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SYNTHESIS AND FUNCTIONALIZATION OF NANOSIZED METAL-ORGANIC FRAMEWORK (MOF) FOR TARGETED CONTROLLED RELEASE CANCER THERAPY

Master Thesis

Maha MORAL

Supervisor
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ÖZET

HEDEF KONTROL SALINIMLI KANSER TEDAVİSİ İÇİN NANO BOYUTLU METAL ORGANİK KAFES YAPILI BİLEŞİK MOF SENTEZİ VE MODİFİKASYONU

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Kanser tedavisi, sadece kanser hücrelerine değil sitotoksisitesi nedeniyle normal vücut hücrelerine de zarar veren ciddi yan etkilere neden olan sitotoksik ilaçların kullanımını gerektirir.

Metal-organik kafes yapılı bileşikler (MOF'ler), tamamen farklı fizyokimyasal özelliklere sahip üç boyutlu yapıda organik köprü ligandları ile birbirine bağlanan metal iyonlarından oluşan koordinasyon polimerleridir.

Çeşitli organik bağlayıcılar ve farklı sentez yöntemleri kullanılarak, çeşitli boyut, bileşim, morfoloji ve kimyasal özelliklere sahip yapılar, istenilen ilaç verme özelliklerine sahip olacak şekilde tasarlanabilir. Yüksek gözeneklilik, yüksek yüzey alanı, yüksek gözenek hacmi ve kontrol edilebilir gözenek boyutu, gözenekleri içinde yüksek ilaç yükleme kapasitesi sağlar. Yüzey modifikasyonlarının kolaylığı, aktif hedeflemede kullanım için kolay fonksiyonelleştirme sağlar. Ayrıca bileşimleri ve yapıları, uyaranlara yanıt veren kontrollü ilaç salımı için kullanılabilecekleri şekilde duyarlı kimyasal ve fiziksel özelliklere sahip olacak şekilde tasarlanabilirler. Sahip oldukları yüksek biyouyumlulukları ve biyobozunurlukları, onları ilaç dağıtımı için mükemmel adaylar yapar. Asidik ortam uyaranlara duyarlı bir MOF tasarlamak, MOF'nin ayrışmasına ve kanser hücrelerinde pasif uyaranlara yanıt veren hedefleme tekniğinde ilacın salınmasına neden olur. Ayrıca, kanser hücreleri üzerinde aşırı eksprese edilen reseptörlere spesifik olarak bağlanan hedefleme molekülleri ile fonksiyonel hale getirilmiş ilaç yüklü MOF, aktif bir hedefleme stratejisinde, ilaç/tanısal ajan MOF'un bozunmasıyla serbest bırakıldığında kanser hücrelerinin içine seçici olarak alınacaktır.

Burada, hidrofobikantikanser Quercetin ile yüklü nano boyutlu CoBDC MOF'yi sentezledik ve folik asit ile fonksiyonelleştirdik. Nano boyutlu Quercetin yüklü MOF'nin sentezi ve fonksinonelleştirilmesi XRD, FTIR karakterizasyonu ve SEM ile doğrulandı. İlaç salınım çalışmaları, MOF'ımızın pH duyarlılığını ve dolayısıyla MOF'nin birleştirilmiş pasif uyaranlara duyarlı hedefleme stratejisini doğruladı.

Anahtar Kelimeler: Metal-organik kafes yapılar (MOF'ler), Uyaranlara duyarlı, Kanser tedavisi, Kanser teşhisi, Quercetin, Pasif hedefleme, Aktif hedefleme, Polidopamin kaplama. Folik asit, Fonksiyonelleştirme.

ABSTRACT

SYNTHESIS AND FUNCTIONALIZATION OF NANOSIZED METAL-ORGANIC FRAMEWORK (MOF) FOR TARGETED CONTROLLED RELEASE CANCER THERAPY

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Cancer therapy requires the use of cytotoxic drugs which results in severe side effects due to its cytotoxicity harming normal body cells being non-targeted to select only cancer cells.

Metal-organic frameworks (MOFs) are coordination polymers formed of inorganic metal ions linked together by organic bridging ligands in a three dimensional structure, acting like a single unit with totally different physiochemical properties.

By using variety of organic linkers and different synthesis methods, structures with various sizes, compositions, morphologies and chemical properties can be designed with desired properties for drug delivery. Their high porosity, high surface area, high pore volume and controllable pore size allow high loading capacity within their pores. Their ease of surface modification enables easy functionalization for use for active targeting. Their composition and structure can be designed and tailored to have responsive chemical and physical properties so they can be used for stimuli-responsive controlled drug release. Their good biocompatibility and good biodegradability makes them perfect candidates for drug delivery. Designing a MOF that is stimuli responsive to cancer acidic environment results in decomposition of MOF and release of drug in cancer cells in a passive stimuli-responsive targeting technique. Also, a drug-loaded MOF functionalized with targeting molecules that specifically binds to overexpressed receptors on cancer cells will be immediately taken selectively inside cancer cells where the drug/diagnostic agent would be released by degradation of MOF, in an active targeting strategy.

Here, we have successfully synthesized a nanosized CoBDC MOF loaded with the hydrophobic anticancer Quercetin and functionalized it with folic acid to active target cancer cells that have overexpressed folic acid receptors. Characterization with XRD, FTIR and SEM confirmed successful synthesis and functionalization of a nanosized Quercetin-loaded MOF, and the drug release study emphasized the pH-sensitivity of our MOF and hence confirmed our MOF's combined passive stimuli-responsive targeting strategy.

Keywords: Metal-organic frameworks (MOFs), Stimuli-responsive, Cancer therapy, Cancer diagnosis, Quercetin, Passive targeting, Active targeting, Polydopamine coating, Folic acid, Functionalization.

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Praise be to Allah, thank Allah first and foremost thanks to him who enabled us to achieve this project.

Islam Prophet Mohammad peace de upon him said: “those who don't thank people, they don’t thank Allah”.

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Finally, I can only offer gratitude and appreciation to my husband and my family, whom without their support, I would not have been able to concentrate on my study and complete my work successfully.

Maha MORAL

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SIMILARS AND ABBREVIATIONS

Ag NCs	: Silver Nanoclusters
AITC	: AllylIsothiocyanate
ALP	: Alkaline Phosphatase
AMP	: Adenosine MonoPhosphate
AMPK	: AMP-Activated Protein Kinase
ARE	: Antioxidant Response Elements
ATM	: Ataxia–Telangiectasia Mutated
BBR	: Building Block Replacement
BMSCs	: Bone Mesenchymal Stem Cells
BMPs	: Bone morphogenetic proteins
BDC	: 1,4 Benzene Dicarboxylic Acid
BTC	: 1,3,5-Benzene Tricarboxylic Acid
Co	: Cobalt
CoBDC	: Cobalt-Benzene-1,4-Dicarboxylic Acid
CP5	: Carboxylatopillar[5]arene
CYP2E1	: Cytochrome P450 2E1
DMF	: N,N-dimethylformamide
DMSO	: Dimethyl sulfoxide
DNA	: Deoxyribonucleic Acid
ECM	: Extra Cellular Matrix
Exo-III	: Exonuclease-III
FA	: Folic Acid
Fn	: Fibronectin
FU	: Fluorouracil
GLUT4	: Glucose Transporter Type 4
GFP	: Green Fluorescent Protein
GOx	: Glucose Oxidase
GSH	: Glutathione
H_2O_2	: Hydrogen Peroxide
ILAG	: Ion-and-Liquid Assisted Grinding
JNK	: c-Jun N-terminal Kinase
LPS	: Lipopolysaccharide

LAG : Liquid-Assisted Grinding
MAPK : Mitogen-Activated Protein Kinase
MPS : Mononuclear Phagocyte System
MOF : Metal-Organic Framework
MPS : Mononuclear Phagocyte System
mRNA : Messenger RNA
NIR : Near-Infrared
NPs : Nanoparticles
Nrf2 : Nuclear factor erythroid 2-related factor 2
PBS : Posphate Buffer Saline
PDA : Polydopamine
PEG : Polyethylene Glycol
PEI : Polyethyleneimine
PI3K : Phosphatidylinositol 3-Kinase
plGFP : Green Fluorescent Protein Plasmid
PNIPAM : Poly(N-isopropyl acrylamide)
PSE : Post-Synthetic Exchange
PSM : Post-Synthetic Modification
PSMA : Prostate Specific Membrane Antigen
PTT : Photothermal therapy
PVP : Polyvinylpyrrolidone
Qu : Quercetin
Qu@CoBDC : Quercetin-Loaded CoBDC
RES : Reticuloendothelial System
RNA : Ribonucleic Acid
ROS : Reactive Oxygen Species
SALE : Solvent-Assisted Linker Exchange
SOD : Superoxide Dismutase
SMC : Smooth Muscle Cells
TCPP : 5,10,15,20-tetrakis(4-carboxyphenyl)porphyrin
TGF-β : Transforming Growth Factor Beta
ZIF-8 : Zeolitic Imidazole Framework-8
ZnTCPP : Zinc 5,10,15,20-tetrakis (4-methoxycarbonylphenyl) porphyrin

LIST OF FIGURES

1. INTRODUCTION

Cancer therapy requires the use of cytotoxic drugs to kill the rapidly multiplying cancer cells which results in severe side effects due to its cytotoxicity and inability to differentiate cancerous cells from normal healthy body cells and so harming normal healthy body cells being non-targeted to select only cancer cells. However, recent progress and research studies have raised opportunities for the development of new drugs that can selectively be targeted to bind with the cancer cells or that can be only be released in response to the cancer cells (Nie et al. 2007).

2. TARGETING IN CANCER THERAPY

Different cancer therapy targeting mechanisms have been employed to prevent the massive toxicity and severe side effects of untargeted unselective cancer therapy. The main targeting strategies include passive targeting, stimuli-responsive triggered release and active targeting (Figure 2.1) (Attia et al. 2019; Nie et al. 2007; Arranja et al. 2017).

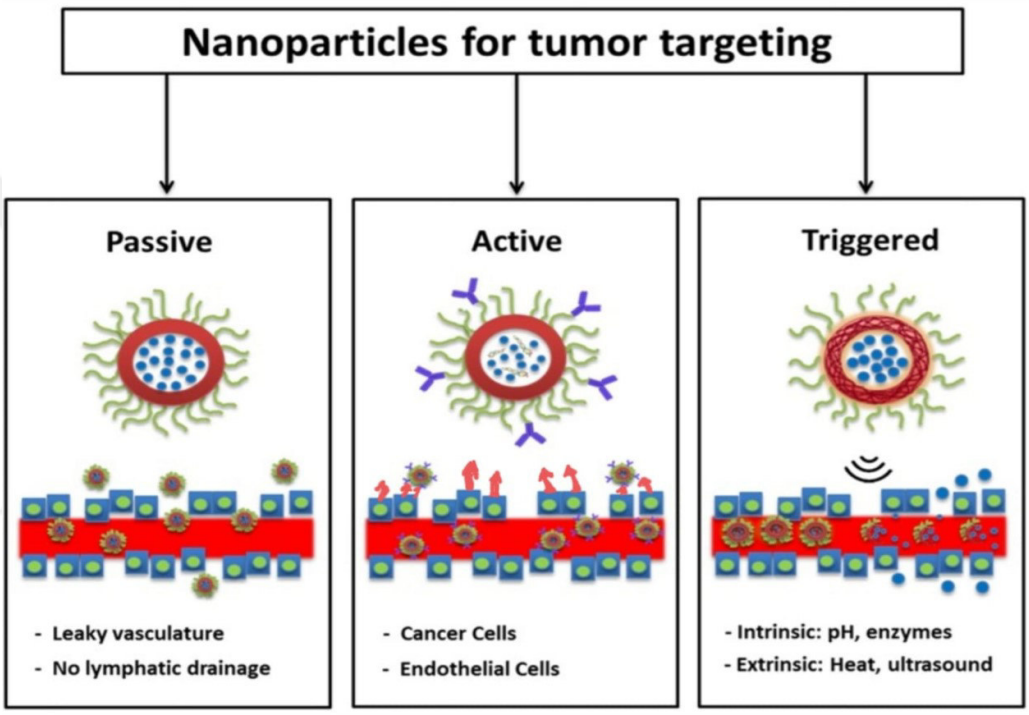

Figure 2.1. Different Targeting Mechanisms in Cancer Therapy (Arranja et al. 2017)

2.1. **Passive Targeting**

Passive targeting in cancer therapy relies on enhanced permeability and retention. Cancer cells are characterized by enhanced permeability and retention effect which results from the rapid vascularization in the rapid-growing cancerous tissues leading to a defective leaky architecture with impaired lymphatic drainage which in turn results in accumulation of nanoparticles (NPs) in the tumor site. Enhanced permeability and retention requires a long blood circulation time of the nanodrug. The most common method in extending the blood circulation time can be achieved by using an optimal nanoparticle size of 20-200 nm and by using a hydrophilic surface coating to prevent plasma protein adsorption. This can be achieved by hydrophilic polymer coating such as polyethylene glycol (PEG) or by the use of block or branched amphiphilic copolymers. These also avoid and prevent

the identification and destruction by the reticuloendothelial system (RES) or circumventing clearance by mirophages, since hydrophobic or charged particles are quickly opsonized by the mononuclear phagocyte system (MPS) and particles more than 300 nm are rapidly taken by the RES. It should be noted also that the nanoparticle size should be also greater than 6 nm to be large enough to escape the renal filtration (Kobayashi, Watanabe, and Choyke 2014; Attia et al. 2019; Nie et al. 2007).

2.2. Active Targeting

Cancer cells are characterized by overexpressed receptors that can be used for ligand-receptor mediated targeting (Akhtar et al. 2014) and by overexpressed antigens that can be used for antibody-antigen mediated targeting (Boinapally et al. 2021; Masood 2016; Nie et al. 2007). Ligand-receptor mediated targeting includes G protein-coupled receptors (Bombesin, Somatostatin and Endothelin receptors), Integrin receptors, Transferrin receptors, Fibroblast Growth Factor receptors, Epidermal Growth Factor receptors, Sigma receptors, Follicle-Stimulating Hormone receptors (overexpressed only in ovarian cancer), Biotin receptors, Nucleolin (that binds to the aptamer AS1411) receptors, CD44 receptors (that binds to hyaluronic acid), Neuropilin receptors and Folate receptors (binding to folic acid) (Akhtar et al. 2014; Masood 2016; Lee et al. 2020). Antibody-antigen mediated strategy includes the prostate specific membrane antigen (PSMA) that binds with its specific antibody (Boinapally et al. 2021).

2.3. Stimuli-Responsive Triggered Release

Stimuli-responsive drug delivery relies mainly on using endogenous or exogenous stimuli to trigger the drug release only in tumor cells, so the drug is released in response to an endogenous tumor environment characteristics like pH, redox or tumor specific enzyme, or in response to an external exogenous stimuli such as magnetic field. This will be explained in details in the section for stimuli-responsive metal-organic frameworks (MOFs) (Arranja et al. 2017; Cai et al. 2019; Wu and Yang 2017).

3. FOLIC ACID AS A TARGETING MOLECULE

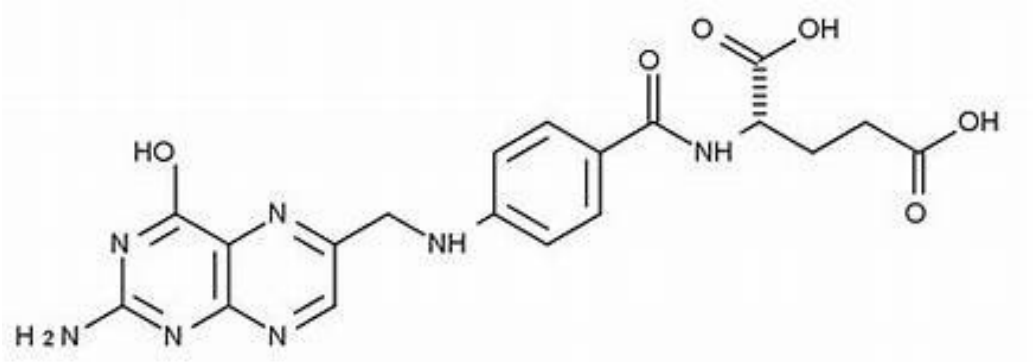

Figure 3.1. Structure of Folic Acid

Folic acid (FA) (Figure 3.1) is vitamin B9. It possesses a vital role in the biosynthesis of amino acids and nucleic acids. FA is the folate receptor ligand and it is a small molecule (around 44 Da). It is stable over a wide range of pH and temperatures values. It is non-immunogenic, non-expensive, and it possesses the ability of binding to the folate receptors when conjugated with drugs and diagnostic markers. Folate receptors are overexpressed in many types of human cancers while being expressed at low level in normal tissues and cells. This upregulation of folate receptor in cancers of several histologies compared to normal tissues together with a relatively low cost of FA have made it an attractive approach for drug targeting strategies for use in cancer-targeted drug delivery and cancer imaging. It has been widely used in recent researches to develop a targeted delivery of chemotherapeutic, anti-cancer drugs and imaging agents (Kharkar et al. 2020; Akhtar et al. 2014; Masood 2016).

4. QUERCETIN

Quercetin (3,3′,4′,5,7-Pentahydroxyflavone or 3,30,40,5,7-pentahydroxy-2-phenylchromen-4-one) (Figure 4.1) is a crystalline yellow solid naturally occurring compound found in many plants that are used as food. It is present in high concentrations in black and green tea leaves, raw onion, apple, spinach and broccoli (Baghel et al. 2012)

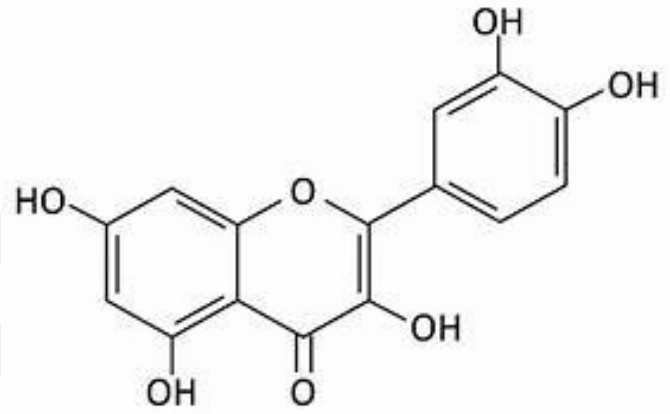

Figure 4.1. Structure of Quercetin

Quercetin is lipophilic, it has very poor solubility in water, moderate solubility in ethanol and very high solubility in dimethyl sulfoxide (DMSO) (Wang, Sun, et al. 2016).

Quercetin has an antioxidant, anti-inflammatory, antibacterial, antiviral, anti-obesity, cardioprotective, hepatoprotective, anti-hypercholesterolemic, anti-atherosclerotic, anticarcinogenic and anticancer effect (Ulusoy and Sanlier 2020; Wang, Sun, et al. 2016). It also helps to maintain normal blood sugar level in diabetes and has shown to have protective effects against neurological diseases (Ulusoy and Sanlier 2020).

4.1. Quercetin as an Antioxidant

Quercetin has shown to be a potent antioxidant through its powerful ability to scavenge reactive oxygen species (ROS). Also, Quercetin exhibits comprehensive protective actions against oxidative stress induced by lipid and lipoprotein fragments oxidation, along with other factors. Quercetin also activates nuclear factor erythroid 2-related factor 2 (Nrf2) binding to antioxidant response elements (AREs) which is an important signaling pathway that induces antioxidant enzymes like glutathione

reductase, catalase, glutathione S-transferase, heme oxygenase-1, superoxide dismutase (SOD) and thioredoxin reductase (Ulusoy and Sanlier 2020).

4.2. Quercetin as an Anti-Inflammatory

Quercetin may suppress lipopolysaccharide (LPS)-induced tumor necrosis factor generation in macrophages and LPS-induced interleukin (IL)-8 generation in lung cells. Quercetin may suppress LPS-induced mRNA levels of cytokines in colloid cells. Moreover, ROS elimination can prevent oxidation and thus Quercetin simultaneously inhibit inflammation (Wang, Sun, et al. 2016). Quercetin may suppress the production of histamine and other mediators responsible for the induction of allergic reactions in mast cells and thus it can be of therapeutic value in respiratory inflammatory diseases treatment. Quercetin also by suppressing eosinophil activation may play a significant role in the control of eosinophil-mediated diseases like asthma and allergic rhinitis (Ulusoy and Sanlier 2020).

4.3. Quercetin in Neurodegenerative Diseases

Since Quercetin has an antioxidant effect, it exhibits neuroprotection against oxidative damage via stimulating Nrf2-ARE pathway. In addition, it exhibits anti-amyloidogenic actions in Alzheimer's disease. Its anti-Alzheimer effect is attributed to its ability to improve mitochondrial morphology, improve memory impairments, reduce neurodegeneration, and protect cognitive deficits. These protective effects against neurological diseases are related to regulating certain pathways such as paraoxonase-2, protein kinase C, c-Jun N-terminal kinase (JNK), mitogen-activated protein kinase (MAPK), and phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT). It protects against the development of Parkinson's disease that is characterized by degeneration of dopaminergic neurons in the substantia nigra pars due to the oxidative damage caused by dopamine metabolism, iron accumulation, depletion of glutathione and lipid peroxidation abnormalities. It also protects against the oxidative stress induced by lipid hydroperoxide or 6-hydroxydopamine in neuronal cell lines. Experiments on rats have shown that administration of Quercetin significantly reduced rapid eye movement sleep behavior disorder probably due to the stimulation of the type-A GABA (c-aminobutyric acid) receptor enhancement (Ulusoy and Sanlier 2020).

4.4. Quercetin in Osteoporosis

Bone mesenchymal stem cells (BMSCs) have been extensively utilized in regenerative medicine due to their regenerative ability and naive cell characteristics. Previous studies revealed that BMSCs aging and apoptosis also play a role in the pathogenesis of osteoporosis since aged BMSCs tend to tansform into adipocytes rather than osteoblasts, and so resulting in age-related bone loss. Also, ROS accumulation in cells induced by oxidative stress can lead to precocious senescence induced by stress and apoptosis of BMSCs. Quercetin also has upregulated significantly the relative activity of SOD which in turn elevates the anti-oxidant responses of BMSCs. Quercetin was found to enhance BMSCs osteogenic differentiation and BMSCs anti-oxidant responses by stimulating the AMP-activated protein kinase (AMPK) and the histone/protein deacetylase SIRT1 (AMPK/SIRT1) signaling pathway. Quercetin also promoted the osteogenic differentiation of BMSCs into osteoblasts and inhibited the adipogenic differentiation of BMSCs into adipocytes (Wang et al. 2021).

4.5. Quercetin in Diabetes

Quercetin helps to maintain normal blood sugar by inducing adenosine monophosphate kinase (AMPK) pathway and activation of glucose transporter type 4 (GLUT4) which is mainly responsible for blood glucose uptake into muscle cells. Quercetin also inhibits carbohydrate absorption from the intestine by suppressing the digestive enzymes responsible for hydrolysis of carbohydrate and glucose carriers in various forms thus reduces glucose absorption in the small intestine (Ulusoy and Sanlier 2020).

4.6. Quercetin as a Hepatoprotective Agent

Quercetin exhibits a hepatoprotective effect because it is a potent antioxidant, free radical-scavenger and anti-inflammatory with antiapoptotic effect and cytochrome P450 2E1 (CYP2E1) inhibitory activities, thus it has shown to reduce the hepatotoxic effect of various hepatotoxic substances (Pingili et al. 2020).

4.7. Quercetin as an Anticancer Agent

Quercetin can protect from cancer by preventing induction of cancer by oxidative stress because of its antioxidant activity and inhibition of many kinases that play important roles in cancer cell growth, proliferation and metastasis (Wang, Sun,

et al. 2016). Quercetin exhibits an anti-carcinogenic action by inducing apoptosis, suppressing the cell cycle, and enhancing matrix metalloproteinase release. It also exhibits telomerase inhibition thus limiting the growth of human cancer cells (Ganesan and Xu 2017) and shows antiproliferative effects. In addition, Quercetin decreases tumor cell adhesion, angiogenesis and metastasis. Quercetin can stop the growth and development of tumor via epigenetic modulations. It is accompanied by inhibition of oncogenes that contribute to the development of cancer and by stimulation of cancer suppressor genes (Ulusoy and Sanlier 2020). (Rauf et al. 2018) has explained in details the different mechanisms of Quercetin anticancer effect in almost all cancer types.

4.8. Quercetin in Radiation Therapy

Quercetin possesses a radiosensitization activity by inhibiting the Ataxia–Telangiectasia Mutated (ATM) kinase the protein critically involved in the DNA damage response. Quercetin also can oppose the immunological response of radiation (Lin et al. 2012). It has been employed in many recent studies for radiosensitization (Ma et al. 2019). Quercetin also suppresses radiation-induced skin fibrosis since it reduces transforming growth factor beta (TGF-β) and tumor necrosis factor-α expression, increases the activity of matrix metalloproteinase-1 and decrease oxidative stress which are involved in the development of radiation-induced fibrosis (Horton et al. 2013).

4.9. Toxicity of Quercetin

Quercetin may form toxic oxidation product in human body when it reacts with free radical, in particular Quercetin-Quinone that is a compound very reactive with thiols and this makes glutathione (GSH) one of its main reactants. So GSH and ascorbate are responsible for trapping its toxic oxidation byproducts. Thus, in absence of enough amounts of glutathione and ascorbate, Quercetin-Quinone can damage proteins containing thiol groups producing toxic effects (Ulusoy and Sanlier 2020; Wang, Sun, et al. 2016).

However, despite all of the mentioned medicinal values of Quercetin, its very diminished water solubility with poor bioavailability has greatly limited its applications, making the development of delivery systems essential to improve its absorption and bioavailability and hence its efficacy (Wang, Sun, et al. 2016).

5. POLYDOPAMINE

Polydopamine (PDA) is generated from the mussel-inspired oxidative polymerization of dopamine hydrochloride. Since it is a major main component of melanin that is naturally occurring and widely distributed in human body, PDA exhibits great biocompatibility. and is found to be promising for tissue engineering and biomedical applications (Lakshminarayanan, Madhavi, and Sim 2018, Ding, Floren, and Tan 2016, Liu et al. 2013).

Medical applications of PDA coating include: antimicrobial surfaces, cell patterning, surfaces for stem and differentiated cell culture, microfluidics, artificial spores, scaffold functionalization for tissue engineering, hydroxyapatite calcium carbonate surface mineralization, bioimaging, theragnostic, photothermal therapy, and capsules for drug delivery (Hyun Ryu, Messersmith, and Lee 2018).

Other applications include: Li-ion battery membranes, immobilization of photocatalysts, carbonization, interplay between PDA and photocatalysts, oil/water separation, organocatalysts, Li-air battery electrolytes, Zn-air cathode materials, Li-sulfur battery cathode materials, atomic transfer radical polymerization, water detoxification, membrane separation technologies, and others (Hyun Ryu, Messersmith, and Lee 2018).

Factors affecting the coating thickness include the presence of dissolved oxygen or oxidizing agents, the initial concentration of dopamine, the pH, the buffer type used, the temperature and the use of ultrasonication. High temperature speeds up the deposition of polydopamine resulting in a rougher and more hydrophilic surface (Ding, Floren, and Tan 2016). Ultrasonication was found to reduce the coating time by 50% and can control polymerization by switching on and off (Mei et al. 2021).

5.1. Characteristics Making PDA Ideal for Use in the Field of Medicine

- Polydopamine coatings offer a simple single-step formation of an ultrastable adherent PDA coatings (Liu et al. 2013; Ding, Floren, and Tan 2016).
- It is based on mussel-inspired oxidative polymerization of dopamine hydrochloride at alkaline pHs on different substrates despite of their

shapes and despite being hydrophilic or hydrophobic surfaces (Liu et al. 2013).

- It is biocompatible.
- It is non-toxic despite of the high cytoxicity of unpolymerized dopamine (Ding, Floren, and Tan 2016).
- It exhibits enhanced cell adhesion (Ding, Floren, and Tan 2016).
- It is highly reactive for secondary functionalization (Ding, Floren, and Tan 2016).
- Since PDA coating is formed from polymerization of its low molecular weight dopamine as a building block and involves covalently/non-covalently polymerization at later stages, this makes PDA coatings ideal coatings for 3D porous materials (like our MOFs) by infusion and in situ polymerization of the low molecular weight dopamine building blocks (Hyun Ryu, Messersmith, and Lee 2018).
- Complexation of polydopamine with Fe^{3+} provide a cost effective strategy for functionalization through Fe^{3+}-mediated coordination chemistry (Shu et al. 2018; Han et al. 2016; Zhang, Hu, et al. 2019).

5.2. Polydopamine Fluorescent Organic Nanoparticles

PDA nanoparticles synthesized by polymerization of dopamine at pH 10.5 then after that these PDA NPs were further oxidized using hydrogen peroxide (H_2O_2) so that they form PDA fluorescent organic nanoparticles (FONs). These FONs are soluble and biocompatible and hence considered to be a promising agent for biomedical imaging (Zhang et al. 2012; Lin et al. 2015).

5.3. Polydopamine Fluorescence Quenching

Polydopamine nanospheres with its energy transfer and electron transfer properties have shown to have a high fuorescence quenching ability that when combined with their affinities towards various DNA conformations make them promising agents for the detection of DNA and proteins (Qiang et al. 2014; Han et al. 2016).

5.4. Polydopamine in Photothermal Therapy

PDA can be used in photothermal therapy owing to its ability to strongly absorb light and high photothermal conversion (Han et al. 2016). Photothermal therapy (PTT) exploit hyperthermia produced from absorbed near-infrared (NIR) light energy by photo-absorbing agents in order to kill cancer cells through thermal ablation thus overcoming chemotherapy resistance effect and suppressing tumor metastasis (Zhang et al. 2021; Poinard et al. 2018).

5.5. Polydopamine Coating in Vascular Tissue Engineering and Stents

Recent researches have demonstrated that PDA coatings showed significant enhanced vascular endothelial cell growth and function; and hence promoted re-endothelialization owing to the presence of quinone group on PDA inducing higher amount of protein adsorption. At the same time, PDA coating simultaneously inhibited smooth muscle cells (SMC) adhesions and proliferation owing to the reactive phenolic groups on dopamine coatings. Also, the PDA coatings on the stents have shown good stability together with resistance to deformational behavior of compression and expansion in stents (Ding, Floren, and Tan 2016).

5.6. Polydopamine for Tissue Engineering and Bone Regeneration

PDA-coated electrospun fibers implanted in mice have shown higher maximized bone regeneration in defected sites due to direct cell migration to the defected area being enhanced by the PDA coating thus enhancing bone regeneration (Ding, Floren, and Tan 2016).

Scaffolds modified by PDA NPs showed enhanced tissue regeneration. The PDA NPs with their intrinsic adhesiveness, and good affinity to different protein and peptide can easily adhere on various porous scaffolds behaving like anchors immobilizing cell adhesive molecules **[**RGD (Arg-Gly-Asp) peptide in Fibronectin (Fn)] and cell differentiation signal proteins [Alkaline Phosphatase (ALP), Bone morphogenetic proteins (BMPs)] on the scaffolds, thus the PDA NPs modifying the scaffolds possesses excellent osteoinductivity in vivo and in vitro. PDA NPs with their adsorbed BMP-2 synergistically stimulate the osteogenic differentiation of BMSCs, and also induce new bone formation. This can be explained by the fact that PDA NPs with their unique adhesiveness recruit BMSCs in the primary stage, and then they also behave as excellent anchors to capture signal proteins secreted by cells

in the following stage, thus establishing positive feedback loop on the signaling cascade and creating suitable extracellular matrix (ECM) microenvironment for cell attachment and differentiation leading to promotion of cell activity and tissue regeneration. And since the PDA NPs with their unique adhesiveness modify the porous scaffolds without compromising the intrinsic properties of the scaffold, they can be used to modify various scaffolds for tissue regeneration (Wang, Wang, et al. 2016).

5.7. Polydopamine in Drug Delivery

PDA NPs have been used as drug carriers due to their hydrophilicity and cell adhesion properties, thus improving the biocomptability and bioavailability of drugs. PDA capsules have been synthesized in an easy stable one-step material-independent adsorption low-cost process. Their high photothermal conversion property has been used in cancer photothermal therapy. The PDA coating has offered an easy single-step cost-effective method for functionalization of drugs with active targeting ligands (Ding, Floren, and Tan 2016; Shu et al. 2018; Han et al. 2016).

6. METAL-ORGANIC FRAMEWORKS (MOFs)

Metal-organic frameworks (MOFs) are nanoporous hybrid materials or coordination polymers that consist of inorganic metal ions linked and connected together by organic linkers that form a bridging ligands in a three dimensional structure which behaves like a single unit with totally different physicochemical property (Figure 6.1) (Beg et al. 2017).

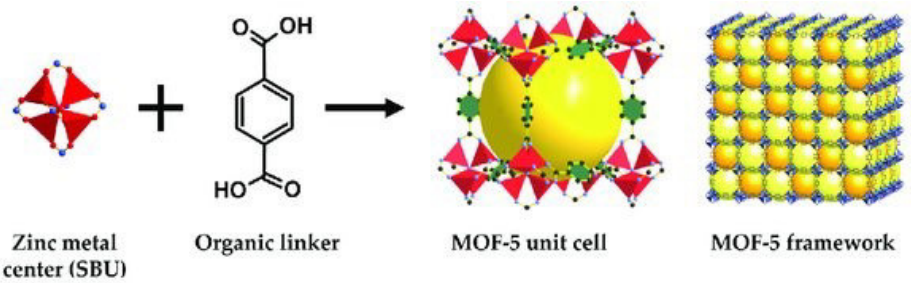

Figure 6.1. MOF synthesis from Zn metal ion and terephthalic acid. The yellow spheres represent the pore volume (Perez et al. 2016)

They have been great candidates for use in drug delivery because of their high porosity and controllable pore size, high surface area and pore volume with high loading capicity and easy surface modification which enables easy functionalization, so they can be multifunctionalized, loaded with drugs and used for active targeted drug delivery. Also their composition and structure can be designed to obtain tailored responsive chemical and physical properties so they can be used for stimuli-responsive controlled drug release. In addition to these, they have good biodegradability and good biocompatibility (Cai et al. 2019; Liu, Zhao, and Chen 2019). In short, their design can be tailored to create functionalized targeted drug carrier and/or deliver a stimuli-responsive drug.

MOF compared to other drug delivery molecules: Over the last years, many various kinds of nanocarriers have been developed for the purpose of drug delivery, such as dendrimers, inorganic mesoporous silica, liposomes, metal nanoparticles (NPs), quantum dots, and organic micelles. However, each of these nanocarriers has its own limitations in biomedical applications. Dendrimers, liposomes and micelles mostly suffer from decreased loading capacities, and inorganic porous materials possess undesirable toxicity with unacceptable degradability in contrast to MOFs which have high loading capacity and can be synthesized from precursors that have good biodegradability and good biocompatibility (Wu and Yang 2017).

MOFs are designed and synthesized from variety of inorganic nodes (metal clusters or ions) and variety of organic linkers through diverse methods and strategies to obtain versatile structures with diverse morphologies, compositions, sizes and chemical properties, that are nearly endless, for the use in different applications (Howarth et al. 2017). In other words, their design can be tailored to serve our desired scientific aim for the use in different applications. These various applications include gas storage and separations, catalysis, removal of toxic and harmful chemicals, sensing, nonlinear optics, magnets, solar and fuel cells, energy conversion and storage, metal corrosion inhibition, biomedical application and drug delivery (Wu and Yang 2017).

6.1. Synthesis of MOF

MOFs have been synthesized employing various methodologies and strategies to obtain various morphologies, compositions, sizes and chemical properties. Green synthesis methods have been also employed using non-hazardous reactants and mild synthetic conditions resulting in less production of wastes (Reinsch 2016). Also, a chromium MOF (Cr-MOF) has been greenly synthesized from waste polyethylene terephthalate (PET) bottles for hydrogen storage applications (Ren et al. 2016). The main synthetic methodologies are summarized here below (Lee, Kim, and Ahn 2013; Giménez-Marqués et al. 2016) together with the most recent promising surfactant-assisted strategy (Zhao et al. 2019). Following the MOF synthesis processes, washing of MOF is done to remove unreacted MOF precursors that can be trapped, and solvent exchange by a lower boiling point solvent can also be done to make our MOF ready for the final activation step done by heating in vacuum ovum in order to evacuate its pores from any solvent and achieve full permanent porosity (Figure 6.2) (Mondloch et al. 2013).

6.1.1. MOF Conventional Solvothermal/Hydrothermal Synthesis

This classic method implicates metal secondary building units precursors, organic linkers and polar high-boiling-point solvents under high temperature and pressure (Figure 6.2.) (Giménez-Marqués et al. 2016). Synthesis can be carried out in a screw-top vial like teflon-lined hydrothermal autoclaves in ovens or by stirring under reflux conditions (Lee, Kim, and Ahn 2013; Kim et al. 2012; Liang et al. 2018). Conditions like pH, temperature, reaction time and stoichiometry are to be

adjusted to have control over the size. Also, other factors, such as modulators which are used to delay the building of structural bonds when the metal ligand bonds are very strong, should be adjusted. Modulators possess a very important role in having control over the nucleation and growth (morphology and crystal size) of the particles, avoiding rapid precipitation for instance. The utilization of polymers and/or surfactants can be done also for controlling the shape and/or the particle growth, therefore, the final morphology and size of the nanosized MOF (Howarth et al. 2017).

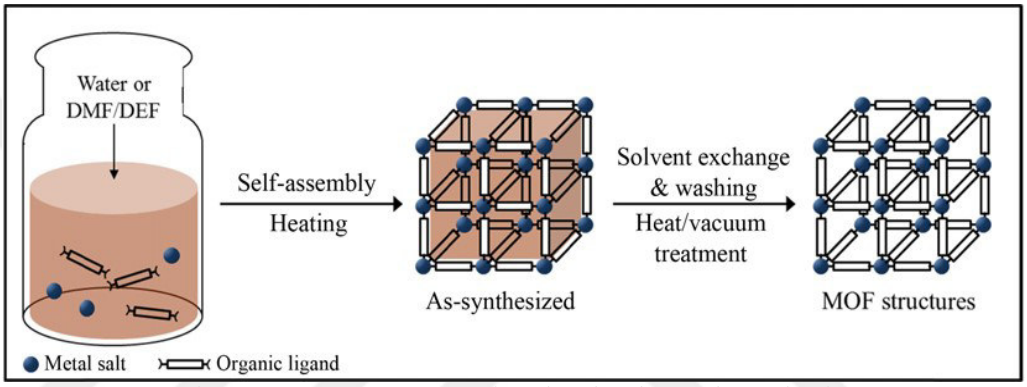

Figure 6.2. MOF Conventional Solvothermal Synthesis

6.1.2. MOF Microwave-Assisted and Sonochemical Synthesis

Microwave and ultrasound sources have been also investigated as an alternative to the conventional heating. These methods usually result in rapid crystallization of MOFs. Microwave-assisted synthesis techniques rely on the interaction between mobile electric charges like that of polar solvent molecules or ions present in the solution and electromagnetic waves. This method has the advantages of being highly efficient, phase selective, offering reduction in particle size and controlling morphology (Sun and Zhou 2015). In specific, microwave heating offers uniform and fast nucleation process due to the creation of local superheated spots and rapid heat transfer, resulting in much homogeneous particle size distribution (Giménez-Marqués et al. 2016). Sonochemical (ultrasonic) techniques through accelerated and homogeneous nucleation can lead to reduction in crystallization time and markedly smaller particle size than those offered by the solvothermal method (Lee, Kim, and Ahn 2013).

6.1.3. MOF Electrochemical Synthesis

The electrochemical method for synthesis of MOFs utilizes metal ions that are supplied continuously via the dissolution of anode as a source of metal instead of metal salts, which react with the solubilized linker molecules and a conductive salt in the reaction medium. The deposition of metal on the cathode can be avoided by the use of protic solvents, but in this process H_2 is released. In the electrochemical method, it can also be possible to run the process continuously to get higher solids yield compared to that offered by normal batch reactions (Lee, Kim, and Ahn 2013).

6.1.4. MOF Mechanochemical Synthesis

In mechanochemical synthesis, the breakage of intramolecular bonds mechanically is followed by the occurrence of chemical transformation. Mechanochemical reactions can take place under solvent-free conditions at room temperature, which is advantageous especially when we want to avoid organic solvents. Mostly, metal oxides are preferred as a starting material than metal salts, since they only result in water as a side product (Lee, Kim, and Ahn 2013). This solvent-free method requires grinding of the precursors directly in a ball-mill or mortar to produce the desired MOF. This technique has recently been used to produce mono-dispersed NPs, without the need to use solvent. The quantitative production of small MOF particles were also produced fast in short time, ranging from 1 to 60 min (Zhang et al. 2018; Leng et al. 2016; Giménez-Marqués et al. 2016). The addition of minute amounts of solvents in liquid-assisted grinding (LAG) can speed up the mechanochemical reactions due to increased reactants mobility at molecular level. The liquid also can act as a structure-directing agent. Recently, this technique was extended to ion-and-liquid assisted grinding (ILAG) which was found to be greatly efficient for the selective formation of pillared-layered MOFs. However, mechanochemical method is limited only to specific types of MOFs whose synthesis is performed under mild conditions and huge amount of product is hard to be obtained (Friščić 2010; Lee, Kim, and Ahn 2013).

6.1.5. MOF Ionothermal Synthesis

Ionothermal synthesis employs ionic liquids as solvent and template. By adjusting the cations and anions of ionic liquids with various reaction conditions, a huge variety of metal-organic frameworks can be obtained. Ionic liquids as a solvent

have attracted attention in chemical synthesis, due to their unique properties like very good solvating properties, essential zero vapor pressure, high thermal stability and ease of their recycling (Oh et al. 2018; Lee, Kim, and Ahn 2013).

6.1.6. MOF Microemulsion Synthesis

A classic synthesis strategy to produce monodispersed nanoMOFs is by reverse micelle technique. This technique utilizes microemulsions as thermodynamically stable dispersions of two immiscible liquids together with the presence of a surfactant or an emulsifier. These microemulsions could be regarded as nanostructured chemical reactors confining MOFs synthesis to the nanoscale, with the advantage of offering possible size tuning (Giménez-Marqués et al. 2016).

6.1.7. Other Synthesis Methods of MOF

Other synthesis methods include dry-gel conversion Synthesis, spray-drying synthesis, electrospinning synthesis, solvent evaporation synthesis, diffusion synthesis and microfluidic synthesis (continuous flow production) methods (Giménez-Marqués et al. 2016; Lee, Kim, and Ahn 2013; Wu and Yang 2017).

6.1.8. Surfactant assisted Strategy for MOF Synthesis

Surfactants can control the dimensions, morphologies and phases of MOFs by acting as dispersants, emulsifiers, foaming agents, wetting agents or detergents. In other words, surfactants have become a promising agents in the field of MOFs development, and surfactant-assisted strategy has been employed in the separation of the pure single-phase of MOFs, synthesis of crystalline MOFs, and in controlling the pore size and morphologies of MOFs (such as core/shell, nanodisks, nanoplates, nanorods and nanosheets). The ability of surfactants to control the pore size of MOF is attributed to the hydrophobic and hydrophilic functional groups of the surfactants which enable them to form various ordered aggregates (like liquid crystals, micelles, reverse micelles and vesicles) in the solution, on which MOF precursors tends to nucleate spontaneously at their edges during the crystallization thus forming crystalline MOF with different pore size and morphology after further growth. Also, the surfactants being dispersants prevent agglomeration of the MOF. Since the hydrophobic long chain hydrocarbon groups of surfactants can be adsorbed on specific MOF crystal face in aqueous solution preferentially, so this crystal face would grow significantly slower than the other crystal faces, thus controlling its

morphology. So the morphology of MOF (eg. nanorods, nanocubes, hexahedron, etc.) can be controlled by choosing different length of hydrocarbon groups, different ionic surfactants or modified groups (Zhao et al. 2019).

6.1.9. Modulators' Role in MOF Synthesis

Modulators are used to delay structural bonds formation when the metal ligand bonds are very strong, and so can play important role in controlling the nucleation and growth (crystal morphology and size) of the particles, avoiding for example rapid precipitation. Usually modulators (like monocarboxylic acids) directly compete with complexation of the ligand, hindering the coordination on the cation (Howarth et al. 2017).

6.1.10. Activation of MOF

During the MOF synthesis, the solvent molecules become trapped within the pores of the framework. Also in some cases, guest molecules like excess linkers can be trapped as well. To achieve the permanent porosity and the high surface areas offered by many framework structures, the solvent molecules must be removed and this process is termed activation. Activation can be done by i) conventional heating and vacuum drying; (ii) solvent-exchange with a lower boiling point solvent proceeded by mild activation under vacuum drying; (iii) freeze-drying; (iv) chemical treatment; and (v) supercritical CO2 (scCO2) exchange (Mondloch et al. 2013; Howarth et al. 2017).

6.1.11. Factors Affecting MOF Crystal size, habits, morphology and topology

These factors include i) the nature of the precursors, ii) synthesis technique, iii) the solvent used and its concentration, iv)the concentration ratio of the building units reagents, v) the pH, vi) the reaction temperature and time, vii) the use of modulator, its composition and concentration and viii) the use of surfactant, its type and the water to surfactant molar ratio (Howarth et al. 2017; Giménez-Marqués et al. 2016; Zhao et al. 2019; Della Rocca, Liu, and Lin 2011).

6.2. Various Applications of MOFs

MOFs have been studied for many different applications including catalysis, gas storage, gas separations, removal of toxic and harmful chemicals, sensing, non-

linear optics, magnets, solar and fuel cells, energy conversion and storage, metal corrosion inhibition, biomedical application and drug delivery (Wu and Yang 2017).

6.3. MOF as a Smart Drug Delivery System

What Makes MOFs Excellent Choice for Drug Delivery?

- Their high porosity and tunable pore size.
- Their high surface area and pore volume with high loading capicity.
- Their easy surface modification which enables easy functionalization, so they can be multifunctionalized, loaded with drugs and used for active targeted drug delivery.
- Their composition and structure can be designed to obtain tailored responsive chemical and physical properties so they can be used for stimuli responsive controlled drug release.
- Their good biocompatibility and good biodegradability.
- Their facile production at low cost.
- They can also be loaded directly either via one-pot synthesis or through post-synthetic diffusion

(Cai et al. 2019; Liu, Zhao, and Chen 2019)

6.4. MOF Functionalization and Surface Modification

6.4.1. Direct Synthesis Approach Using Functionalized Ligand

Here, functionality is incorporated into the MOF by firstly modifying its organic linker with specific substituents and then utilizing the modified linker directly in the solvothermal method to synthesize the desired MOF (Figure 6.3). This 'prefunctionalization' technique has lead to MOFs with functional groups like–NH2, –CH3, –Br and other simple substituents lining the pore channels. This functional groups' scope within the MOFs' pores has remained relatively limited by this technique, since not all functional groups would be stable within the solvothermal conditions, which usually employ high pressure and temperature to obtain the required crystalline structure as a common method in the synthesis of MOF (Tanabe and Cohen 2011).

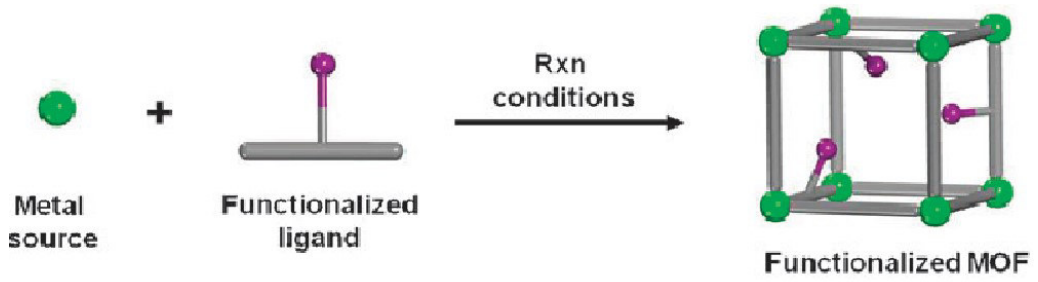

Figure 6.3. Functionalization by Direct Synthesis Using a Prefunctionalized ligand (Tanabe and Cohen 2011)

6.4.2. Post-Synthetic Approaches for Functionalization

Sometimes, introduction of functionality to MOFs through direct synthesis strategy is not convenient because of various challenges like thermal stability, limited linker solubility, functional group compatibility, chemical stability, and undesired interference between linker functional groups and metal ions during MOF assembly. To overcome this, post-synthetic strategies were employed to modify the preassembled MOFs functionality, like post-synthetic exchange (PSE), post-synthetic modification (PSM) and post-synthetic deprotection (PSD) (Sun and Zhou 2015).

- Post-synthetic modification (PSM): Most PSM techniques for MOFs functionalizing (Figure 6.4) include covalent and coordinate covalent modification. Covalent modification involves the chemical modification of the organic linker constituent of the MOF. In contrary to covalent modification, the coordinate covalent modification implies variations in the coordination environment of metal binding groups (secondary building units) within the framework which do not change the overall metal binding groups or the topology of the framework. Two main methodologies are employed for coordinate covalent modification: One is by introducing coordinating ligands into the framework by binding to the metal nodes which have unsaturated metal sites. The other is when having metal binding groups (e.g. –OH) in the organic linker of the MOF which do not contribute in the framework structure, and so they could be metallated in a PSM approach. Coordinate covalent and covalent modifications were also utilized together in conjugation. By using coordinate covalent and covalent modification in combination, the organic linker of the framework could be modified with a ligand and so becomes metallated, when the metal binding groups lacks unsaturated metal sites built into the organic

linkers. This strategy has the advantage of being able to introduce metal ions and various combinations of chelators (like –COOH and–OH) (Tanabe and Cohen 2011).

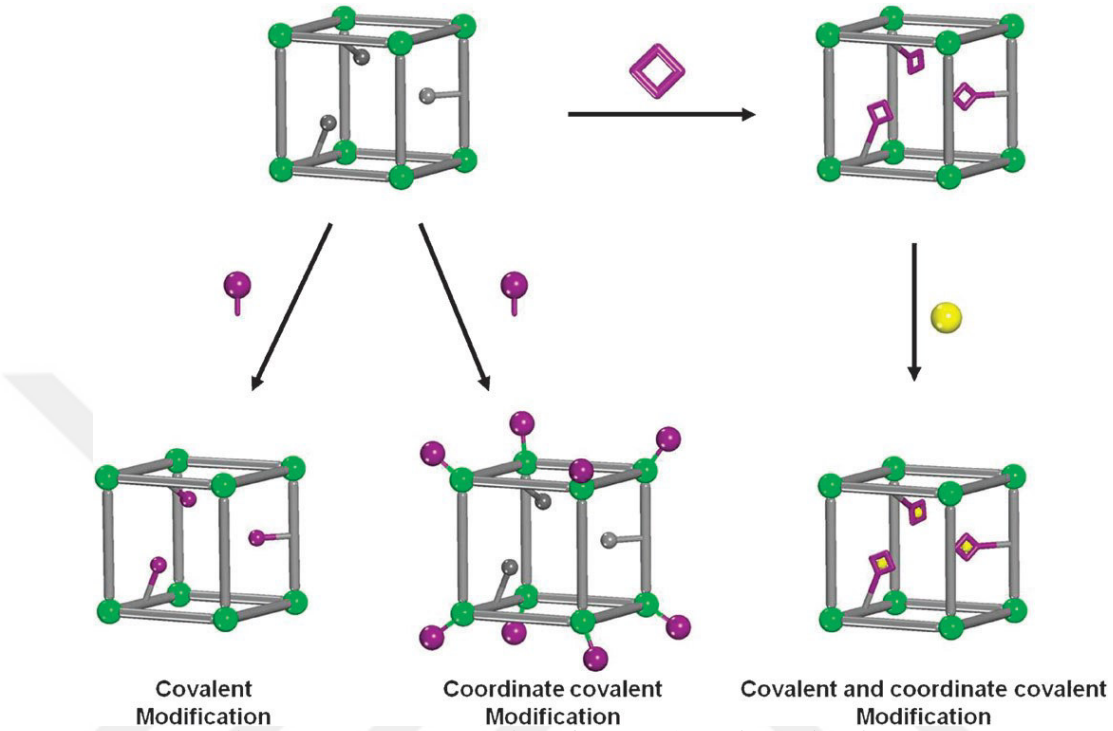

Figure 6.4. PSM Strategies of MOFs (Tanabe and Cohen 2011)

• Post-synthetic exchange (PSE)**:** Post-synthetic exchange, also known by building block replacement (BBR), implies the replacement of a key structural component of the preassembled MOF, by various methods including (i) non-bridging ligand replacement, (ii) solvent-assisted linker exchange (SALE), (iii) solvent-assisted linker incorporation and (iii) transmetalation at nodes or within linkers. This strategy has been successfully employed in MOFs synthesis with both desired functionality and high stability (Deria et al. 2014; Sun and Zhou 2015).

6.5. Coating of MOFs

To optimize the performance of nanoparticles in vivo, surface modification by coating strategy has been mainly utilized for improving water dispersity, decreasing binding to plasma protein, escaping the reticuloendothelial system, adding affinity molecules to target specific cells or tissues and functionalizing of the coat to target specific receptors for active cell targeting (Figure 6.5). Surface modification is particularly useful for nanoMOFs, due to the intrinsic instability of many nanoMOFs under physiological conditions, so surface coatings can delay the dissociation of nanoMOFs and thus preventing premature drug release. Two main general methods

have been employed to modify the surfaces of the nanoMOF: (i) Coating by organic polymers like the previously discussed polydopamine coatings, polyvinylpyrrolidone, phospholipid bilayers and polyethylene glycol, and (ii) silica encapsulation (Della Rocca, Liu, and Lin 2011).

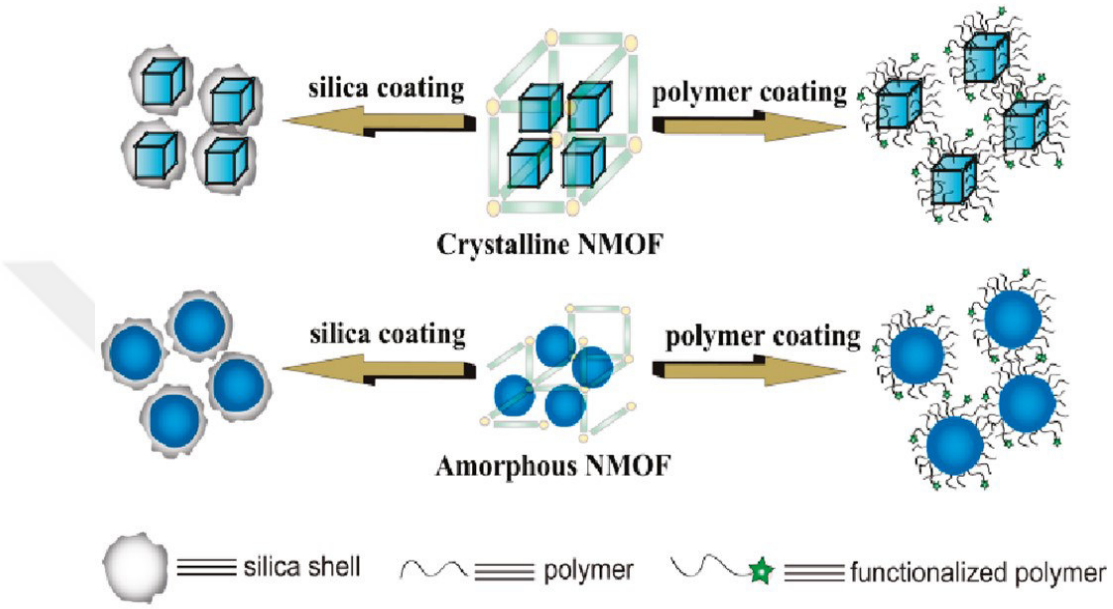

Figure 6.5. Surface coating of crystalline and amorphous nanoMOFs with either a thin shell of silica or with polymer coatings (Della Rocca, Liu, and Lin 2011)

6.5.1. Polyvinylpyrrolidone (PVP) Coating

Modifying the surface of several nanoMOFs with the hydrophilic PVP stabilizes the nanoMOF and increases its dispersibility and can be used as a way to increase the efficiency of the subsequent coating step by preventing aggregation (Della Rocca, Liu, and Lin 2011).

6.5.2. Silica Coating

A silica shell as a surface coating possesses several advantages which include stability, increased water dispersibility, ease of functionalization with silyl-derived molecules and biocompatibility. The silica shell thickness can be managed by adjusting reactants concentrations or the time of the reaction. The release profile studies have shown that silica shell has slowed down significantly the rate of nanoMOF dissolution and the release of encapsulated drug. Generally, nanoMOFs are firstly coated with a hydrophilic polymer to keep the nanoMOF particles well dispersed so that individual nanoMOF particles are coated with silica shells without

aggregation (Liu and Han 2010; Rieter, Taylor, and Lin 2007; Della Rocca, Liu, and Lin 2011; Beg et al. 2017).

6.5.3. Polyethylene Glycol (PEG) Coating

PEG oligomers/polymers form a highly hydrated hydrophilic shell that can hinder the interaction of the coated nanoparticles with biomacromolecules like opsonins. Densely tethered PEG chains also create an entropic barrier against the biomacromolecules, thus PEG chains prevent the interaction with the penetrating proteins. PEG coatings can subsequently prolong the blood circulation time of the nanoparticles and decrease their opsonization as well. For smaller nanoparticles, the hydrated PEG layer widens the hydrodynamic radius of these NPs and so decreases their renal excretion. PEG coating offers control over the MOFs' interaction with the biological surface and increase the circulation half-life time of the loaded drug in the circulation from several hours to days. Similar to other nanocarriers, PEG-coated nanoMOFs also exhibit 'stealth' imparted by the PEG molecules, that tend to circumvent their uptake by the RES. Alternatives to PEG like polyvinylpyrolidone, polyoxazoline, polyglycerols, polyacrylamides, polyaminoacids, polysaccharides, biomembranes and zwitter ions can also be used for the same purpose (Hadjesfandiari and Parambath 2018; Beg et al. 2017).

6.5.4. Phospholipid Bilayers Coating

Phospholipid bilayers coating is a highly efficient method for stabilizing NPs in physiological environment. It should be noted that the phospholipid bilayers do not only make the systems biocompatible but also make it becomes feasible through internalization of the phospholipid-coated NPs by cells. Here, the nanocore serves as a reservoir carrying drugs and also acts as a supporting tool for the fragile phospholipid bilayers avoiding its detachment, while the supported bilayer shell makes the nanobiocomposites biocompatible and dispersible (Sheno et al. 2019).

6.5.5. Exosome Coating

Exosomes are endogenous liposomes found in body liquids. They are supposed to be non-immunogenic and are utilized by cells for communication purposes. Thus, they merge the advantages of a lipid bilayer together with potential shielding from the immune system. This enables them to overcome the challenges of drug delivery leading to a leakage-free delivery and release, together with endosomal escape of the

drug, being shielded from the immune system and thus having long circulation time and total biocompatibility. Exosome-coated MOF NPs act as an efficient "onboard-trigger" smart drug delivery system. Thus it combines synergistically the features of exosomes and MOF NPs, and thus yields a promising system that offers efficient and easy loading with sealing. In addition, it exhibits high therapeutic efficacy without any premature leakage. Intracellular drug release is possibly controlled by a combination of the release mechanism of the endogenous exosomes and the nanocarrier degradation, which decomposes into naturally existing substances in our body. Further advantages of this system will be studied and analyzed in the future, including the incorporation of targeting ligands into the exosomal bilayer. The utilization of autologous exosomes will avoid any susceptibility to responses from the immune system, thus offering longer circulation times (Illes et al. 2017).

6.6. Drug Loading Strategies of MOFs

The loading strategies fall into three general categories which can be used separately or mixed together in synergistic drug delivery systems for multimodal therapy.

6.6.1. Direct Incorporation as Metal Connecting Points or Bridging Ligands

In the direct incorporation method, the drug is utilized as either the metal connecting nodes or the bridging ligand to form nanoMOF (Figure 6.6). For instance, the paramagnetic metal ions like Fe^{3+},Mn^{2+} and Gd^{3+} would not only join the ligands together to form the nanoMOF but also would act as magnetic resonance imaging (MRI) contrast-enhancing agents either in intact nanoMOF particles or upon dissolution of the nanoMOF particles. Another example, the prodrug disuccinatocisplatin (DSCP) and I_4-BDC were utilized to link metal centers forming a nanoMOF that provide the anticancer therapeutic cisplatin and the high Z element iodine for CT imaging, consecutively. The direct incorporation method has the advantage of achieving very high drug loading with uniform distribution throughout the NPs (Wang, Zheng, and Xie 2018; Della Rocca, Liu, and Lin 2011; Shen et al. 2017).

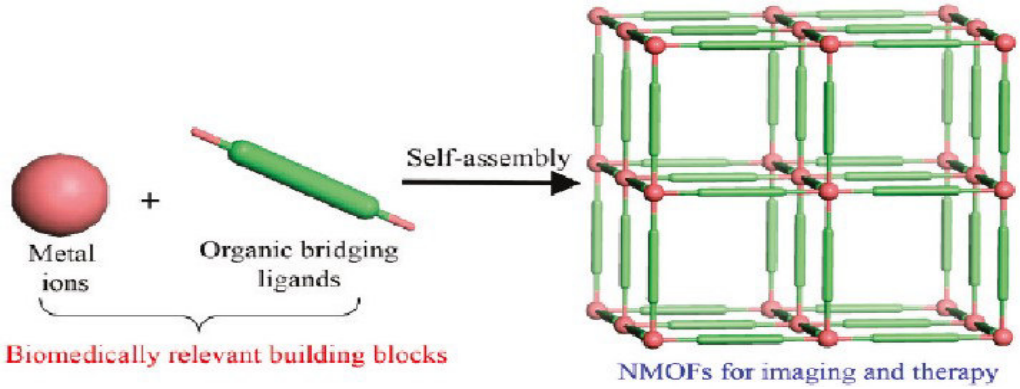

Figure 6.6. Drug Loading Strategy for Direct Incorporation of Drugs as Metal Connecting Nodes or Bridging Ligands (Della Rocca, Liu, and Lin 2011)

6.6.2. Post-Synthetic Encapsulation via Non-covalent or Covalent Interactions

Here, the drug is loaded to the nanoMOF by non-covalent or covalent interactions after the synthesis of porous nanoMOFs (Figure 6.7). The nanoMOFs' pore size should be greater than the incorporated drug to offer high loading capacity. Non-covalent drug loading was primarily demonstrated using bulk phase MOFs, resulting in excellent loading. Because non-covalent drug loading is a constitutionally reversible process, so subsequent processing of the nanoMOF could result in premature release of the drug. Post-synthesis covalent attachment of the drug offers a more strong technique, as the drug will only be released upon decomposition of the nanoMOF. In this strategy, functional groups within the frameworks of the nanoMOFs are covalently attached to the active drug. This method creates effectively a prodrug, and thus the functionality of the drug should be maintained. The active drug should be cleavable from the nanoMOF under the specified biological conditions. However, since functional groups near or at the NP surface are more accessible kinetically, drug loading may not be uniform throughout the nanoMOF particles (Wang, Zheng, and Xie 2018; Della Rocca, Liu, and Lin 2011; Shen et al. 2017).

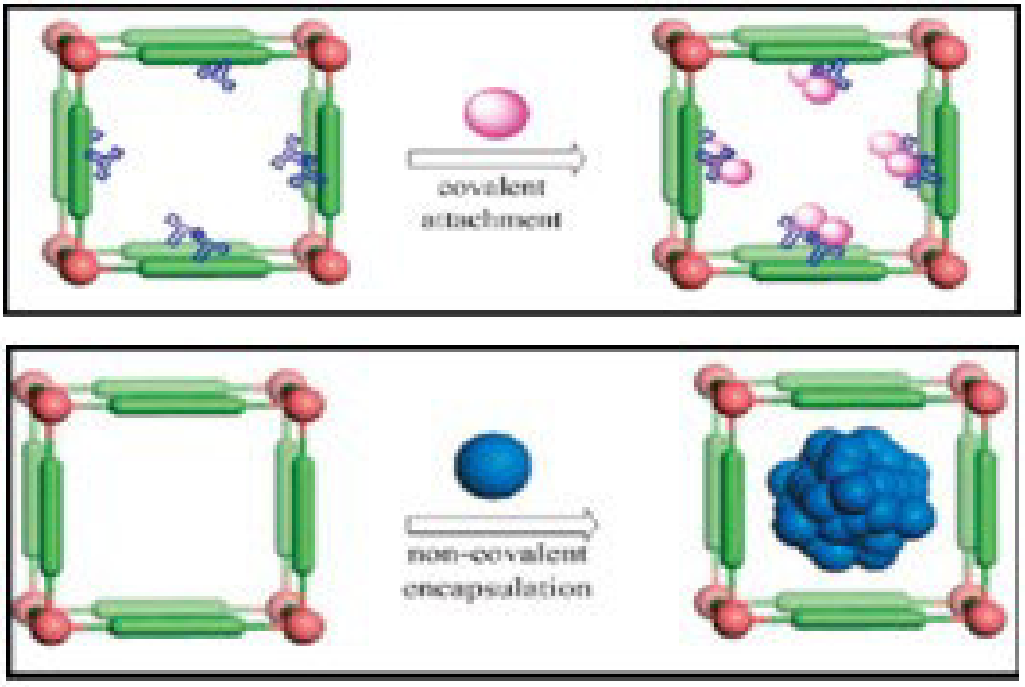

Figure 6.7. Drug Loading Strategies for Post-Synthesis Incorporation via Non-Covalent Interactions and Covalent Attachment (Della Rocca, Liu, and Lin 2011)

Also, there should be size matching between the selected drug and pore structures of MOF carriers which is the key factor for successful incorporation. That's why time-consuming studies have been done to design and prepare MOFs with larger pores for accommodating large-molecule drugs and for enhancing the loading capacity. However, increasing the pore size can result in bursting release of the drug and decreased delivery efficiency (Wang, Zheng, and Xie 2018).

6.6.3. Drug Loading Using Post-Synthetic Functionalization

In this method, MOF nanoparticles with the desired morphology, size, and physiochemical properties are firstly synthesized and isolated. Then drugs are then incorporated into the nanoMOF carriers in a following step by covalent bonds with the functional sites of the linkers or by coordination bonds formation with the metal nodes (Figure 6.8). Here, the drugs are attached to the outer surface of the presynthesized nanoMOF particles (Wang, Zheng, and Xie 2018).

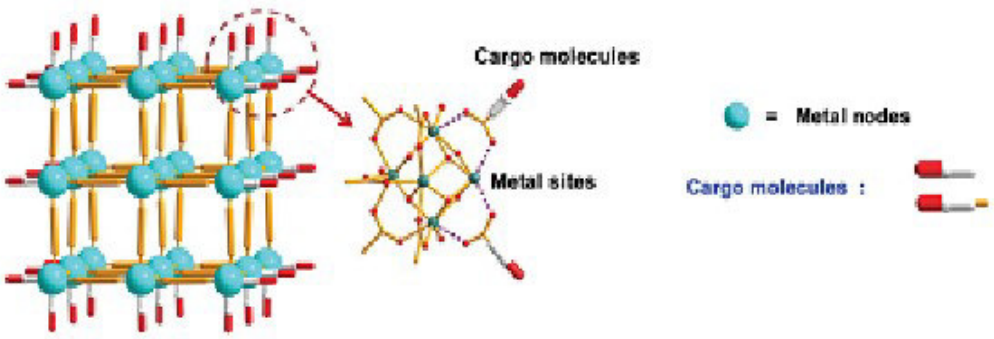

Figure 6.8. Drug Loading Using Post-Synthetic Functionalization (Wang, Zheng, and Xie 2018)

The post-synthesis sites utilized in this method can be divided into 3 types: (i) The functional sites of the linkers, (ii) coordinatively unsaturated (open) metal sites and (iii) ligand defects within the metal nodes. Coordinatively unsaturated metal sites represent a unique feature of MOFs which mainly results from the metal-coordinated solvents during the solvothermal process. Coordinatively unsaturated metal sites with a Lewis acid nature could be achieved simply by mediating the final performance of the MOFs in catalysis, removing solvents, gas adsorption and separation, and heating under low pressure. These Coordinatively unsaturated metal sites could be used as hooks to determine the effectiveness of drug incorporation and overcome many synthetic challenges (Wang, Zheng, and Xie 2018).

6.6.4. Drug Loading Using One-Pot Self Assembly Method

Here the drug to be loaded is included together with the MOF precursors in an appropriate solvent and the drug is loaded during the processing of MOF synthesis (Shu et al. 2018; Laha et al. 2019).

6.7. Different Application Aims for MOFs in Drug Delivery

6.7.1. Enhance the Dissolution and Absorption of Hydrophobic Oral Drugs

If we incorporate a drug molecule in a water-reactive MOF, we will have an amorphous drug trapped within the nanoscale pores and upon hydrolytic degradation of the water-reactive MOF, rapid release of the drug occurs in the dissolution media. In this case, the MOF acts as a drug carrier that prevents drug crystallization through confinement within its nanosized pores while undergoing rapid hydrolytic degradation in simulated gastric media and phosphate buffer saline (PBS) media resulting in spontaneous release of the amorphous drug. This amorphous drug

delivery system possesses an increase in the drug free energy relative to that of the crystalline form which is required for achieving high supersaturation. This rapid drug release generating high supersaturation represents a potential for enhanced absorption of the drug and rapid onset of its therapeutic effect. A convenient MOF that can act as a stabilizer for an amorphous drug and a rapid release host should meet the following criteria (i) have acceptable toxicity profile, (ii) reactively decompose in the desired media, and (iii) have suitable pore size to host the target drug. Drugs with very low water solubility like Curcumin and Sulindac were selected as guest molecules. The drug molecules were loaded using post-synthetic incorporation via soaking activated MOF-5 crystals in saturated solution of the drug in methylene chloride for Curcumin and acetonitrile for Sulindac. MOF-5 crystals were then immersed in simulated gastric media and PBS media at 37ºC in presence of methyl cellulose to prevent drug molecules precipitation at high supersaturation and to serve as a proxy for this extensive formulation process to this supersaturating drug delivery system. Curcumin@MOF-5 and Sulindac@MOF-5 achieved superior significant supersaturation higher than that generated during the dissolution of their solid polymeric amorphous dispersions (Suresh and Matzger 2019).

6.7.2. Controlled Extended Drug Release

MOFs has shown to be very good candidates for controlled extended slow drug release. A study for a multivariate MOF reports the control of drug release profiles by controlling desirable interactions between the guest drug molecules and pores in a stable MOF. The quantitatively correlated rate constants to the proportion and type of functional groups were used to drive interactions in the MOF, allowing estimated and programmed release of the guest drug molecules. Doxyrubicin and Ibubrufen were studied in a series of mesoporous MOFs bearing multiple functional groups [MIL-101(Fe)- (NH2)x, MIL-101(Fe)-(C4H4)x, and MIL-101(Fe)-(C4H4)x(NH2)1−x]. It was found that the drug release peak time was shifted by up to 12 days through a 40-day release period in [Doxorubicin from MIL-101(Fe)-(C4H4)x(NH2)1−x], which could not be achieved in the physical mixture of a single component MOF nor in other porous materials. The release of the two prodrug molecules (Doxyrubicin and Ibubrufen) was also accomplished. The single probe release test enabled us to screen different functional groups in the linkers, identifying the one that could pair up with the probe via dominant interactions like NH_2-BDC to Ibubrufen and C_4H_4-BDC to

DOX. By controlling the exact ratio of these paired-up functional groups in the multivariate MOFs, the behavior type of each probe in these co-release systems can be programmed. The establishment of an accurate correlation between the functional groups and the guest drug molecules opens up the possibility of designing carrier materials for the controlled release of multiple drugs in a programmed way (Dong et al. 2017).

In another study, Oridonin (the natural active ingredient with strong anticancer activity showing very poor water solubility, low bioavailabity and short half-life time restricting its use) was loaded into MOF-5. It showed high drug loading capacity and good sustained release of Oridonin with significant in vitro cytotoxicity and apoptosis effect on HepG2 cancer cells (Chen, Luo, Cai, et al. 2019).

6.7.3. Stabilize Oral Drugs (Insulin) Against Degradation by Stomach Acidity

Insulin was loaded and immobilized in 30 minutes only in the mesoporous crystalline MOF (NU-1000), with a high loading capacity of 40 wt%. The acid-stable MOF crystals were found to effectively prevent insulin degradation protecting it from stomach acidity and from pepsin the digestive enzyme. Moreover, under simulated physiological conditions, the incorporated insulin was released from the MOF (Chen et al. 2018).

6.7.4. Enhance the Bactericidal Effect of Antibiotics and Overcome Resistance

A study based on a synthesized chitosan-coated MOF showed enhanced interaction with the cell surface of Staphylococcus aureus. Chitosan, which is the deacetylated derivative of chitin, is capable of forming non-bonding interaction with the negatively charged bacterial cell resulting in enhanced contact of MOFs with staphylococcus aureus. Vancomycin bactericidal activity was markedly increased upon incorporation in chitosan-coated MOFs leading to increased inhibition of resistant staphylococcus aureus. Atomic force microscopy analysis of staphylococcus aureus strains showed clearly complete disruption of the morphology of those treated by chitosan-modified drug-loaded MOFs. Results of this study suggest that chitosan-coated MOFs have the potential for beating and overcoming the bacterial resistance

to Vancomycin, opening up new avenues for better antibiotic therapy of infections accompanied by multiple drug resistance (Ghaffar et al. 2019).

6.7.5. Vaccines Stabilization with Providing Potency and Long-Lasting Immunity

Oral antigen vaccination faces numerous challenges, including gastrointestinal proteolysis and mucosal barriers. A biomimetically mineralized aluminum-based MOF (Al-MOF) system resistant to ambient temperature and pH, that can act synergistically as a delivery vehicle, was developed to limit gastrointestinal proteolysis. To act as armor, an adjuvant is fabricated over a model antigen ovalbumin (OVA). A yeast-derived capsule is utilized as a "Trojan Horse"-like transport platform, as a carrier for the Al-MOF-armored OVA, to overcome mucosal barriers. In vitro trials revealed that Al-MOFs, by forming an armor on OVA, protect it from the highly acidic and degradative gastrointestinal conditions. Yet, in simulated phosphate-ion-containing intracellular fluid, the Al-MOFs decompose gradually, slowly releasing their incorporated OVA. In vivo experiments revealed that the "Trojan Horse"-like transport platform targets specifically the intestinal M cells, supporting the Al-MOF-armored OVA transepithelial transport, preceded by endocytosis later in local macrophages and eventually accumulating in mesenteric lymph nodes, thus resulting in long lasting, high levels of mucosal S-IgA and serum IgG antibodies. This designed delivery platform could be a promising technique for prophylactic or therapeutic vaccine antigens oral administration, providing potent long-lasting immunity (Miao et al. 2019).

In another study, the ability of zeolitic imidazolate framework-8 (ZIF-8) to protect a model viral vector against denaturing conditions was studied, and it demonstrated enhanced thermal and chemical stability to the conformational structure of the incorporated viral vector. The biological activity of this virus-ZIF nanocomposite on long-term was also examined in animal models to better understand the integrity of the incorporated virus, together with the biosafety and immunogenicity of the whole drug delivery system. Following a serious of subcutaneous administrations, histological analysis revealed no observable tissue damage on the skin or vital organs of mice. This experiment demonstrated that ZIF-based protein nanocomposites are robust candidates for improved proteinaceous drug

preservation. They are also biocompatible and capable of controlling in vivo drug release and adsorption (Luzuriaga et al. 2019).

6.7.6. Osteoarthritis Treatment

Magnesium (Mg) is a mineral that is abundant in the human body and contributes to bone structural development. Mg deficiency affects several pathways that play an important role in the pathology of osteoarthritis, including articular cartilage loss progression, inflammation of tissue and abnormal bone formation, all of which result in pain, joint function loss, and disability.

A study performed showed stability of Mg/HCOOH-MOF in various physiological media with long-term slow release of Mg^{2+}. Its results revealed that administration of Mg/HCOOH-MOF with appropriate dose resulted in beneficial biocompatibility and showed a beneficial effect when administered for a long period. This advantage is not found in currently available drugs. Mg/HCOOH-MOF has the potential to be used as a long-acting drug for osteoarthritis relieving (Li et al. 2020).

6.7.7. Protein Delivery

Since proteins have large size, charged surfaces and are environmentally sensitive leading to their denaturation, so proteins naturally do not cross cell-membranes in their intact form making their delivery difficult for both therapeutic and diagnostic purposes. A facile strategy using DNA functionalized insulin-loaded MOF NPs was successfully designed in a study to deliver insulin, as a protein model, at high payloads and negligible cytotoxicity across cell membranes. Future designs will enable encapsulation of different proteins by tuning the MOF's pore size as well as potentially co-deliver protein and nucleic acid targets, which are important for a variety of applications such as in vivo imaging, therapeutics, study of fundamental cellular processes and gene regulation (Wang et al. 2019)

6.7.8. Gene Delivery

MOFs have proven to be efficient carriers for gene delivery. In a study done to to examine their intracellular gene delivery and expression, large plasmid DNA molecules (pDNA) expressing enhanced green fluorescent protein (pEGFP-C1) was loaded and encapsulated within the MOF zeolitic imidazole framework-8 (ZIF-8) by the rapid and economical one-pot method, with polyethyleneimine (PEI) 25 kD as a capping agent. The synthesized nanostructures demonstrated high loading capacity,

improved pH-responsive release, and pDNA high binding affinity. In vitro studies also revealed improved cellular uptake and endosomal escape of the protected pDNA within the ZIF-8-PEI 25 kD vector, resulting in successful gene expression together with high transfection efficacy, compared to expensive commercial agents, implying future prospects for the development of cost-effective avenues to develop MOF-based nonviral vectors for efficient gene delivery and gene expression (Li et al. 2019).

In another study, a green fluorescent protein plasmid (plGFP) was utilized as a model for genetic macromolecule and was loaded into ZIF-8 MOF. It successfully transfected mammalian cancer cells with plGFP for up to 4 days. The feasibility of DNA-MOF for use as intracellular gene delivery vehicles was also confirmed by cell transfection assays together with soft X-ray cryo-tomography (cryo-SXT). The nucleic acid cargo is gradually slowly released over relatively long time to maintain sustained gene expression (Poddar et al. 2019).

6.7.9. Stimuli-Responsive MOF for Drug Delivery

One of the most important properties of MOF is that their composition and structure can be designed to obtain tailored responsive chemical and physical properties so they can be used for stimuli responsive controlled drug release. In this context, recent scientific research has designed single and multiple-stimulus-responsive MOFs. Different stimuli have been studied for regulation of drug delivery like pH, magnetic, ions, temperature, pressure, competitive binding, light, humidity, H_2S, ATP, liposomes, redox and enzymes (Cai et al. 2019; Liu, Zhao, and Chen 2019).

6.7.9.1. pH Responsive

Because of the acidic nature of the tumor microenvironment and coordination bonds sensitivity to external pH in MOFs, variety of pH-responsive MOFs have been employed for drug delivery in cancer therapy. The nanocarrier polyacrylic acid@ZIF-8 was fabricated through ion exchange reaction between Zn^{2+} ions and polyacrylic acid sodium salt followed by a reaction with 2-methylimidazole in methanol solution. The electrostatic interactions between the negatively charged polyacrylic acid and the positively charged Doxyrubicin as well as the coordination interaction in Zn^{2+}–DOX, resulted in high DOX loading capacity for the nanocarrier

polyacrylic acid@ZIF-8. The DOX-loaded polyacrylic acid@ZIF-8 exhibited faster drug release in a PBS buffer at pH 5.5 than that at neutral pH 7.4. These drug nanocarriers, which are pH responsive, were endocytosed by MCF-7 cells and exhibited a low toxicity in the living cells, thus demonstrated their potential for cancer therapy (Ren et al. 2014; Cai et al. 2019).

6.7.9.2. Magnetic Responsive

Magnetic-responsive drug delivery is a novel strategy that employs the influence of an external magnetic field, to not only accurately drive the magnetic NPs-loaded drug to the intended site to increase its therapeutic efficacy, but also to serve as an external stimulus to switch on a controllable release of the drug. Moreover, the MOF-based magnetic nanocarriers can be utilized simultaneously as a theragnostic system for imaging-guided therapy and MRI. Core-shell nanostructures are frequently utilized in magnetic-responsive MOF-based drug delivery nanocarriers. Magnetic NPs are commonly used as the magnetic core in these nanocarriers, with the MOF forming the shell in this drug delivery system (Cai et al. 2019).

In a study, Nimesulide, an anti-inflammatory and a promising anticancer drug, was loaded in a magnetic $Fe_3O_4/Cu_3(BTC)_2$ nanocomposite MOF by soaking the nanocomposite MOF in the Nimesulide trichloromethane solution. The magnetic measurements revealed that the synthesized MOF possessed the desirable magnetic performance required for stimuli targeted drug delivery (Ke et al. 2011; Wu and Yang 2017)

6.7.9.3. Ion Responsive

Here, the strong electrostatic interactions between ionic drugs and MOFs control the drug diffusion and release in the ion-responsive MOF-based drug delivery system (Cai et al. 2019). A zinc-based anionic MOF was fabricated by mixing adenine and zinc acetate dihydrate together in the presence of biphenyldicarboxylic acid. The synthesized anionic bioMOF-1 was loaded by procainamide HCl (a cationic anti-arrhythmatic drug), supported by the electrostatic interaction between the cations and anions achieving a good loading capacity. The release profile was examined by immersing the procainamide.HCl-loaded MOF in PBS buffer solution of pH 7.4 or deionized nanopure water as a control, which demonstrated that the

buffer cations stimulates the drug release from this ion-responsive MOF (An, Geib, and Rosi 2009; Cai et al. 2019).

6.7.9.4. Temperature Responsive

In temperature-responsive MOFs, changes in the surrounding temperature cause alterations in the utilized thermoresponsive materials, which in turn regulate the drug release. Poly(N-isopropyl acrylamide), or PNIPAM, was used among the different thermoresponsive materials to modify the MOF UiO-66. The PNIPAM-modified MOF nanocarrier (UiO-66-PNIPAM) when loaded with caffeine, resorufin and procainamide showed a release that is thermally controlled, driven by simple change in the temperature, and the drug-loaded UiO-66-PNIPAM demonstrated rapid release of the incorporated drugs at a lower temperature (25ºC), but the release was inhibited at a higher temperature (40ºC) exceeding its *T*c (31ºC), thus emphasizing its temperature responsiveness (Nagata, Kokado, and Sada 2015; Cai et al. 2019).

6.7.9.5. Pressure Responsive

Pressure has been also utilized for controlling drug release. A zirconium-based MOF (ZJU-800) which is composed of zirconium metal ions and (2E,2E′)-3,3′-(2-fluoro-1,4-phenylene)diacrylic acid was synthesized and loaded with Diclofenac Sodium achieving a high loading capacity to design a pressure-responsive drug release. An adjustable controlled release of Diclofenac Sodium was achieved over a time from 2 to 8 days by controlling the extent of compaction which results in applied pressure between the MOF and drug. When the pressure was greater than 30 MPa, the release time was constantly prolonged as predicted (Jiang et al. 2016; Cai et al. 2019).

6.7.9.6. Competitive Binding Responsive

Supramolecular materials like β-cyclodextrin and pillararenes act as switches or gatekeepers that can be employed to modify MOF surfaces to obtain a smart stimuli-responsive drug delivery strategy. When a competitive binding agent and a supramolecular material on the surface of a MOF possess a higher binding affinity between each other, the drug release from the MOF can be stimulated by the addition of a competitive binding agent (Meng et al. 2016; Tan et al. 2015; Cai et al. 2019).

6.7.9.7. Light Responsive

Because of its spatiotemporal precision using specific light wavelength and its non-invasiveness, light-controlled therapy has proven to be far superior in achieving on-demand diagnostics and therapeutics in targeting areas both in vivo and in vitro. NanoMOFs have also been employed to design light-responsive drug delivery systems. A thin layer of MOF (UiO-66) film was synthesized on an optical fiber substrate with 5-Fluorouracil (5-FU) encapsulated into the MOF pores. The delivery of light at an appropriate (1050 nm absorption) activated the MOF thus overcoming the adsorption enthalpy of 5-FU and enabling its in situ release via the fiber catheter, triggering the release of 5-FU on demand (Nazari et al. 2016; Cai et al. 2019).

6.7.9.8. Humidity Responsive

Humidity has been used as well in stimuli-responsive drug release. In a recent study, three MOFs were selected and loaded with the volatile potent natural antimicrobial agent allylisothiocyanate (AITC) based on AITC molecular characteristics and the structural properties of the MOFs. The results demonstrated that these MOFs could incorporate and preserve AITC molecules within their nanopores under low relative humidity conditions (30–35%). In the contrary, AITC molecules release was triggered under high relative humidity conditions (95-100%) from these MOFs, thus indicating that humidity could trigger AITC release from these MOFs (Lashkari et al. 2017; Cai et al. 2019).

6.7.9.9. H_2S Responsive

A significant high content of hydrogen sulfide (H_2S) is found in human colon adenocarcinoma cells (Szabo et al. 2013). A study based on the synthesis of an H_2S-responsive MOF was performed using Cu^{2+} ions as the metal node and zinc 5,10,15,20-tetrakis(4-methoxycarbonylphenyl)porphyrin (ZnTCPP) as a bridging photosensitive ligand with photodynamic activity for the construction of single-component MOF NP with the Cu^{2+} ions as metal nodes of the framework being the H_2S-responsive site in the H_2S fluorescence probes. The paramagnetic Cu^{2+} ions have both completely quenched the MOF NPs ligand-based fluorescence and reduced significantly the photosensitive ligand ROS production efficiency. The Cu^{2+} ions were released out from the MOF nodes upon interaction with H_2S, and the luminophor photosensitive bridging ligand in (ZnTCPP) was released simultaneously

thus achieving potent cancer therapy combined with controllable photosensitive ligand release for photodynamic therapy (Ma et al. 2017; Cai et al. 2019)

6.7.9.10. ATP Responsive

The ATP-responsive nanoMOFs strategy was suggested since ATP is upregulated and overexpressed in cancer cells. Based on this, a study reported the synthesis of a nanoMOF comprising of Zr^{4+} ions and amino-triphenyldicarboxylic acid loaded with Rhodamine 6G fluorescent agent and Doxorubicin anticancer agent, and locked by stimuli-responsive DNA caps (aptamer sequence AS1411) that also specifically binds to the nucleolin receptors present on the cell membrane of cancer cells and so acting as bifunctional nanoparticles exhibiting both cancer cells ligand targeting and ATP-responsive release. The nanoMOF, through forming ATP-aptamer complexes, were unlocked in the presence of ATP, leading to the release of their loaded drugs. AS1411-functionalized DOX-loaded nanoMOF exhibited high cytotoxic efficacy and targeted drug release (Chen et al. 2017; Cai et al. 2019).

6.7.9.11. Liposome Responsive

Liposomes are considered a true biomimetic system because they mimic cell membranes. Electrostatic interactions between the liposomes and the drug can trigger drug release from a drug-loaded MOF. Two DOX-incorporated MOFs (Fe-BTC and Zn-BTC) were prepared by a one-step simple reaction at room temperature. Stronger electrostatic interactions between the DOX and liposomes triggered the release of DOX from the MOFs in the presence of biocompatible liposomes. Additionally, in the acidic environment, the carboxylate anions becomes protonated breaking down the coordination between the carboxylic acid group and metal center, leading to DOX release through MOF destruction. These MOFs offer new strategies for liposome-responsive drug release (Adhikari and Chakraborty 2016; Cai et al. 2019).

6.7.9.12. Redox Responsive

Redox reactions or oxidation reduction reactions can be also utilized to trigger drug release and different promising studies on GSH-responsive MOFs, Glucose-responsive MOFs and H_2O_2-responsive MOFs based on redox reponses demonstrate their promising use for smart-stimuli drug release (Cai et al. 2019).

- **Glucose Responsive:**

A glucose-responsive MOF has been experimentally developed for self-regulated insulin release depending on glucose concentration. The MOF ZIF-8 was loaded with insulin and glucose oxidase (GOx) to form the insulin-GOx/ZIF-8 composite. When a high glucose concentration is detected, it enters the composite pores and comes in to contact with the GOx, causing glucose to be oxidized to gluconic acid and H_2O_2. The released gluconic acid lowers the pH in the composite leading to its degradation and thus insulin release. This insulin-GOx/ZIF-8 composite has the potential to be a self-regulating drug delivery system for insulin (Duan et al. 2018; Cai et al. 2019).

- **GSH Responsive**

Human cancer tissue cells are characterized by significantly high concentrations of reducing agents such as glutathione (GSH) than that of normal tissue cells. The redox-responsive disulfide bond (S-S) can be readily broken in presence of GSH, and so it has become an attracting approach for the design of a redox-sensitive drug delivery system.

GSH-responsive MOF was synthesized from manganese ions (Mn^{2+}) and the disulfide (SS)-containing organic ligand dithiodiglycolic acid. DOX was incorporated through hydrophobic interactions into the Mn–SS MOF to produce Mn-SS/DOX NPs, that were then coated with a polydopamine (PDA) layer and then was further modified with PEG forming spherical Mn-SS/DOX@PDA/PEG NPs. Because of the disulfide linkage (SS) within dithiodiglycolic acid, cleavage in presence of GSH occurs resulting in degradation of the MOF and thus releasing the drug. In addition, when compared to free DOX, these Mn-SS/DOX@PDA/PEG NPs have demonstrated improved in vivo therapeutic efficacy (Zhao et al. 2017; Cai et al. 2019).

6.7.9.13. Enzyme Responsive

The MOF surface can be functionalized by DNA to cap it for enzyme-responsive drug delivery. The enzymes used are exonuclease-III (Exo-III), endonuclease (EcoRI), DNase-I and nicking enzyme (Nt.BbvCI). The drug-loaded nanoMOFs were capped with sequence-specific DNA duplex units. The biocatalytic degradation of the DNA duplex capping units by enzymes would result in unlocking

of the nanoMOF and release of the loaded drug from it. In a study, a nanoMOF containing camptothecin was capped with a hairpin DNA nucleic acid. The high levels of ATP in cancer cells combined with the biocatalytic Exo-III triggered the unlocking of the DNA-capped nanoMOFs, leading to cooperative release of the incorporated drug. The tailored hairpin DNA-capped camptothecin-loaded MOF demonstrated selective cytotoxicity against MDA-MB-231 cancer cells which have overexpressed exonuclease-III. Low apoptosis was also observed in epithelial MCF-10A breast cells with low exonuclease III expression (Chen, Luo, Sohn, et al. 2019; Liu, Zhao, and Chen 2019).

6.7.10. MOF Ligand Targeting Drug Delivery

Because of the large surface area and ease of surface modification which enables easy functionalization, MOFs can be multi-functionalized, loaded with drugs and used for ligand targeting drug delivery, especially in cancer therapy and diagnosis. Based on this, a method for designing an active tumor-targeted nanoMOF drug carrier was developed by functionalization with folic acid (FA) molecules onto nanoMOFs. Two zirconium-based MOFs (MOF-808 and NH2-UiO-66) were selected as models and were loaded with the anticancer drug 5-fluorouracil (5-FU), then folic acid molecules were anchored on the MOF by their carboxylate terminal. The drug loading capacity was determined and the drug release behavior was measured at normal physiologic pH 7.4 and at cancer acidic pH levels. Confocal laser scanning microscopy confirmed the excellent selectivity of the 5-FU-loaded/FA-nanoMOFs demonstrated by the efficient cellular uptake of FA-nanoMOFs and so it can be potentially used for cancer therapy (Dong et al. 2018).

In another study, a targeted anticancer drug delivery system that relies on the utilization of AS1411 aptamer as a targeting ligand with its affinity to bind to nucleolin receptors on the cell membrane of cancer cells was developed. The nanocomposite zirconium MOF (Zr-MOF, UiO-66) was incorporated with bioactive silver nanoclusters (Ag NCs) and functionalized by AS1411 aptamer for targeting. This targeting anticancer drug delivery system was easily synthesized by one-pot incorporation of the anticancer drug DOX and Ag NCs formation. It demonstrated high loading capacity for DOX and enhanced controlled sustained release. Confocal laser scanning microscopy confirmed that the MOF functionalized with AS1411 aptamer was taken up effectively and was highly selective in targeting cancer cells.

The in vitro study showed that the drug delivery and cellular uptake studies for cancer MCF-7 and normal L929 cells confirmed enhanced selective cancer-targeted delivery of DOX for targeting of cancer cells only. The results demonstrated that our drug delivery system was taken up and endocytosed through AS1411-mediated endocytosis into the inside of cancer cells, where the released DOX was delivered effectively to the nucleus, and thus it can be used as a targeted drug delivery system in vivo. The results suggested high potentiality of the designed functionalized drug delivery system as a promising targeting therapeutic (Su et al. 2019).

6.8. Toxicity and Safety Considerations of MOFs

The metal ions utilized in the synthesis of MOF mostly possess their own toxicity and can accumulate in the body. Therefore, the metal ions level in the MOF should be kept within the biologically permissible limits for avoiding toxicity. To avoid toxicity, metal cations having higher daily requirements and permissible limits in the body would be ideal for selection for MOFs synthesis. Metals with known low toxicity profiles such as Zn, Ca, Mg and Fe are regarded safe for diagnostic and drug delivery applications. Aside from metals, the organic component of the MOFs should be also biocompatible. Furthermore, the strategy for bioMOFs synthesis has become more acceptable by using biomolecules within their structure to modify their degradation behavior, improve their biocompatibility, and reduce their toxicity. Metal peptide frameworks have recently been reported in the literature as innovative 'bioinspired' materials with little toxicity when compared to MOFs (Beg et al. 2017).

The in vitro and in vivo stability and degradation processes of different MOF carriers require additional systematic exploration. Moreover, currently available imaging and drug delivery application studies are far from being clinically applicable, and full understanding of absorption-distribution-metabolism-excretion mechanisms is required (Wu and Yang 2017).

7. COMBINED TARGETED AND STIMULI-RESPONSIVE MOF IN CANCER THERAPY AND IMAGING EXAMPLES

7.1. Multiple-Stimuli-Responsive MOF for Cancer Therapy

As a triple-responsive hybrid theragnostic platform, a multiple-stimuli-responsive drug carrier formed of carboxylatopillar[5]arene (CP5)-gated Zr-MOF loaded with 5-Fu was fabricated consisting of MOF and pillararene-based supramolecular nanovalves. Through post-synthetic modification, positively charged stalks of quaternary ammonium salt were substituted to the MOF, and subsequently the negatively charged CP5 was capped on the stalks located on the MOF surface via host-guest complexation. Because of low pH in tumor cells and higher Ca^{2+} concentrations in osteoclasts due to osteolysis in bone cancer, the Ca^{2+}-competitive responsiveness and the pH-responsiveness triggered the release of the therapeutic agent and made it a promising platform for potential bone regeneration and bone cancer therapy. In addition, high temperatures in thermal therapy can destruct and kill tumor cells and weaken host-guest interactions to trigger gradual drug release of (5-Fu). As a result, this triple-responsive drug delivery system exhibited desirable features such as negligible premature release, good biocompatibility and non-cytotoxicity (Tan et al. 2016; Wu and Yang 2017).

7.2. MultiFunctionality for Cancer Therapy and Imaging

Here, DNA-functionalized nanoscale PCN-224 MOF was synthesized from 5,10,15,20-tetrakis(4-carboxyphenyl)porphyrin (TCPP) and biocompatible Zr^{4+} ions in presence of benzoic acid, N,N-dimethylformamide (DMF) by a stirring method then loaded with DOX then functionalized with the A549 lung cancer aptamer. The aptamer was substituted by fluorescein and carboxyl at the two terminals. Fluorescein could trace the A549 cells by targeting of PCN-224-DNA into the cells and could be used for cell imaging. When coming in contact with the A549 cells, our drug delivery system can trace cancer cells presenting efficient targeting therapy by the aptamer specific binding. In cancerous acidic conditions (pH 5.0), the DOX was released relatively rapidly, indicating that DOX@PCN-224-DNA displays a capacity for chemotherapeutic effect. The bridging linker of the MOF TCPP is a highly effective photosensitive agent for photodynamic therapy. By combining chemotherapy and photodynamic therapy, DOX@PCN-224-DNA can track cancer

cells and provide effective targeting therapy. This simple PCN-224 functionalization with aptamer open the door for the development of MOF-based target-directed treatment combined with detection and imaging (Zhang, Wang, et al. 2019).

7.3. MultiFunctionality Magnetic Core-shell MOFs for Cancer Therapy and Imaging with triple modality

A multifunctional Fe_3O_4/polyacrylic acid/Au nanoclusters/zeolitic imidazolate framework-8, denoted as (Fe_3O_4/PAA/AuNCs/ZIF-8 NPs) was fabricated and loaded with DOX for tumor diagnosis and visualized-combined therapy. The developed nanomedical system featured numerous therapeutic properties, like high DOX loading capacity, easy magnetic separation, dual pH-responsive drug release, tri-modal imaging, and high in vivo cancer suppression efficacy. To combine the advantages and overcome the limits of diverse imaging techniques, tri-modal imaging of magnetic resonance, optical imaging and X-ray computed tomography were used. Thus, such multifunctional core-shell nanocarriers demonstrated a promising potential for efficient theragnostic applications (Bian et al. 2015; Wu and Yang 2017).

8. LITERATURE REVIEW

Quercetin has an anticancer effect combined with being a potent antioxidant and anti-inflammatory while possessing a radiosensitization activity and suppressing radiation-induced skin fibrosis at the same time. That give it a high potential for being a successful drug for use in cancer therapy; however, its very poor solubility in water and low bioavailability have made it essential to develop a drug delivery system to enhance its absorption and bioavailability and hence its efficacy (Wang, Sun, et al. 2016; Ulusoy and Sanlier 2020)

MOFs, having the potential to be designed to be stimuli-responsive and to be functionalized with targeting molecules along with having high loading capacity within its pores together with its biocompatibility, have become an excellent candidates to be utilized as a drug delivery system for cancer therapy (Cai et al. 2019; Wu and Yang 2017).

(Ma et al. 2019) group have successfully synthesized a Zr-BDC structure loaded with Quercetin to perform a radiotherapy with a synergetic dual sensitization. Their study showed that Qu-loaded Zr-BDC exhibited an excellent radiotherapy sensitization potential in vivo and in vitro through reducing hypoxia-induced resistance and sensitization of cancer tissues to improve cell apoptosis where 1,4-benzenedicarboxylic acid produced from decomposition of Zr-BDC MOF acted as a carbonic anhydrase IX inhibitor and Quercetin acted as a radiosensitizer boosting sensitivity in radiation therapy, with no significant systemic toxicity over the treatment duration assessed in animal models.

(Shu et al. 2018) group have synthesized a Doxorubicin-loaded ZIF-8 MOF by one pot self assembly method using Zn nitrate and 2-methylimidazole with doxorubicin then they coated it with polydopamine to functionalize it with hyaluronic acid through chelation with Fe^{3+} to PDA. The synthesized hyaluronic-acid functionalized DOX-loaded ZIF-8 MOF has shown in vitro targeting ability towards prostate cancer PC3 cells which possess hyaluronic acid overexpressed receptors. The in vitro release study showed release of DOX in a pH-responsive manner. The in vitro MR imaging ability study showed that Fe^{3+} chelation to PDA made the fabricated MOF a good contrast agent for MR imaging.

(Laha et al. 2019) fabricated a folic acid-functionalized Curcumin-loaded IRMOF-3 using Zinc Nitrate, 2-aminoterphethalic acid, Curcumin and Folic acid in a one pot self-assembly technique. The drug release study has shown to be pH-responsive. The in vitro and in vivo studies of folic acid-conjugated MOF in mice have proven to be of higher efficacy compared to the untargeted Curcumin-loaded MOF and provided promising results for the use of Folic-acid functionalized targeted therapy.

9. EXPERIMENTAL SECTION

9.1. Materials and Methods

Cobalt(II) nitrate hexahydrate $Co(NO_3)_26H_2O$, terephethalic acid (1,4-benzenedicarboxylic acid) (BDC), ferric chloride, phosphate buffer tablets pH 7.4, Trizma (Tris HCl)(hydroxymethyl aminomethane) and N,N-dimethylformamide (DMF) were purchased from Sigma-Aldrich. Folic acid was purchased from Alfa Aesasr. Quercetin, Dopamine Hcl (dopasel ampoule 40 mg/ml) and ethanol were also purchased. Filters of 0.45 μm pore were also purchased from Millipore. All reagents were used without further purification. Deionized water was used throughout the whole experiment.

9.2. Synthesis of CoBDC MOF

CoBDC was synthesized by the conventional solvothermal method in accordance to our reference (Ma et al. 2021) with slight modification. Here, we dissolved 0.73 g of $Co(NO_3)_2.6H_2O$ in 20 ml DMF and we dissolved 0.42 g terephethalic acid (BDC) in 30 ml DMF and then we mixed both solutions together and stirred them for one hour. Then, we transferred the mixture to a Teflon-lined autoclave [we used two 45 ml teflon-lined autoclaves (acid digestion vessel), each filled with 25 ml of our mixture]. We then heated it in the oven at 120°C for 24 hours. The purple crystalline precipitate was collected by centrifugation and washed twice with 10 ml DMF each and then washed three times with 10 ml ethanol each. The final precipitate in ethanol was centrifuged and the supernatant was collected and removed. The precipitate (CoBDC) was dried overnight in a vacuum oven at 60°C for activation (Zha et al. 2020). A dark violet powder of activated CoBDC was obtained. XRD and FTIR of CoBDC were taken confirming the successful synthesis of CoBDC. CoBDC was kept in a tightly closed bottle away from air, light and humidity to prevent its oxidation and calcination.

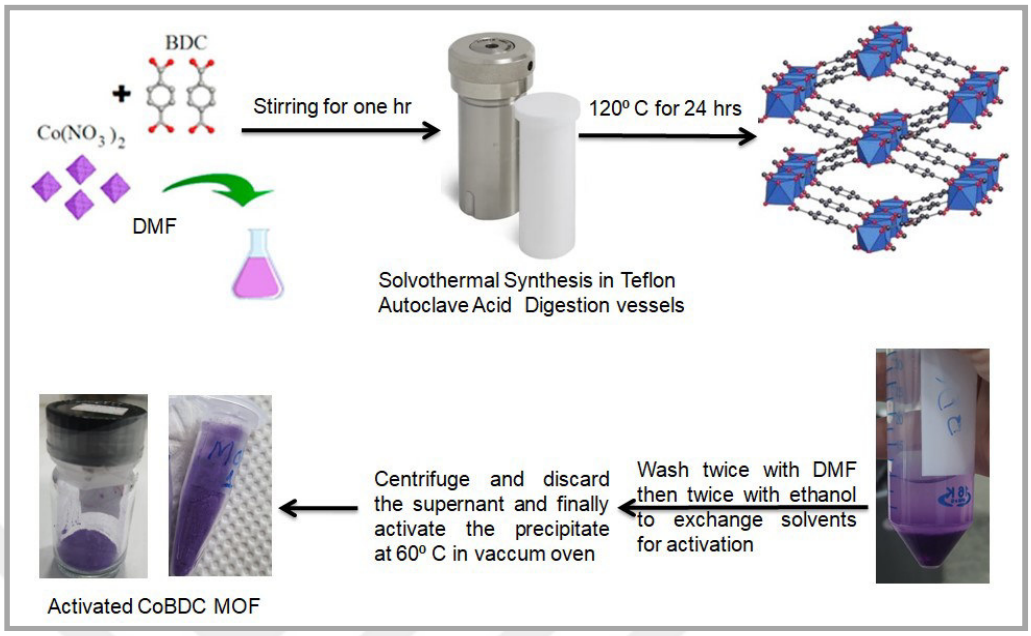

Figure 9.1. Synthesis Steps of CoBDC

9.3. Preparation of Quercetin-Loaded CoBDC

Loading was done twice in accordance to our reference (Ma et al. 2019). The first time: 104 mg Quercetin were dissolved in 10 ml ethanol then 52.2 g of our synthesized CoBDC MOF were added and dispersed in the solution and stirring was continued for 6 hours in ice bath protected from light. Quercetin-loaded CoBDC nanoparticles were collected by centrifugation and the supernatant was taken to measure the amount of unloaded Quercetin to calculate the loaded amount to determine the loading capacity of our CoBDC MOF. The collected Quercetin-loaded CoBDC nanoparticles were dried in vacuum oven at temperature 40°C (Yang et al. 2019). The first time loading was used to perform the drug release study of the Quercetin-loaded CoBDC (the uncoated unfunctionalized one).

For the second time loading: 500 mg Quercetin were dissolved in 50 ml ethanol then 250 mg of our synthesized CoBDC MOF were added and the same procedure for the first time loading was followed. The second time loading was used for coating and functionalization of the Qu-loaded CoBDC, and the drug release study of the FA-functionalized Qu-loaded CoBDC.

A standard solution of known concentration of Quercetin in ethanol was prepared and used to construct a calibration curve for Quercetin in ethanol by measuring the absorbance of various concentrations at λ 375 nm using UV-visible

spectroscopy to determine the amount of unloaded Quercetin in both loading experiments and hence get the amount loaded.

9.4. Coating of Quercetin-Loaded CoBDC with Polydopamine

Coating of Quercetin-loaded CoBDC with polydopamine was done using mussel-inspired oxidative polymerization in accordance to our references (Shu et al. 2018; Zhang, Hu, et al. 2019) with slight modification. Tris-HCl buffer solution (pH 9.1, 7.7 mM) was prepared by dissolving 0.1211 g of Tris.HCl reagent in 80 ml deionized water and adjusting pH by NaOH drops till reaching a pH of 9.1 then completing the final solution volume to 100 ml. A 30 mg Qu-loaded CoBDC MOF was dispersed in 30 ml Tris-HCL buffer solution (pH 9.1, 7.7 mM) using sonication for 15 minutes. Then an amount equivalent to 15 mg Dopamine Hydrochloride was added and the solution was stirred for 4 hours to enable oxidation and self polymerization of dopamine hydrochloride forming a coat. Then the black polydopamine-coated Quercetin-loaded CoBDC (PDA-coated Qu-loaded CoBDC) was collected by centrifugation and was washed with 10 ml deionized water twice. Finally, it was dried in vacuum oven at 40°C.

9.5. Functionalization of our PDA-Coated Qu-Loaded CoBDC with Folic Acid

Folic acid was conjugated onto PDA-coated Qu-loaded CoBDC (PDA-Qu@CoBDC) through an Fe^{3+}-mediated coordination reaction of PDA-Qu@CoBDC (Zhang, Hu, et al. 2019; Shu et al. 2018). A 20 mg of PDA-Qu@CoBDC was dispersed in 20 ml Tris-HCL buffer solution (pH 8.5, 7.7 mM) using sonication for 15 minutes. Then 0.2 ml of iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H2O$) solution in water 100 mg/ml was added and stirred for one hour. Then 20 mg Folic acid was added to the mixture and stirred for another three hours protected from light. Finally, our particles were collected by centrifugation and washed twice with 10 ml Tris HCl (pH 8.5,7.7 mM) buffer solution then washed twice with 10 ml deionized water then dried in vaccum oven overnight at 25°C.

10. CHARACTERIZATION

10.1. X-ray Diffraction (XRD) characterization

Rigaku Smart Lab XRD was used to characterize the composition and crystal structure of the CoBDC MOF, Quercetin, Quercetin-loaded CoBDC MOF, polydopamine-coated Quercetin-loaded CoBDC and Folic acid-functionalized Quercetin-loaded CoBDC. The measurements were performed at 40 kV and 40 mA, with CuKα radiation.

10.2. SEM and EDX Characterization

Jeol JSM-7001F SEM equipped with energy-dispersive X-ray spectroscopy (EDX), wavelength-dispersive X-ray spectroscopy (WDX), secondary electron (SE) detector, backscatter electron detector (BSD) and electron backscatter diffraction (EBSD) was used. SEM was used to characterize the morphology and size of CoBDC MOF, Quercetin, Quercetin-loaded CoBDC, polydopamine-coated Quercetin-loaded CoBDC and Folic acid-functionalized Quercetin-loaded CoBDC after coating them with gold. SEM images and EDX elemental maps were used to determine the elemental composition.

10.3. Fourier-transform infrared spectroscopy (FTIR)

Fourier-transform infrared spectroscopy (**FTIR**) was done using a Perkin Elmer Spectrum Two FTIR for CoBDC MOF, Quercetin, Quercetin-loaded CoBDC, polydopamine-coated Quercetin-loaded CoBDC and Folic acid-functionalized Quercetin-loaded CoBDC from 450 cm^{-1} to 4500 cm^{-1}.

10.4. UV-Visible Spectroscopy

Spectroscopy using Thermo Scientific GENESYS 10S Vis spectrophotometer operating from λ 200 -800 was done for Quercetin to create calibration curves for Quercetin in ethanol to determine the amount of unloaded Quercetin and hence calculate the loaded Quercetin, and for Quercetin in phosphate buffer saline (PBS) solution pH 7.4 and PBS solution pH 6.6 to measure the amount of released Quercetin in the drug release study.

11. DRUG RELEASE STUDY

An in vitro drug release study was performed to determine the cumulative release of Quercetin from both the Qu-loaded CoBDC and from the FA-functionalized Qu-loaded CoBDC using phosphate buffer saline (PBS) solution of pH 7.4 (normal body cells physiologic pH) and phosphate buffer saline (PBS) solution of pH 6.6 (acidic pH of cancer cells). A 10 mg of our Qu-loaded CoBDC was dispersed in 25 ml of freshly prepared PBS solution of pH 7.4 which is the same as normal body cells physiologic pH. Another 10 mg of Qu-loaded CoBDC was dispersed in a 25 ml of freshly prepared PBS solution of pH 6.6 which is the same as that of cancer cells (Zhang et al. 2021). Both of them were placed in a water bath of temperature 37º C, which is the same as our body temperature, with continuous stirring using magnetic stirrer. At specified periods 2.5 ml of the supernatant were taken from each solution for measurement of the amount of the released Quercetin and replaced immediately by a 2.5 ml PBS solution of the corresponding pH with continuous stirring each time (Laha et al. 2019). The 2.5 ml taken for measurement was filtered using a 0.45 μm pore filter then its absorbance was measured using UV-Visible spectroscopy at λ 370 for pH 6.6 and at λ 375 for pH 7.4, to determine the amount of the released Quercetin in the drug release experiment.

A standard solutions of known concentration of Quercetin in PBS solution pH 6.6 and PBS solution pH 7.4 were prepared and used to construct calibration curves for Quercetin in PBS solution 6.6 at λ 370 nm and in PBS solution 7.4 at λ 375 nm by measuring the absorbance at various known concentrations using UV-Visible spectroscopy to determine the amount of the released Quercetin in the drug release experiment.

12. RESULTS AND DISCUSSION

12.1. Synthesis

Synthesis of CoBDC was done using the conventional Solvothermal Method using $Co(NO_3)_2.6H_2O$ and terephethalic acid (BDC) in DMF followed by washing with DMF to remove unreacted reactants then washing by ethanol for solvent exchange to replace the high boiling point solvent DMF by the low boiling point solvent ethanol in order to get rid completely of the low boiling point solvent ethanol in the activation process of CoBDC MOF by drying at relatively low temperature in vacuum oven at 60°C.

The activated CoBDC was then soaked in a solution of Quercetin in Ethanol 10mg/ml in ice bath with stirring to allow diffusion of Quercetin into the pores of our MOF and its entrapment through interaction of functional groups of Quercetin with that of the CoBDC.

Since CoBDC lacks functional groups that can be conjugated and functionalized, so functionalization of the Qu-loaded CoBDC was done by coating it with polydopamine through an easy single-step mussel-inspired oxidative polymerization of dopamine hydrochloride then the functional groups of polydopamine was easily successfully conjugated to Folic Acid through Fe^{3+} complexation coordination reaction leading to successful synthesis of FA-functionalized Qu-loaded CoBDC (Fn-Qu@CoBDC) (Zhang, Hu, et al. 2019; Shu et al. 2018).

12.2. Characterization

12.2.1. XRD Analysis

XRD pattern confirmed the successful synthesis of CoBDC showing the characteristic peaks of our synthesized CoBDC MOF provided and supported by our references (Li et al. 2015) and (Bigdeli et al. 2017) at 2θ 8.25 of intensity 438.3, 2θ 10.02 of intensity 361.67 and 2θ 16.54 of intensity 638.33. After loading, the characteristic peaks of Quercetin at 2θ 13.67 and 2θ 26.25 becomes prominent in the Quercetin-loaded CoBDC XRD pattern with very minimal shifting compared to that of Quercetin confirming successful loading. After coating and functionalization, the XRD shows alteration of the crystalline structure, thus confirming successful coating of our Qu-loaded CoBDC and successful functionalization (Figure 12.1).

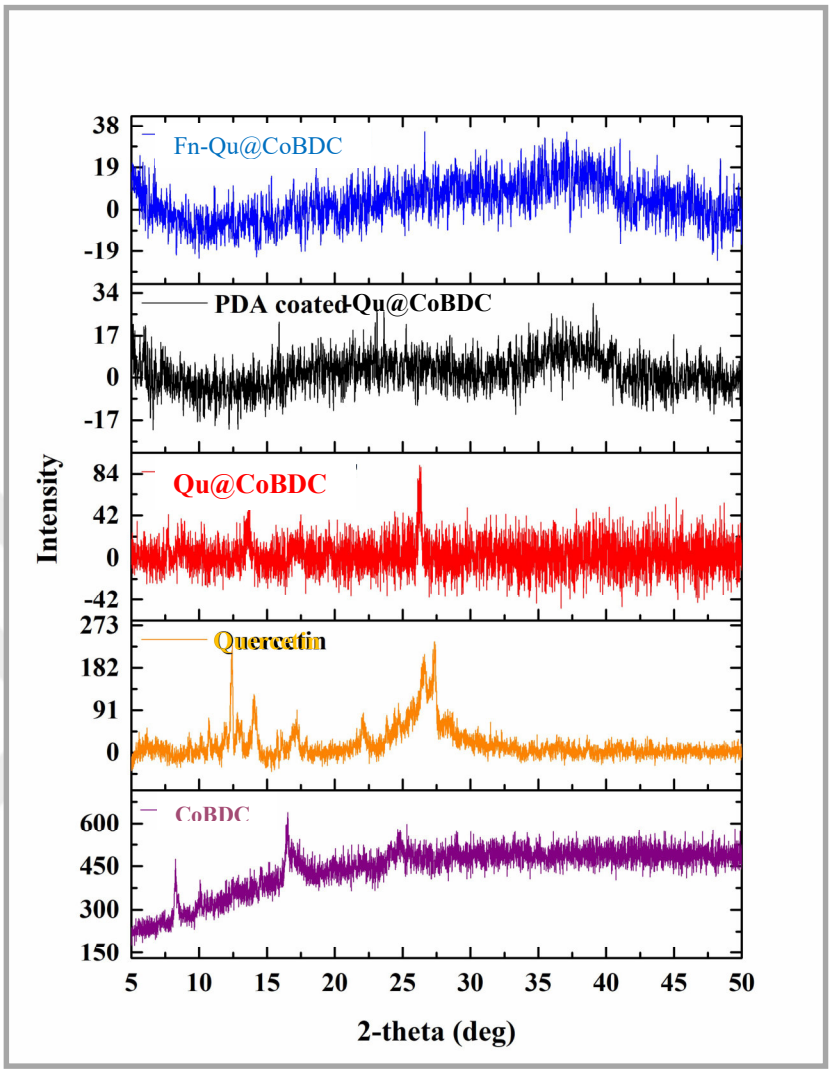

Figure 12.1. XRD of CoBDC, Quercetin, Qu@CoBDC, PDA coated-Qu@CoBDC and Fn-Qu@CoBDC

12.2.2. FTIR Analysis

The FTIR of CoBDC shows a series of bands at 742, 1104, 1360, 1578 and 1646 cm^{-1}, consistent with that of our references (Bigdeli et al. 2017) and (Li et al. 2015) and thus confirming successful synthesis of our CoBDC.

After loading, the bands of the CoBDC were dominant but with lower intensity and some broadening due to the interaction of Quercetin functional groups loaded within the CoBDC pores with that of CoBDC with the appearance of a band at 621 cm^{-1} corresponding to out-of-plane bending bands of C–H in aromatic hydrocarbon in Quercetin (Catauro, 2015), suggesting successful drug loading (Figure 12.2).

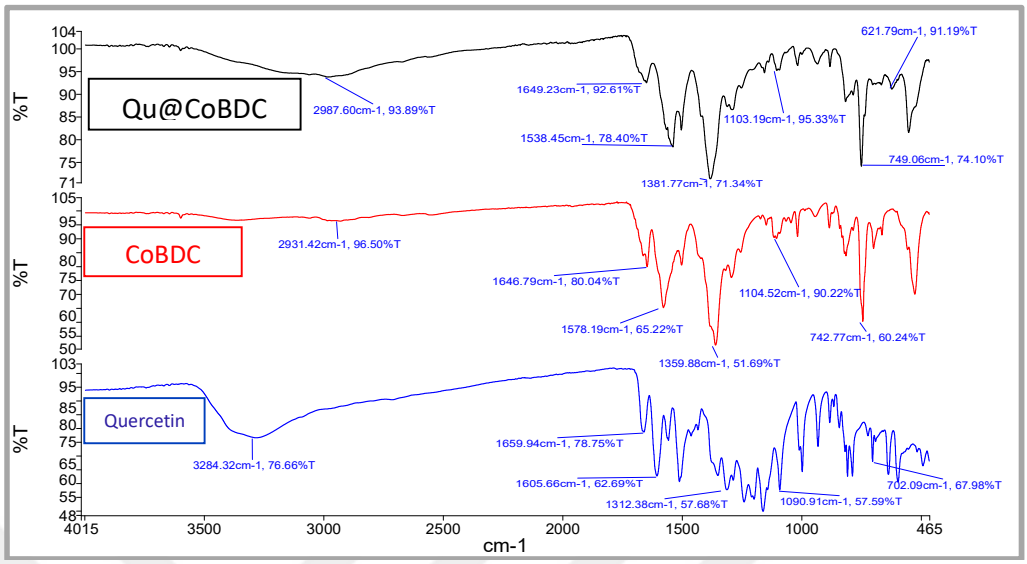

Figure 12.2. FTIR Analysis of CoBDC, Quercetin and Qu-Loaded CoBDC

After coating with polydopamine, the band at 3335 cm^{-1} becomes more prominent with higher intensity corresponding to polydopamine stretching vibrations of –OH and N–H groups (Luo et al. 2015) with alteration of the remaining bands due to interaction between the functional groups of the polydopamine with that of Quercetin-loaded CoBDC MOF. Functionalization results in lowering and broadening of the bands of polydopamine-coated Quercetin-loaded CoBDC together with diminishing of other bands confirming successful functionalization.

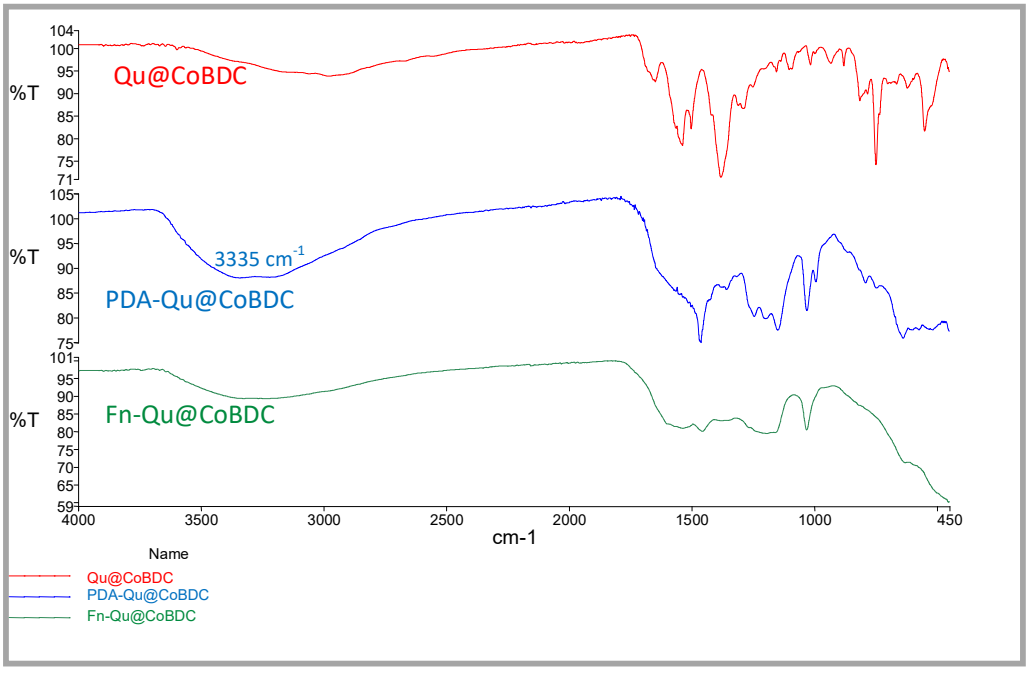

Figure 12.3. FTIR analysis of Qu-Loaded CoBDC, PDA-coated Qu-Loaded CoBDC and FA-Functionalized Qu-Loaded CoBDC

12.2.3. Electron Microscopy Analysis

SEM analysis has shown that the CoBDC synthesized by the solvothermal method has a particle size range of 188- 311 nm with an average particle size of 245 nm, which is relatively large but this is because the solvothermal method, that we used, results in large particle size. The Qu-loaded CoBDC has shown a particle size range of 135-350 nm with an average particle size of 253 nm. Because we have used sonication for dispersion of the Qu-loaded CoBDC before coating and before functionalization as well, this resulted in reduction of the particle size. The PDA-coated Qu-loaded CoBDC has shown a particle size range of 92-212 nm with an average particle size of 135 nm. The FA-functionalized Qu-loaded CoBDC (Fn-Qu@CoBDC) showed a particle size range of 52-110 nm with an average particle size of 80 nm. The particle size distribution of CoBDC, Qu@CoBDC, PDA-Qu@CoBDC and Fn-Qu@CoBDC is shown in figure 12.5. Coating resulted in a rough irregular appearance of particles. After functionalization, the margin of the particles shows slight illumination suggesting successful functionalization with Folic acid (Figure 12.4).

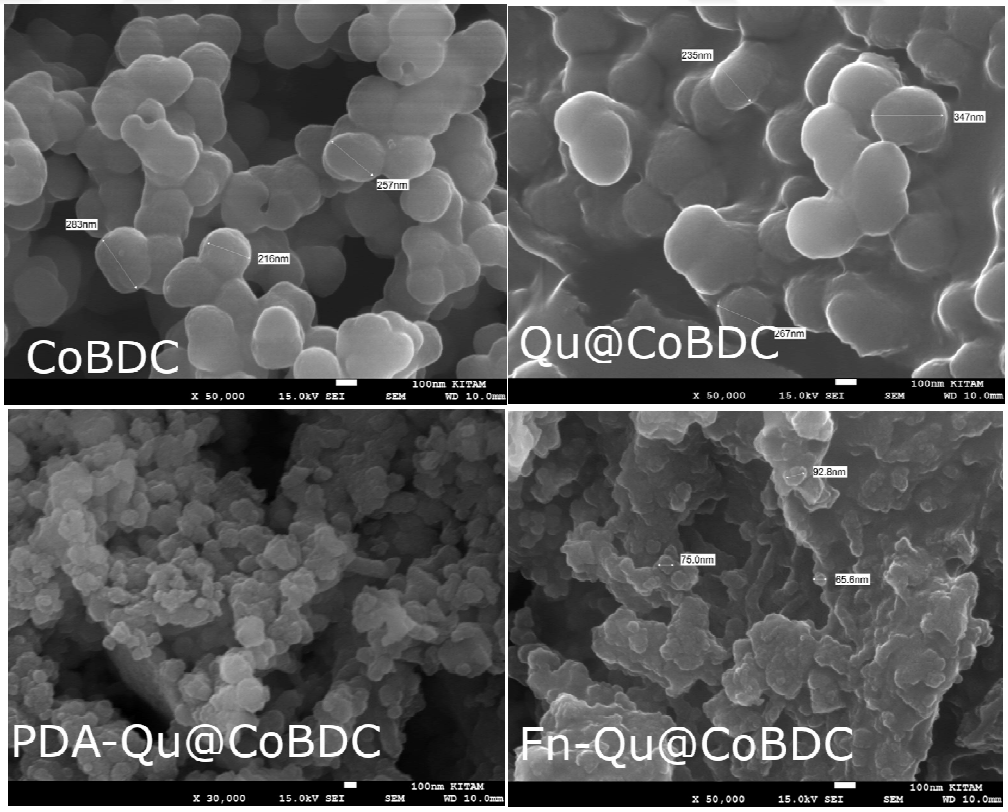

Figure 12.4. Electron Microscope Image of CoBDC, Qu@CoBDC, PDA-Qu@Loaded CoBDC and Fn-Qu@CoBDC

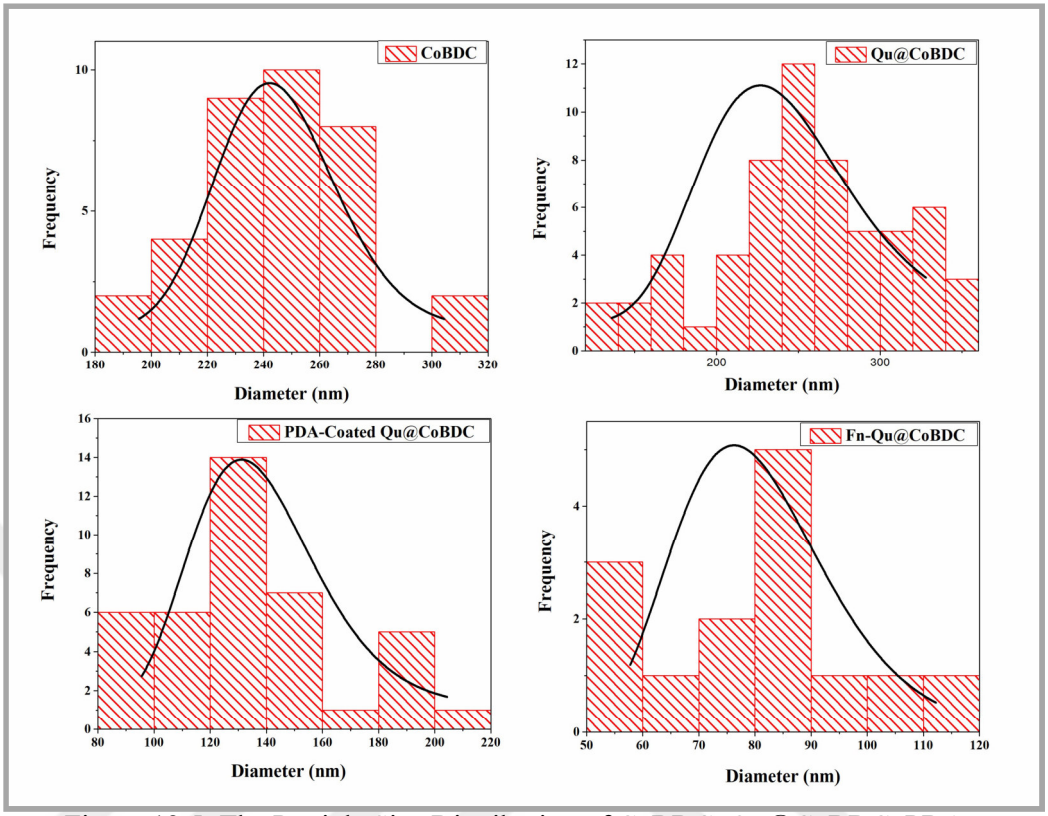

Figure 12.5. The Particle Size Distribution of CoBDC, Qu@CoBDC, PDA-Qu@Loaded CoBDC and Fn-Qu@CoBDC

EDX elemental analysis shows the detection of cobalt in the synthesized CoBDC, Qu-loaded CoBDC and PDA-coated Qu-loaded CoBDC only while being undetectable in the FA-functionalized Qu-loaded CoBDC suggesting full particle surface functionalization with Folic acid in a way that blocks cobalt detection and identification in the elemental analysis, in addition to the presence of Fe in the FA-functionalized Qu-loaded CoBDC, confirming the success of our functionalization procedure. Traces of Sulfur were present in the elemental analysis of PDA-coated Qu-loaded CoBDC and FA-functionalized Qu-loaded CoBDC due to the use of Dopasel ampoules in our coating procedure which contains sodium metabisulfite as an excipient.

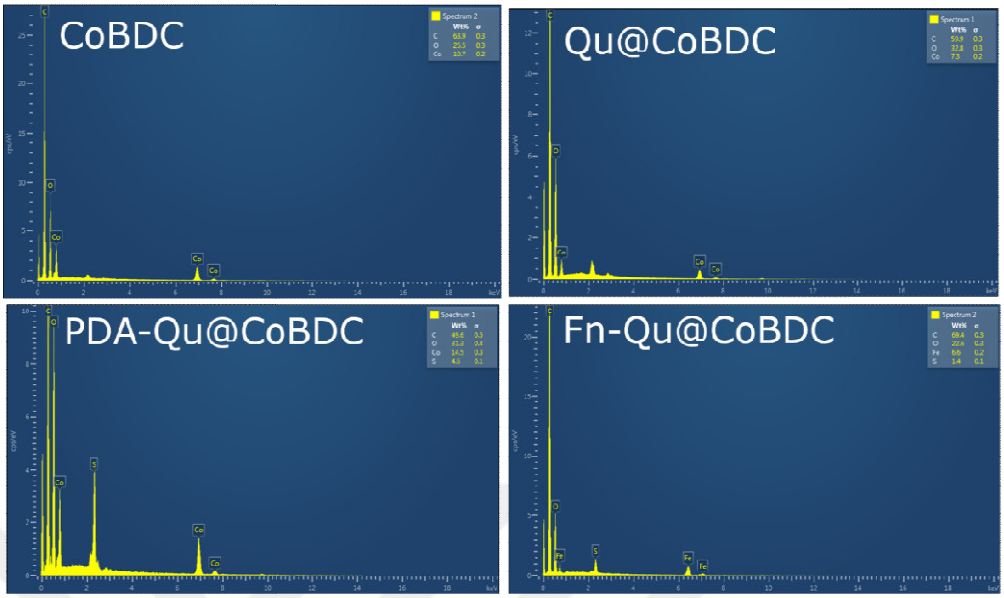

Figure 12.6. EDX Elemental Analysis of CoBDC, Qu@CoBDC, PDA-Qu@Loaded CoBDC and Fn-Qu@CoBDC

12.3. Drug Loading

The amount of loaded drug was calculated. Accordingly, the loading capacity of MOF was determined to be 51% for the first loading and 28% for the second loading. The reason for a higher loading capacity in the first loading experiment could be attributed to using a freshly prepared and freshly vacuum activated CoBDC for loading, while in our second loading experiment, the prepared CoBDC had been stored for a couple of weeks.

12.4. Drug Release Study

The cumulative drug release percent for the unfunctionalized uncoated Qu-loaded CoBDC was found to be 25% after 20 minutes and reached 31% after 6 hours in the PBS solution pH 6.6, while for the PBS solution pH 7.4, the cumulative drug release percent was 2.71% after 20 minutes and reached 5.66% after 6 hours. This emphasizes the sensitivity of CoBDC MOF to the acidic pH and the release of our drug in a pH-responsive manner (Figure 12.7).

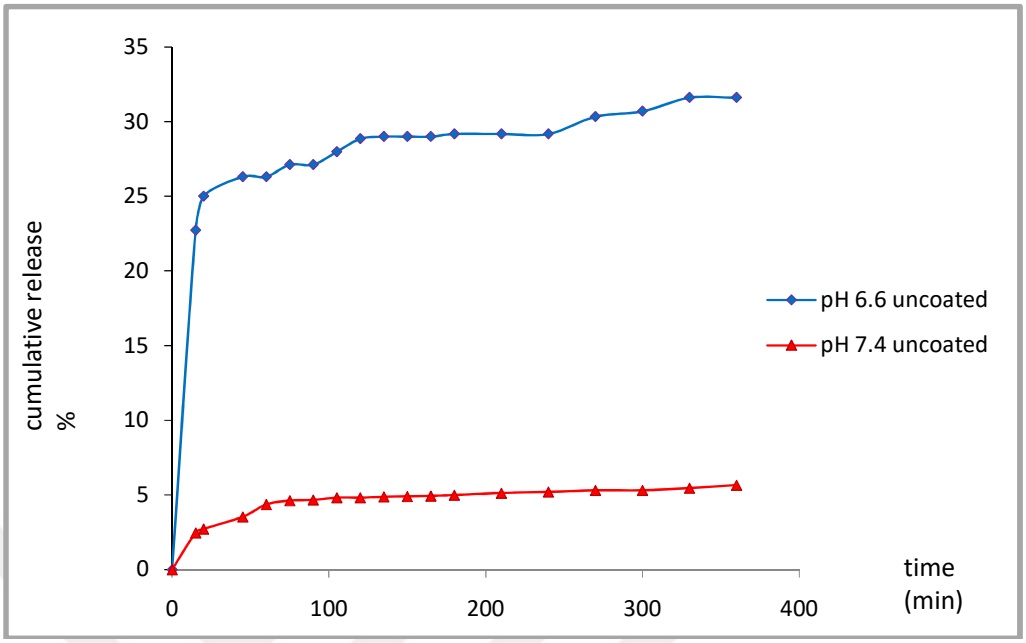

Figure 12.7. Drug Release Curves from Uncoated Unfunctionalized Qu-Loaded CoBDC

The cumulative drug release percent for our FA-functionalized PDA-coated Qu-loaded CoBDC MOF was found to be 8.23% after 15 minutes and reached 18.33% after 6 hours in PBS solution pH 6.6, while in the PBS solution pH 7.4, the cumulative drug release percent was 1.57% after 15 minutes and reached 6.24% after 6 hours, again emphasizing the pH-responsiveness of CoBDC but with a little bit delayed release due to the coating which slows down the release (Figure 12.8).

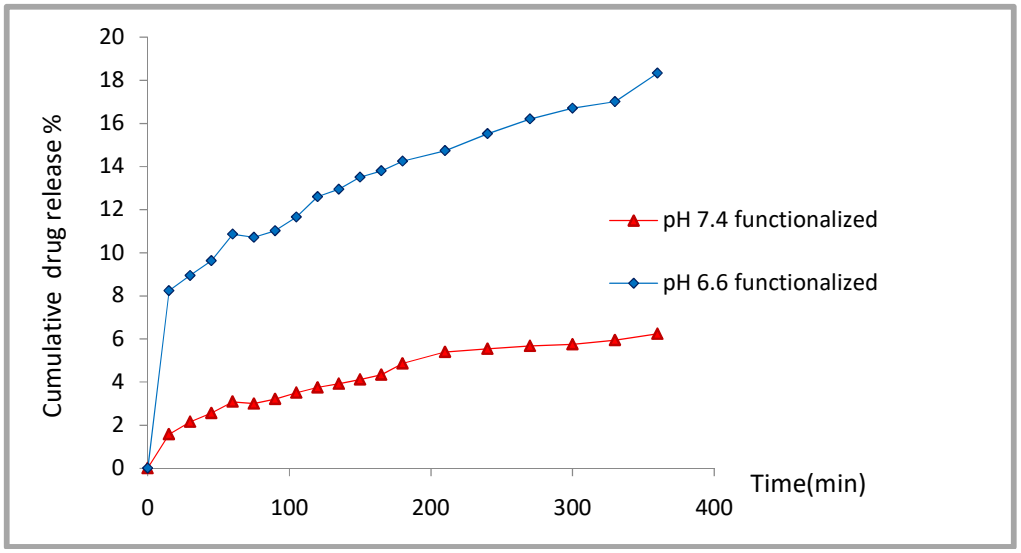

Figure 12.8. Drug Release Curves from FA-functionalized PDA-Coated Qu-Loaded CoBDC

13. CONCLUSION

The use of a naturally extracted compound like Quercetin that has anticancer effect associated with radiosensitization activity while suppressing radiation-induced skin fibrosis as a therapeutic agent is a promising approach, being commercially available at low cost and at the same time having antioxidant and anti-inflammatory effects. However, the very low solubility of Quercetin and its poor bioavailability are limiting its use. To overcome this very low bioavailability, we have successfully implied a strategy for the delivery of this very poorly soluble natural compound by encapsulating it in CoBDC nanoparticles functionalized with folic acid, to accumulate in cancer cells by both passive targeting through enhanced permeability and retention, being nanosized, and by active targeting through binding to FA overexpressed receptors. In cancer cells due to its sensitivity to the low pH characteristic to cancer cells, the CoBDC MOF is dissociated and Quercetin is released. We have successfully developed a drug delivery system for Quercetin as anticancer agent with a combined active and passive stimuli-responsive targeting approach. Using polydopamine enabled us to functionalize our CoBDC MOF using Fe^{3+}-mediated coordination chemistry which is simple and cost effective rather than having to use N, N dicyclohexyl carbodiimide (DCC) or 1-ethyl-3-(-3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) together with N-hydroxysuccinimide (NHS) for coupling and conjugation of Folic acid and the need of presence of amino group in our MOF to functionalize it (Kharkar et al. 2020). Also, polydopamine would be benificial in photothermal therapy. Moreover, polydopamine would increase the hydrophilicity and biocomptability of our synthesized drug-loaded MOF which would result in increasing the circulation time and hence the bioavailablity of our designed nanoparticulized drug delivery system.

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