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Mesoporous Titania in Local Drug Delivery

Master of Science Thesis in the Master Degree Program, Material Chemistry and Nanotechnology

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CHALMERS UNIVERSITY OF TECHNOLOGY
Gothenburg, Sweden, 2013

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1. Abstract

There is an ongoing development within the area of bone anchored implants to achieve an improved healing. An interesting approach is to administrate drugs with the aim of improving the implant integration and reducing the inflammatory responses after the surgery. One of the most recent approaches for enhancing the integration ability of implantable materials is to design a local drug delivery system. Such systems are designed to regulate the drug delivery by a sustained release at the site of healing.

In the present study, mesoporous titania thin films have been formed and applied as drug delivery matrices. The loading and releasing behaviour of different drugs using an *in vitro* method have been investigated. It is shown that by controlling the synthesis conditions and choice of the structure directing agents, the system can be engineered to fit the desired release behavior.

The evaporation-induced self-assembly (EISA) method was applied as synthesis route. Synthesis parameters such as type and amount of template, swelling agent volume ratio and aging environment were varied in order to obtain mesoporous titania thin films with variable pore sizes (3-7nm). Synchrotron small angle X-ray scattering (SAXS) measurements showed that the titania films had a long-ranged order and transmission and scanning electron microscopy (TEM and SEM) demonstrated a well-defined porous structure. X-ray diffraction (XRD) measurements displayed that the mesoporous matrices were semi-crystalline with anatase as the crystalline phase. A surface modification was performed to tune the polarity of the surface by treating the materials in dimethyldichlorosilane (DCDMS), which resulted in more hydrophobic surfaces, shown by contact angle measurements.

A successful loading of drugs into mesoporous titania films were proved by XPS and the adsorption and release behaviour for different drugs such as AMD-3100, Alendronate (ALN), Raloxifene (RLX), Strontium Ranelate (SR) and SDF-1alpha were evaluated by quartz crystal microbalance with dissipation monitoring (QCM-D). A much higher loading of drugs was demonstrated for the mesoporous samples compared to their nonporous counterpart. The drug release from the mesoporous surfaces was shown to be sustained for the investigated drugs.

Contents

2. Introduction

Titanium is an attractive choice as material for implantation purposes since it possesses unique properties such as excellent biocompatibility and mechanical suitability. ［1］ The biofunctionality of titanium implants is highly dependent on their surface topography, which can be modified in order to improve the implant osseointegration. Osseointegration refers to the anchoring of a surgical implant to the bone by the growth of bone around it leading to a firm intelocking between the bone and implant. ［2］

Several surface modification technologies exist that are applied on titanium implants to achieve a good interaction between the implant and the bone tissue ［3］. Rougher surfaces are proved to have a superior function in bone and implant interlocking. Applying a mesoporous coating on the implant surface is one method with the aim of improving the bioactive properties of the implant. [4-6］ Previous studies have highlighted the use of mesoporous silica in implantology to achieve improved bone integration. ［7, 8］

Patients suffering from bone diseases are prescribed to take drugs, the dosage form of the drug is normally the oral route, to avoid the most common problems contributed to implant replacement such as failure to fixation to tissue and inflammatory/inflectional responses after surgery. [9]

Delivery of drugs by means of controlled release technology began in the 1970s and has continued to expand rapidly. [10] A recent approach in nanomedicine is by implementing the drugs into mesoporous materials followed by a sustained and controlled local release. This will result in preventing the unwanted distribution of the drugs throughout the body, minimizing the side effects, improving the therapeutic efficiency as well as reducing toxicity and the cost for the patient in the case of expensive drugs. ［7, 8, 11, 12］

Large surface area, large pore volume and relatively narrow pore size distribution (2-50nm) are significant properties of mesoporous materials, which enable them to act as drug reservoirs. Moreover, tunable pore size, tailorable textural properties and possibility of functionalization of the pore walls makes mesoporous titania a suitable host of variety of drug molecules. [11]

In this present study, the synthesis of mesoporous titania was regulated to design efficient drug delivery carriers which can be later applied on implantable materials and improve their integration ability. Cubic mesoporous titania with variable pore sizes (3-7nm) was obtained by the evaporation-induced self-assembly (EISA) method with and without modification of the surfaces by silanization. The properties of the films were evaluated by several characterization methods such as transmission and scanning electron microscopy (TEM and SEM), X-ray diffraction (XRD), Synchrotron small angle X-ray scattering (SAXS) and contact angle measurements. Different types of drugs such as AMD-3100, SDF-1alpha, Alendronate (ALN), Raloxifene (RLX) and Strontium Ranelate were loaded and released from both hydrophilic and hydrophobic mesoporous titania. The loading and releasing behavior of drugs from these surfaces were monitored and studied by quartz crystal microbalance with dissipation monitoring (QCM-D). XPS measurements were performed in order to confirm a successful loading of drug into mesoporous titania surfaces.

3. Materials and methods

3.1 Synthesis of mesoporous titania

Cubic mesoporous titania with different textural properties were synthesized by the evaporation-induced self-assembly process. A precursor solution was obtained by adding titanium (IV) ethoxide (TEOT, 20% Aldrich) to concentrated hydrochloric acid (HCl, 37 % Aldrich) under stirring forming a homogenous solution. The acid helps preventing TiO_2 from fast sedimentation and let it hydrolyze in the precursor solution. To form a variety of pore sizes, different types of templates such as Pluronic P123 (triblock copolymer EO20PO70EO20, Aldrich), CTAB (CH_3 $(CH_2)_{15}N$ (Br) $(CH_3)_3$, Aldrich) and Brij-S10 ($C_{18}H_{37}$ $(OCH_2CH_2)_nOH$, Aldrich) were dissolved in ethanol under vigorous stirring. The template solutions were added to the precursors and the final solutions were left stirring over-night to achieve homogeneity homogenous mixture. The specific amount of each species to form a cubic structure is shown in Table 1. These amounts are obtained from surfactant to water ratio giving a cubic phase in water-surfactant binary phase diagram of each template (figure 1). [13]

Table 1: The type and amount of species to make cubic mesoporous titania.

Template	Template (g)	TEOT (g)	HCl (g)	Ethanol (g)
P123	0.5	2.1	1.6	8.5
CTAB	0.74	2.1	1.65	12
Brij-S10	0.52	2.1	0.7	12

To obtain uniform films of mesoporous titania, 100μl of the final solutions were spin-coated for 1min on glass slides and titanium discs (8mm diameter and 3mm thickness). The spin-coating speed was 7000r.p.m for all the samples. The coated disks and glass slides underwent different aging processes from 1 to 7 days to allow the self-assembly and cross-linking of titanium oxide films. The films were calcinated by heating to 350° C with a ramping temperature of 1C/min to remove the templates and allow more cross-linking of the titania framework.
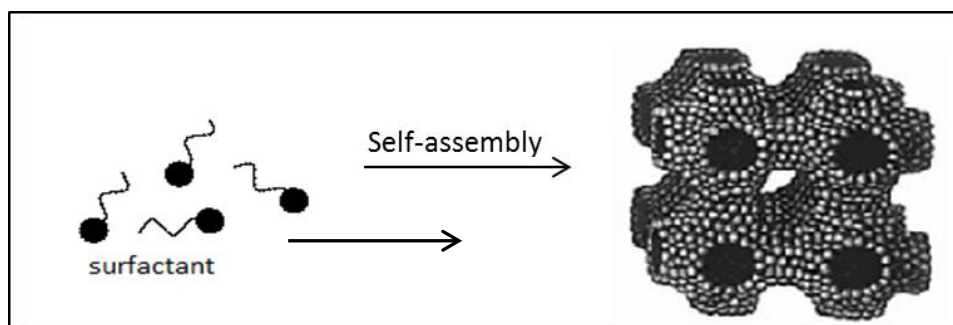
Figure 1: A schematic illustration of a cubic structure formed by surfactant self-assembly.

3.2 Tuning the pore size

The pore diameter of mesoporous titania with the purpose to serve as a drug delivery system is a crucial factor. For instance it determines how big the drug molecule could be in order to be hosted. One important factor to direct the pore size is the type of template used in the synthesis route. Surfactants with increased chain length produce materials with bigger pores and vice versa. In this work, three types of templates were used (P123, Brij-S10 and CTAB) which resulted in specific diameters ranged from 3 to 6nm. To enable loading of bigger molecules, such as proteins, into the mesoporous matrices a larger pore size is desired. This can be done by the use of swelling agents. Swelling the surfactant micelles by the aid of an organic additive such as poly propylene glycol (PPG) gives bigger pores up to 7.2nm when combined with P123 [14, 15]. Here, the titania sols were prepared by the same synthesis procedure as cubic mesoporous titania with P123 as template and in order to regulate the pore sizes, two different volume ratios of poly propylene glycol (PPG) as swelling agent was added (see Table 2) and after two hours of constant stirring the solutions were spin-coated on glass slides and titanium discs followed by aging at moderate humidity environment (RH=54%) provided by saturated KNO3 aqueous solutions in a refrigerator (T=4±1˚C) and eventually the samples were calcinated in order to remove the template and PPG.

Table2: The amount of each component to make mesoporous titania, when adding swelling agent

PPG:P123 (volume ratio)	1:1	0.5:1
PPG (gr)	0.65	0.32
P123 (gr)	0.65	
TEOT (gr)	2.1	
HCl (gr)	1.53	
Et-OH (gr)	6	

3.3 Procedures for improved self-assembly

3.3.1 Aging environment and time

It is desirable for mesoporous matrices to have a high degree of periodicity in their pore structure to control the performance. Aging environment is one of the parameters that can be tuned to obtain more order in the material. Prolonged aging under controlled humidity can increase the periodicity of the porous structure [16]. The films were aged in a sealed chamber containing saturated NaCl solution (RH=70%). The aging time was extended from 1 day to 7 days, which was expected to reveal higher degree of ordering in the mesoporous structure by prolonged exposure time to the humid environment. [17]

3.3.2 Calcination process

Different calcination atmospheres can readily affect the degree of ordering of mesoporous matrices. N_2 added to the calcination atmosphere can result in a more ordered mesostructure compared to calcination performed in air [18]. In this work a N_2 gas flow was added to the oven during the calcination process.

3.4 Surface modification

The surface energy of mesoporous titania is a parameter that can be altered in order to enhance the affinity to water-insoluble drugs and reduce the attachment of the polar drugs. Modifying the mesoporous titania surfaces using an organosilicon compound give them a more hydrophobic character which is expected to alter the drug delivery process.

Glass slides and QCM-D discs coated with mesoporous titania thin films were treated in 5 wt-% dimethyldichlorosilane (DCDMS) in methanol solution for one hour. In order to make the surface less polar, the mesoporous titania coated on glass slides were pre-treated in water baths in order to graft more hydroxyl groups on the surfaces. The glass slides was then placed in DCDMS solution for 15 min and thereafter flushed with chloroform and followed by another 45min treatment in DCDMS. ［19, 20］ The films were dried with nitrogen gas. Contact angle measurements were performed to evaluate whether the surface modification was successful. The QCM-D discs were also modified by silanization to analyze whether the hydrophobic surfaces could affect the adsorption and release rate of drugs compared to hydrophilic surfaces.
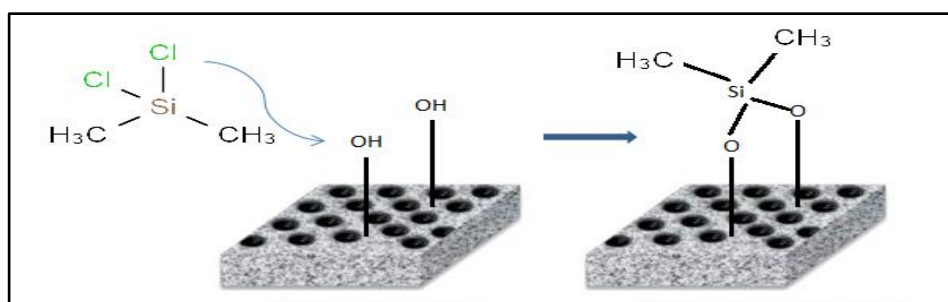

Figure 2: Schematic of the silanization of mesoporous titania using 5 wt.-%DCDMS

3.5 Drugs

Alendronate (ALN), Raloxifene (RLX), Strontium Ranlate (SR), AMD-3100 and SDF-1alpha are the five different drugs evaluated in this study and tested when using mesoporous titania as drug delivery system. ALN, RLX and SR are all osteoporosis drugs which have been shown to increase the bone mass density and have a positive effect in reducing the bone fracture. [21, 22] AMD-3100 can act as a stem cell mobilizer and it is used to stimulate the release of stem cells from the bone marrow into the blood. [23] Stromal cell-derived factor SDF-1alpha (CXCL12) is a small protein cytokine belonging to the chemokine family produced by a variety of tissues, including bone marrow stromal cells. [24] The drug solution concentration used in this study is shown in Table 3 and the molecular structures of the drug candidates are shown in figure 3.

Table 3: Molar mass and drug solution concentrations of the drugs used in this work.

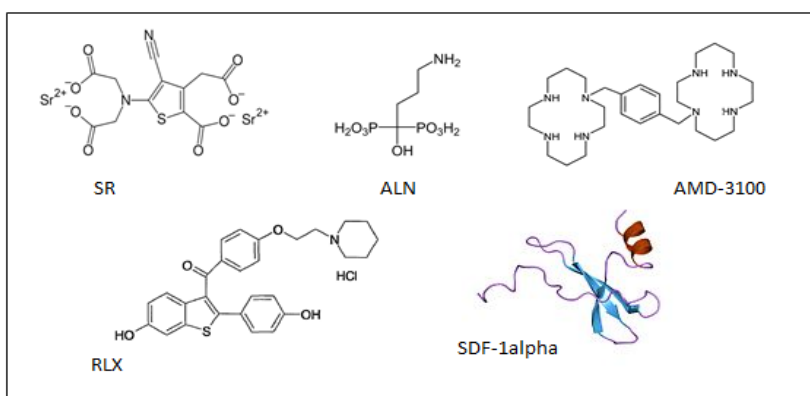| Drug	Solvent	Concentration (mg/ml)	Molar mass (mg/mol)
ALN	water	0.8	352.12
RLX	methanol	0.8	473.58
SR	water	0.8	513.491
SDF-1alpha	water	$0.8*10^{-3}$	$8*10^3$
AMD-3100	water	0.4	795.5

Figure 3: Molecular structures of drug molecules used in this work.

3.6 Material characterization

3.6.1 Scanning electron microscopy

In scanning electron microscopy (SEM), a focused beam of electrons scans over the solid sample surface and a high resolved image is created by detecting the signal from the secondary electrons that leave the surface. [25] In this study a Leo Ultra 55 electron microscope was used in order to obtain morphological information of the mesoporous titania coating as well as to estimate the pore size.

3.6.2 Transmission electron microscopy

In transmission electron microscopy (TEM), microstructural information of the sample can be revealed when the accelerated high energy beam of electron pass through the ultra-thin specimen and create a high resolution image on a fluorescent screen. ［26］ In this study the samples were prepared by scraping off the mesoporous titania from coated glass slides. The collected powder was dispersed into ethanol (proof 200). Droplets of the dispersion were added on the TEM grids (carbon 300 mesh, caspilor, Sweden) and left to dry before inserting the grids into the sample holder of the microscope. The TEM used was a JEOL-1200EX II, operated at 120 kv (JEOL Tokyo, Japan).

3.6.3 Synchrotron small angle X-ray scattering

By means of small-angle x-ray scattering (SAXS) the structural information of mesoporous materials can be revealed by the scattering of X-ray beams at low angles ($<10°$). Existence of long range orders and determination of pore sizes can be attained from scattering patterns obtained from the SAXS measurements. By using this method it is possible to determine the phase of the mesoporous matrix. The mesoporous titania was grinded into powders and inserted to the SAXS sample holders. The SAXS measurements were performed at MAX-lab, beam station 1911 (Lund, Sweden)

3.6.4 X-ray diffraction

X-ray Diffraction (XRD) is a method to determine the crystalline structure of a sample. When exposing a specimen to an X-ray beam, diffraction will occur from the planes in the crystallites, which happen to be oriented in the correct angle to fulfill the Bragg condition, (Equation 1) where d is the lattice spacing, θ is the angle and λ is the wavelength of the incident beam [27]. The results obtained from XRD were compared and matched against the patterns of known crystalline phases in the database MINCRYST.

Equation 1 (Bragg's equation): $n\lambda = 2d\sin\theta$

3.7 Drug loading and release evaluation

3.7.1 X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy (XPS) also known as electron spectroscopy for chemical analysis (ESCA) is a surface analysing technique based on irradiating the sample with low energy X-ray and determining the bonding energy of the photoelectrons leaving the surface. The amount of bonding energy together with the intensity of the peaks, allows quantitative analysis of elements present on the surface as well as elemental identity and chemical state of surface components. [28]

In this study, XPS was used to verify whether the drug loading was successful into mesoporous titania surfaces for the different drug candidates.

3.7.2 Quartz crystal microbalance with dissipation monitoring

The quartz crystal microbalance with dissipation monitoring (QCM-D) is a mass sensitive analytical technique, which is widely used in the field of biomaterials, cell adhesion, material science and biophysics. [29, 30] In QCM-D a thin quartz crystal disc is attached to a pair of gold electrodes, which causes the piezoelectric crystal to oscillate due to appliance of an AC voltage over the electrodes. The outcome of the experiment is the changes in the resonance frequency (Δf) as a function over time which can be converted into mass adsorption or desorption by applying the Sauerbrey equation (equation 2) The equation implies on a linear relationship between frequency and the mass adsorbed where C is the mass sensitive constant, C=17.7ng.cm^{-2}.HZ^{-1}. [25]

Equation 2 (Sauerbrey equation): $$\Delta m = -C \times \frac{1}{n}\Delta f$$

In addition Δf, which is a mass dependent property, this technology enables the measurement of an energy dependent parameter called dissipation of the system (ΔD), which is correlated to the viscoelastic properties of the adsorbed layer. [31]

In this study, QCM-D was used to monitor the absorption and release behavior of different drugs from the surfaces of mesoporous titania (hydrophilic and hydrophobic surfaces) spin

coated on 14mm quartz crystal discs (Q-sense AB, Gothenburg, Sweden). In the measurements with Alendronate, the rinsing media was changed to buffer to observe the changes in releasing behavior compared when using milli-Q H_2O as media. The buffer used was Phosphate buffered saline (PBS, PH= 7.4)

By the aid of QCM-D, the accessible volume of the pores that can be filled with water was also calculated by the amount of absorbed deuterium oxide (D_2O) on the mesoporous titania thin films.

All frequency shifts presented are based on data recorded at the 7th overtone and the collected data were processed using Q-tools software (Q-sense).

4. Results

4.1 Surface evaluation

The images obtained from SEM illustrate a porous structure for all the different mesoporous titania films formed on titanium discs, figure 4. Some periodicity in the pore alignments was detected and the pores are pointing out from the surfaces. Cross-sections of the coatings was also visualized with SEM, images shown in figure 5, this to measure the film thickness. The film thicknesses were 250 nm for the films synthesised with the templates P123, CTAB and Brij-S10, and for the films produced by adding PPG as swelling agent together with P123 the obtained thicknesses were 750 nm.
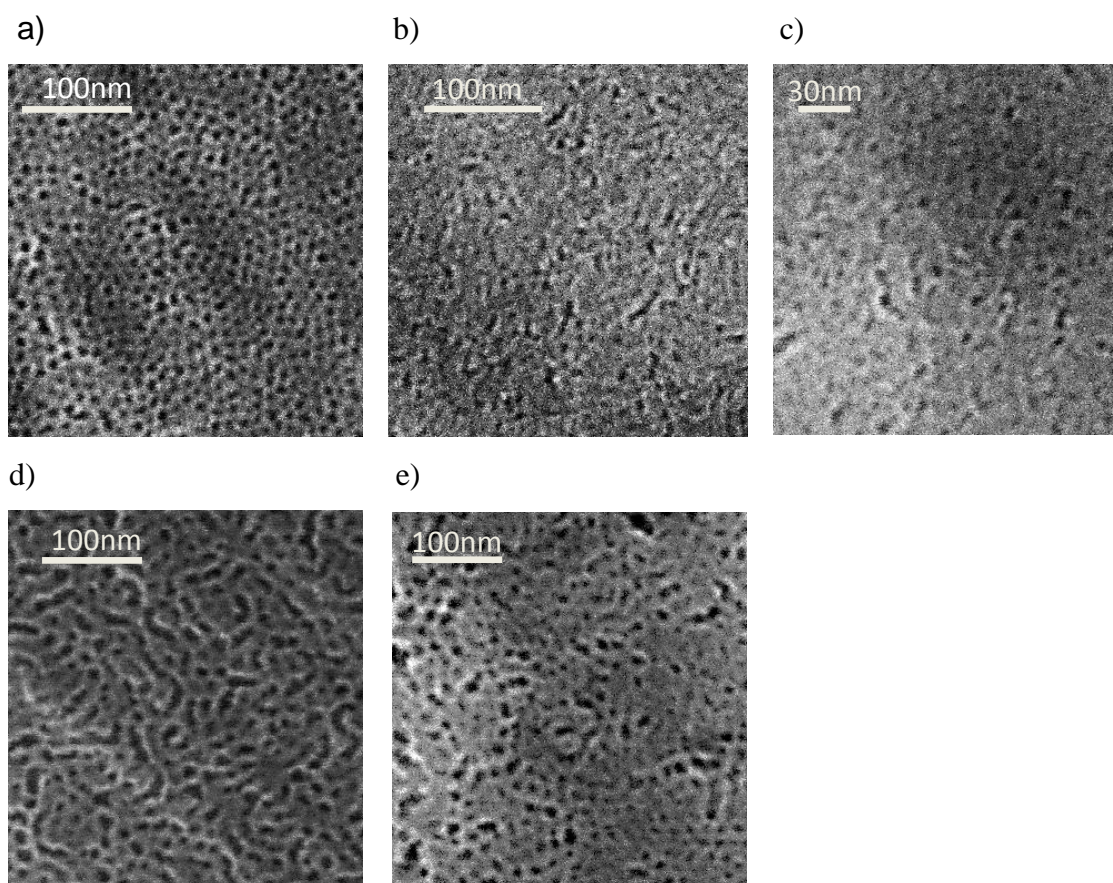

Figure 4: SEM images of mesoporous titania using different templates a) P123 (6 nm), b) C-TAB (4.4 nm), c) Brij-S10 (3.4 nm), d) P123 with PPG, 1:0.5 (7.2nm) e) P123 with PPG, 1:1 (6.8 nm).
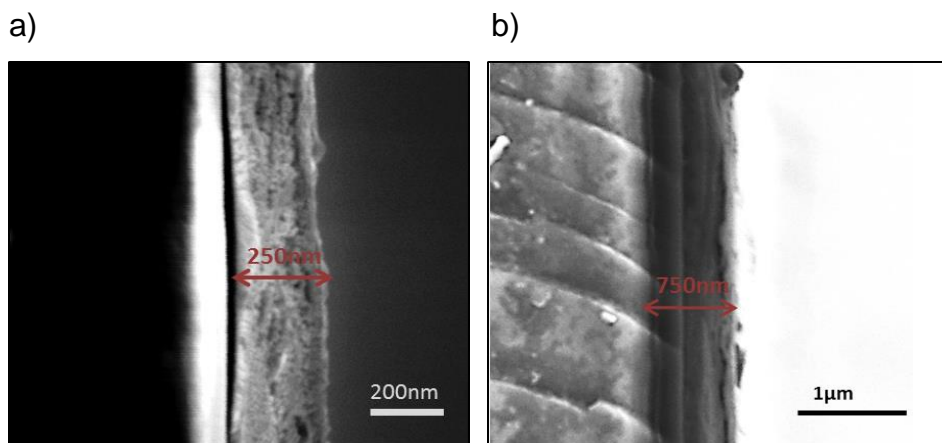
a)   b)

Figure 5: SEM images showing the mesoporous titania coating cross-section on glass slides for a) film with the pore size 6nm and b) film with the pore 7.2nm

The images obtained from TEM, for the different mesoporous titania films are shown in figure 6.

The size of the pores measured by the aid of both TEM and SEM images show similar results which range from 3.4 nm to 7.2 nm corresponding well to the size of templates used in each synthesis see Table 3.

Table 4: Estimated average pore sizes calculated from SEM and TEM images.

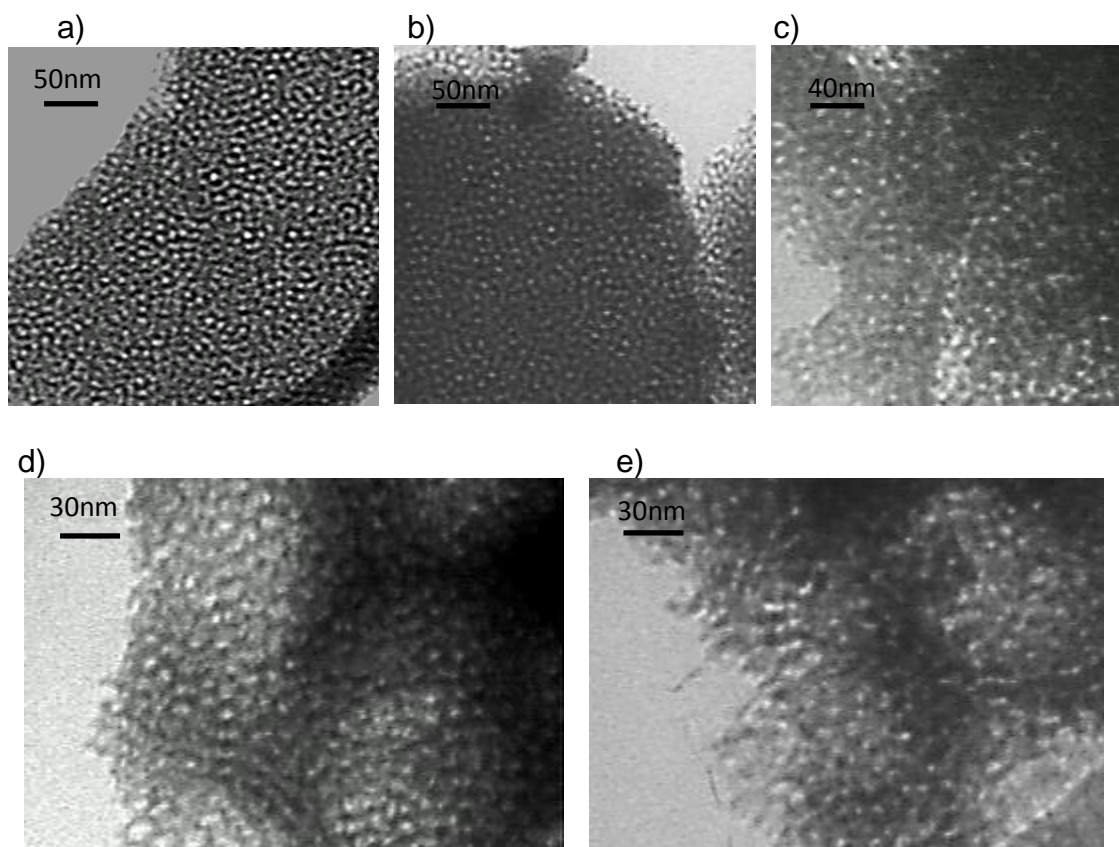| Template	Average pore size (nm)
Brij-S10	3.4
CTAB	4.4
P123	6
P123 + PPG (1:1)	7.2
P123 + PPG (1:0.5)	6.5

Figure 6: TEM images of mesoporous titania synthesied by different templates: a) P123, b) C-TAB, c) Brij-S10 and with adding swelling agents: d) P123 + PPG (1:1) and e)P123 + PPG (1:0.5).

With the purpose of confirming the cubic structure of the mesoporous titania films and existence of long range orders of the pores in the film, SAXS measurements were performed. As shown in figure 7, the results illustrate peaks appearing at different positions for the different cubic mesoporous titania powders while nonporous samples did not show any peak in SAXS measurements, as expected.

An anatase semi-crystalline phase was detected using XRD for all the films with different pore sizes (figure 8).
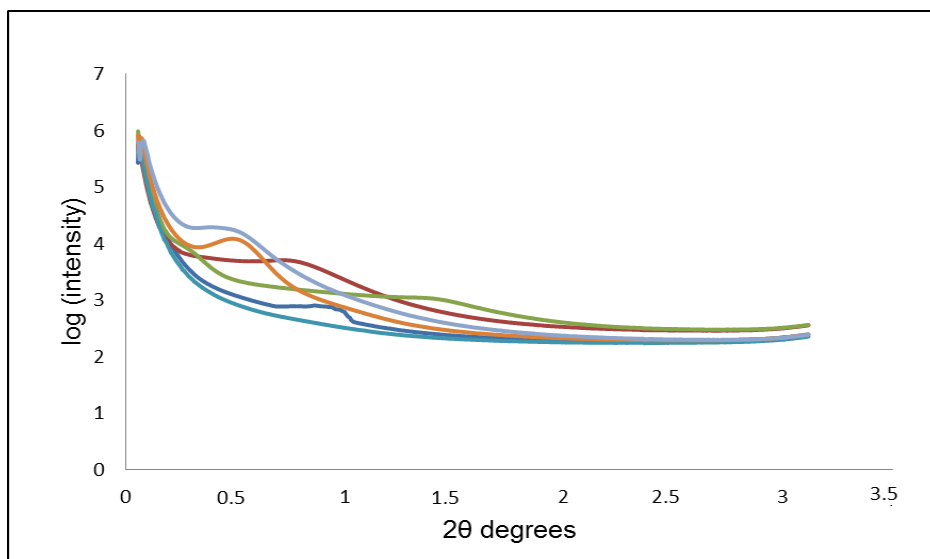
Figure7: SAXS results for the different mesoporous titania matrices synthesized by different templates.
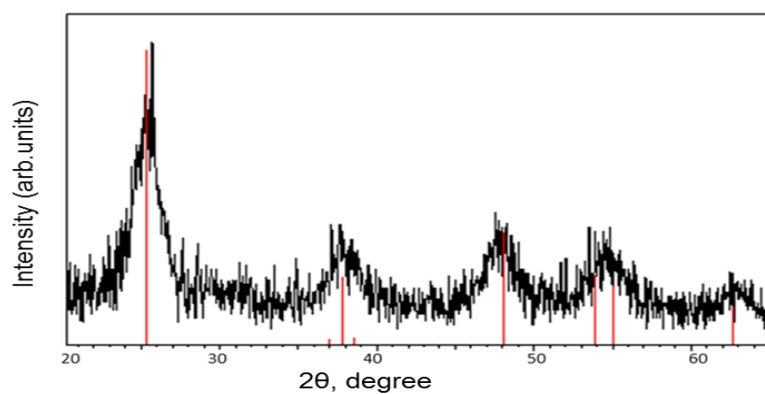

Figure 8: XRD patterns obtained from mesoporous titania powders shows anatase phase according to #191 (MINCRYST).

4.2 Surface energy evaluation

According to the results obtained from contact angle measurements, the surfaces of mesoporous titania are super hydrophilic (<5˚) and modifying the surface by grafting silanol groups on it results in much higher contact angles, see Table 5.

Table 5: Contact angle measured by mili-Q water before and after silanization.

Template	CA before silanization	CA after silanization
P123	5˚	80˚
C-TAB	4˚	85˚
Brij-S10	Too small to measure	85˚
P123+PPG (1:1)	Too small to measure	110˚
P123+PPG (1:0,5)	3.5˚	107˚
none	7.5˚	100˚

4.3 Pore volume measurement

The QCM-D data collected from absorption of deuterium oxide on the mesoporous titania coatings and compared to the nonporous counterparts together with the film thicknesses measured by SEM, were used to calculate the amount of the deuterium oxide absorbed into the pores. Subsequently, the accessible pore volume that can be filled with water compared to the whole film volume was calculated and is reported in Table 6. The calculated pore volumes were larger for the coating having a larger pore size. Moreover, the surface modification slightly decreased the pore volume capacity of the coatings.

Table 6: Pore capacity for mesoporous titania thin films calculated from D_2O experiements.

template	Pore volume percentage	
	Hydrophilic films	Hydrophobic films
P123+PPG(1:1)	75%	71%
P123+PPG(1:0,5)	60%	57%
P123	57%	55%
CTAB	55%	49%
Brij-S10	42%	37%

4.4 Drug loading and release results

4.4.1 XPS results

The results from XPS show a successful loading of the drug molecules into the mesoporous titania surfaces. According to Table 7 there are specific elements detected which can be found in the structure of each drug molecule (figure 2). These data confirm the presence of the drug molecule on the mesoporous titania surfaces.

Table 7: XPS results from loading the drugs into mesopoorus titania coated titanium discs

Mesopoorus titania + drug	C1s	N1s	O1s	Ti2p	P2p	S2p
MP Titania (ref)	16.28	-	58.75	24.26	-	-
MP Titania + SDF	17.55	1.78	56.02	23.75	-	-
MP Titania + AMD3100	15.94	1.17	58.58	23.75	-	-
MP titania + ALN	13.73	2.40	60.25	19.40	4.23	-
MP titania + RLX	18.91	2.72	14.41	19.21	-	2.68
MP titania + SR	18.64	0.85	54.13	18.64	-	-

4.4.2 Drug loading and release rate

Quartz crystal microbalance with dissipation (QCM-D) was used to monitor the absorption and release behaviour of different drugs from both modified and non-modified mesoporous titania surfaces with different pore sizes as shown in figures 9-13.

Figure 9 demonstrates the loading and releasing behaviour of Alendronate from hydrophilic and hydrophobic mesoporous titania surfaces. Hydrophobically modified surfaces give rise to a faster release rate.
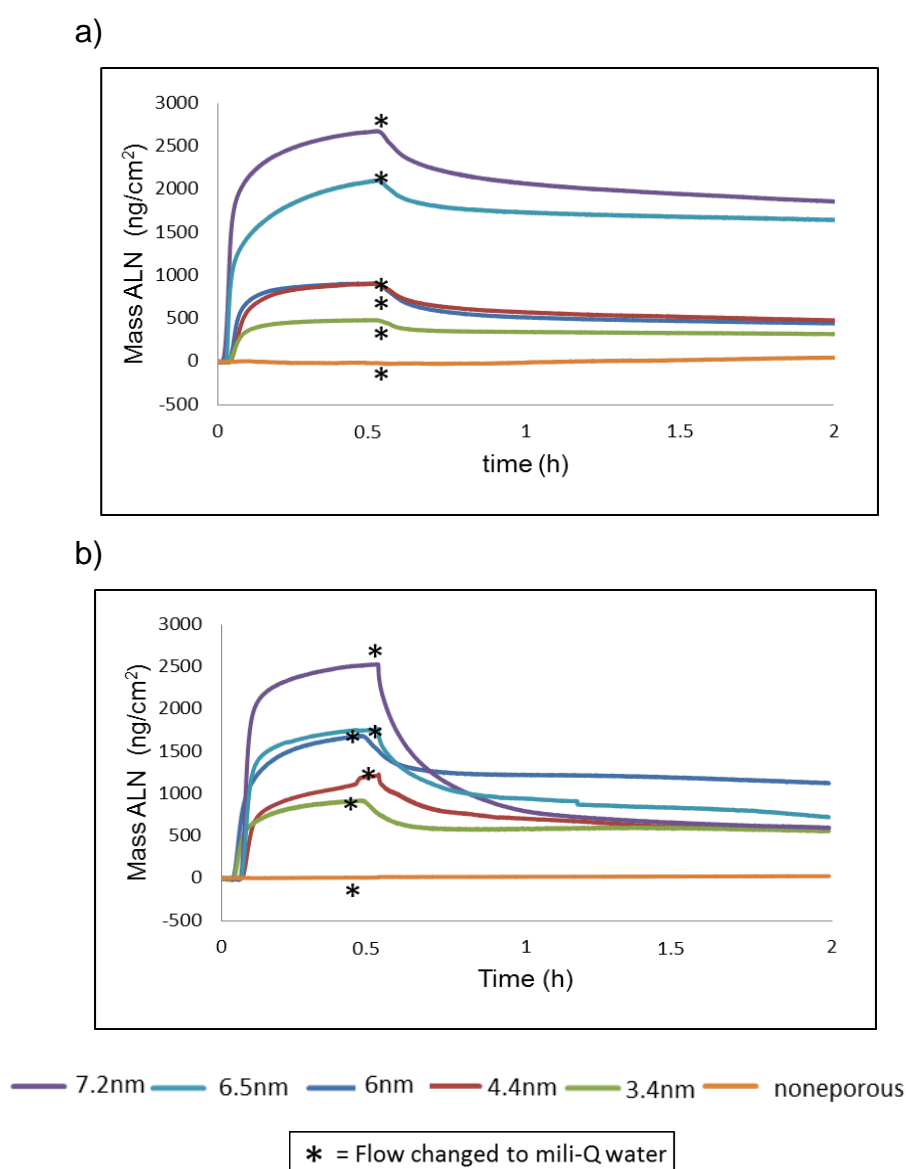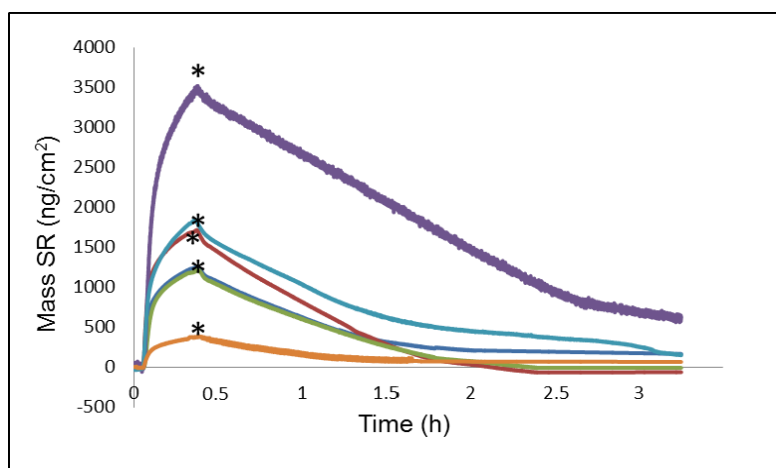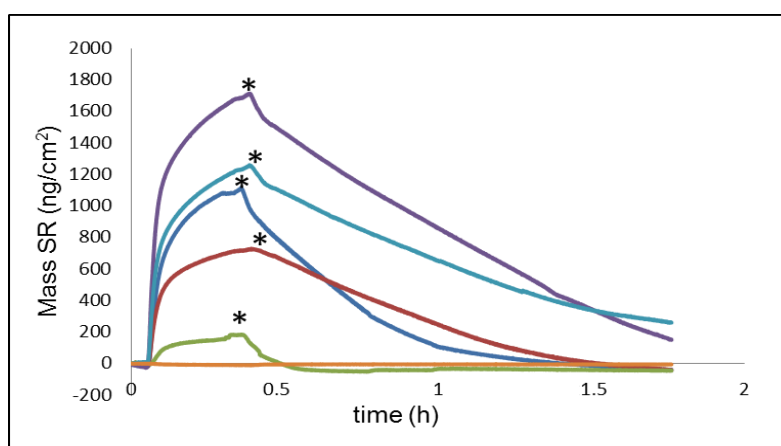

Figure 9: QCM-D results showing the absorption and release of Alendronate from a) hydrophilic and b) hydrophobic mesoporous titania thin films.

According to figure 10, Strontium Ranelate shows a different release behaviour compared to what was seen for Alendronate. The loading of the drug is much higher into the hydrophilic matrices and a slower release was displayed for the hydrophilic surfaces.
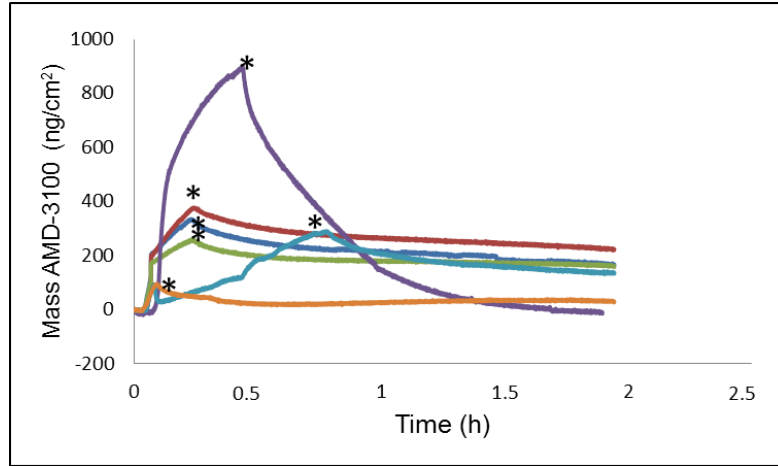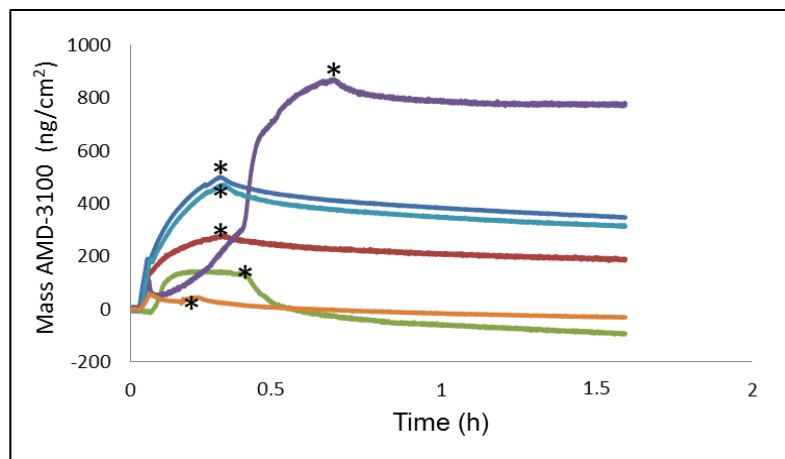
a)

b)

Figure10: QCM-D results showing Strontium Ranelate absorption and release from a)hydrophilic and b) hydrophobic mesoporous titania thin films.

The release behaviour of the unpolar drug AMD-3100 is shown in figure 11. An extremely slow release of AMD-3100 was observed from hydrophobic surfaces while the release behaviour from hydrophilic surfaces is much faster.
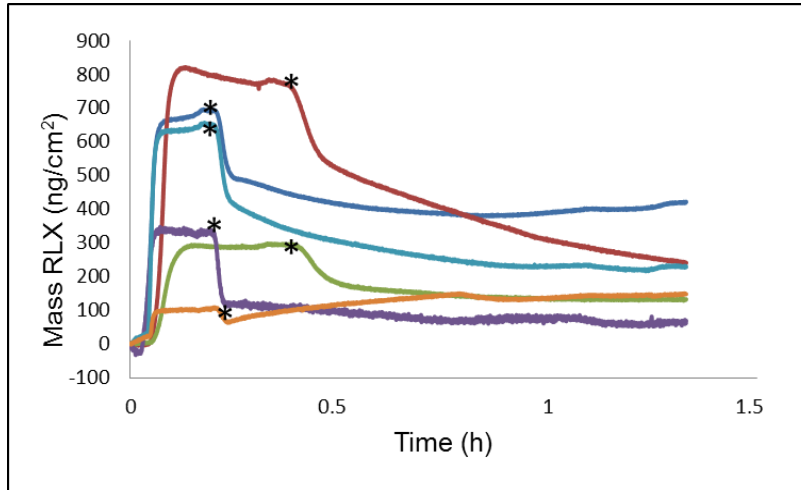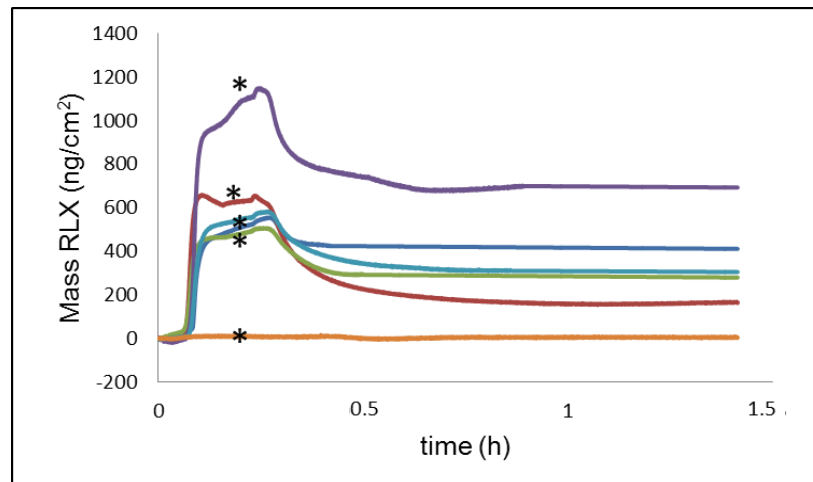
a)

b)

Figure 11: QCM-D results showing AMD-3100 absorption and release from a) hydrophilic and b) hydrophobic mesoporous titania thin films.

The loading of RLX (figure 12), being a small molecular hydrophobic drug, was shown to be high on the hydrophobic surfaces. The release was sustained from these surfaces. The hydrophilic surfaces had also a sustained release of the drugs but with faster release kinetics compared to release from the hydrophobic surfaces.

a)

b)

Figure 12: QCM-D results showing Raloxifene absorption and release from a) hydrophilic and b) hydrophobic mesoporous titania thin films.

SDF-1alpha is a protein and its size is bigger compared to the other evaluated drugs. In figure 13, it can be seen that a significant increase in the loading into the biggest pore size (6.5 and 7.2nm) is obtained and the releasing patterns are very similar for the modified and non-modified surfaces.
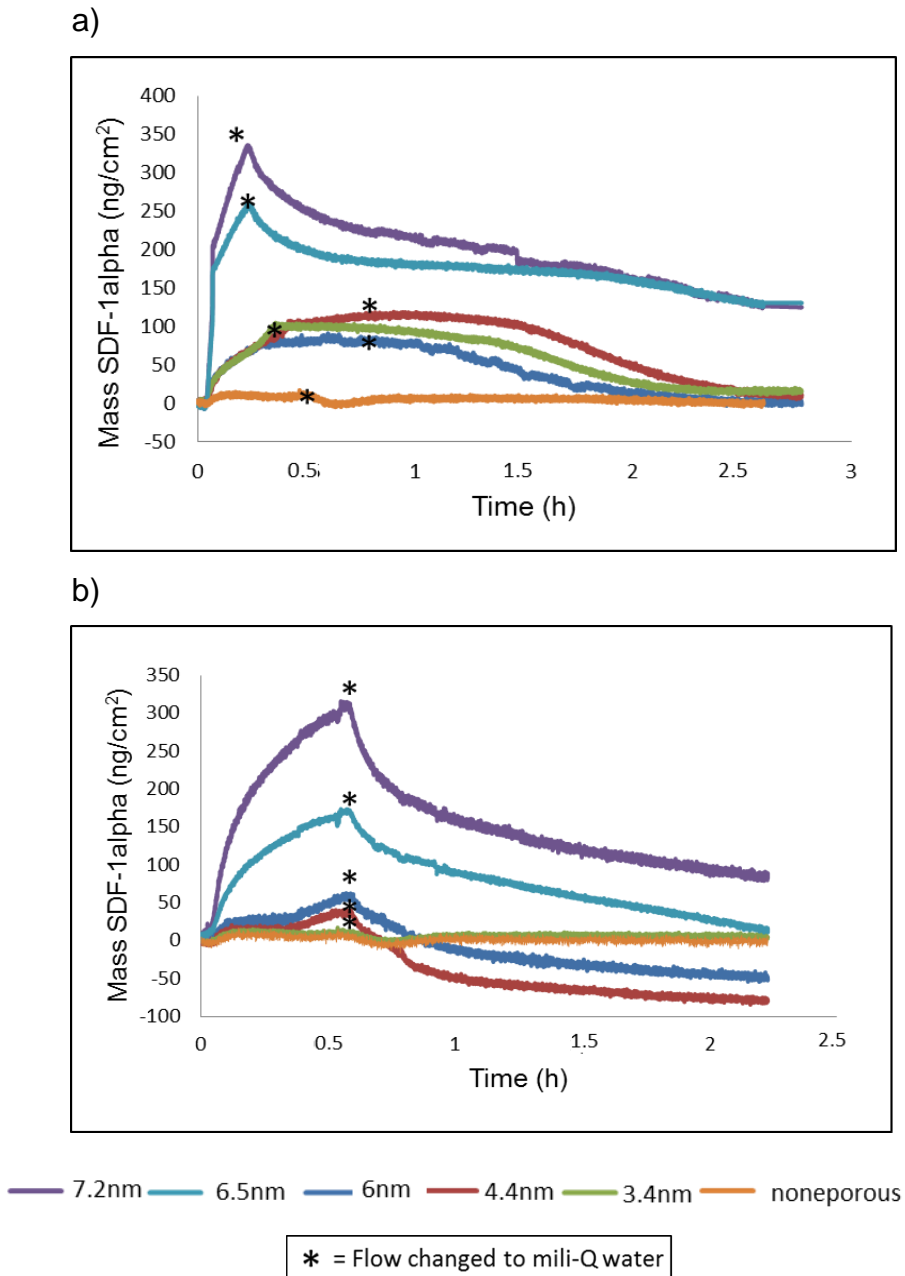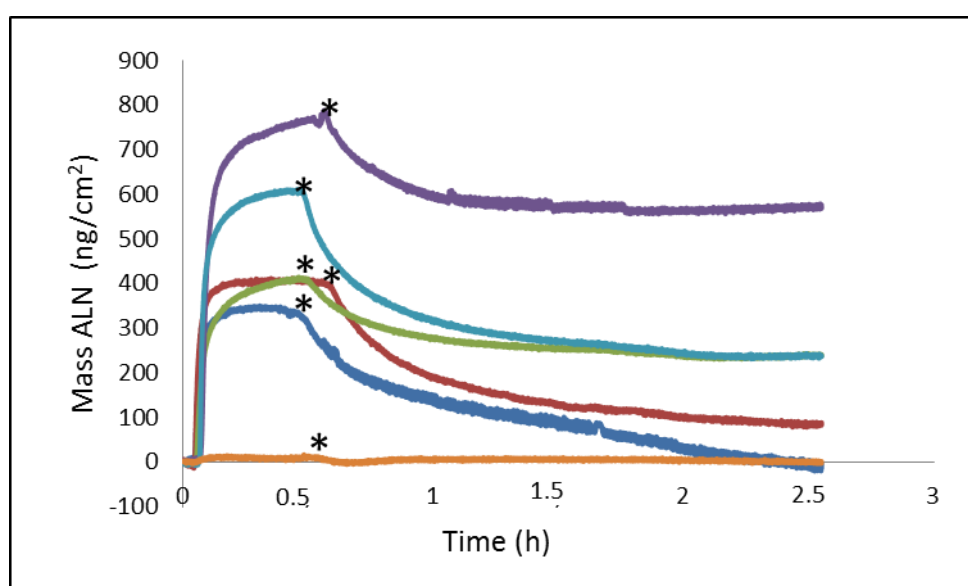
a)

b)

Figure 13: QCM-D results showing SDF-1alpha absorption and release from a) hydrophilic and b) hydrophobic mesoporous titania thin films.

4.4.3 Alendronate release in PBS buffer

The flow media in the QCM-D measurements was changed to buffer (PH=7.4) to evaluate its effect of the drug loading and subsequently the release rate. As shown earlier, Alendronate had a sustained release in the water media. Figure 14 shows the mechanism of the drug loading and release of Alendronate when using buffer as media instead of water. A more sustained release of the drug from the mesoporous titania surfaces is observed although the amount of the loaded drug is much less when dissolved in the buffer.

✳ = Flow changed to mili-Q water

Figure 14: QCM-D results for ALN release from mesopoorus titania in PBS.

5. Discussion

5.1 Surface evaluation

The material characterization using scanning and transmission electron microscopy illustrated that the mesoporous titania surfaces with intended pore diameters and a relatively high degree of porosity were successfully obtained. From the SEM analysis, it could be seen that the pore exits were pointing out from the surfaces, which is an essential prerequisite for the surfaces to be used as drug delivery systems.

The pore diameters were evaluated by SEM and TEM and it was shown that using different sizes of templates it resulted in matching pore sizes. Using bigger templates like P123 resulted in a 6nm pore size, while smaller surfactants like CTAB and Brij-S10 resulted in smaller pores with the diameter of 4.4 and 3.4nm, respectively. Hence, tuning the pore sizes in the range of 3-6nm by changing the type of template was successfully achieved.

Adding swelling agent (PPG) resulted in clearly larger pores as illustrated by SEM and TEM images, which are a result of that PPG interacts with the PO domains of the P123 micelles and swelling them into larger aggregates [32]. By changing the volume ratio of the surfactant to PPG, the degree of the swelling and consequently the diameters of the pores were tuned and gave rise to the films with pore sizes of 6.5 and 7.2nm. It was shown that adding swelling agents also resulted in a thicker coating as measured by SEM. As the spin coating parameters, such as the speed and the time, was kept constant for all the solutions, the increase in the thickness of the coated mesoporous titania on the substrates were expected to be governed by the viscosity of the solution to be deposited. Adding PPG gave a more viscous solution and subsequently thicker films after the spin coating process.

According to the SAXS analysis the cubic order was detected for all the surfaces. Relatively broad peaks appeared at higher angles indicate that the degree of order is less for the smaller pore sizes while the bigger pore sizes showed more order in related peaks at lower angles.

The pore volume measurement from D_2O tests on mesoporous titania surfaces by QCM-D indicated that the pore volumes increase directly with increasing pore diameters. This also

clarifies the higher drug loading in the surfaces with bigger pore sizes that was shown in the results from QCM-D.

The results from contact angle measurements showed that the mesopoorus titania surfaces were super hydrophilic and modifying the surfaces by DDCMS increased the contact angle. The highest contact angles were obtained for the surfaces with largest pore sizes, 6.5 and 7.2nm. This can be due to the fact that the bigger pores can create a higher specific surface for the organosilane groups attached to and also that more air pockets can be trapped inside the pore area and give these films more hydrophobic character. Hydrophobically modified surfaces had to some extent less pore volume compared to the non-modified surfaces since the hydrophobic groups introduced to the pores occupy some space and thus decrease the available capacity of the pores.

The results obtained from XRD showed the same anatase patterns for all the porous surfaces and the nonporous reference, indicating that the presence of pores with variable diameters did not alter the crystal structure of the materials.

5.2 Drug delivery evaluation

The results from QCM-D showed the dependency of altered properties of the mesoporous titania thin films in optimizing the loading and releasing behaviour of different drugs.

The optimization implies on reducing the initial burst effect in the drug delivery process and receive a more sustain release of the drug from the mesoporous titania surfaces. Initial burst release was shown to occur more readily for carrier systems with high surface area and during the release of small and hydrophilic drugs. This can be due to diffusion of the drug from a surface layer, which is poorly interacting with the matrix [26]. The sustained release of drugs implies on slow release of the drug during an extended period of time. This type of release is slower since the drug molecules interact more strongly with the pore walls.

The results from QCM-D demonstrated that mesoporous surfaces had a much higher loading of drug compared to the nonporous counterparts for both hydrophilic and hydrophobic surfaces. Moreover, tuning the surface energy of the mesoporous titania surfaces affected the loading and releasing trends for most of the investigated drugs.

The amount of the drug loaded into each matrix, varied according to the pore size and the pore volume of the mesoporous titania thin film. The properties of the drug molecules that were loaded also had a determining role in loading and release tendencies, such as molecular size and polarity.

Much higher amount of Alendronate was loaded to the surfaces with larger pore sizes and pore volumes. The Alendronate molecules are small and polar and can fill all the pores while bigger pores provide more surfaces for the drugs to be attached to. Due to high affinity of Alendronate to the pore walls of hydrophilic mesoporous titania a certain amount of drugs could not be released by a mili-Q water flow, while hydrophobically modified surfaces gave rise to a faster release rate due to decreased attraction of the drug molecules to the mesoporous titania pore walls

Strontium Ranelate showed a different release behaviour compared to what was seen for Alendronate. The amount of the drug loaded on the surfaces increased with increasing pore size and consequently increased pore volume. Much higher loading of the drug into hydrophilic surfaces compared to the hydrophobic surfaces is an indication of higher

affinity of the drug to the hydrophilic surfaces. This affinity is much less for the hydrophobic surfaces and is not as sufficient to load as high amount of drug. However, slower release displayed for the hydrophilic surfaces compared to the hydrophobic counterparts implied on slower diffusion of highly attached Strontium Ranelate polar molecules to the pore walls.

The loading of AMD-3100 into mesoporous titania surfaces was higher for the bigger pores and is significantly higher for the 7.2nm sized pores having the highest pore volume. AMD-3100 is an unpolar drug, and it has therefore a low affinity to the hydrophilic surfaces, as can be seen in its release behaviour. High attachment forces between the drug molecules and hydrophobically modified surfaces gave an extremely slow release of AMD-3100 while a faster release was observed when they released from hydrophilic surfaces.

The loading behaviour of RLX, a small molecular hydrophobic drug, changes independent of the pore sizes and volumes into hydrophilic surfaces. The explanation to such behaviour can be attributed to the hydrophobic character of the methanol as the drug solvent compared to the mili-Q water which was used for the other drugs. The diffusion of methanol as a less polar solvent is less favourable in the hydrophilic substrates when the pores are bigger and there will be less chance for the drug to absorb into these pores. Relatively high amount of RLX was loaded on hydrophobic surfaces since the drug molecules could create stronger attachments to the pore walls and it was released in a slow sustained pattern from these surfaces because strong forces created between the drug molecule and the mesoporous titania surfaces. The hydrophilic surfaces also showed a sustained release of the drugs, but with faster release kinetics compared to release from the hydrophobic surfaces, which most probably is due to low affinity of the drug molecules.

SDF-1alpha is a protein and its size is bigger compared to the others, therefore it was a pore size selectivity that determined in the amount to be loaded of the drug molecule into mesoporous titania. The big drug molecules cannot penetrate into pore sizes of 3 to 6nm while a significant increase in the loading for the biggest pore sizes, 6.5 and 7.2nm, was observed. From these observations it can be assumed that the drug molecular size is larger

than 6nm. Release patterns were shown to be identical for the modified and the non-modified surfaces. This behaviour can be due to the ability of the protein molecules to change its configuration and exposing both polar and unpolar segments, allowing attachment to both hydrophilic and hydrophobic surfaces.

According to the results obtained from loading and releasing of Alendronate with buffer it was illustrated that changing the surrounding media can change the release rate of the drug molecules into the media. Buffer changes the pH in the media and creates a specific concentration of buffering species that can decrease the interaction of Alendronate with the surface. The less amount of Alendronate loaded on the surfaces when it was dissolved in buffer can be due to the presence of ionic species in the buffer which migrate into the pores and prevent the strong interaction of the drug molecules with the pore walls.

6. Conclusions

The structural and morphological properties of mesoporous titania thin films as well as their surface energy were tailored by adjusting the geometry and dimensions of the system and applying surface modifications in order to turn mesoporous titania into a suitable matrix to serve as a drug delivery system.

Five different kinds of drugs were successfully loaded into mesoporous titania surfaces and much higher loading of the drugs was demonstrated on the mesoporous surfaces compared to their non-porous counterpart and the mesoporos surfaces was shown to serve for a sustained release for the different drug molecules.

In addition to textural properties of mesoporous titania matrices, the properties of the drug molecules such as their sizes and interactions with the mesoporous pore walls play an important role in the drug delivery process.

The loading and releasing behavior of Alendronate from mesoporous titania surfaces were evaluated when changing the media to PBS buffer and resulted in less loading and sustained release, but with a faster release compared to using water as media.

7. Future work

This project has opened up many possibilities on how to design a proper drug delivery system using mesoporous titania thin films.

It would be of interest to characterize the mesoporous titania matrices by nitrogen adsorption method, which would provide information about their surface area and pore volume. The challenge then is to create a proper method to collect a considerable amount of mesoporous titania in the form of powders.

When it comes to the drug loading and release behaviour from the surfaces, it would be of interest to change the surrounding media to simulated body fluid to examine how it would affect the diffusion behaviour.

An *in vivo* study would be a great complement to this work, in order to see how these systems will act in the more complicated biological environments and follow their therapeutic responses.

8. Acknowledgement

9. References

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