

Table of Contents

Chapter 1 General Introduction	1
1.1 Nanotechnology and nanomedicine.	3
1.2 Mesoporous silica materials in advanced applications.	5
1.2.1 Synthesis of mesoporous silica nanoparticles.....	7
1.2.2 Functionalisation of mesoporous silica materials.....	9
1.3 Stimuli-responsive gated materials.	11
1.3.1 Endogenous stimuli-responsive materials.	13
1.3.2 Exogenous stimuli-responsive materials.....	21
1.3.3 Gated mesoporous silica nanoparticles as drug delivery systems in biomedical applications.	27
1.3.4 Clinical relevance of gated mesoporous silica nanoparticles.....	30
1.4 Gold nanoparticles.	31
1.4.1 Synthesis and functionalisation of gold nanoparticles.....	32
1.4.2 Clinical relevance of gold nanoparticles.....	36
1.5 Biocompatibility and biodistribution of nanoparticles.....	37
1.6 Breast cancer.....	45
1.6.1 Breast cancer intrinsic subtypes.	45

1.6.2	Current therapeutic approaches for breast cancer treatment.	47
1.6.3	Bcl-2 protein family and drug resistance in breast cancer.....	49
1.6.4	Nanomedicine-based approach for breast cancer treatment.	55
1.7	References.....	61
Chapter 2 Objectives.....		85
Chapter 3 Navitoclax resistance overcoming using mesoporous silica nanoparticles		89
3.1	Abstract.	95
3.2	Introduction.....	95
3.3	Results and Discussion.....	97
3.3.1	Synthesis and characterisation of aptamer-capped nanoparticles... .	97
3.3.2	Cargo controlled release and biocompatibility studies.....	102
3.3.3	Targeted cellular uptake studies.....	104
3.3.4	Navitoclax resistance overcoming in TNBC cells.	106
3.3.5	Platelets protection assay.....	107
3.4	Conclusions.....	110
3.5	Experimental section.....	111
3.5.1	Synthesis of the mesoporous silica nanodevices.	111

3.5.2	Synthesis of APTES-MSNs(RhB).....	111
3.5.3	Synthesis of apMUC1-MSNs(RhB).....	111
3.5.4	Synthesis of drug-loaded apMUC1-gated MSNs.....	112
3.5.5	Standard characterisation procedures of the prepared materials.	113
3.5.6	Cargo delivery studies.....	114
3.5.7	Cell culture conditions.	114
3.5.8	Protein expression characterisation by western blot.	114
3.5.9	Cytotoxicity cell studies with apMUC1-MSNs.	115
3.5.10	Navitoclax resistance overcoming TNBC cells.	116
3.5.11	Targeted cellular uptake studies.....	116
3.5.12	Platelets protection assay.....	117
3.6	References.....	118
3.7	Supporting information.....	121
Chapter 4 CRISPR/Cas9 machinery and model drug co-delivery as one-shot treatment strategy	129	
4.1	Abstract.	135
4.2	Introduction.....	135
4.3	Results and Discussion.....	137

4.3.1	Assembly and characterisation of CRISPR-MSNs	137
4.3.2	Controlled release, biocompatibility, and internalisation studies. .	141
4.3.3	Gene editing of GFP and cargo delivery cellular studies.....	145
4.4	Conclusions.....	149
4.5	Materials and methods	150
4.5.1	Materials.	150
4.5.2	General methods.	150
4.5.3	Synthesis of mesoporous silica nanoparticles (MSNs).	151
4.5.4	Synthesis of PEI-MSNs.....	152
4.5.5	Synthesis of CRISPR-MSNs.	152
4.5.6	Synthesis of PEI-RhB-MSNs.	152
4.5.7	Synthesis of CRISPR-RhB-MSNs.....	153
4.5.8	Synthesis of CRISPR-RhB*-MSNs.....	153
4.5.9	Preparation of the CRISPR/Cas9 vector.	153
4.5.10	Assembly and characterisation of CRISPR-RhB-MSNs.....	154
4.5.11	CRISPR-RhB-MSNs delivery studies.....	154
4.5.12	Stability studies of the CRISPR/Cas9 vector in MSNs complexes ...	154
4.5.13	Toxicity studies with CRISPR-RhB-MSNs.	155

4.5.14	Cellular uptake studies with CRISPR-RhB*-MSNs.	155
4.5.15	Gene editing of GFP in U-2 OS-GFP cells with CRISPR-MSNs.	156
4.5.16	Gene editing of GFP in U-2 OS-GFP cells with CRISPR-RhB-MSNs. .	157
4.6	References.....	158
4.7	Supporting information.....	160

Chapter 5 | Enzyme prodrug therapy for breast cancer treatment 171

5.1	Abstract.	177
5.2	Introduction.....	177
5.3	Results and Discussion.....	180
5.3.1	Synthesis and characterisation of HRP-AuNCs.....	180
5.3.2	Activity and stability of HRP-AuNCs.	183
5.3.3	Biocompatibility and cellular uptake of HRP-AuNCs.....	184
5.3.4	HRP-AuNCs for EPT in breast cancer cells.	187
5.3.5	HRP-AuNCs for EPT in breast cancer multicellular tumour spheroid-like cultures (MCTS).	188
5.4	Conclusions.....	190
5.5	Experimental section.....	192
5.5.1	Synthesis of gold nanoparticles (AuNPs).....	192

5.5.2	Synthesis of HRP-functionalised gold nanoconjugates (HRP- AuNCs).	192
5.5.3	Standard characterisation procedures of HRP-AuNCs.	193
5.5.4	HRP activity assay.....	193
5.5.5	Cell culture conditions.	195
5.5.6	Biocompatibility studies with HRP-AuNCs.	195
5.5.7	Cellular uptake studies.....	195
5.5.8	HRP-AuNCs for EPT in breast cancer cells.	196
5.5.9	HRP-AuNCs for EPT in triple-negative breast cancer MCTS.	196
5.6	References.....	198
5.7	Supporting information.....	204
Chapter 6 Conclusions and future perspectives		209