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Grafting cyclodextrins to calcium phosphate ceramics for biomedical applications

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Abstract The grafting of hydroxyapatite/beta-tricalcium phosphate with \(\beta\)-cyclodextrin was achieved using a two step reaction with (3-glycidyloxypropyl)trimethoxysilane as a linker. Firstly, the silane group was brought to react with the hydroxyl groups at the surface of the hydroxyapatite/beta-tricalcium phosphate and secondly, the epoxy group was brought to react with the \(\beta\)-cyclodextrin. The amount and distribution of covalently bound \(\beta\)-cyclodextrin were investigated by thermogravimetric analysis, colorimetric total sugar assay, fluorescence spectroscopy and confocal laser scanning microscopy. The concentration of grafted \(\beta\)-cyclodextrin was found to be approximately 3.5 \(\mu\)mol per gram of material corresponding to an almost complete surface coverage, when assuming a closed hexagonal packed monolayer.

Keywords Cyclodextrin · Hydroxyapatite · Grafting · Drug delivery

Introduction

Infections at the site of implantation in orthopaedic procedures is a major cause of post operative problems [1] and may lead to the need of further medical treatments [2]. The risk of infection can be reduced by treating the patient with suitable antibiotics, either systemically or locally at the site of the implant by incorporation of drugs into the device [3]. However, mainly due to low blood circulation in the bone tissue, systemic administered antibiotics often have a limited effect [4]. Therefore, it can be necessary to increase the amount of administered drugs to ensure that the necessary therapeutic concentration is reached in the intended area [2]. The systemic dose of an administered drug has an upper limit due to toxicity and other side effects, thus minimizing the therapeutic window [4]. Local release of drugs from the implant is a promising method to achieve an effective dose of a drug at the site where the infection risk is imminent. However, Duan and Wang [4] state that it can be very challenging to load a surface of an implant with the sufficient amount of drug, and at the same time control the release rate of the drug to have a significant effect. It is known that cyclodextrins form inclusion complexes with various antibiotics [5] and other drug molecules [6] and that cyclodextrins therefore can be used as efficient drug carriers. This property may be utilised to increase the load of certain drugs and control the release of the drug from the implant to the recipient and thereby reduce the risk of a post operative infection.

Several methods for immobilizing cyclodextrins on surfaces, where the cyclodextrin does not contain guest molecules, have been reported [5, 7–16]: Lepretre et al. [5] and Liu et al. [8] employed a cyclodextrin polymer to physically coat hydroxyapatite, El Ghoul et al. [9] and Blanchemain et al. [12] used citric acid as a cross linking agent to physically fix polymer-cyclodextrin coatings to a surface. Lagrost et al. [14] covalently modified a silicon surface with a \(\beta\)-cyclodextrin derivate with seven 1-alkene chains using a photochemical reaction. Silanisation reactions are also suitable for the modification of silica gels as demonstrated by Ponchel et al. [15] and Nielsen et al. [16] who covalently grafted (3-glycidyloxypropyl)trimethoxysilane to the
surface of silica gel via the silane group and then let the epoxy group react with a primary or secondary hydroxyl group of \(\beta\)-cyclodextrin.

Silanisation can also be used to modify hydroxyapatite as shown by Durrieu et al. [13] who used a silanisation to covalently bind peptides to the surface.

Direct covalent modification by grafting cyclodextrins onto hydroxyapatite, beta-tricalcium phosphate or a biphasic material of the two has, to our knowledge, not previously been reported in the literature.

We here report the modification and addition of a drug delivery capability to an implant material which is known to be osteoconductive and biodegradable [13, 17–20]. We propose to use (3-glycidyloxypropyl)trimethoxysilane to graft \(\beta\)-cyclodextrin to a sintered biphasic ceramic material consisting of 70 mol% hydroxyapatite and 30 mol% beta-tricalcium phosphate (HA/\(\beta\)TCP), where the silan group covalently bind to one or more hydroxyl groups on the surface of HA/\(\beta\)TCP forming ether bonds and then allowing the epoxy group to react with a primary or secondary hydroxyl group of \(\beta\)-cyclodextrin.

**Materials and methods**

The implant material employed was a biphasic material, consisting of hydroxyapatite \((\text{Ca}_5(\text{PO}_4)_3(\text{OH}))\) and beta-tricalcium phosphate \((\text{Ca}_3(\text{PO}_4)_2)\) in 70:30 molar ratio and was obtained from FinCeramica, Faenza, Italy as a fine powder with a mean surface area of 27 m\(^2\) per gram. The porous structures were prepared using \(\text{CO}_2\) foaming to produce a ceramic slip with high polymer content, followed by binder removal and sintering at 1200 °C. Prior to use, the sintered porous material was crushed in a mortar, washed with ethanol soxhlet extraction for 2 days and dried in a vacuum oven at 110 °C overnight.

\(\beta\)-Cyclodextrin was purchased from Wacker Chemie GmBH, Germany and dried in a vacuum oven at 60 °C overnight. \(N,N\)-dimethylmethanamide (DMF) was obtained from Fluka, Germany and dried before use with molecular sieves. (3-glycidyloxypropyl)trimethoxysilane was obtained from Fluka, Germany. 1-anilinonaphthalen-8-sulfonat (1,8-ANS) was produced by Fluka, Switzerland. NaOH was produced by J.T.Baker, Holland.

Grafting of HA/\(\beta\)TCP with \(\beta\)-cyclodextrin

The synthesis was optimized from Ponchel et al. [15] and illustrated in Fig. 1. In the first step 4.0 g of HA/\(\beta\)TCP were mixed with 200 ml of DMF in a two-neck-flask with a flow of nitrogen and a Dean Stark Trap. The solution was kept at 175 °C for 2 h and the distillate, approximately 10 ml, was removed. When the solution had cooled to 95 °C, the trap was changed into a condenser. 3.0 ml of (3-glycidyloxypropyl)trimethoxysilane were added and the solution was kept at 95 °C and left to react for 48 h. In a separate flask, 2.0 g of \(\beta\)-cyclodextrin were dissolved in 250 ml of DMF and 2.0 g of sodium hydride were added and left to react for 4 h. Finally, the solution was filtered. The filtrate was added to the flask containing HA/\(\beta\)TCP, and left to react for 72 h at 95 °C. After this, the mixture was filtrated and subsequently washed two times with DMF and two times with demineralised water. Finally, the powder was washed using soxhlet extraction with water at reflux temperature for 5 days and afterwards dried in a vacuum oven at 70 °C.

Quantification of grafted \(\beta\)-cyclodextrin

**Thermal gravimetric analysis**

Thermal Gravimetric Analysis (TGA) was performed on a Netzsch STA449C (Erich NETZSCH GmbH & Co., Germany). An empty alumina crucible was weighed and the sample was placed in the crucible and the weight of the sample was recorded. The crucible was placed in the oven and heated in atmospheric air from 50 to 800 °C at a heating rate of 10 °C per minute.

**Total sugar assay**

The spectrophotometric quantification of total sugar, the phenol assay, was modified from Taylor et al. [21].

Eight solutions of \(\beta\)-cyclodextrin in a concentration ranging from 0 to 30 mg/L was prepared and 0.6 ml of each standard were transferred to six test tubes. 2.5 ml of concentrated sulphuric acid were added to each test tube and the tubes were placed in a 50 °C water bath for 3 h. After the tubes have reached room temperature, 75 µL of 90% phenol solution were added to 3 tubes per standard and none to the remaining three tubes, which were used as blank controls. After mixing, the tubes were left to react for 1 h. The samples where measured twice at 487 nm on a spectrophotometer (Cary 50 Bio UV–Visible spectrophotometer, Varian).
Samples of HA/βTCP with β-cyclodextrin and samples of HA/βTCP without β-cyclodextrin were measured by adding 5.0 mg of material and 0.6 mL of demineralised water to a test tube. The tubes were treated and measured as described for the standard solutions. Subtracting the two measurements and correlating via the standard curve gave the concentration of cyclodextrin in the sample.

Adsorption of 1,8-ANS

To quantify the adsorption of the fluorescence probe to the surface, the concentration of the probe in the solution was quantified using a fluorescence spectroscopy: Samples of 15.0 ± 0.1 mg modified and unmodified HA/βTCP were placed in a 11.5 mL centrifuge tube together with 10 mL of a solution containing between 5.0 × 10⁻⁷ and 1.0 × 10⁻³ M of 1,8 ANS. The tubes were shaken for 40 h to ensure equilibrium and afterwards centrifuged at 1620 g for 10 min. The standard curve was constructed from solutions containing between 5.0 × 10⁻⁷ and 5.0 × 10⁻³ M of 1,8 ANS. The samples were measured in quartz cuvettes on a fluorescence spectrophotometer (Cary Eclipse Fluorescence Spectrophotometer, Varian) with an excitation wavelength of 355 nm and an emission wavelength scanning from 450 to 600 nm where the emission at 522 nm was collected. The gain was set at 770 V. For some samples dilution with demineralised water was necessary in order to stay within the limits of the standard curve.

Release of 1,8-ANS

60 mg of HA/βTCP with and without cyclodextrin were placed in separate 1L blue cap bottles containing 0.5 L of a 5 × 10⁻⁵ M 1,8-ANS solution. The bottles were left shaking for 48 h, where after the solution was filtered and the powder dried in a vacuum oven at 40 °C over night.

25.0 mg of HA/βTCP with and without cyclodextrin was placed in a small mesh bag and submerged into 75 mL of demineralised water. Samples were taken at intervals by removing 3 mL of the solution and replacing it with 3 mL demineralised water. A standard curve was made with concentrations ranging from 1 × 10⁻⁷ to 5 × 10⁻⁵ M. All samples were measured by fluorescence spectroscopy with an excitation wavelength of 340 nm and emission was measured between 420 and 600 nm where the emission at 522 nm was collected.

Confocal laser scanning microscopy

To visualize the presence of cyclodextrin on the particles and the cyclodextrins ability to form inclusion complexes with 1,8-ANS, Confocal Laser Scanning Microscope (CLSM) was employed. 15 mg of sample were added to 10 mL 1.0 × 10⁻⁵ M 1,8-ANS and stirred for 24 h. Suspended particles was then withdrawn from the suspension and placed on a glass slide which was placed in the CLSM (LSM510 META, Zeiss, Germany). A wavelength of 351 nm was used for excitation and the emission was collected between 452 and 484 nm. The resolution of the image was 1024 × 1024 pixels using a 40× objective which gives a size of the image of 230 × 230 µm.

HA/βTCP with and without (3-glycidyloxypropyl)trimethoxysilane was included as references.

Results and discussion

Quantification of grafted β-cyclodextrin

Amount of hydroxyl groups

As the applied syntheses utilize hydroxyl groups on the surface it is of interest to know the amount of hydroxyl groups that are available for the reaction. Several different methods for the quantification of the total amount of hydroxyl groups in the material have been developed: Meyer [22] used a titration method where calcium phosphates were dissolved using hydrochloric acid and he concludes that the amount of titratable hydroxyl groups in the dissolved samples were between 13 and 21% of the theoretical amount for pure hydroxyapatite. It is known that stoichiometric hydroxyapatite has a total amount of 2 mmol hydroxyl groups per gram of hydroxyapatite [23], which means that the material Meyer [22] investigated contained between 0.26 and 0.42 mmol titratable hydroxyl groups per gram of dissolved hydroxyapatite. Arens et al. [24] determined by Fourier transform infrared spectroscopy (FTIR) and Magic-Angle-Spinning NMR (MAS-NMR) the total amount of hydroxyl groups in hydroxyapatite to be 10 wt% lower than for stoichiometric hydroxyapatite, corresponding to 1.8 mmol hydroxyl groups per gram of material. Cho et al. [25] used two-dimensional solid-state NMR to determine the amount of hydroxyl groups in bones and found 20 wt% hydroxyl groups lower than the amount expected in stoichiometric hydroxyapatite corresponding to 1.6 mmol hydroxyl groups per gram of hydroxyapatite.

From the above mentioned studies it is clear that it is not possible to generalize on the quantities of hydroxyl groups on hydroxyapatite and related materials. This may be caused by differences in materials being used, the origin, surface area and porosity, as well as differences in analysis techniques.

Only one paper was found that describes the measurement of hydroxyl groups on the surface of hydroxyapatite. Tanaka et al. [26] describes a method to measure the
content of surface active hydroxyl groups by measuring the amount of methane produced by a reaction with methylmagnesium iodide and found a concentration of 2.6 hydroxyl groups per nm². This value is therefore the best estimate of the amount of hydroxyl groups on the surface available to react with the silane group of the linker molecule. As one β-cyclodextrin covers 1.8 nm² it is concluded that the amount of hydroxyl groups will not be a limiting factor in the grafting process.

Qualification of cyclodextrin

To prove that cyclodextrins are covalently bound to the HA/βTCP surface, two different syntheses were made. The first synthesis contained HA/βTCP, (3-glycidoxypropyl)trimethoxysilane and β-cyclodextrin and the second one contained only HA/βTCP and β-cyclodextrin. The synthesis route and cleaning procedures are the same as described for the grafting of HA/βTCP with β-cyclodextrin in the material section. When employing the phenol assay to determine the amount of β-cyclodextrin in samples from each synthesis, it is clear that no cyclodextrin can be detected in the sample without (3-glycidoxypropyl)trimethoxysilane, whereas the sample containing (3-glycidoxypropyl)trimethoxysilane is positive for carbohydrates. This means that β-cyclodextrin is covalently bound to the surface of HA/βTCP and the cleaning method employed is sufficient to remove any β-cyclodextrin not covalently bound to the surface.

Quantification of grafted β-cyclodextrin

Due to the applied reaction conditions the silan group of (3-glycidoxypropyl)trimethoxysilane will primarily react with hydroxyl groups on HA/βTCP and the epoxy group will only react with the activated hydroxyl groups of cyclodextrins which suggests that a monolayer is formed. Repeated measurements of the surface area with BET suggested a surface area of 3.6 ± 1.1 m² per gram of HA/βTCP but with a poor fit to the isotherm (data not shown) which is partly reflected in the high standard deviation. If cyclodextrins are placed in a hexagonal close packed structure, the maximum theoretical concentration will be 3.0 ± 0.9 μmol β-cyclodextrins per gram of HA/βTCP corresponding to 0.34 ± 0.1 wt%.

Measurements using thermo gravimetric analysis showed, as expected, that the amount of cyclodextrin relative to the amount of HA/βTCP was low and the concentration of cyclodextrin was calculated to 2.6 μmol per gram of HA/βTCP (data not shown). Furthermore, the results from unmodified HA/βTCP showed a small increase in weight as function of temperature but with poor reproducibility. This increase was also seen when using an inert gas as argon and it was not possible to find an explanation for the weight gain. The poor reproducibility of the HA/βTCP baseline together with the low content of the organic part precluded determination of the amount of grafted cyclodextrin with the desired precision using this technique. Therefore our focus was shifted to the phenol essay as the primary method to quantify the cyclodextrins, as this method quantifies total sugars with a very low detection limit and a good reproducibility. The phenol essay showed that concentrations of cyclodextrins in solution as low as 2.5 μmol with a standard deviation in the whole range of no larger than 0.4 μmol could be determined. The concentration of cyclodextrins on HA/βTCP was determined to be 3.5 ± 0.7 μmol per gram of HA/βTCP.

When considering that the cyclodextrins will only be able to form a monolayer, it is possible to calculate how large an area the cyclodextrins will cover, assuming a hexagonal close packed monolayer. The covered area can be calculated to 4.2 ± 0.8 m² per gram of HA/βTCP. This area corresponds well to the area estimated using BET and it is therefore reasonable to state that HA/βTCP is nearly completely covered by a monolayer of cyclodextrins.

As no previous records are available concerning the covalent grafting of cyclodextrins to the surface of calcium phosphate, it is difficult to evaluate the efficiency of the performed modification. However, as mentioned earlier, a similar synthesis route has been used to modify different silica gels and as long as the larger surface area of silica gel, which is between 200 and 600 m² per gram depending on the type of silica gel, is taken into account, these results can be used to assess the efficiency of the performed synthesis. Therefore, all concentrations of cyclodextrins are normalised with regard to its surface area to give cyclodextrins per m² and are shown in Table 1.

The amount of cyclodextrins bound to the surface of HA/βTCP is relative high compared with what was found in literature. Nielsen et al. [16] found for the applied silica gel, which had 0.076 μmol cyclodextrins per m², that the coverage of cyclodextrin is not evenly distributed and that the highest concentration of cyclodextrins was close to the surface of the particle. It was proposed that this effect was due to the pores being blocked by the cyclodextrins and that this effect therefore is a limiting factor in achieving a

<table>
<thead>
<tr>
<th>Material</th>
<th>μmol CD per m²</th>
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<tbody>
<tr>
<td>Silica gel</td>
<td>0.076</td>
</tr>
<tr>
<td>Silica gel</td>
<td>0.076</td>
</tr>
<tr>
<td>HA/βTCP</td>
<td>0.97</td>
</tr>
</tbody>
</table>

Table 1: Yields of different syntheses normalized with regard to the surface area.
higher degree of coverage for silica gels. As the pores of HA/βTCP are large, it is not expected that clogging of the pores is an issue and the lack of blockage is the most plausible explanation to the observed difference in cyclodextrins per m².

Adsorption of 1,8-ANS

To investigate the adsorption properties of HA/βTCP with and without β-cyclodextrins towards a guest molecule, samples were placed in different concentrations of 1,8-ANS followed by quantification of 1,8-ANS in the water phase. 1,8-ANS displays a relatively low fluorescence intensity in water and the lower and upper detection limits were found to be below $5 \times 10^{-7}$ and $5 \times 10^{-5}$ M, respectively. By adding free β-cyclodextrins to the solution, it is possible to increase the fluorescence intensity and thereby obtain a higher sensitivity.

The inclusion complex formation constant, $K$, can be calculated from a fit of the data with a suitable adsorption isotherm. For the adsorption of 1,8-ANS to unmodified HA/βTCP, the Langmuir isotherm was found to give a reasonably good fit (see Fig. 2). However, for the adsorption of 1,8-ANS to cyclodextrin modified HA/βTCP, the Langmuir isotherm did not give a good fit whereas a fit to the Bi-Langmuir isotherm resulted in a better fit (see Fig. 3). It was anticipated that the Bi-Langmuir provided the best fit to the modified HA/βTCP since this material contains two types of very distinct adsorption sites, the cyclodextrin and the calcium phosphate surface.

From the fit of the Langmuir isotherm, the concentration of binding sites for 1,8-ANS adsorption to unmodified HA/βTCP is calculated to be 34 µmol per gram of HA/βTCP with a $K_{HA/βTCP} = 15000 \pm 1400$ M⁻¹. To better evaluate the result, the number of molecules of 1,8-ANS per nm² is calculated and results in 5.6 molecules of 1,8-ANS per nm². This value is in the high range, but not unlikely as it is possible that more than one layer is formed.

When fitting the Bi-Langmuir isotherm to the measured adsorption data from modified HA/βTCP, both the result from the Langmuir fit and the known concentration of β-cyclodextrin are used as input for the fitting process. This results in $K_{CD} = 400000$ M⁻¹ and the concentration of binding sites to 3.6 and 2.6 µmol per gram of HA/βTCP for cyclodextrins and unmodified surface, respectively. The change in the concentration of binding sites with regards to modified HA/βTCP was anticipated because cyclodextrins occupy most of the surface.

Literature studies have shown that the stability constant between 1,8-ANS and β-cyclodextrin dissolved in water is between 100 and 300 M⁻¹ [14, 28–30]. This value is considerably lower than the above measured binding constant of 400000 M⁻¹, but the difference was expected as both Lagrost et al. [14] and Beulen et al. [28] determined stability constants for 1,8-ANS to 390000 and 289000 M⁻¹, respectively, for β-cyclodextrins attached to a surface in a monolayer. It is proposed that this large increase in binding constant is a combination of a cooperative effect between the cyclodextrin and the surface...
together with a reduced loss of entropy upon formation of
the inclusion complex compared to the complex formed in
solution.

Release of 1,8-ANS

The release of 1,8-ANS shows that HA/βTCP with cyclo-
dextrin is releasing considerably more 1,8-ANS than
unmodified HA/βTCP (see Fig. 4). HA/βTCP with cyclo-
dextrin is, in the current setup, releasing a total of 3.4 μmol
1,8-ANS per gram of HA/βTCP which is approximately
equal to the theoretical maximum of approximately
3.5 μmol per gram of HA/βTCP. Whereas unmodified HA/
βTCP releases 1.6 μmol 1,8-ANS per gram of HA/βTCP.

This result clearly demonstrates that cyclodextrins can
be used to increase the amount of a drug and change the
release rate of a drug that can be released from a porous
material like HA/βTCP. This effect will be highly depen-
dent on the used drug, but a thorough study of the release
functionality and consideration of effectiveness is outside
the scope of this article.

Confocal laser scanning microscope

A useful property of 1,8-ANS and other isomers of ANS, is
their ability to emit fluorescence in hydrophobic environ-
ments such as inside the cavity of a cyclodextrin [30]. It is
therefore possible to use the change in fluorescence
intensity of 1,8-ANS as a marker for the 1,8-ANS cyclo-
dextrin inclusion complex and thus distinguish between

![Fig. 4 Accumulated concentration of 1,8-ANS from HA/βTCP with and without cyclodextrin](image)

![Fig. 5 Light transmission
microscopy (left) and CLSM
(right) images of HA/βTCP
particles in a suspension of 1,8-
ANS. a Unmodified HA/βTCP
particle. b HA/βTCP coated
with β-cyclodextrin. The scale
bar is 40 μm for all pictures](image)
surface adsorption and inclusion complex formation with cyclodextrins.

Samples containing 1,8-ANS with and without cyclodextrins were studied both using normal light transmission microscopy revealing the shape of the actual particle and with the CLSM revealing focal distribution of fluorescence. Figure 5a shows the light transmission microscopy image (left) and the corresponding CLSM image (right) where fluorescence should appear as bright areas. In the fluorescence image, it is not possible to see any bright areas which document that 1,8-ANS does not emit significant fluorescence when adsorbed to the surface of unmodified HA/βTCP or in solution. The CLSM images for the control, HA/βTCP coated with (3-glycidyloxypropyl)trimethoxysilane in a solution of 1,8-ANS (data not shown) yielded comparable results showing that (3-glycidyloxypropyl)trimethoxysilane does not affect the fluorescence of 1,8-ANS. The samples coated with β-cyclodextrin are seen in Fig. 5b, where the bright areas indicate the fluorescence from 1,8-ANS (right).

When comparing the three CLSM images, it is clear that the presence of cyclodextrins on the surface of the particle together with 1,8-ANS gives rise to a significant increase in the fluorescence signal and it is therefore evident that the surface of the samples contains cyclodextrins and that 1,8-ANS indeed is forming an inclusion complex with β-cyclodextrin. The fluorescence intensity varies on the surface due to structural variations of the material but also a heterogeneous distribution of cyclodextrins will contribute to this variation.

Due to the rather high density of the particle it was not possible to measure the distribution of cyclodextrins inside the particles as the excitation light cannot fully penetrate the particle and thus the 1,8-ANS cyclodextrin inclusion complexes situated deep inside the pores of the particles will not emit fluorescence. Some particles were therefore grinded to see if a difference in fluorescence was visible, thereby indicating a variation in distribution of cyclodextrin and 1,8 ANS. However, no differences were apparent which is either because no fragments from the interior of the particle was found and examined or, which is more likely, that cyclodextrin and 1,8-ANS is present throughout the particle.

To test if the cyclodextrins are present inside the particle and still capable of forming complexes with 1,8-ANS, smaller particles were analysed (see Fig. 6). As seen from the light transmission microscopy image the size of this particle was approximately 15 μm long, 12 μm wide and 16 μm high and the fluorescence was emitted throughout the interior of the particle.

All of the CLSM images revealed a uniform distribution of fluorescence with only small local areas with somewhat lower emission revealing that inclusion complexes between β-cyclodextrin and 1,8-ANS is distributed throughout the interior of the particle, even though the intensity is varying. It is therefore evident that cyclodextrins have been covalently bound both at the surface but also inside the porous particles, at least to a depth of 8 μm.

For silica gel with a mean pore size of 10 nm, we have previously reported that inclusion complexes between β-cyclodextrin and 1,8-ANS can mainly be detected near the surface due to blocking of the pores [16]. However, as HA/βTCP and silica gel have very different pore structures, it is difficult to draw any conclusion, but it is likely that the bigger interconnected pores of HA/βTCP will decrease the likelihood of pore blocking drastically and thereby a near total coverage of the entire surface is possible.

Conclusion

The amount of β-cyclodextrins grafted to HA/βTCP was quantified to 3.5 μmol per gram of HA/βTCP corresponding to an almost complete surface coverage. The adsorption of a model molecule with affinity towards β-cyclodextrin (1,8-ANS) could be fitted using the Bi-Langmuir binding isotherm. Thus, the modified material contains two distinct binding sites for 1,8-ANS, the unmodified surface and the cyclodextrins with binding constants of 15000 and 400000 M⁻¹ respectively.
The results from CLSM demonstrate the presence of cyclodextrins both at the surface but also at least 8 μm inside the particles. However, it is not possible to see if the cyclodextrins are situated in the core of larger particles with a particle size of 80 μm because the excitation light has a limited penetration into HA/FTCP, but grinded samples indicates that cyclodextrins are present throughout the particles. The penetration depth of the excitation light was found to be at least 16 μm but well below 80 μm. The fluorescence experiments furthermore showed that the surface bound cyclodextrins are available to form inclusion complexes with molecules and, as demonstrated for 1,8-ANS, with dramatically increased binding constants compared to complex formation in solution. The molecules that interact with the cyclodextrins can be released again, and it is therefore concluded that low concentrations of cyclodextrins grafted to a surface will increase the amount of drug that can be released to the recipient.

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