Study of cell effects, release and adsorption of doxycycline by carbonated hydroxyapatite microspheres

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Introduction

Bone substitutes are now part of many orthopedic and dental procedures. Carbonated hydroxyapatite (CHA), for example, is a bone substitute with great similarity with natural bone, with high biocompatibility and osteointegration capacity. Due to Its visible therapeutic benefits CHA has become a good candidate as vehicle for several drugs for local delivery, for example Doxycycline (DOXY) [1]. DOXY is a broad-spectrum antibiotic from the tetracycline family, And has recently demonstrated biological activities in human cells, especially in bone tissue, promoting greater bone formation. The present study aims to understand the kinetics of DOXY release and its cellular effects using CHA and alginate microspheres as a vehicle loaded with different concentrations of DOXY

Powder Preparation and physical chemical characterization

CHA are produced true wet method and the CO_3 substitution was done true the addition of ammonium carbonate during the synthesis. The Dry powder of CHA was mix with different concentrations of DOXY for 4 hours to build a isotherm Model. With the result the concentrations of 17mg/g (D17), 8,5mg/g (D8,5), 4,25mg/g (D4,25), and 1,7mg/g (D1,7) were chosen to continue further analysis. The Physical chemical characterization was performed Using the following analyses: Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) Brunauer-Emmett-Teller Analyzer (BET). The microsphere was created by a mixture of 2,5% of sodium alginate and the extruded in Calcium chloride 0.3M

The release kinetics was made during 10 days in phosphate buffered saline (PBS) using the ratio of 10ml of solution for 200mg of microspheres.

The biological Assay was made in Human Saos-2 cell line, via indirect method, by exposing the cell to the 24h release in cell culture Medium (Mc'Coys). After expose the cell for 24h, a MTT and Live and dead assay were conducted, and the Mineralization capacity was measure after 24h of exposure during 14 days and 21 days. The upernatant were collect to quantify proinflammatory and anti-inflammatory factors vi Luminex

The minimum inhibitory concentration (MIC) was performed in *Enterococcus faecalis* bacteria to determine the inhibitory capacity of the material

Results and discussion:

XRD result was compared with the HA crystallographic record JCPDS 9-432 and showed a similar crystal profile, with a slight broadening of the peaks indicating a lower crystallinity expected due to the presence of the carbonate (figure 1)

FTIR results demonstrated a similar pattern with a stoichiometric CHA and a presence of DOXY caused an increase in the amine related peak in the same spot were is located the carbonate group bands related Also the increased hydroxyl band indicate an incorporation of the antibiotic without material. (figure 2)

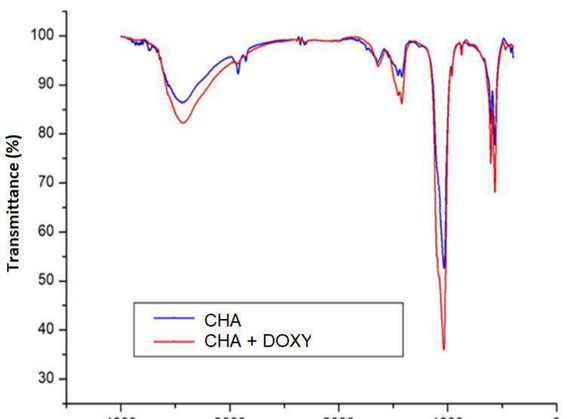

Figure 1: FTIR Spectrum of CHA and DOXY loaded CHA

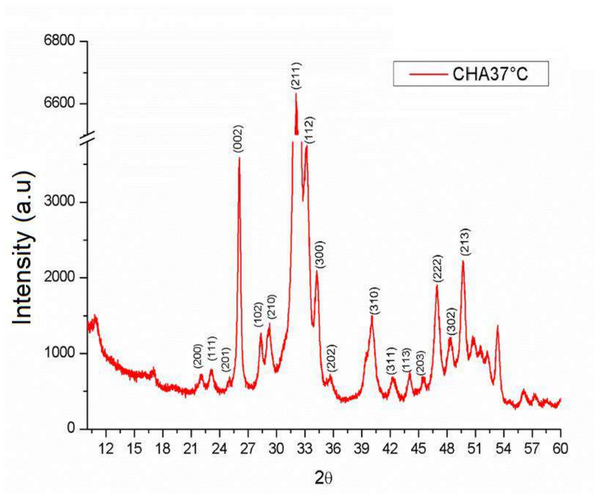

Figure 2: XRD spectrum with Miller Index

BET results shown a surface with a average surface area of 66,537m²/g with mesopores (20,10nm of diameter and 0,332cm³/g of volume)

The experimental model has shown a max adsorption capacity of 17mg/g, a indication of a great adsorption capacity, and it was more correlated with the Langmuir Model with a R^2 of 0,979 indicating a monolayer (figure 3)

Release kinetics demonstrated a controlled release over the 10 day. after 30 minutes all loaded microsphere was able to release Doxy Above the Mic concentration for Enterococcus faecalis with exception of the D1,7 samples that only reach the Mic concentration after 24h (figure 4)

MTT result shown a cytotoxic effect by the released from D17 microsphere as the other groups kept the reduction activity above 75%, D8,5 demonstrate a significant reduce and D4,25 and D1.7 show little effect on the metabolic activity (figure 5) A similar to the live and dead imagens, where its possible to see a proportional number of death cell as the DOXY concentration rises, indicated by the red cells labeled with propidium iodide.

Its also possible to see a reduced cell surface area in D17, D8,5 and D4,25 but D1,7 kept the original cell morphology A similar to the live and dead imagens, where its possible to see a proportional number of death cell as the DOXY concentration rises, indicated by the red cells labeled with propidium iodide. Its also possible to see a reduced cell surface area in D17, D8,5 and D4,25 but D1,7 kept the original cell morphology

Mineralization results demonstrated a capacity the mineralization capacity was proportionally affected by the amount of DOXY present in the culture medium, where it is possible to see that all samples with the exception of D1.7 had a significantly lower mineralizing capacity compared to the control, however by extending for 21 days the mineralization is stabilized

Mic results show that the amount of mass of material needed to inhibit bacterial growth is smaller in more loaded materials, the Mic value were 0,391-0,195 for the D17 sample, 0,781-0,391 for the D8,5, 3,125-1,536 for the D4,25 and D1,7. the release in PBS were tested and all the 24h extract had be able to inhibit bacterial growth.

Luminex quantification showed that the DOXY presente in the microspheres D1.7 AND D4.25 are able to significantly reduce the expression of proinflammatory factores in relation to the Control and the material.

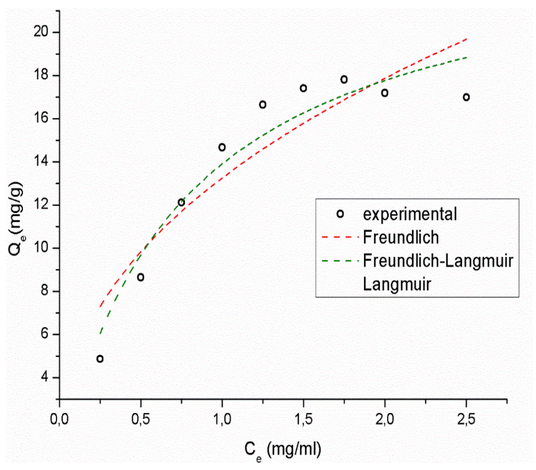

Figure 3: experimental modeling fit with the Langmuir and Freundlich isoterm

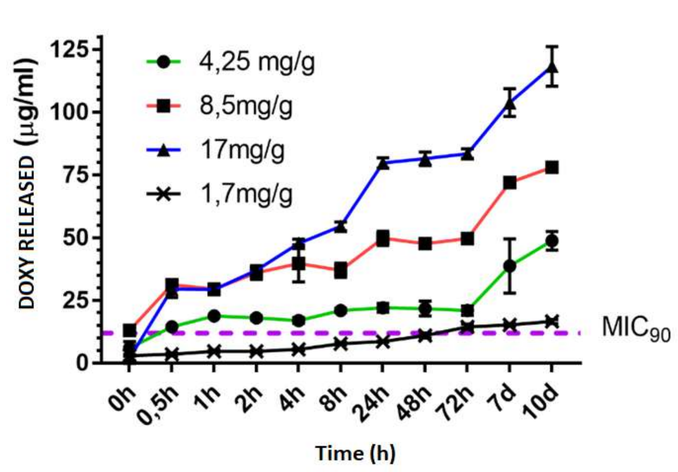

Figure 4: Amount of doxy released compared to DOXY Mic value for *E. faecalis*

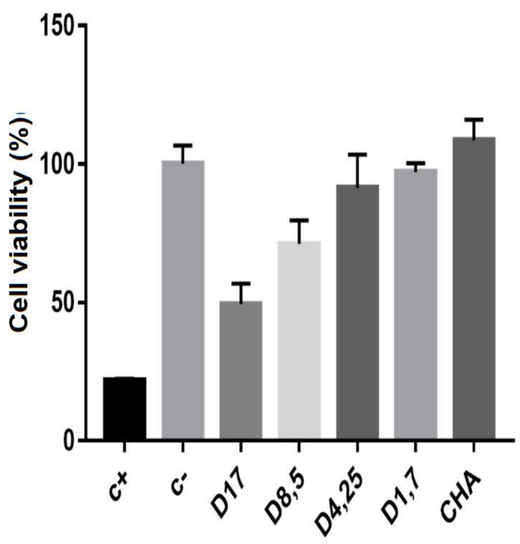

Figura 6: MTT viability assay

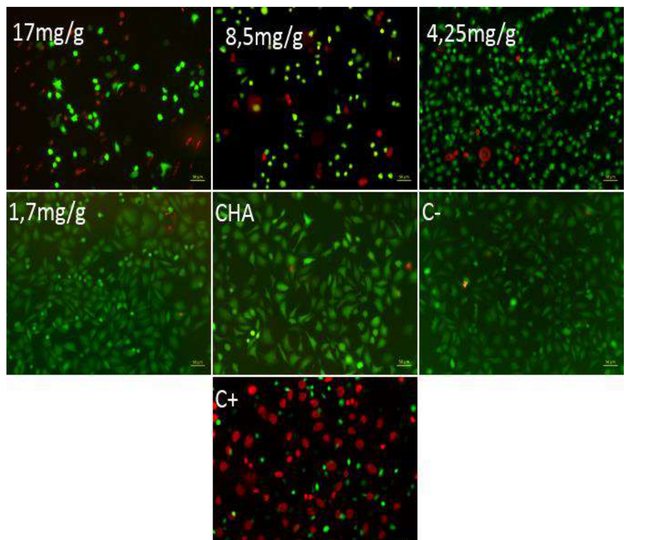

Figura 7: Live and dead fluorescence images in 200x Magnification

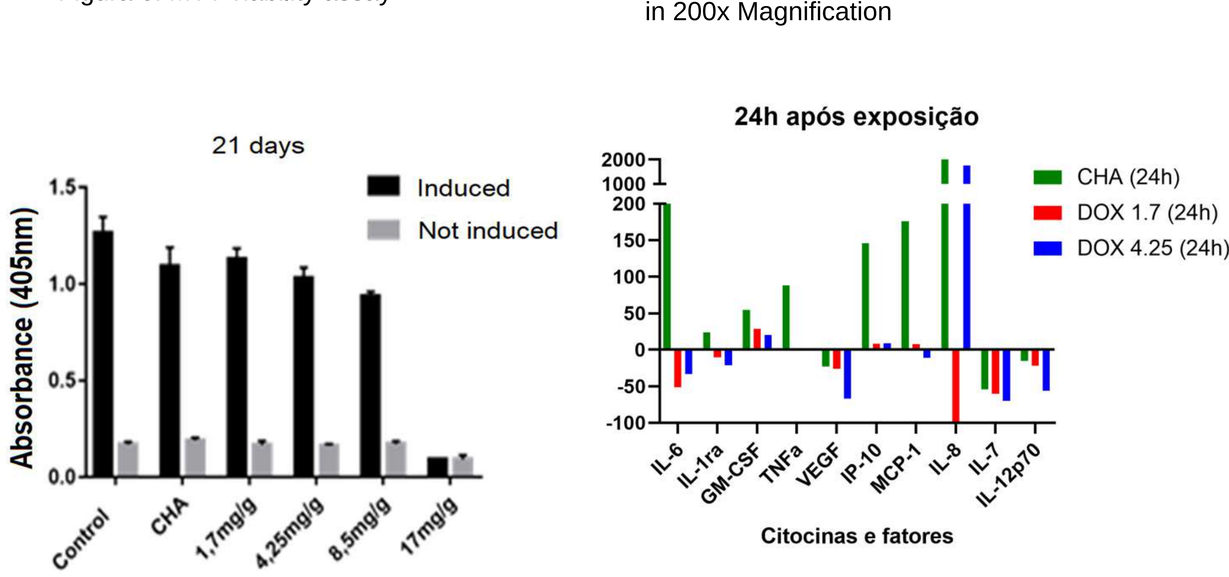

Figure 8: Calcium deposits marked by alizarin red and compare OD 405

Conclusion:

Our results indicate that we were able to create a carbonated suggest that samples D1,7 and D4,25 can be good candidates for material with capacity to inhibit bacterial growth, showing high inhibitory capacity and low impact on cell viability. In particular the D1.7 samples which have been shown to maintain preserved mineralizing capacity.

ACKNOWLEDGMENTS

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