Overcoming Chemoresistance in Colorectal Cancer by FcRn-Driven Nucleic Acid Delivery

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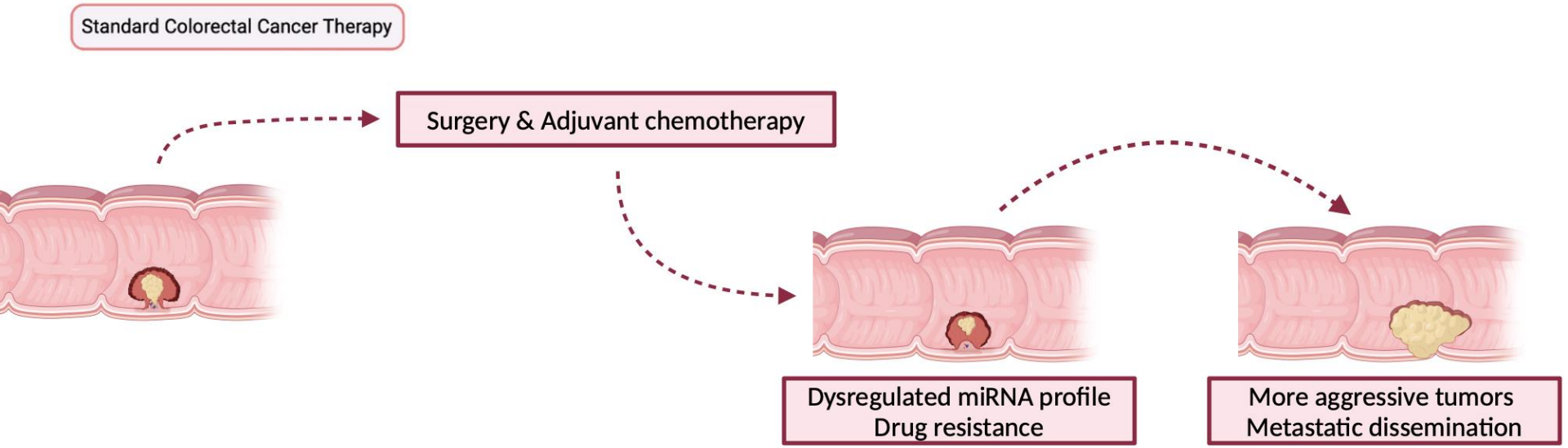
NEXT-GENERATION DELIVERY INNOVATIONS

Introduction

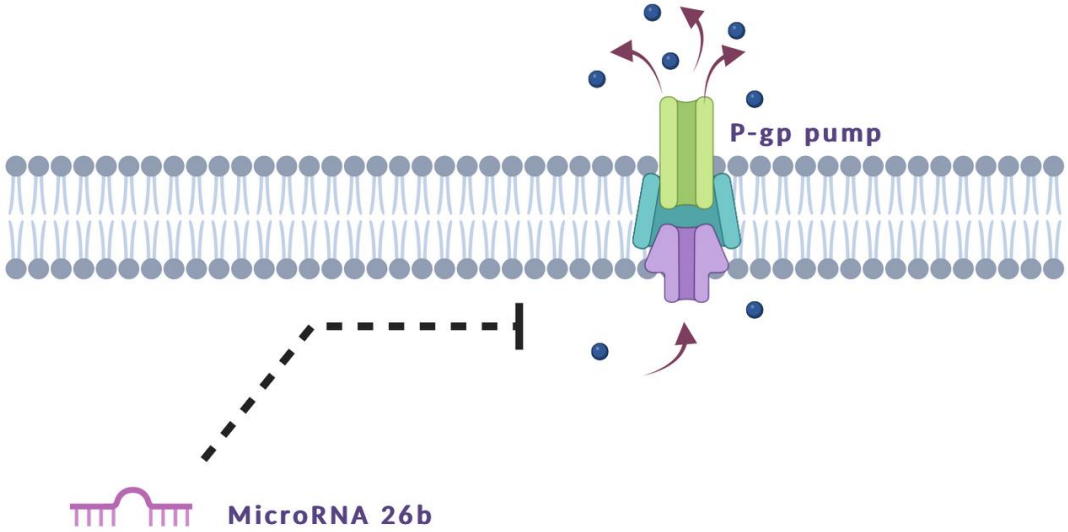
Colorectal cancer therapy challenges

Colorectal cancer is globally among the most lethal malignancies & second cause of cancer death



Introduction

microRNAs as therapeutic agents



Susceptibility to degradation
Poor cell membrane penetration
Difficult endosomal escape
Possible off-target effects

Introduction

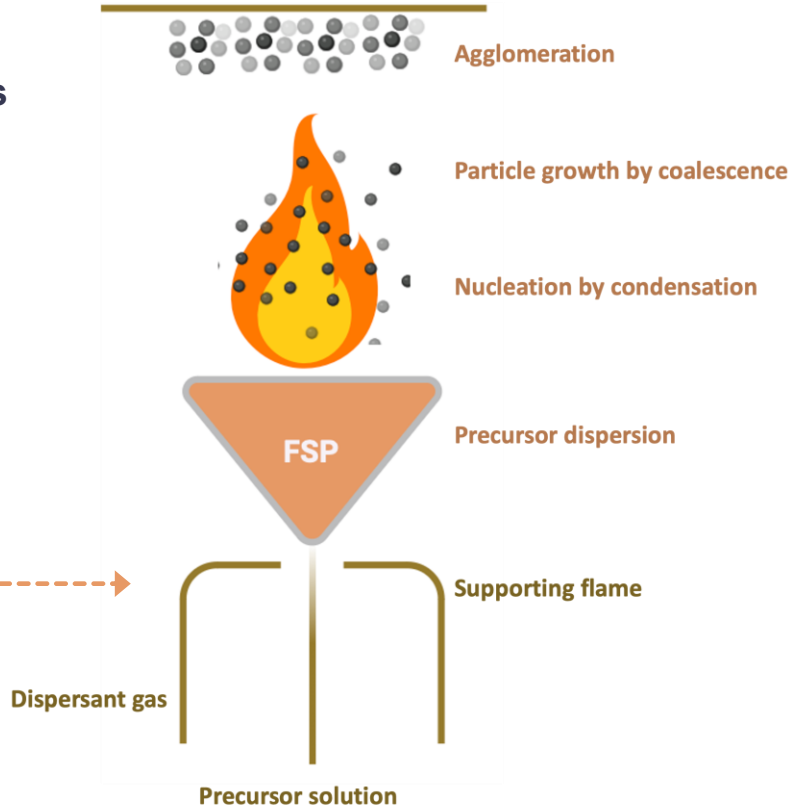
Calcium Phosphate nanoparticles as nucleic acid nanocarriers

Calcium Phosphate Nanoparticles

Biocompatibility & biodegradability

Strong affinity to nucleic acids & other biologics

Scaled-up synthesis by flame spray pyrolysis

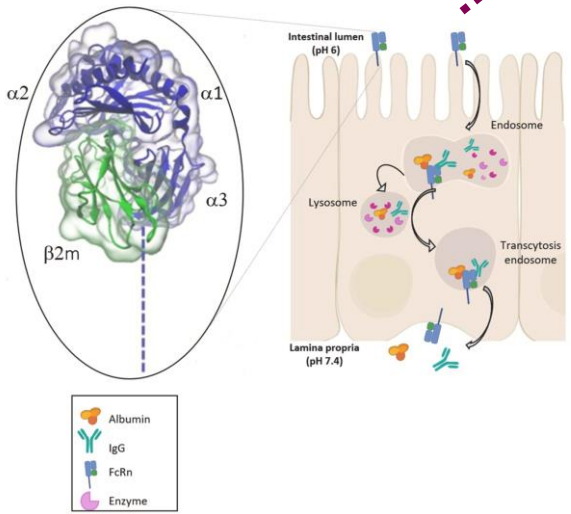


Introduction

Neonatal Fc Receptor as CRC target

Understanding FcRn role in tumor microenvironment

FcRn crystallographic illustration



Peptides with FcRn pH-dependent affinity

Ligand	Sequence	Fusion molecule for SPR affinity evaluation	Kd at pH 6.0 (nM)	Kd at pH 7.4 (nM)
SYN 746	QRFCTGHFGGLYPCNGP	———	5,100 [1]	30,000 [1]
SYN 1327	RF-Pen-TGHFG-Sar-NMeLeu-YPC	———	31 [1;2]	170 [1;2]
Linear derived-SYN746	QRFVTGHFGGLYPANG	Fab moiety	4,300 [3]	21,600 [3]
		mKate protein	9,250 [4]	154,000 [4]

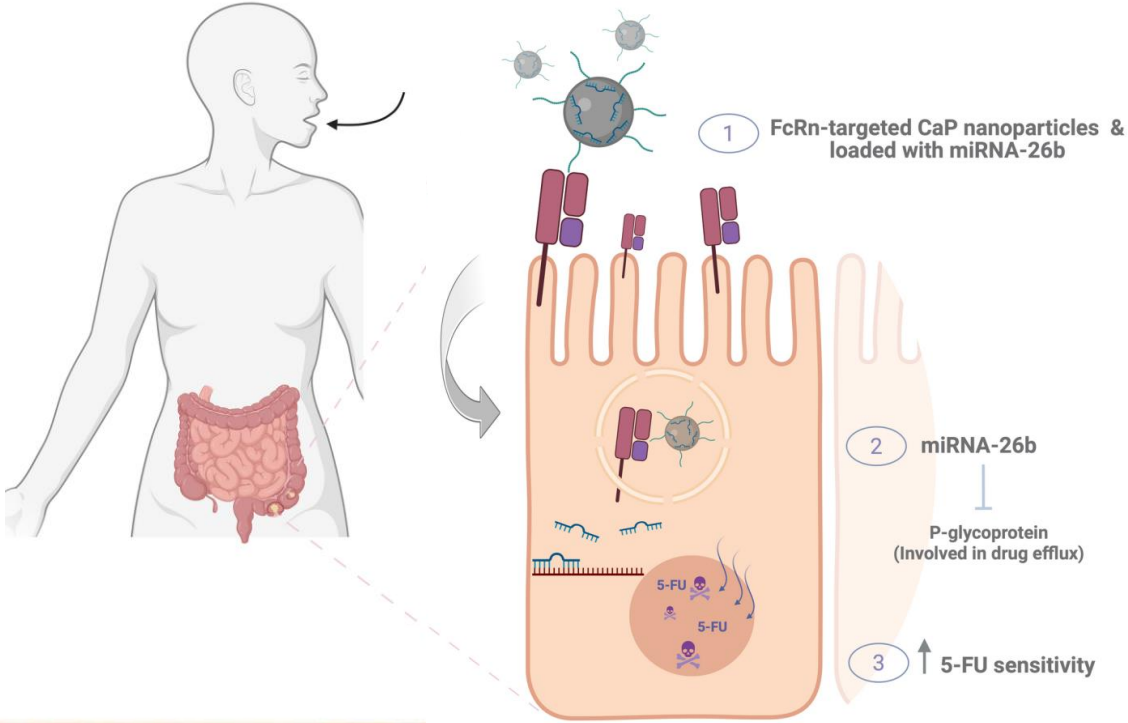
1. Mezo, A. R.; McDonnell, K. A.; Tan Hehir, C. A.; Low, S. C.; Palombella, V. J.; Stattel, J. M.; Kamphaus, G. D.; Fraley, C.; Zhang, Y.; Dumont, J. A.; Bitonti, A. J. Reduction of IgG in Nonhuman Primates by a Peptide Antagonist of the Neonatal Fc Receptor FcRn. Proc. Natl. Acad. Sci. U. S. A. 2008, 105 (7), 2337–2342. https://doi.org/10.1073/pnas.0708960105.
2. Mezo, A. R.; McDonnell, K. A.; Castro, A.; Fraley, C. Structure–Activity Relationships of a Peptide Inhibitor of the Human FcRn:Human IgG Interaction. Bioorg. Med. Chem. 2008, 16 (12), 6394–6405. https://doi.org/10.1016/j.bmc.2008.05.004.
3. Datta-Mannan, A.; Boyles, J.; Huang, L.; Jin, Z. Y.; Peariso, A.; Murphy, A. T.; Ellis, B.; Douglass, N.; Norouziyan-Cooper, F.; Witcher, D. R. Engineered FcRn Binding Fusion Peptides Significantly Enhance the Half-Life of a Fab Domain in Cynomolgus Monkeys. Biotechnol. J. 2019, 14 (3), 1–12. https://doi.org/10.1002/biot.201800007.
4. Sockolosky, J. T.; Tiffany, M. R.; Szoka, F. C. Engineering Neonatal Fc Receptor- Mediated Recycling and Transcytosis in Recombinant Proteins by Short Terminal Peptide Extensions. Proc. Natl. Acad. Sci. 2012, 109 (40), 16095–16100. https://doi.org/10.1073/pnas.1208857109.

CQRFVTGHFGGLYPANG
FcRn affinity in-house
Kd at pH 6.0 2,094 ± 900 nM
No binding at pH 7.4

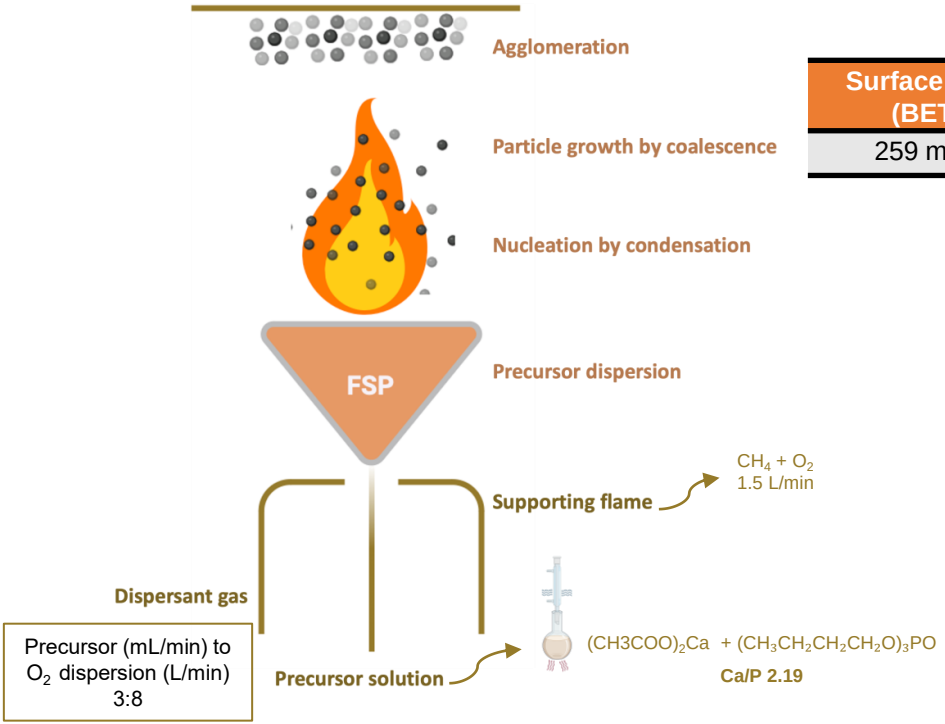
Hameedat, F., Pizarroso, N. A., Teixeira, N., Pinto, S., & Sarmento, B. (2022). Functionalized FcRn-targeted nanosystems for oral drug delivery: A new approach to colorectal cancer treatment. *European journal of pharmaceutical sciences.*

Graphical Abstract

FcRn-targeted Nanomedicines for combined Gene and Chemotherapy

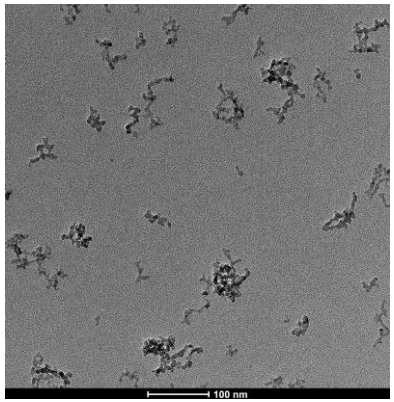


CaP Nanoparticles by Flame-spray pyrolysis



Precursor (mL/min) to O_2 dispersion (L/min) 3:8

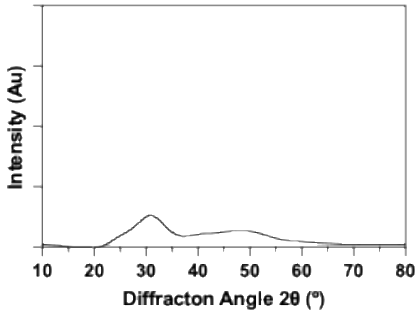
Surface Area (BET)	Average Particle Size (BET)
259 m^2/g	7.3 nm



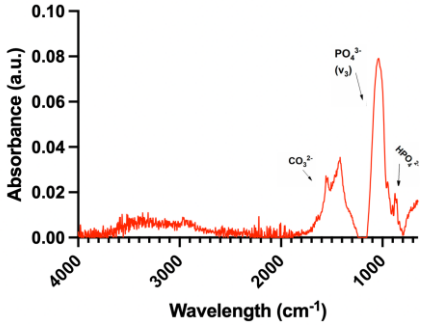
TEM of CaP NP 0.5 mg/mL in ethanol

Crystallographic Structure (XRD)

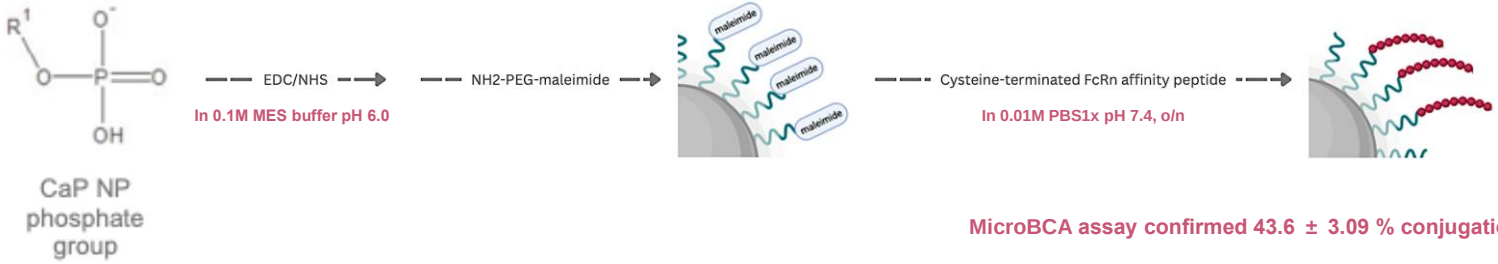
Amorphous NP



CaP NP functional groups (FTIR)



Hydrodynamic Characterization

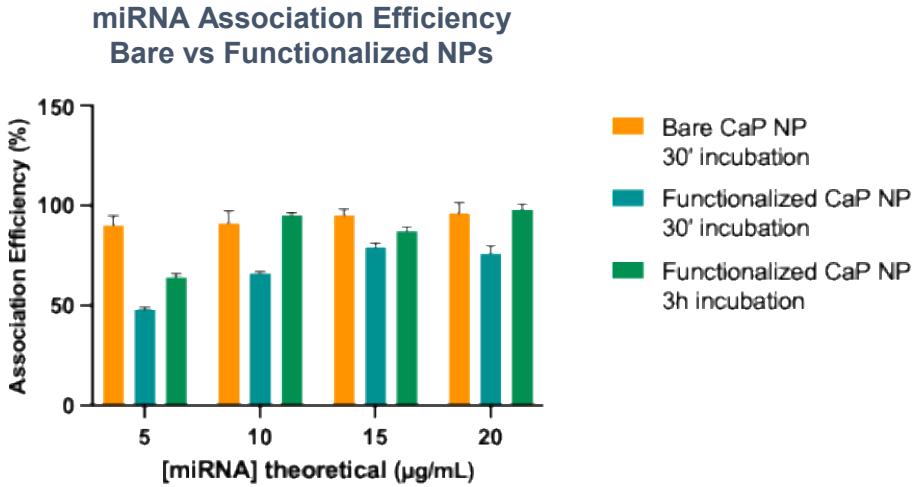


MicroBCA assay confirmed 43.6 ± 3.09 % conjugation efficiency for FcRn-peptide

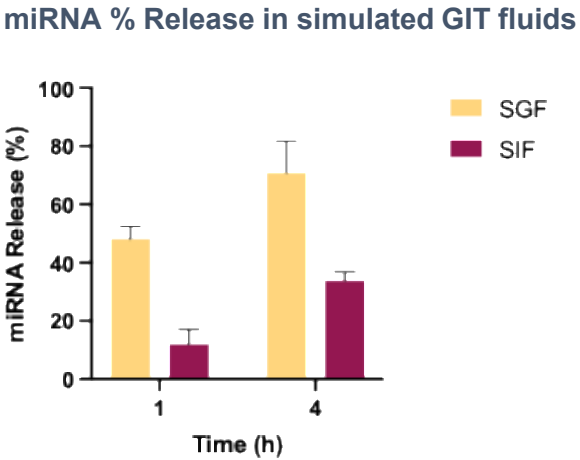
	Size (nm)	PdI	ζ potential (mV)
CaP NP (H_2O)	4442 ± 173.4	0.28 ± 0.22	2.25 ± 0.27
CaP NP (0.1M MES buffer)	1305 ± 117.4	0.32 ± 0.12	1.07 ± 0.16
CaP-PEG(5KDa) NP (PBS 0.01M)	1072 ± 77.3	0.56 ± 0.10	0.98 ± 0.37
CaP-PEG(5KDa)-FcRnpeptide NP	816 ± 118	0.51 ± 0.25	1.79 ± 0.29

1. MES buffer maintains mildly acidic pH avoiding CaP dissolution at lower pH, but also avoids flocculation and aggregation in aqueous suspension;
2. After pegylation CaP NP are more stable in aqueous solution without the need to maintain pH constant;
3. Site-oriented conjugation of FcRn-affinity peptide to CaP-PEG NP was well-succeeded and able to stabilize CaP NP.

Nucleic Acids Loading & Release

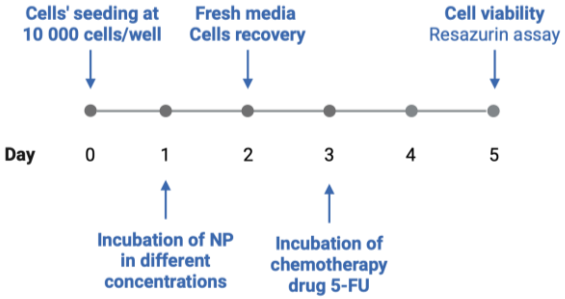


Bare NP load miRNA faster than functionalized NP, with values of AE % after 30 minutes incubation compared with 3h incubation in functionalized NP.

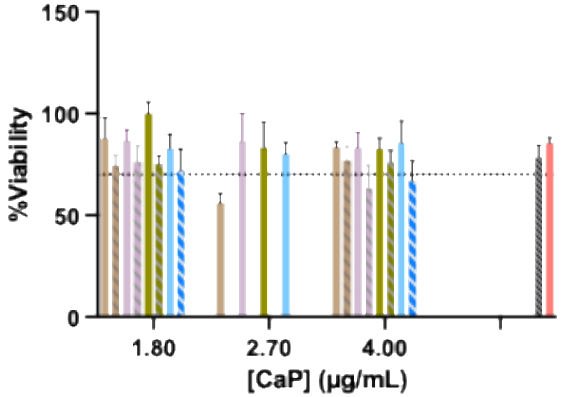


MicroRNA release from CaP NP is higher in simulated gastric fluid compared to simulated intestinal fluid release.
This was attributed to the faster dissolution of CaP, which can be advantageous to escape intracellular acidic organelles.

Cytotoxicity & Efficacy Assays

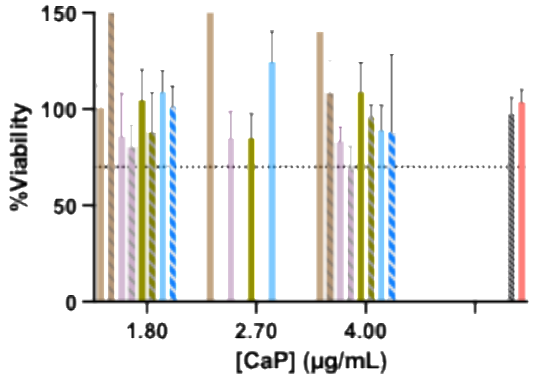


HCT116

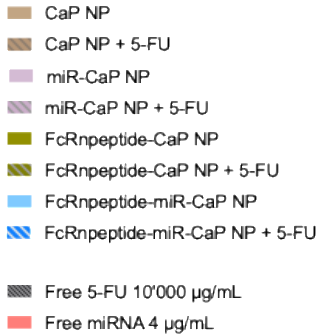


- Observed trend at **higher miRNA concentrations**: **miRNA-CaP NPs** & **$FcRn_{peptide}$-miRNA-CaP NPs** may **sensitize cells to 5-FU**.

HIF

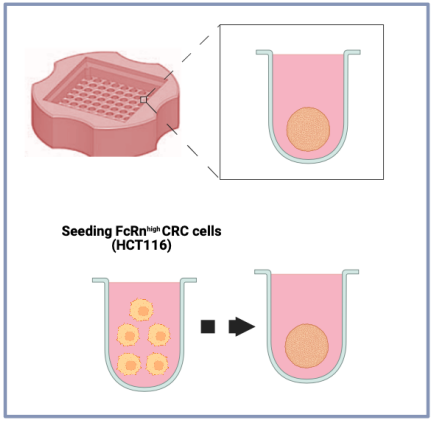


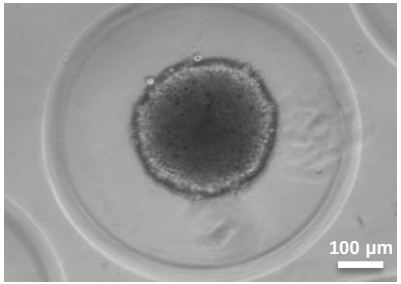
- **No significant sensitization** to 5-FU observed under any condition.
- **Longer doubling time** of HIF cells; 5-FU targets **actively dividing cells**.



CRC Spheroids: Monoculture and Triple Co-culture

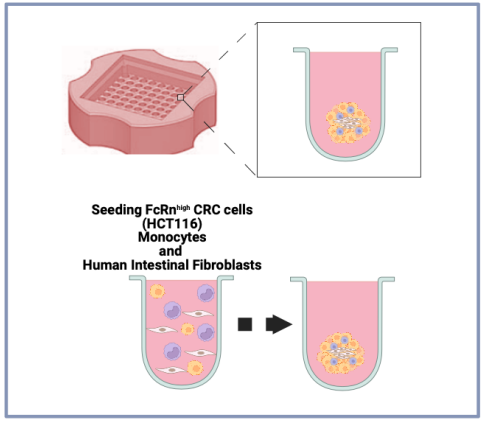
Monoculture CRC 3D spheroid

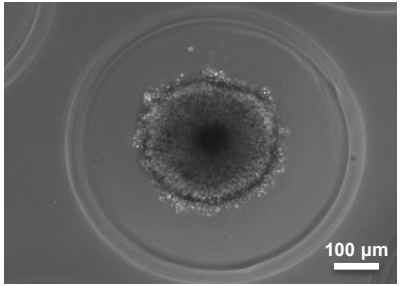




Initial cell density of 5000 cells/spheroid

Triple Co-culture CRC 3D spheroid





Initial cell density of 5000 cells/spheroid,
1:4:4
HCT116 : monocytes : HIF

Morphology comparison between **monoculture (HCT116)** and **triple co-culture (HCT116, HIF and monocytes)** multicellular tumor spheroids using brightfield microscopy on day 4 development.

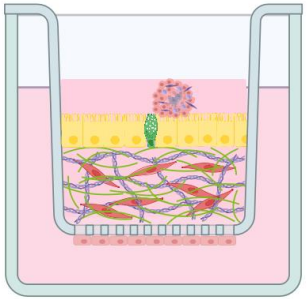
Future Perspectives

1. Validation of uptake and transfection in 2D & 3D cell models

   A. Cell transfection efficiency of CaP NP in comparison to other transfection vectors

   B. Uptake studies using labeled NP and cy5-miRNA by flow cytometry & confocal microscopy

2. Evaluate the efficacy of combined therapy in sensitizing advanced 3D CRC chemoresistant *in vitro* models



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