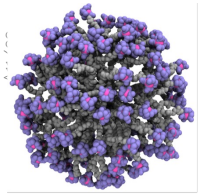

COST ACTION CA 17140

NANO2CLINIC

CANCER NANOMEDICINE - FROM THE BENCH TO THE BEDSIDE

Old drugs in new nanomedicines for gastrointestinal tumors

Bruno Sarmento

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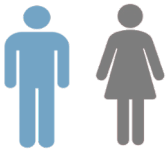
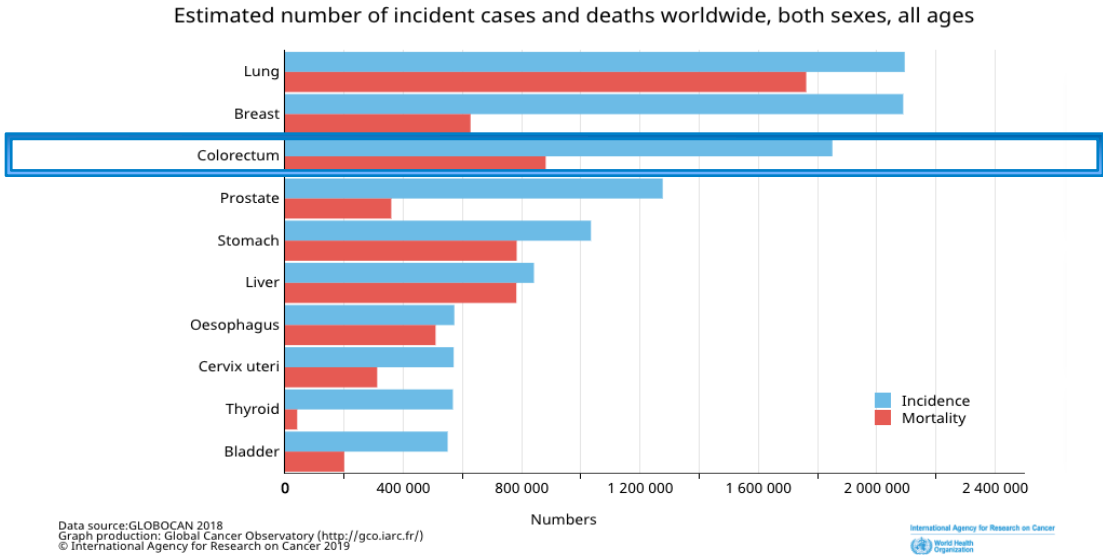
Colorectal cancer

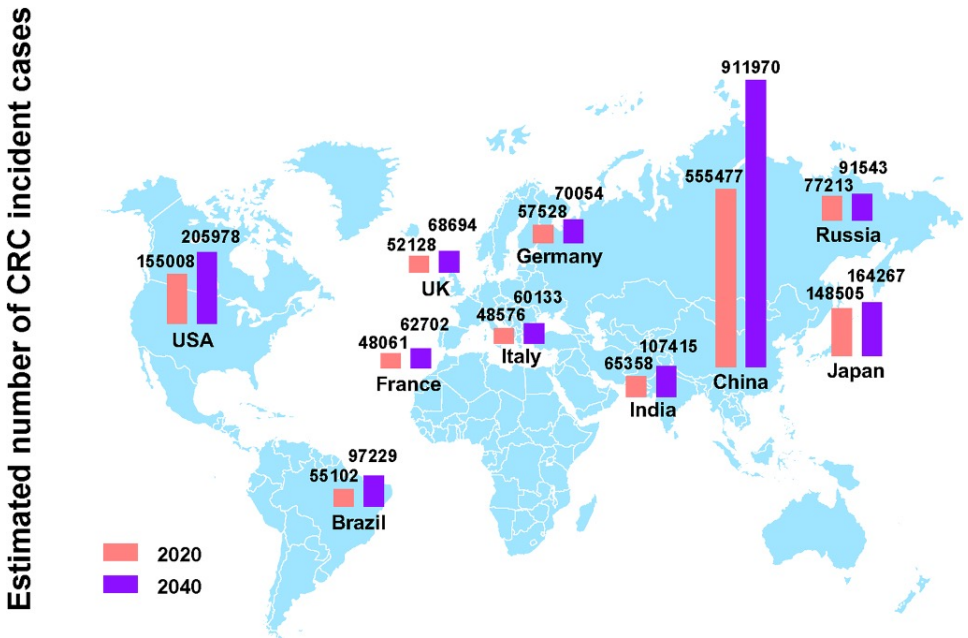
Worldwide

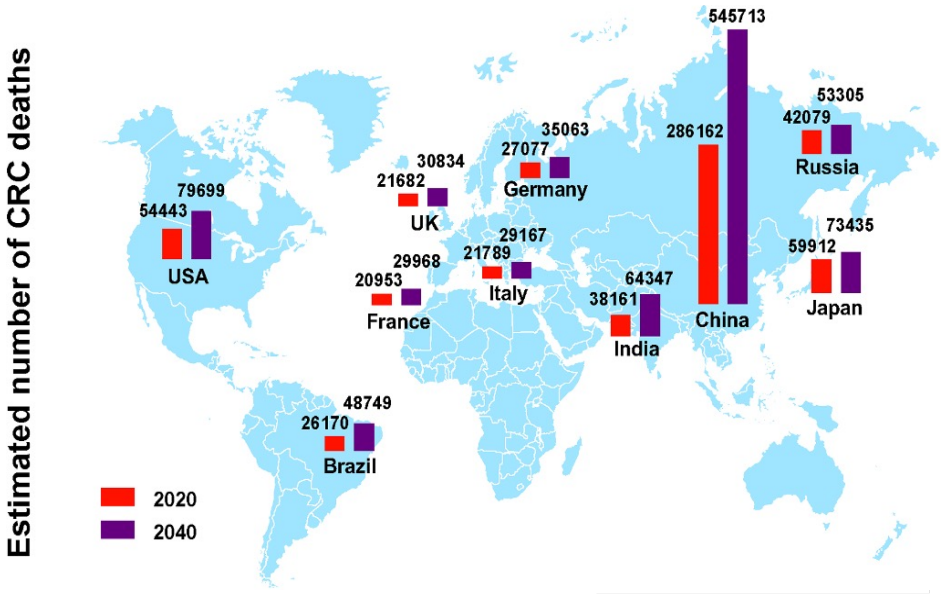




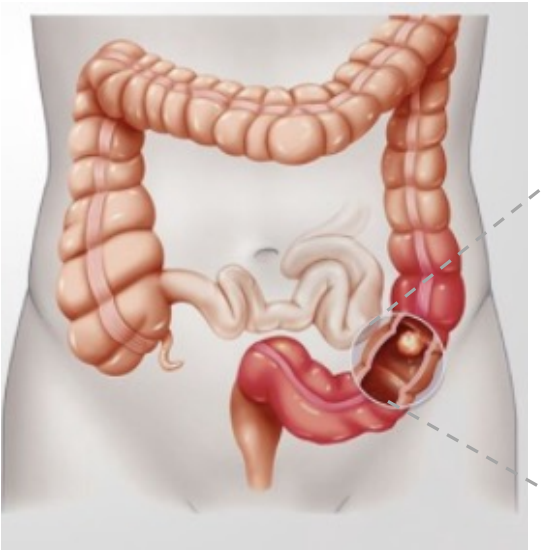
- 3rd most common
- 2nd most deadly

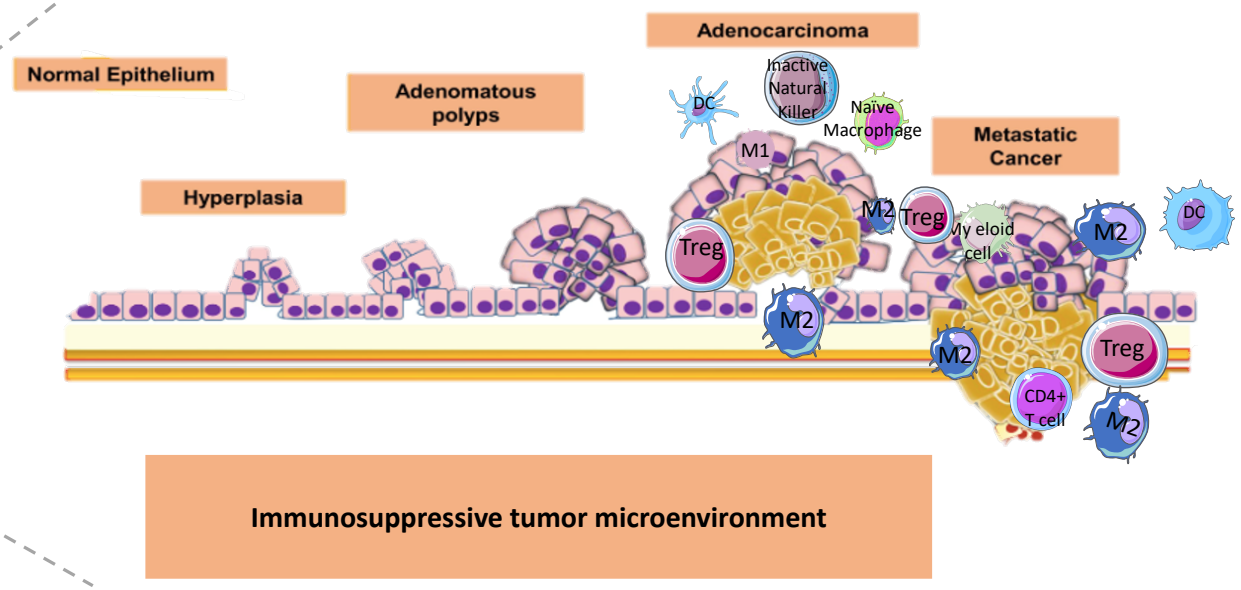
Over **1.9 million new cases** in 2020
and **0.9 million deaths** in 2020





Colorectal cancer





Major mechanisms of tumorigenesis

- Chromosomal instability (CIN)
- Microsatellite instability (MSI)
- CpG island methylator phenotype (CIMP)

Treatment failure in CRC is mainly due to the development of **metastases** (liver metastases)

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Colorectal cancer

SURGERY

RADIOTHERAPY

CHEMOTHERAPY / IMMUNOTHERAPY

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Colorectal cancer

Treatment regimens

- FOLFOX: leucovorin, 5-FU, and oxaliplatin
- FOLFIRI: leucovorin, 5-FU, and irinotecan
- CAPEOX or CAPOX: capecitabine and oxaliplatin
- FOLFOXIRI: leucovorin, 5-FU, oxaliplatin, and irinotecan
- One of the above combinations plus either a drug that targets VEGF (bevacizumab, ziv-aflibercept or ramucirumab) or a drug targets EGFR (cetuximab or panitumumab)

Colorectal cancer

- Anti-cancer chemotherapic drugs are, nowadays, highly effective. Cancer have high cure rates when detected early and treated according to best practices
- But:
    - The lack of selectivity compromise the viability of normal cells
    - Most of anticancer drugs present solubility or permeability issues, requiring excipients with toxicological drawbacks

Nanotechnology has provided the possibility of delivering drugs to specific cells in a tailored and engineered manner

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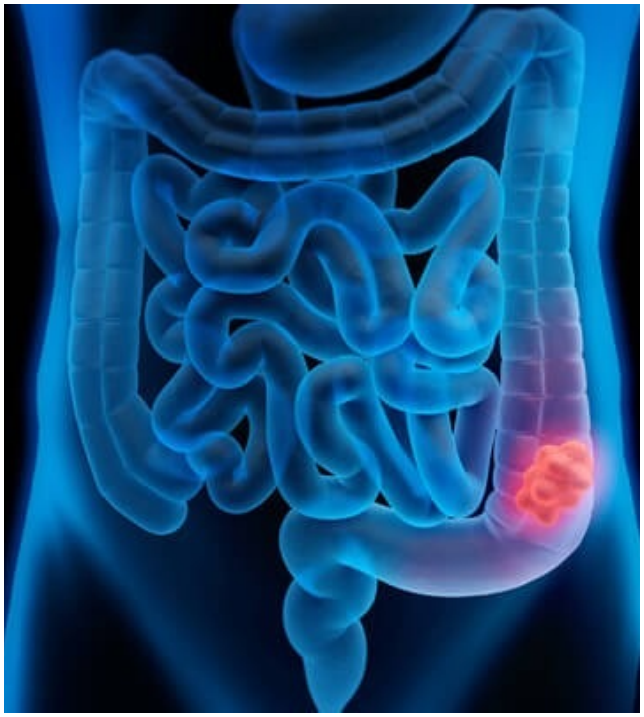
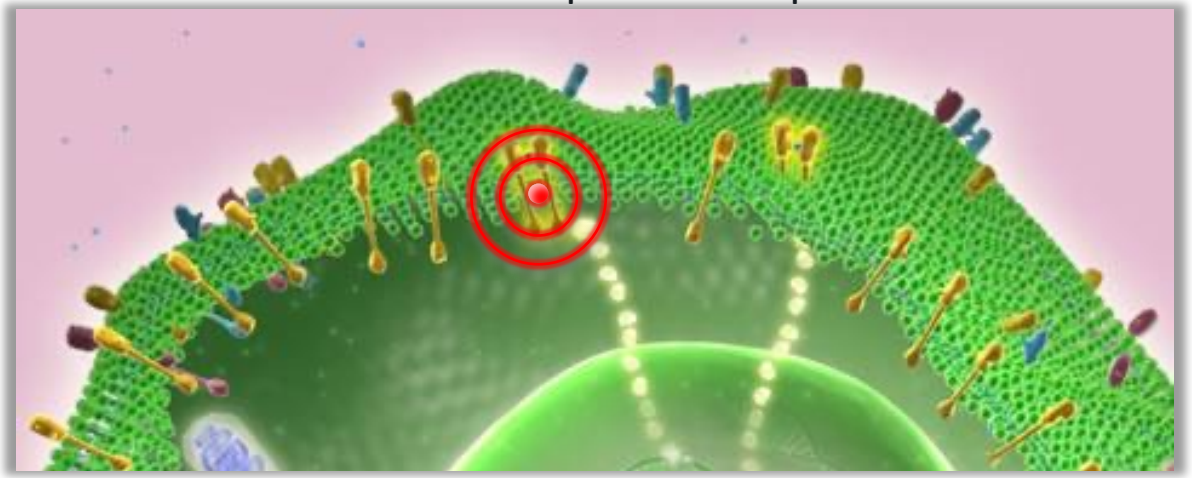
Nanomedicines for modulating cancer



Cell surface receptor overexpression



In need of advanced technologies for drug/diagnostic delivery

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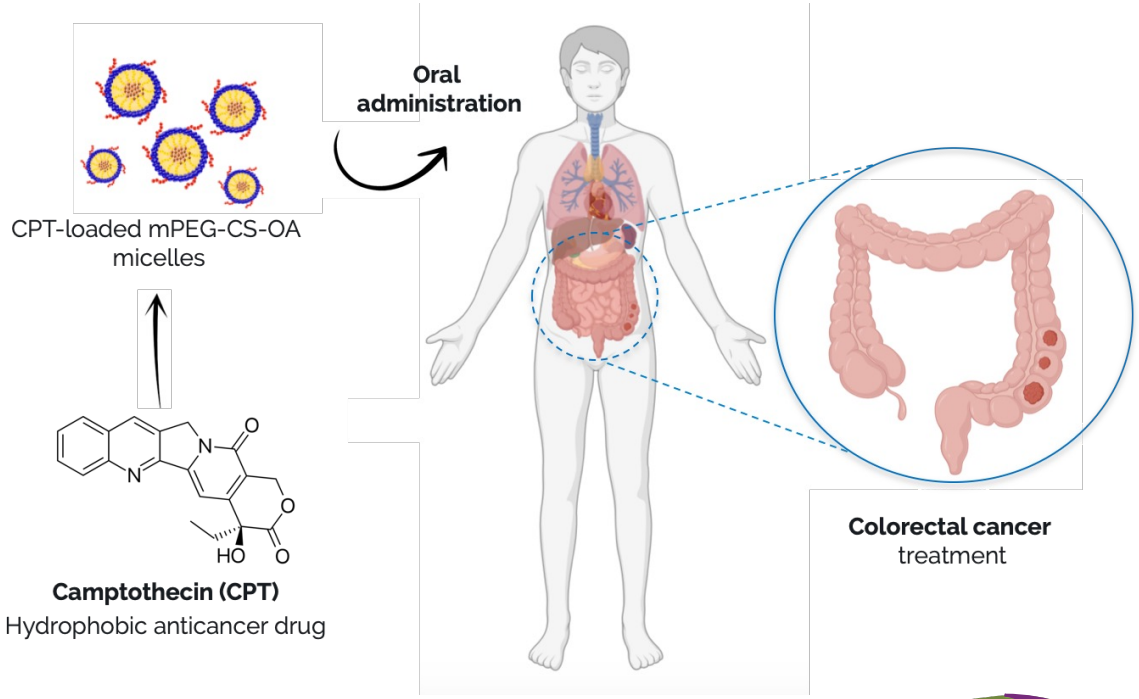
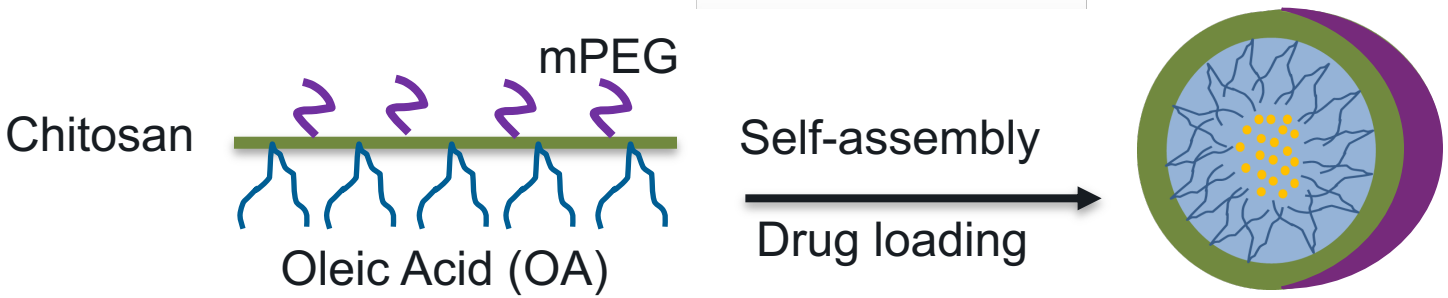
Adapted from nagpurtoday.in and National Cancer Institute

Polymeric micelles for oral chemotherapy to treat colorectal cancer





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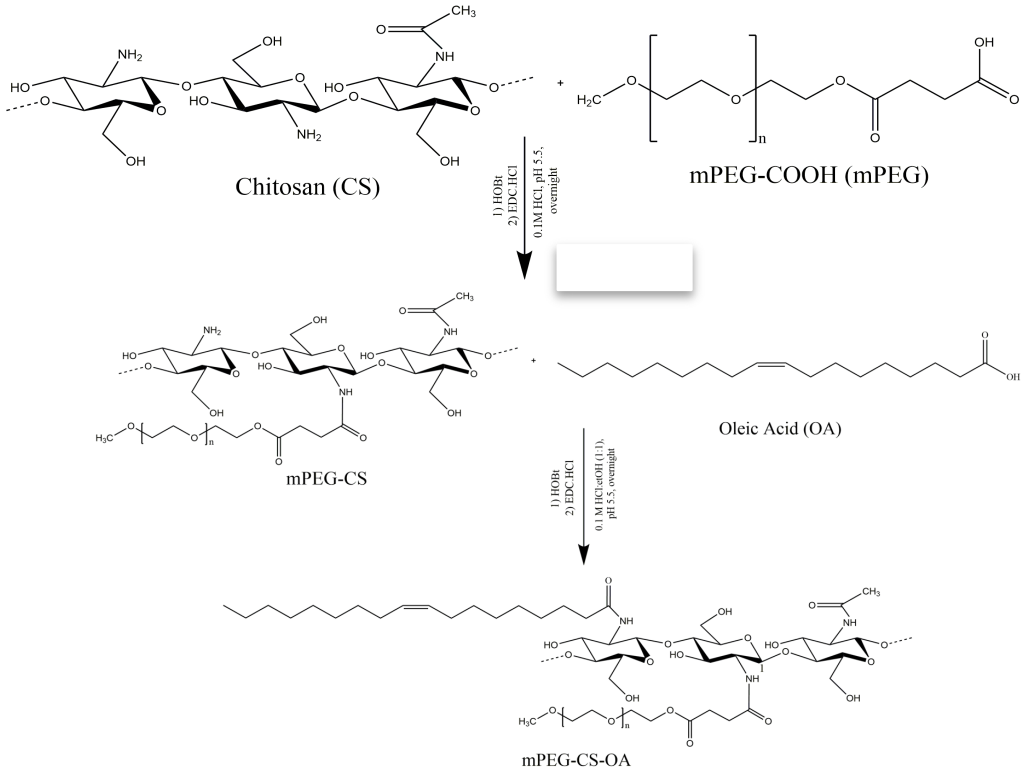
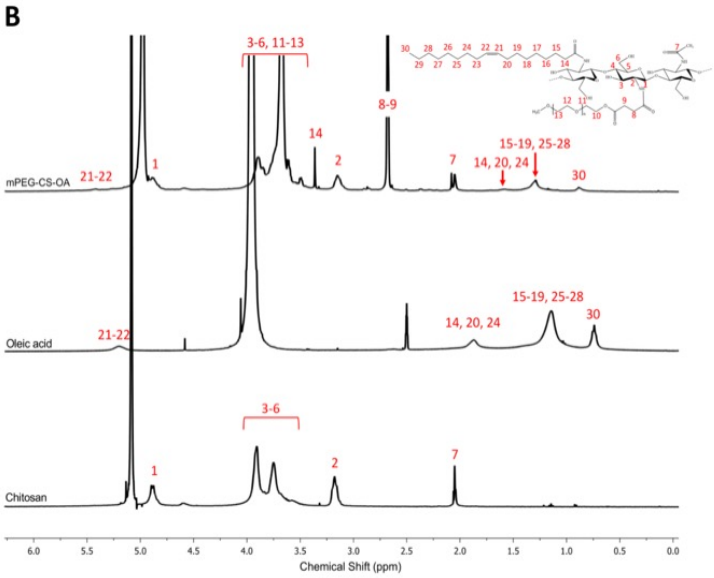
Polymeric micelles for oral chemotherapy to treat colorectal cancer





Almeida, A., et al., (2020), Materials and Science Engineering C, 2020

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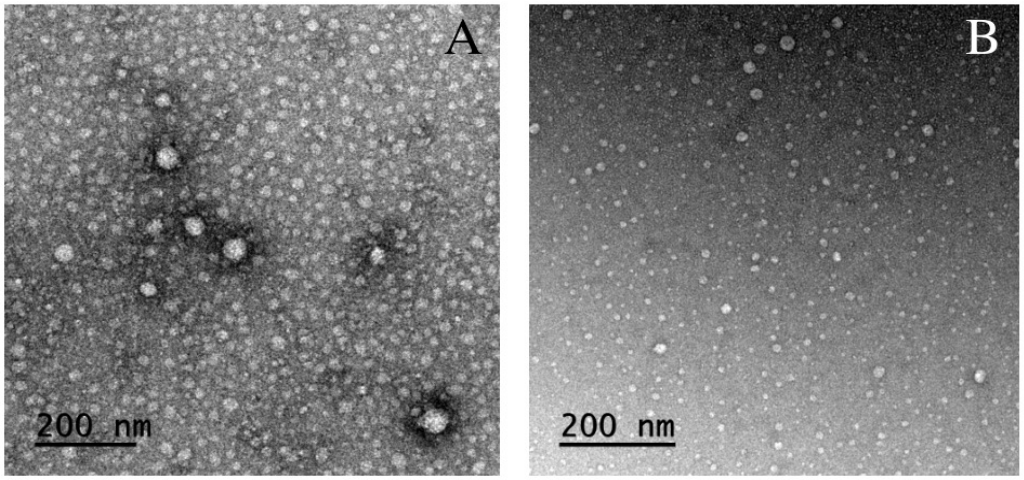
Polymeric micelles for oral chemotherapy to treat colorectal cancer

	Size (nm)	PdI	Zeta potential (mV)	DL (%)	AE (%)
Unloaded mPEG-CS-OA	137 ± 5	0.233 ± 0.025	+ 33.7 ± 1.8	0.0	-
CPT-loaded mPEG-CS-OA	146 ± 3	0.229 ± 0.005	+ 41.8 ± 3.0	5.0	77.6 ± 7.7



TEM images of empty (**A**) and CPT-loaded micelles (**B**)

Almeida, A., et al., (2020), Materials and Science Engineering C, 2020

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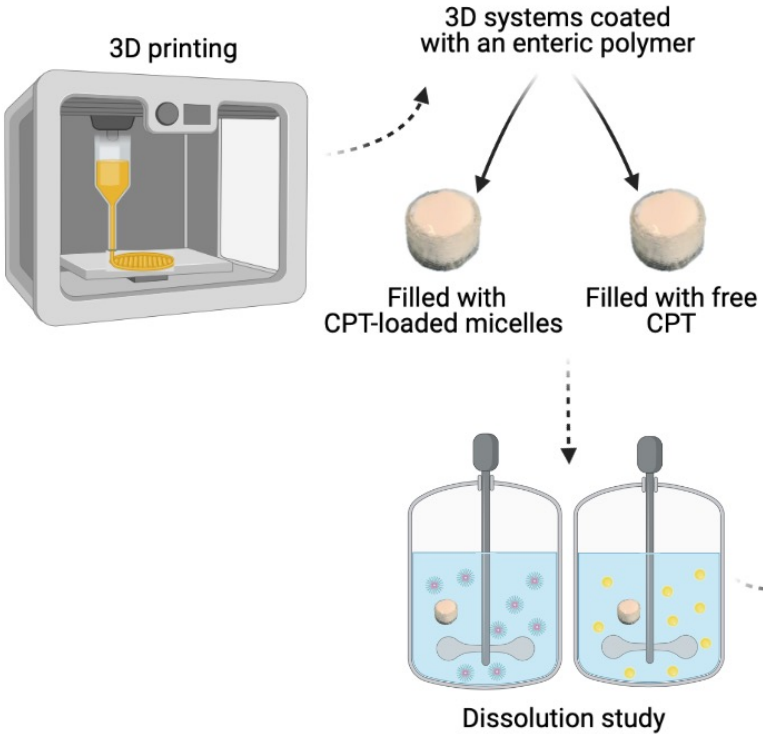
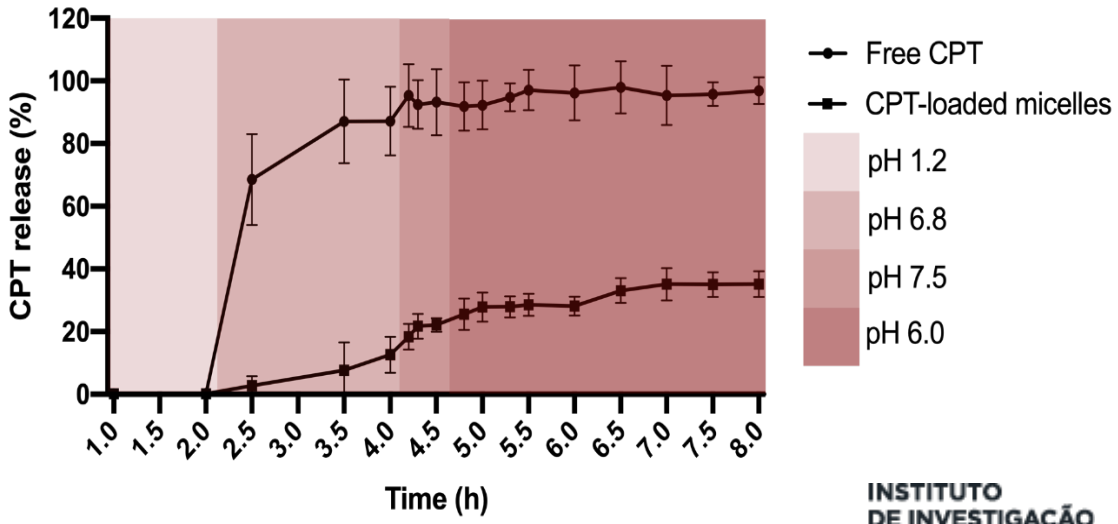
Polymeric micelles for oral chemotherapy to treat colorectal cancer





Almeida, A., et al, Eur J Pharm Biopharm, 167, 48-56, 2021

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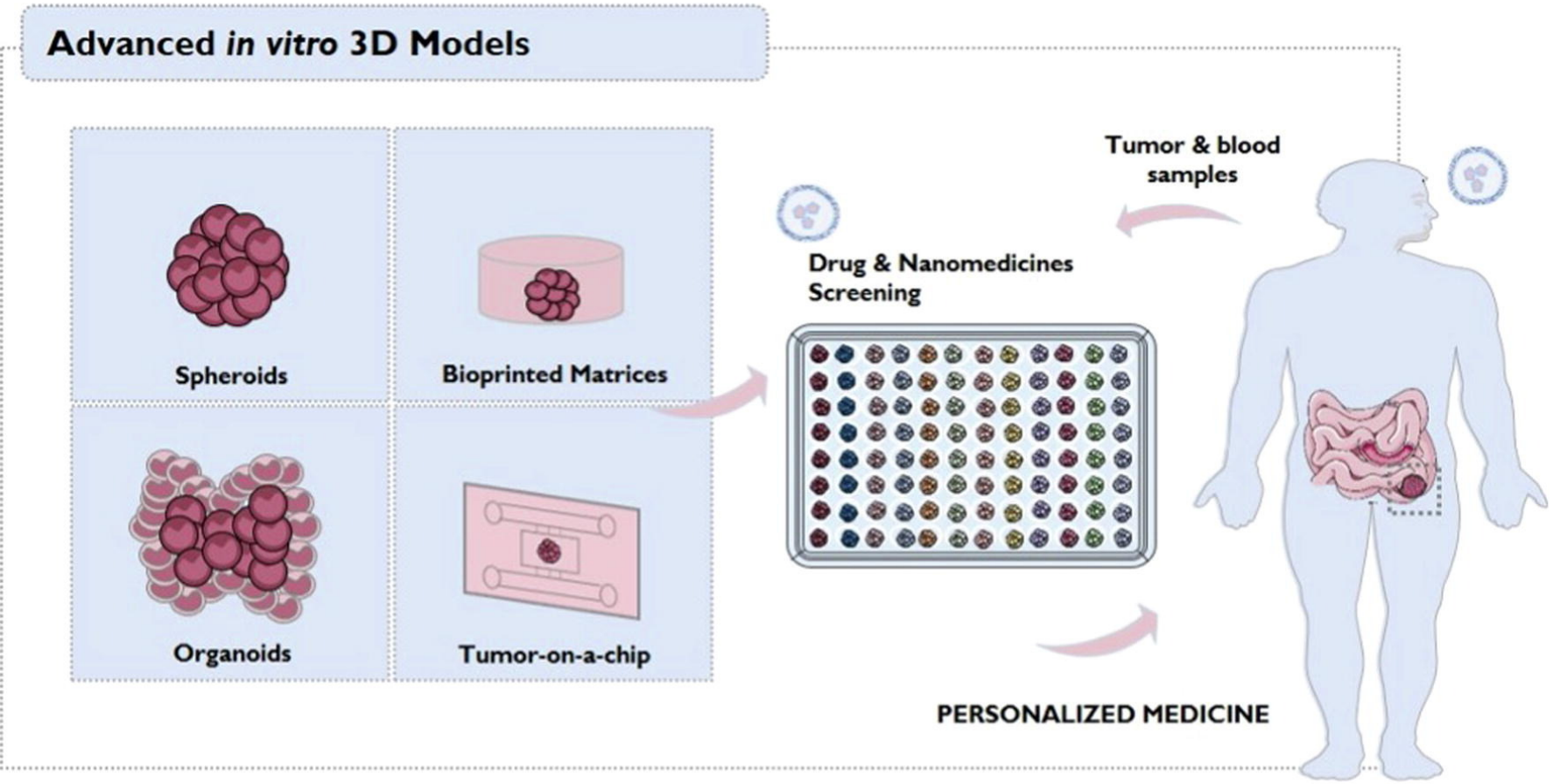
Colorectal cancer 3D models



Castro et al., Advanced Drug Delivery Reviews 175 (2021) 113824

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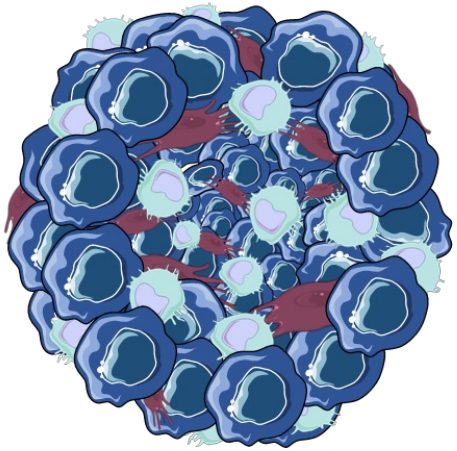
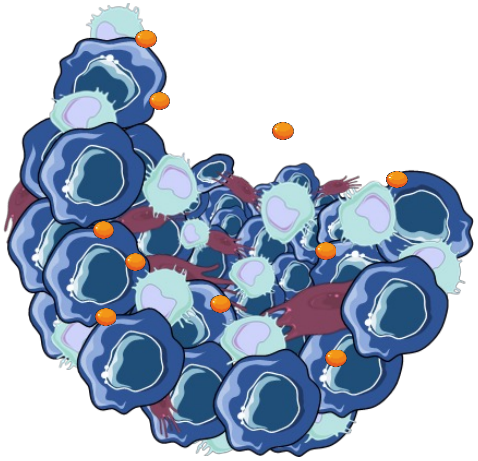
CRC multicellular tumor spheroids model

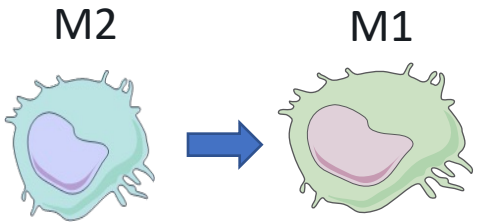
1) Develop model closer to TME

2) Study interaction of NPs and antiproliferative ability

3) Study macrophage polarization







Tumor associated macrophages (TAMs)

M1 macrophages

Epithelial colon cancer cells (HCT116)

Cancer associated fibroblasts (CAFs)

Co-loaded NPs

T. Bauleth-Ramos et al., J Controlled Rel, 323, 398-411, 2020

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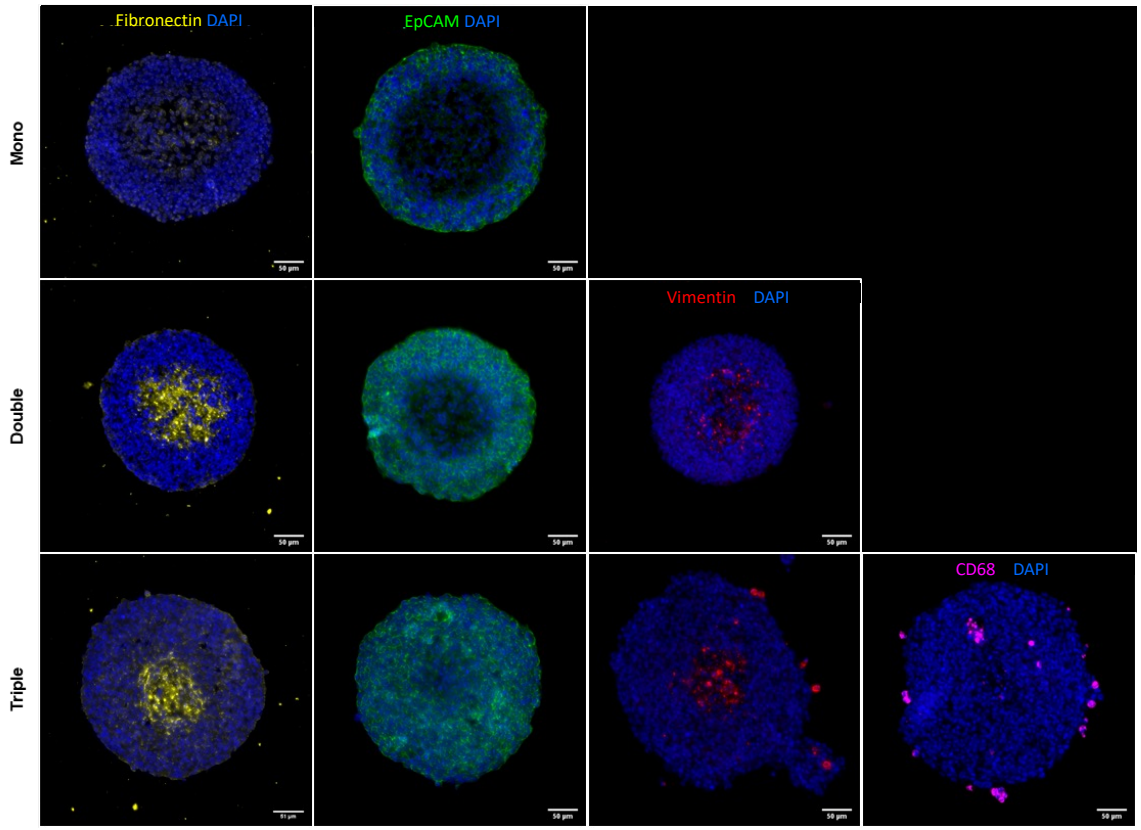
CRC multicellular tumor spheroids model



Fibronectin - Extracellular matrix
EpCAM - Tumor cells
Vimentin - Fibroblasts
CD68 - Macrophages

D7 spheroid constitution

Cells	%
Tumor cells	90.8±2.4
Fibroblasts	5.6±1.6
Macrophages	7.5±1.2

- Fibronectin production by HIF
- Necrotic core and viable outer rim
- Spatial organization
- Majority of epithelial cells

T. Bauleth-Ramos et al., J Controlled Rel, 323, 398-411, 2020

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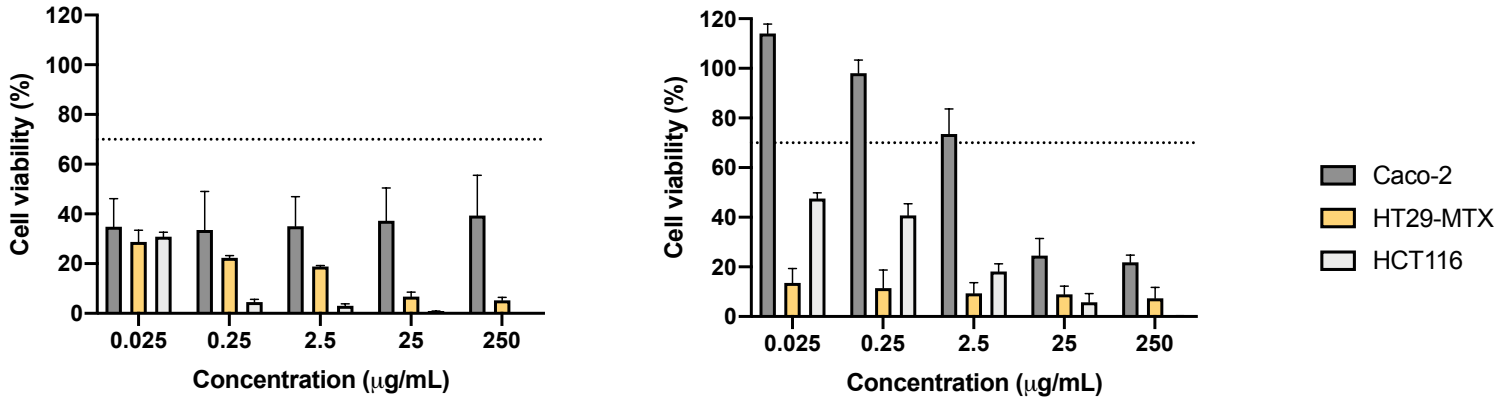
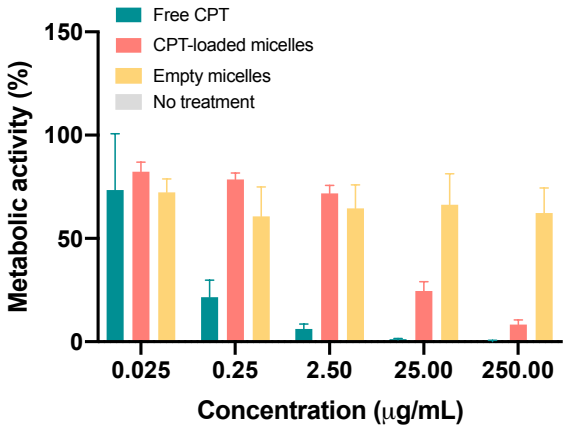
Polymeric micelles for oral chemotherapy to treat colorectal cancer - CRC spheroid

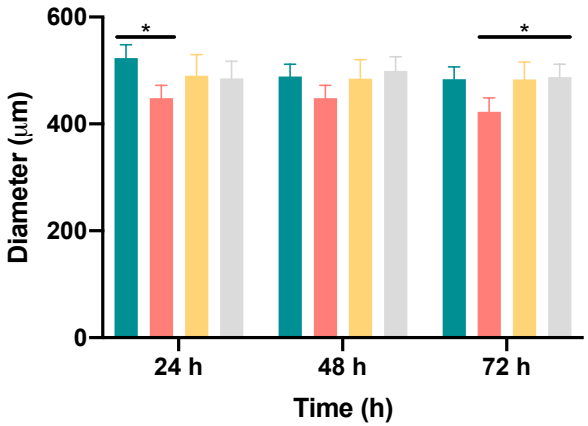


Cell viability after 72 h of incubation with (**left**) free CPT and (**right**) CPT-loaded micelles against Caco-2, HT29-MTX and HCT116 cell lines

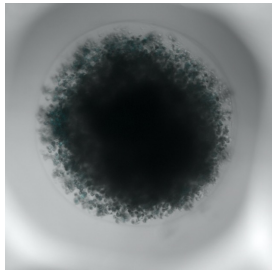
Cytocompatibility on a 3D spheroid model



Cytocompatibility of free CPT, CPT-loaded micelles and empty micelles against PBMCs



Size reduction on spheroids after 72 h in contact with free CPT, CPT-loaded micelles



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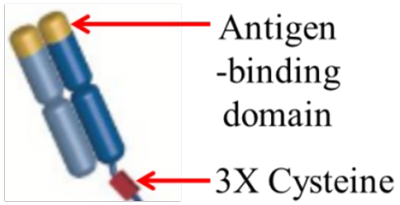
CD44v6 as target for functional nanoparticles

Non-specific Fab or mAb used as negative controls

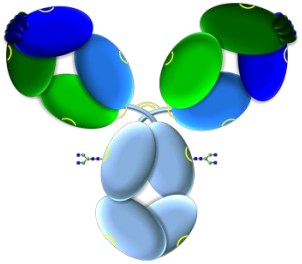
Anti-human CD44v6 antibodies

Engineered HuCAL® Fab

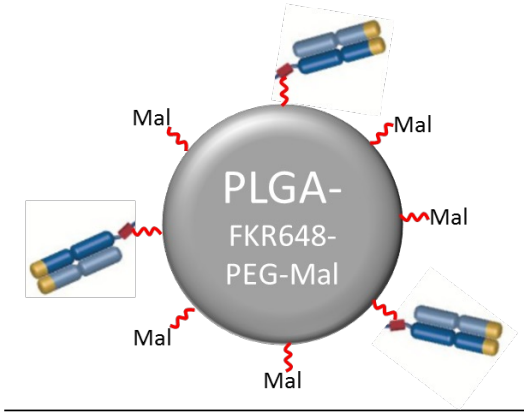


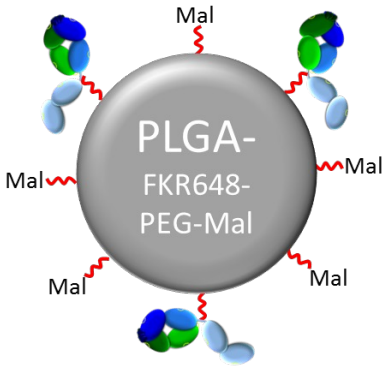
Mild TCEP treatment to reduce cysteines (Fab) or hinge disulfide bridges (mAb)

Commercial mAb



Fluorescent, PEGylated PLGA nanoparticles





Kennedy *et al*, Pharmacology and Therapeutics, 2017;177:129-45

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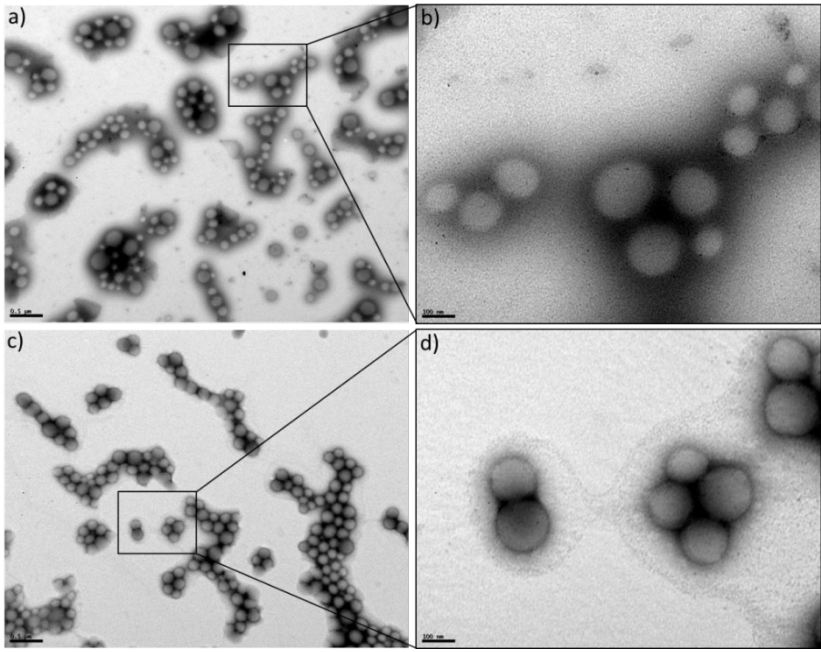
CD44v6 as target for functional nanoparticles

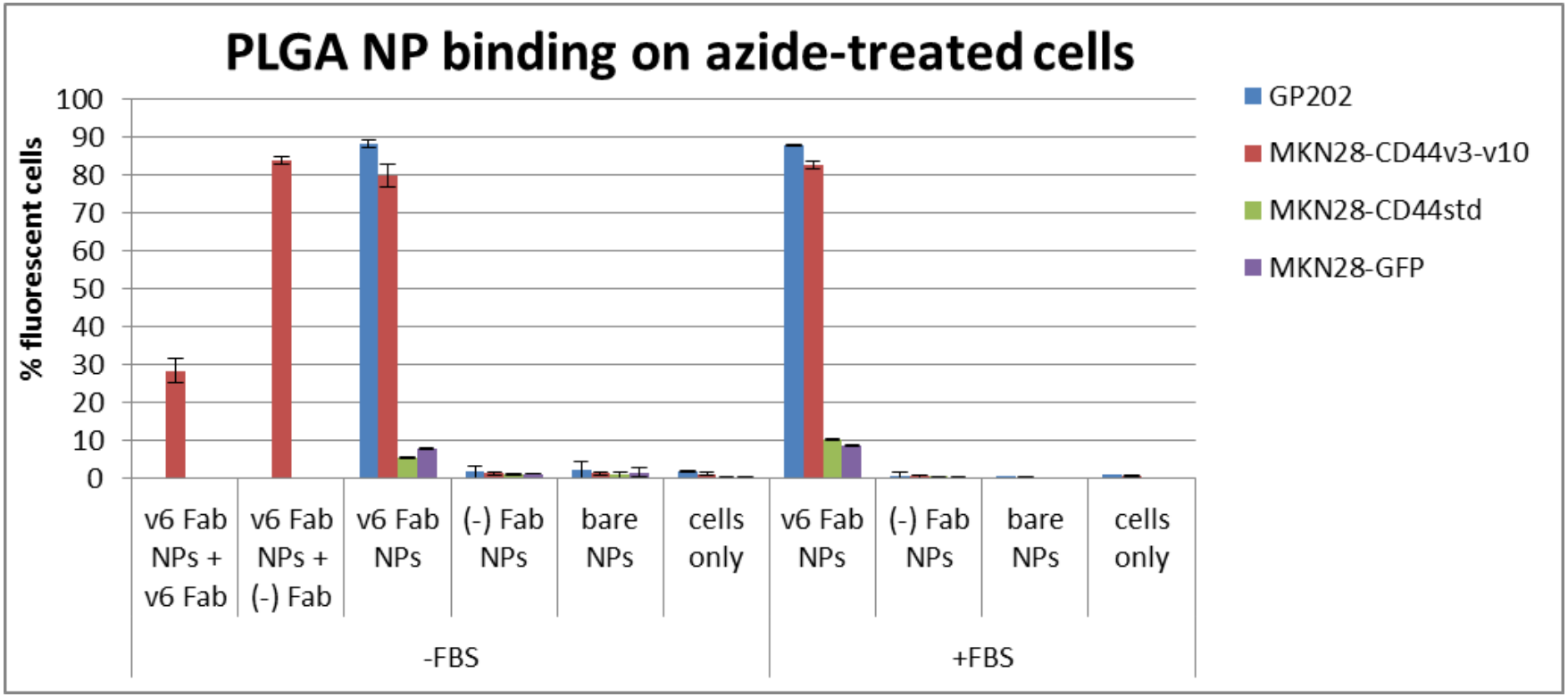
PLGA NPs	PTX	Z-average (size, nm)	polydispersity index (PdI)	Zeta Potential (charge, mV)
v6 Fab-PLGA	-	379 ± 48	0.40 ± 0.05	-19.9 ± 0.4
	+	293 ± 15	0.32 ± 0.04	-20.0 ± 0.4
(-) Fab-PLGA	-	234 ± 27	0.21 ± 0.04	-18.2 ± 2.0
	+	234 ± 34	0.22 ± 0.06	-19.6 ± 0.1
bare PLGA	-	205 ± 6	0.16 ± 0.01	-15.6 ± 0.8
	+	217 ± 10	0.24 ± 0.03	-17.4 ± 0.1



TEM images of the v6 Fab- (a and b) and bare (c and d) PLGA NPs. a and c = 25,000X magnification (scale bar = 0.5 µm). b and d = 120,000X magnification (scale bar = 100 nm).

Kennedy *et al*, Acta Biomaterialia, 81, 208-218, 2018

CD44v6 as target for functional nanoparticles



Kennedy *et al*, Acta Biomaterialia, 81, 208-218, 2018

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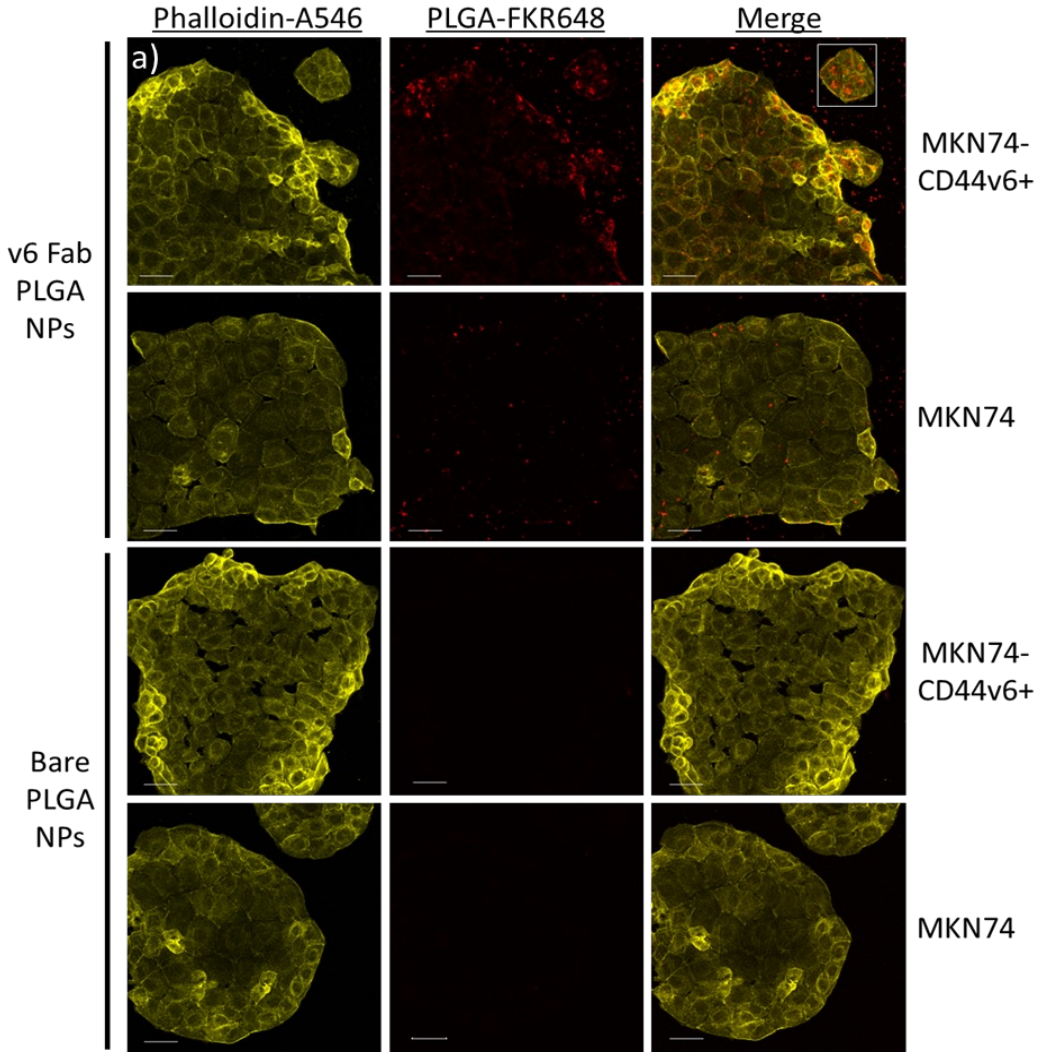

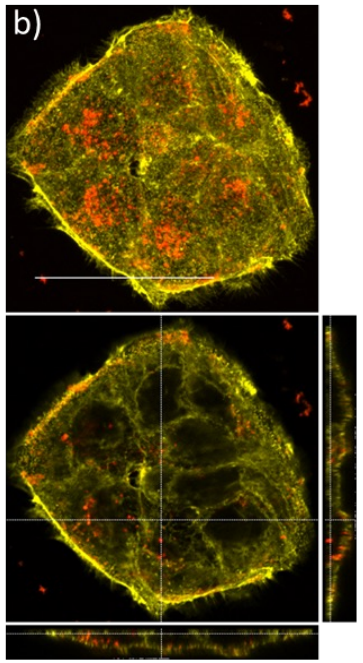

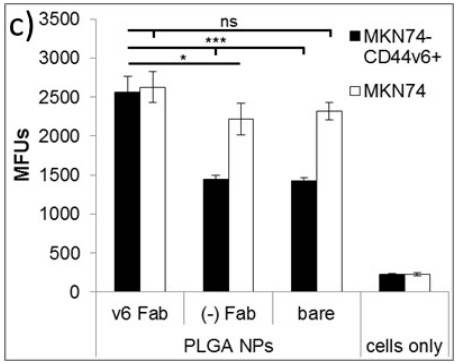

Kennedy *et al*, Acta Biomaterialia, 81, 208-218, 2018

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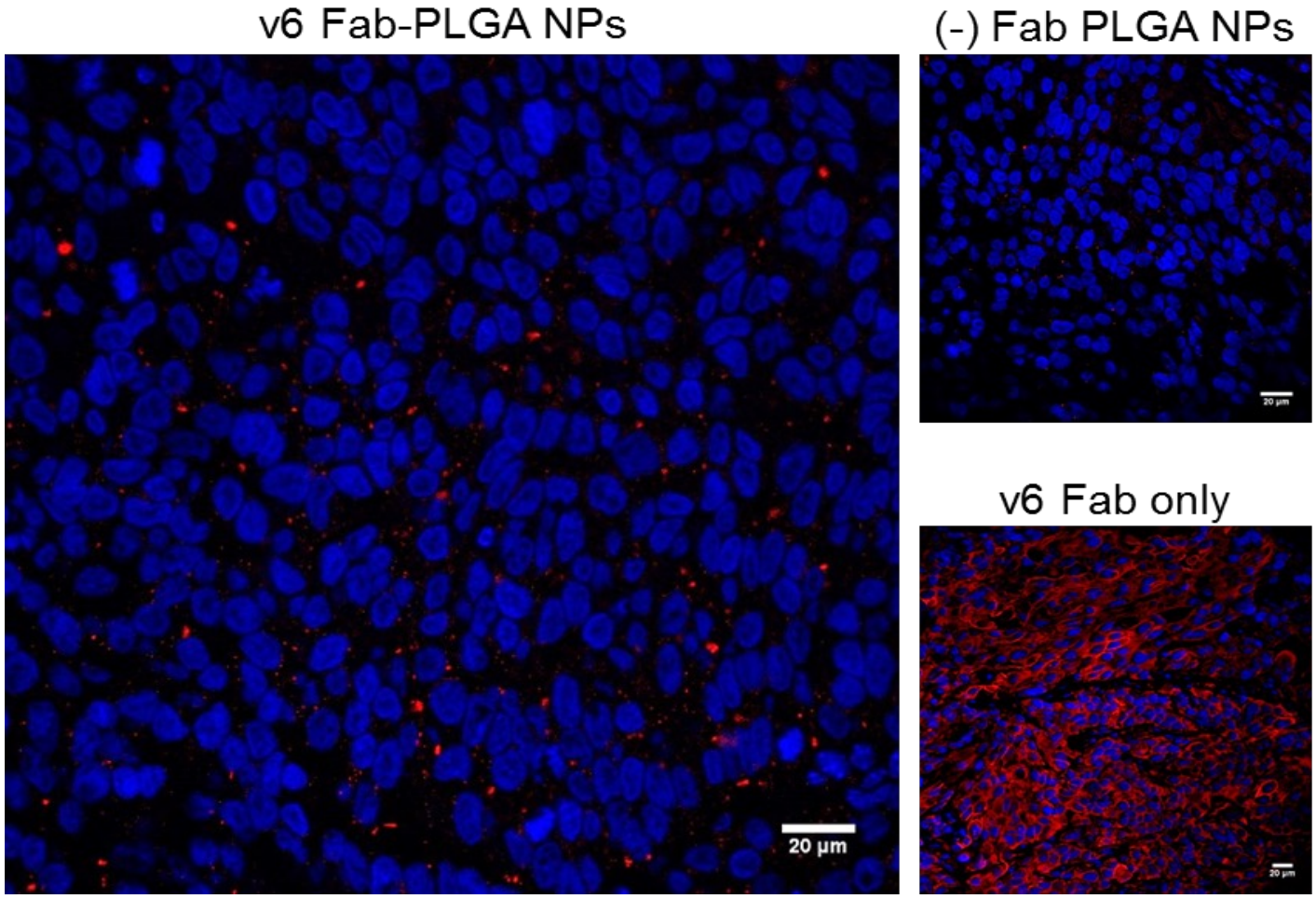
CD44v6 as target for functional nanoparticles

Confocal microscopy of v6 Fab-PLGA NP binding to MKN74-CD44v6 tumor sections



Kennedy *et al*, Acta Biomaterialia, 81, 208-218, 2018

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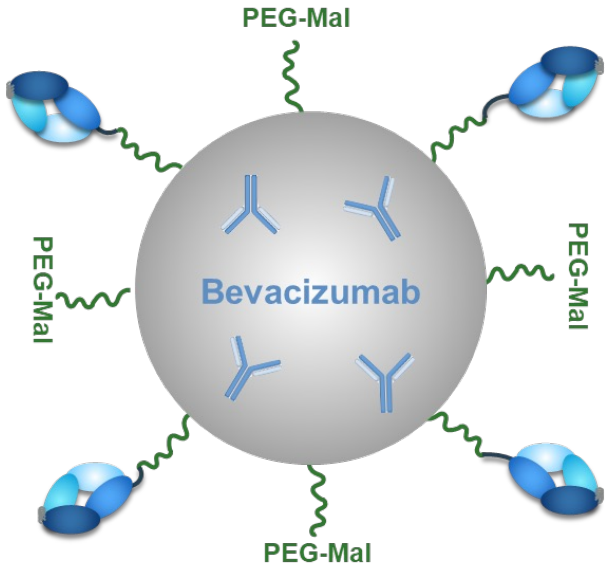
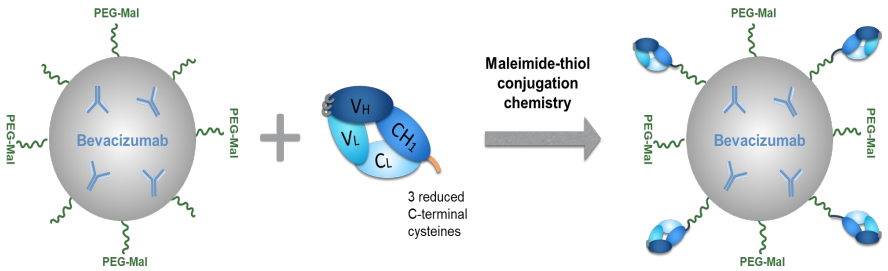
CD44v6 as target for anti-angiogenic nanoparticles

Produce PLGA-PEG nanoparticles loaded with bevacizumab and functionalized with a human antibody fragment with specificity for CD44v6



- ✓ Biodegradable
- ✓ Biocompatible
- ✓ Safe

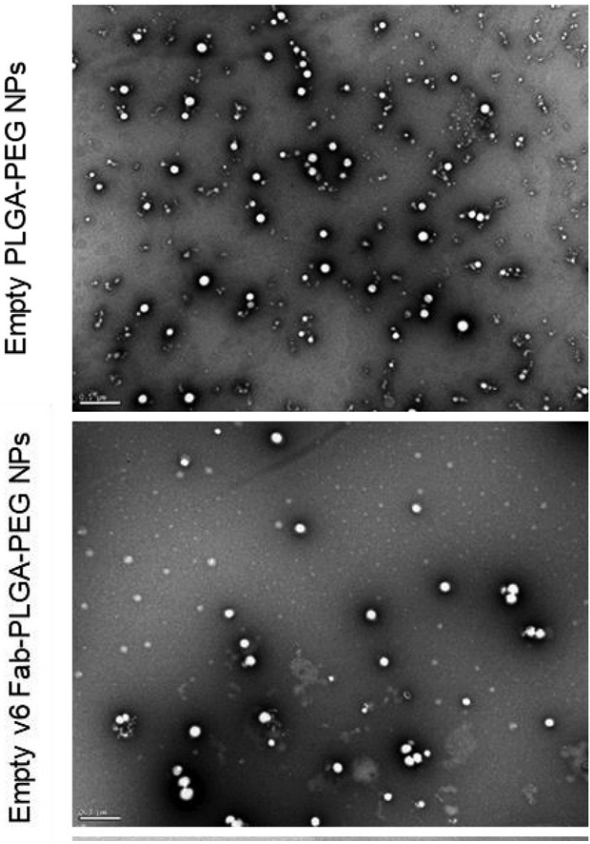


Baião *et al*, Biomater. Sci., 8, 3720–3729, 2020

CD44v6 as target for anti-angiogenic nanoparticles

Formulation	BVZ	Z-average (size, nm)	Polydispersity Index (PdI)	Zeta Potential (charge, 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

Dilution 1:100 NaCl pH 7.4



Functionalization and bevacizumab encapsulation increases size and PdI

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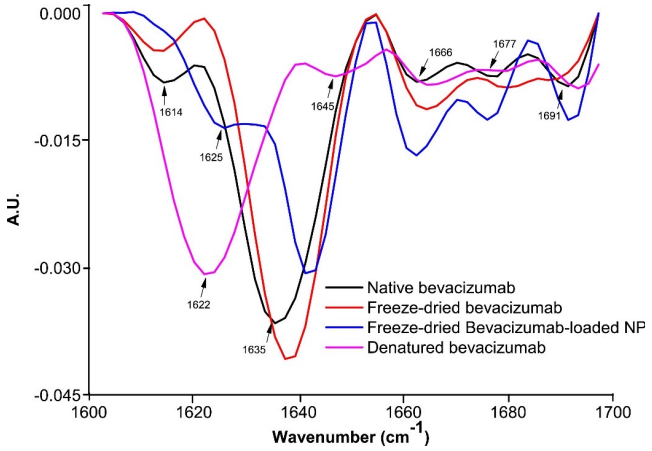
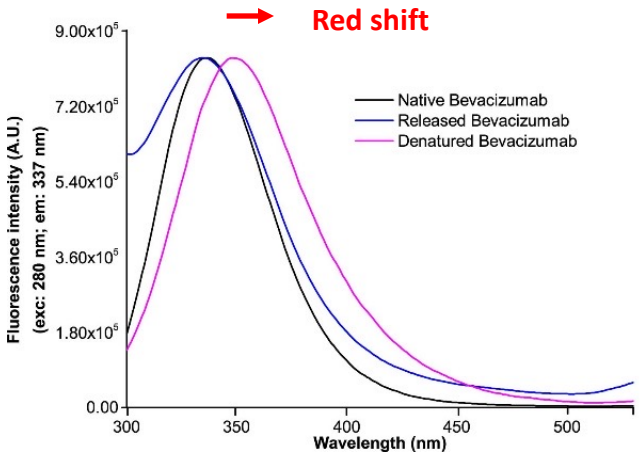
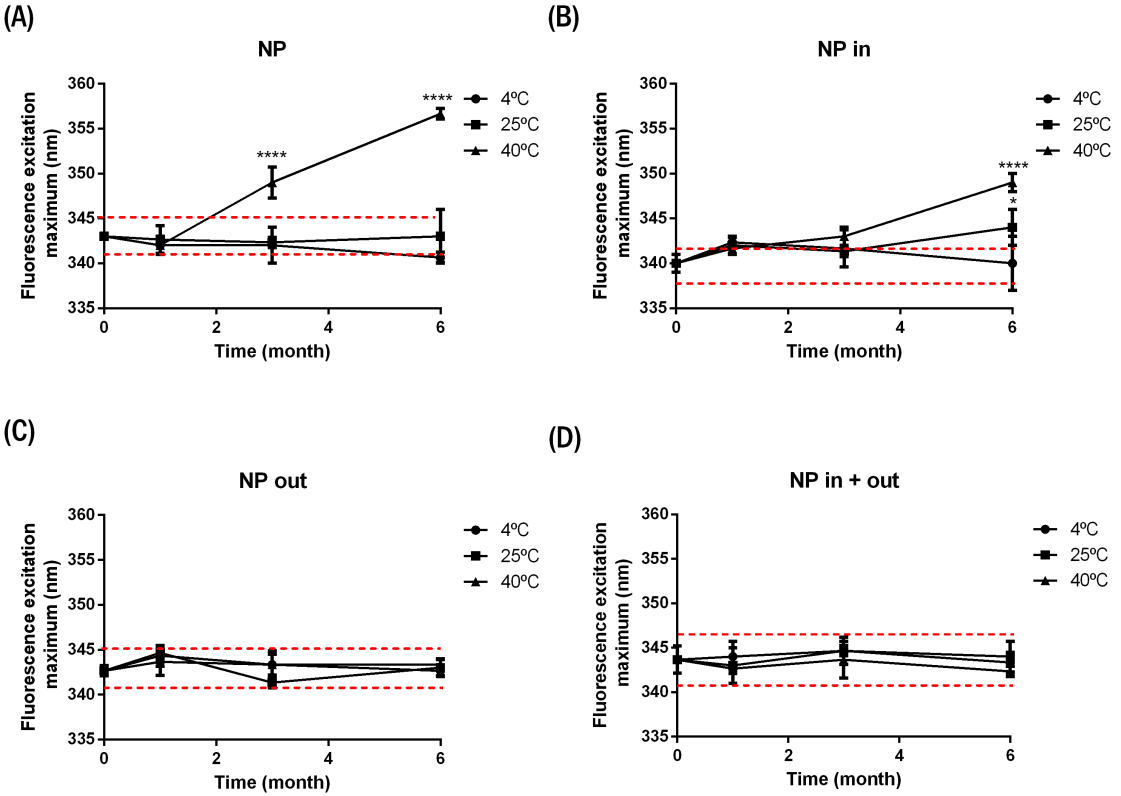
Baião *et al*, Biomater. Sci., 8, 3720–3729, 2020

CD44v6 as target for anti-angiogenic nanoparticles

Maintenance of bevacizumab structure after encapsulation







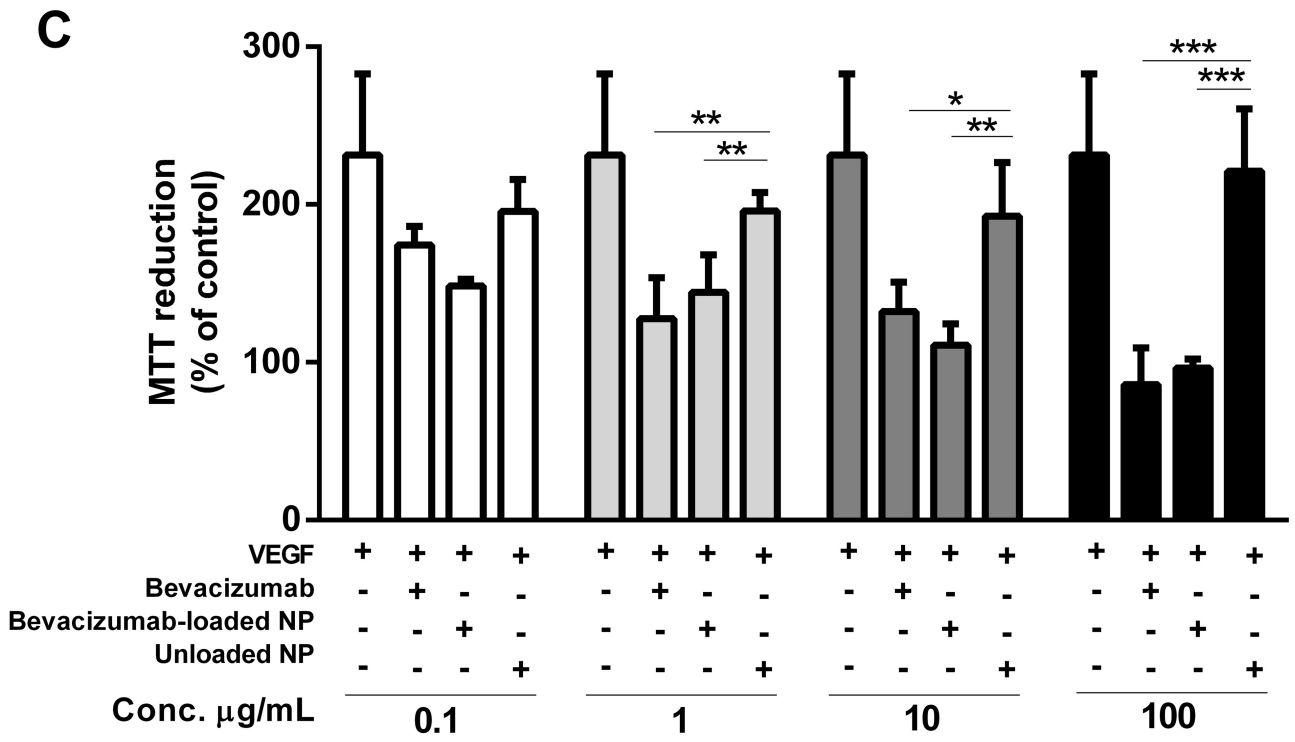
6-month long-term stability

F. Sousa et al., **Sci. Rep**. 2017, 7, 3736

F. Sousa et al., **Acta Biomat.**, 2018, 78

CD44v6 as target for anti-angiogenic nanoparticles

Bevacizumab bioactivity after encapsulation



HUVEC cell line

Biological activity of bevacizumab was maintained even with a slow release profile from PLGA NP.

F. Sousa et al., **Sci. Rep**. 2017, 7, 3736

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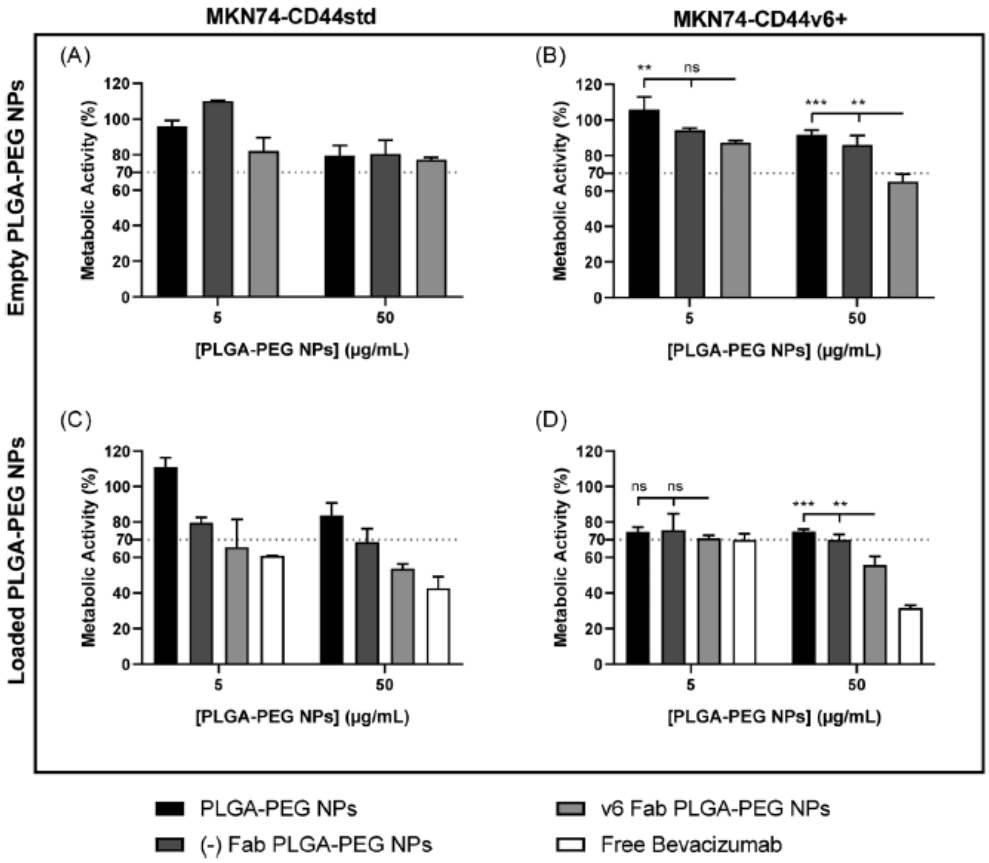
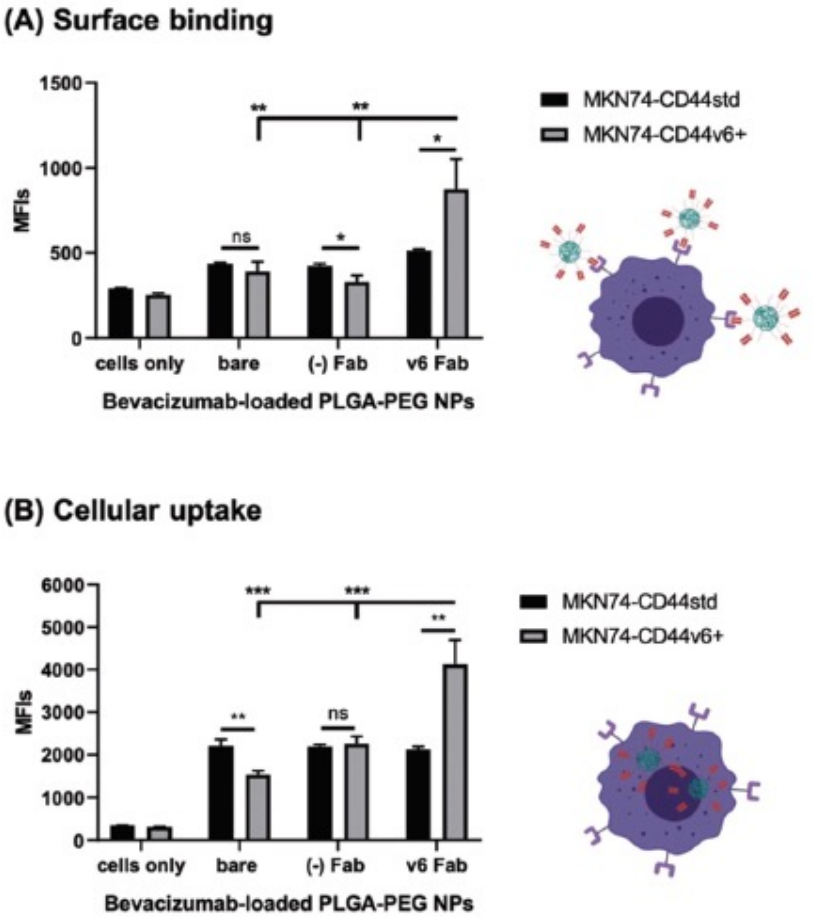
CD44v6 as target for anti-angiogenic nanoparticles





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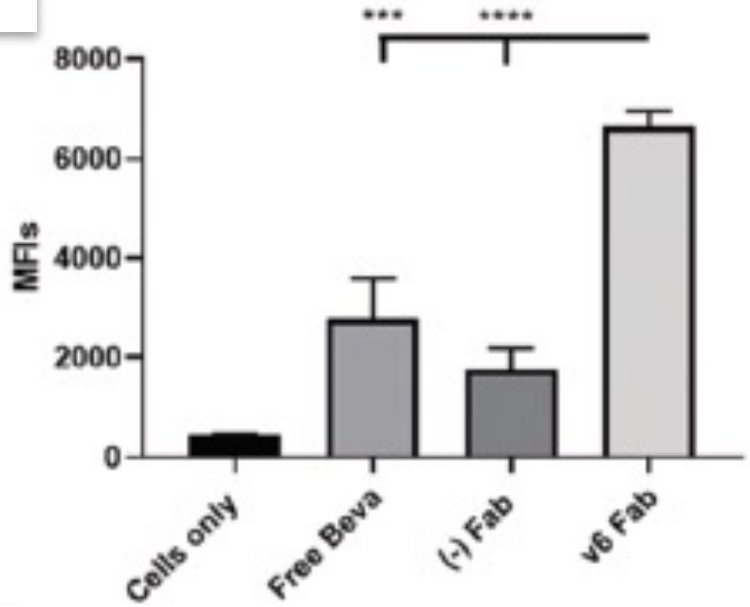
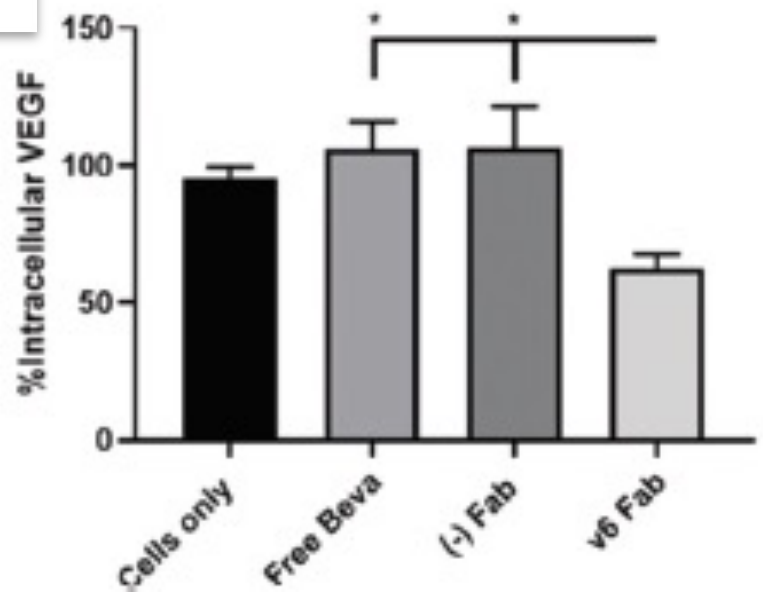
Baião *et al*, Biomater. Sci., 8, 3720–3729, 2020

CD44v6 as target for anti-angiogenic nanoparticles

Intracellular levels of bevacizumab



Intracellular VEGF



MKN74-CD44v6+ cells

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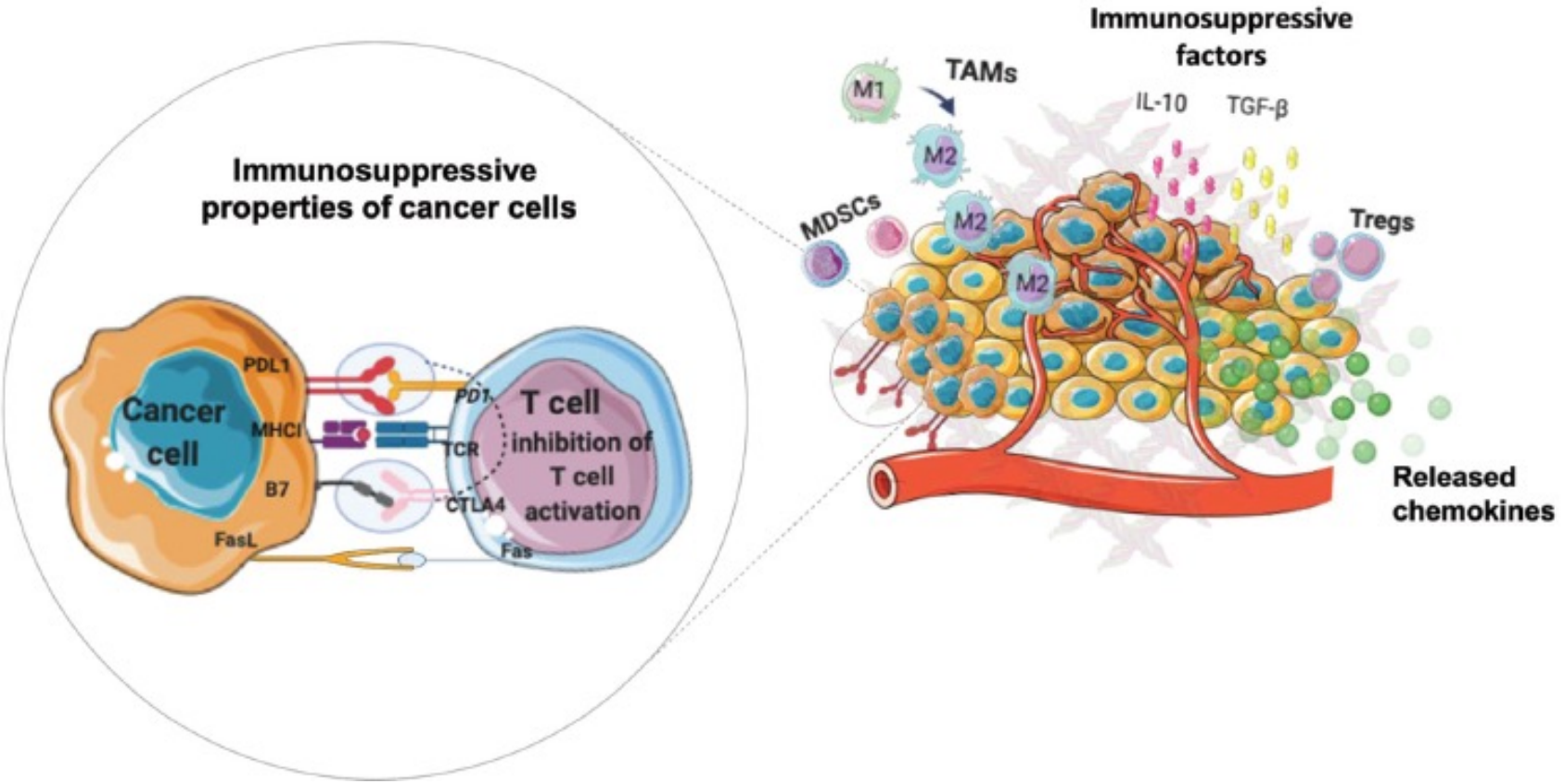
Baião *et al*, Biomater. Sci., 8, 3720–3729, 2020

Immunosuppressive mechanisms in the CRC



Silveira *et al*, Biomater. Sci., 9, 3228-3243, 2021

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In summary...

- Nanotechnology is a key enabling technology with potential to modulate the biopharmaceutics of anticancer drugs
- Nanoparticles can be proposed to encapsulate and delivery a wide variety of anticancer drugs with solubility/permeability issues, relevant in cancer treatment, namely in colorectal cancer
- Nanoparticles can be easily functionalized in order to develop target nanosystems, with superior ability for specific cellular moieties

Nanotechnology has provided the possibility of delivering drugs to specific cells in a tailored and engineered manner