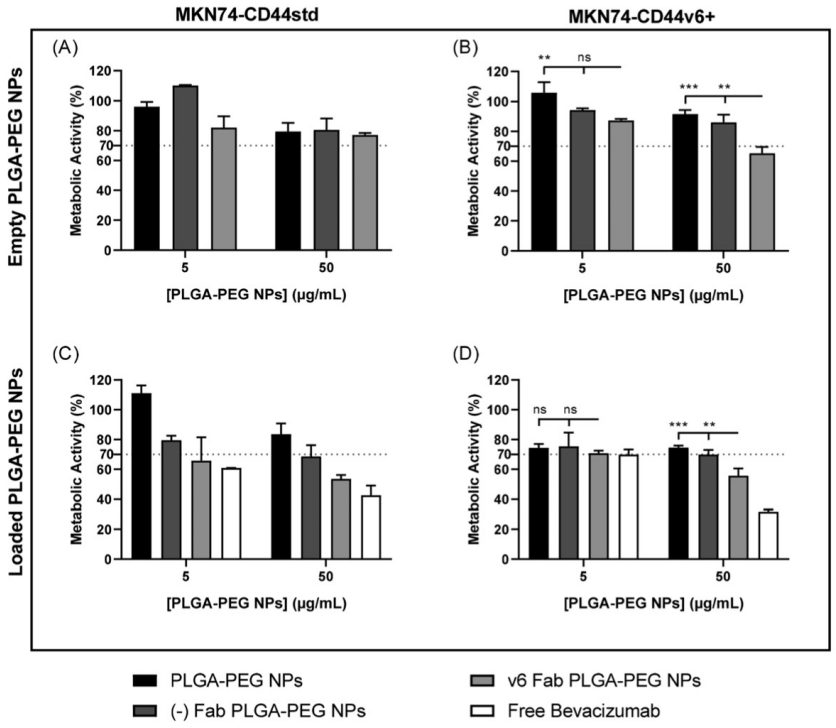
Formulation	Bevacizumab	Z-average (size, nm)	Polydispersity Index (PdI)	Zeta Potential (mV)
Bare PLGA-PEG NPs	-	124.1 ± 0.1	0.098 ± 0.015	-4.5 ± 0.2
	+	183.5 ± 4.9	0.388 ± 0.044	-6.4 ± 1.1
(-) Fab-PLGA-PEG NPs	-	167.2 ± 2.5	0.235 ± 0.005	-6.1 ± 0.8
	+	253.5 ± 1.4	0.353 ± 0.003	-9.8 ± 0.1
v6 Fab-PLGA-PEG NPs	-	245.4 ± 2.9	0.186 ± 0.013	-8.2 ± 0.5
	+	345.8 ± 16.4	0.382 ± 0.072	-12.0 ± 0.9

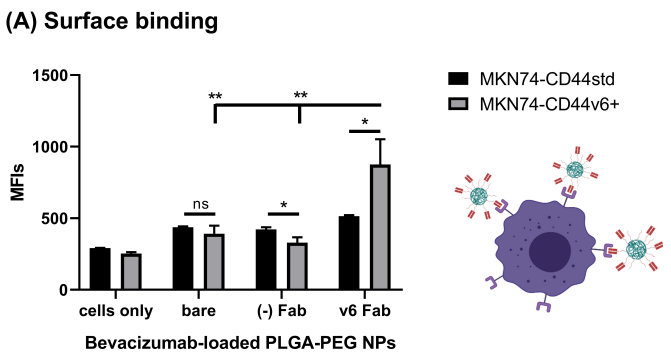
v6 Fab conjugation efficiency: 86 ± 5%

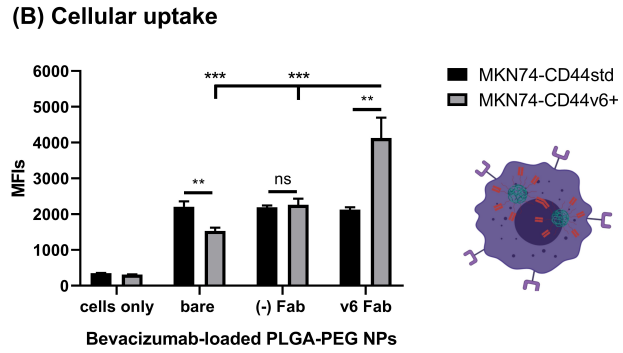
Bevacizumab

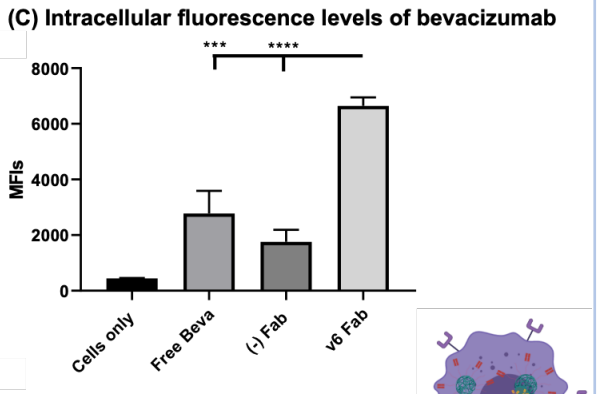
- **Association efficacy (AE)**: 86.5 ± 1.8%
- **Drug loading (DL):** 7.9 ± 0.2%

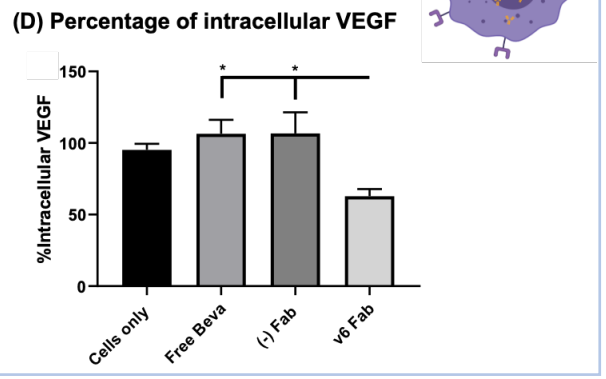
RESULTS AND DISCUSSION











CONCLUSIONS

It is shown that v6 Fab-PLGA-PEG NPs have the potential to intracellularly deliver bevacizumab into CD44v6 expressing cancer cells.

This targeted delivery system may result in higher bioavailability of a therapeutic agent at its site of action, which simultaneously increases the effectiveness of a drug, reduces the total dose needed and the side effects associated with the drug.

The nanocarrier developed in this study present clinical potential. Though, its use in drug delivery requires further investigation and optimization.

ACKNOWLEDGEMENTS

This work was financed by the project NORTE-01-0145- FEDER-000012 by Norte Portugal Regional Operational Programme (NORTE 2020), and COMPETE 2020 – Operacional Programme for Competitiveness and Internationalisation (POCI), under the PORTUGAL 2020 Partnership Agreement, through the FEDER – Fundo Europeu de Desenvolvimento Regional, and by Portuguese funds through FCT – Fundação para a Ciência e a Tecnologia/Ministério da Ciência, Tecnologia e Ensino Superior in the framework of the project "Institute for Research and Innovation in Health Sciences" UID/BIM/04293/2019.