DOI: 10.1002/chem.201000137

Functionalizing Mesoporous Bioglasses for Long-Term Anti-Osteoporotic Drug Delivery

Adolfo López-Noriega,[a, b] Daniel Arcos,[a, b] and María Vallet-Regí*[a, b]

Abstract: Mesoporous bioactive glasses (MBGs) associated with an anti-osteoporotic drug (ipriflavone) have been prepared. With this aim, MBGs were functionalised with different organic groups by following a post-grafting method, thus retaining the mesoporous network of the bioactive substrates. Drug-delivery tests were carried out by using ipriflavone as a hydrophobic model drug. Our results revealed that by means of the proper functionalisation, most of the drug is retained in the mesoporous network. By tailoring the hydrophobicity of the surface with functional groups, the drug–material link can be tuned, thereby ensuring the long-term delivery of ipriflavone. In vitro bioactive tests demonstrate that these systems exhibit the same excellent behaviour of non-functionalised MBGs. The possibility to add a bone resorption inhibitor such as ipriflavone to highly bioactive materials confirms functionalised MBGs as very promising bone-tissue regeneration systems.

Keywords: bioactivity · drug delivery · functionalization · mesoporous materials · nanotechnology

Introduction

Bioactive glasses (BGs) are well-known biomaterials that have been used as bone-tissue regeneration systems since their discovery some decades ago.[1] In 2004, a significant evolution in this field was developed by Yan et al., who synthesised for the first time bioactive glasses that showed an ordered mesoporous arrangement.[2]

The inclusion of mesopores in SiO_2–CaO–P_2O_5-based glasses opened a wide range of new potential applications for BGs. Mesoporous bioactive glasses (MBGs) show the outstanding textural properties of classical silica-based mesoporous materials such as MCM-41 and SBA-15, that is, high surface area and pore volume and well-defined mesoporous diameter.[3] The large surface area of MBGs results in higher chemical reactivity compared to that of BGs. Thus, bioactive kinetics are enhanced, which improves the role of silica-based bioactive glasses as devices for bone-tissue regeneration.[4] In this sense, several groups have proposed MBGs as ideal components of scaffolds for bone-tissue engineering.[5]

On the other hand, a well-ordered mesoporous arrangement opens the possibility to use MBG as reproducible drug-release agents analogously to pure silica mesoporous materials.[6] Some tests have already been carried out and have shown promising results.[7] Nevertheless, MBGs have important limitations due to their chemical nature. The surface of MBGs is covered by silanol groups, which imbue them with a hydrophilic nature. In the case of very hydrophobic drugs, the active principle would lead to chemical repulsion and thus the impossibility to carry the drug. This drawback may be solved by a chemical modification of the surface of MBGs that could ease the interaction between the hydrophobic drug and hydrophilic carrier.

Ipriflavone (IP), a drug of the family of flavonoids that improves bone-tissue regeneration by the inhibition of osteoclasts, is a highly hydrophobic molecule. It was chosen as a model drug for the present work because its biodispensibility in plasma after oral administration is 20% and thus a local delivery is desirable on damaged bone tissue.[8] It is estimated that around 120 mg per day of ipriflavone is required in plasma.[8d] In the cases of local drug-delivery systems, this amount would be much lower and loadings of 1.5–2 g would ensure the biodispensibility of ipriflavone for sev-

[a] A. López-Noriega, Dr. D. Arcos, Prof. Dr. M. Vallet-Regí
Departamento de Química Inorgánica
y Bioinorgánica Facultad de Farmacia
Universidad Complutense de Madrid Pza Ramón y Cajal s/n
28040 Madrid (Spain)
Fax: (+34) 913941786
E-mail: vallet@farm.ucm.es

[b] A. López-Noriega, Dr. D. Arcos, Prof. Dr. M. Vallet-Regí
CIBER de Bioingeniería, Biomateriales
y Nanomedicina (CIBER-BBN)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201000137.

eral weeks if it is not released before. Incorporating a bone-resorption inhibitor into mesoporous bioactive glasses would reinforce these materials as excellent potential candidates to be used for bone grafting in osteoporotic-derived fractures and defects.

MBGs are intended for bone-tissue regeneration and are resorbable in time inside the body. In this sense, a quick delivery of the therapeutic agent (such as few hours or days), in the case of drugs used for helping bone regeneration, is not appropriate from a clinical point of view. These agents might be present in the medium during the replacement of the implant by new-forming bone, which is a long-term process of several weeks. Thus, an efficient drug carrier should attach the drug to its surface until colonisation by the new tissue takes place.

To the best of our knowledge, this work presents for the first time a controlled incorporation of a drug in a mesoporous bioactive glass by means of surface functionalisation. Moreover, a stable drug–MBG attachment for long-term delivery is also described. This work demonstrates that by tailoring the drug-surface interactions, an anti-osteoporotic drug with long-term dispensibility can be incorporated into osteoregenerative matrices.

Results and Discussion

Figure 1 collects the small-angle XRD patterns of as-synthesised and functionalised MBGs. As expected from previous characterisation, the pattern of non-functionalised MBG (Si85) shows three maxima at $2\theta = 1.2°$, 1.98° and 2.29°, which can be indexed to the 211, 400 and 322 reflections of a 3D cubic phase with space group $Ia\bar{3}d$.[4a] XRD patterns of functionalised materials also show the same maxima.

The N_2 adsorption isotherms and the pore-size distribution of the different materials before and after the loading of ipriflavone are summarised in Figure 2. All of them show type IV isotherms with H1 hysteresis loops in the mesopore range, which are characteristic of cylindrical pores open at both ends. Table 1 collects textural parameters calculated from the sorption isotherms. Surface area, pore diameter and pore volume decrease after the functionalisation of Si85. A further decrease in the textural properties is observed in ipriflavone-loaded materials.

Table 1. Textural parameters obtained by N_2 porosimetry of non-functionalised Si85 and ipriflavone-loaded materials. Values in parenthesis are those of functionalised materials before ipriflavone incorporation.

Group/sample	S_{BET} [$m^2 g^{-1}$]	Pore diameter [nm]	Pore volume [$cm^3 g^{-1}$]
non-functionalised/Si85	441.3	4.9	0.52
phenyl/Si85Ph	260.9 (322.4)	3.6 (3.8)	0.32 (0.39)
OH-propyl/Si85OH	246.8 (326)	4.2 (4.4)[a]	0.36 (0.39)
NH_2-propyl/Si85NH_2	245.1 (321)	4.2 (4.5)	0.31 (0.39)
SH-propyl/Si85SH	263.9 (335.7)	3.1 (3.4)	0.31 (0.36)

[a] Si85OH and loaded Si85OH show a minor maximum in the pore-diameter distribution graphic at 4.9 nm.

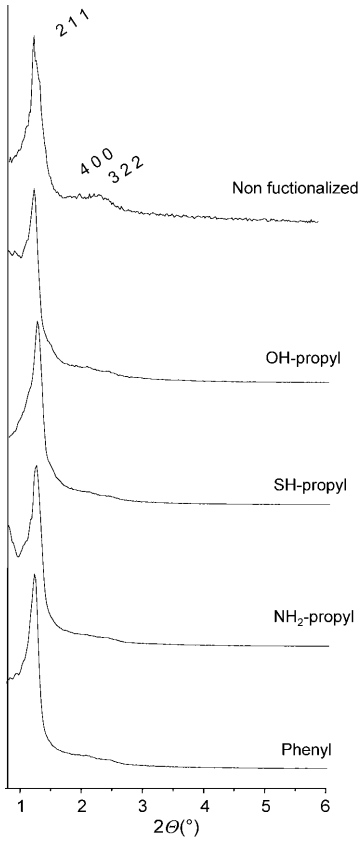


Figure 1. Small-angle XRD patterns of as-synthesised mesoporous bioactive glasses and functionalised materials.

Figure 3 shows FTIR spectra of Si85 and ipriflavone-loaded Si85Ph. The Si85 MBG spectrum shows absorption bands at around 1050, 800 and 450 cm^{-1}, which are characteristics of the stretching, rocking and bending, respectively, of Si-O-Si bonds. A low-intensity band that corresponds to amorphous phosphate is observed at 600 cm^{-1}. New absorption bands between 550–750, 1150–1250 and 2800–3050 cm^{-1} are observed in the spectrum of the material after functionalisation and ipriflavone loading. Similar spectra are observed for the rest of loaded functionalised materials (see the Supporting Information). All these new bands can be assigned to $-CH_2-$, $-CH_3$ and aromatic carbon groups from the organic groups from both functionalising group and ipriflavone.

The mesoporous arrangement is not affected after the chemical functionalisation, according to the small-angle XRD results. Functionalised materials keep the $Ia\bar{3}d$ cubic mesoporous ordering of Si85. Functionalising groups cover the inner surface of the mesopores, as revealed by the nitrogen-adsorption characterisation, since Brunauer–Emmett–Teller (BET) surface, pore volume and pore diameter decrease after the functionalisation of the material. After the loading process with ipriflavone, these three parameters undergo a further drop. Taking into account the percentages of loaded IP (4–12% in weight), pore-volume decrease would agree with IP incorporation within the pores. However,

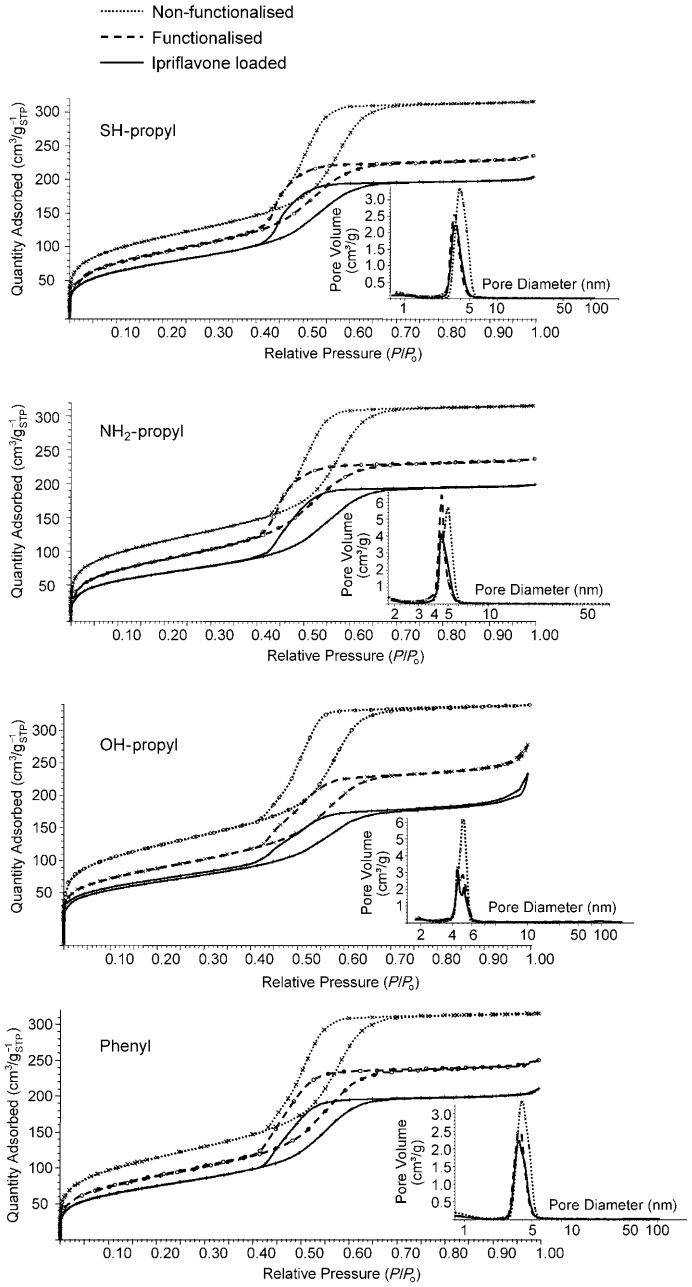


Figure 2. N_2 adsorption isotherms and pore-size distribution of mesoporous bioactive glasses (<L), functionalised and ipriflavone-loaded materials.

pore-size decrease is not as large as expected according to the size of the molecule of ipriflavone (ca. 1.2 nm). Thus, in concordance with FTIR spectra, the drug is probably being incorporated both inside the mesopores as well as in the outer surface of the materials. The mean pore size (shown in Table 1) is the average value of those filled, unfilled and partially filled pores. This scenario has been previously observed for mesoporous silica-based materials with drugs, mainly due to the high reactivity of silanol groups on the outer surface.[6a,c]

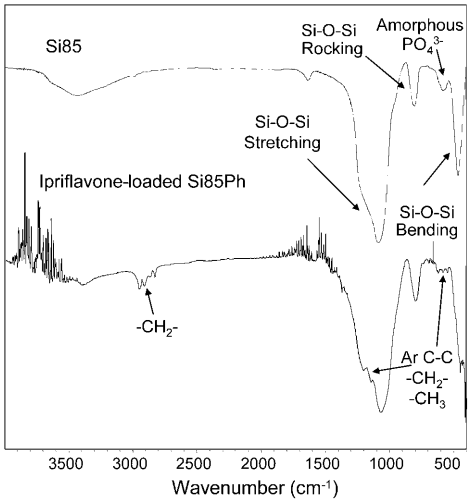


Figure 3. FTIR spectrum of as-synthesised mesoporous bioactive glass (Si85) and ipriflavone-loaded phenyl-functionalised material (Si85Ph).

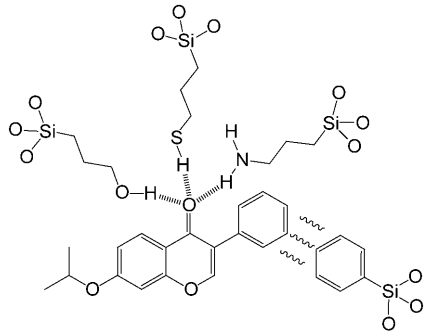
Important differences among functionalised materials can be observed in the amount of ipriflavone loaded and are summarised in Table 2. Phenyl-functionalised MBG loads around 12% weight of the drug; Si85OH and $Si85NH_2$ load 6% of ipriflavone whereas Si85SH loads 4%. No significant variations in the carbon content or differences in the thermogravimetric profile (data not shown) were observed in the analyses before and after the ipriflavone-loading process in non-functionalised mesoporous glasses, Si85Cl and Si85C4, though it is worth pointing out that insignificant drug amounts (around 1% weight) are loaded in these samples.

Table 2. The percentage of functionalisation (by mass) and ipriflavone loading obtained by thermogravimetric differential thermal analysis (TG-DTA) and CHN elemental analysis and the percentage of loaded ipriflavone released from the different materials.

Group/sample	Functionalised material [%]	Ipriflavone loaded [%]	Ipriflavone released [%]
non-functionalised/Si85	–	1.29	N/A
phenyl/Si85Ph	4.56	11.7	3
OH-propyl/Si85OH	4.79	6.08	6.02
NH_2-propyl/$Si85NH_2$	6.78	6.14	7.27
SH-propyl/Si85SH	4.29	4.05	13
Cl-propyl/Si85Cl	4.16	1.68	N/A
butyl/Si85C4	3.44	1.06	N/A

The amount of ipriflavone incorporated strongly depends on the chemical nature of the surface of MBGs. Two main factors determine the degree of drug charge: the hydrophobicity of the surface and the presence of a chemical group able to form a stable interaction with the drug molecule. Ipriflavone is a very hydrophobic drug and thus the charging solution must be carried out in an organic solvent such as acetone. The surface of non-functionalised MBG (Si85) is covered by silanol groups, which confer a hydrophilic profile to these materials. Although these silanol groups have hy-

droxyl terminals that are able to form hydrogen bonds with the oxygen atoms of ipriflavone, the difference in hydrophobicity between Si85 and acetone causes ipriflavone to remain in the solvent rather than interact with the biomaterial. Functionalisation of the surface of MBGs with organic chains may diminish this difference in hydrophobicity between solvent and biomaterial. However, functionalised materials such as Si85C4 and Si85Cl do not load ipriflavone, thereby indicating that chemical bonding between the functionalising group and ipriflavone is required. In this sense, Si85OH, Si85SH or Si85NH_2, which bond to drug by means of hydrogen interaction, or Si85Ph, which bonds ipriflavone through π–π stacking interactions between phenyl groups as shown in Scheme 1 charge ipriflavone from the solution. It



Scheme 1. Molecule of ipriflavone and interactions with the different functionalising groups (hydrogen bonds or π–π stacking interactions).

is well known that among other factors (pore size, surface area or pore volume, for example) the amount of drug loaded in mesoporous materials strongly depends on the strength of the newly formed bond between the drug and the surface.[6b] π–π stacking interactions seem to be very efficient bonds since phenyl-functionalised materials load more ipriflavone than Si85OH, Si85NH_2 and Si85SH and they show almost identical textural properties.

At this point, it must be mentioned that surface modification with organic groups is an appropriate method for regulating both the uptake and delivery of drugs in mesoporous materials.[6b] However, the potential toxicity of organic compounds should be considered. Avoiding the transference of the organic functional groups to the systemic circulation through the formation of a strong covalent bond with the matrix is an appropriate strategy. In this sense, the covalent bond between organic groups and MBGs achieved by the post-grafting method ensures that is links to the mesoporous matrix. Some recent studies have demonstrated that certain surface functionalisations with groups such as amino or mercapto can even reduce the cytotoxicity of silica mesoporous materials.[9a,b] Regarding phenyl and hydroxypropyl groups, some studies have been carried out with very limited information about their cytotoxicity.[9c,d]

The in vitro release profile of ipriflavone from the different materials as a function of time is shown in Figure 4. A similar pattern that consists of a first fast release followed

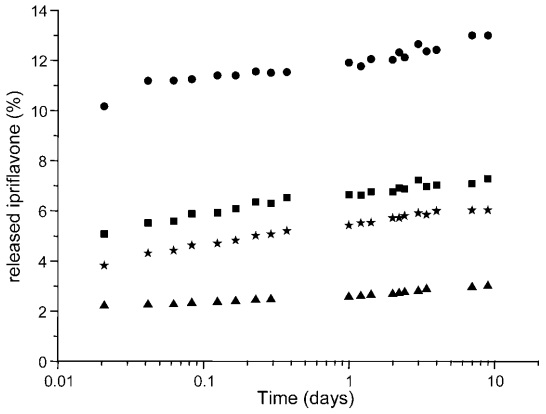


Figure 4. Ipriflavone delivery profiles of different functionalised materials (% of loaded ipriflavone released vs. time; ●=SH-propyl, ■=NH_2-propyl, ★;=OH-propyl and ▲=phenyl).

by a slow delivery is observed in all the materials. Nevertheless, the amount of ipriflavone released from the materials shows differences. Whereas Si85SH delivers up to 13% of the loaded drug after ten days of essay, phenyl-functionalised materials release only 3%, thus revealing that ipriflavone is strongly attached to the material. The small initial burst release confirms that most of the drug is chemically attached to the functional groups and not physisorbed to the surface of the MBG.

Drug-delivery profiles are in agreement with ipriflavone-loading capability, thereby demonstrating the high chemical affinity of the drug toward the new functionalised surface. During the drug-release essay, ipriflavone must be solved in an aqueous medium such as an isopropanol/water mixture. Isopropanol/water is the usual delivery medium chosen for release tests of ipriflavone.[10] This experimental medium, very far from biological conditions, is used only for comparative purposes among different materials. Ipriflavone is almost completely insoluble in water and thus is not expected to be easily solved in plasma. This feature would ease the permanence of the drug in the biomaterial until the colonisation by the newly formed tissue in in vivo conditions.

In in vitro delivery tests, ipriflavone must leave a hydrophobic environment to be delivered into a hydrophilic solvent. In this case, the strength of the bonding molecule biomaterial will determine the amount of ipriflavone to be released. Si85Ph releases less drug than those that have hydrogen bonds with the drug since, as also observed from drug loading, π–π stacking creates more efficient bonds to link this molecule and the biomaterial. Within Si85SH, Si85NH_2 and Si85OH the strength of the hydrogen-bonded ipriflavone–biomaterial depends on the electronegativity (χ) of the atom bonded to H. Consequently, ipriflavone will be released more easily from Si85SH than Si85NH_2 and Si85OH (χ is 2.58, 3.04 and 3.44 for S, N and O, respectively). Ipriflavone delivery tests determine that the drug will not be released quickly into the aqueous medium and it will thus be present during the formation of the new bone tissue. Moreover, results shown in the present work demonstrate that ap-

propriate chemical functionalisation of the surface is compulsory to use MBGs as hydrophobic drug-carrier systems (Table 2). In addition, by choosing a specific functionalising agent, it is possible to tune the dosage of ipriflavone in the material.

Figure 5 shows the FTIR spectra of the different ipriflavone-loaded materials before and after being soaked in simulated body fluid (SBF) for one day. SBF contains the

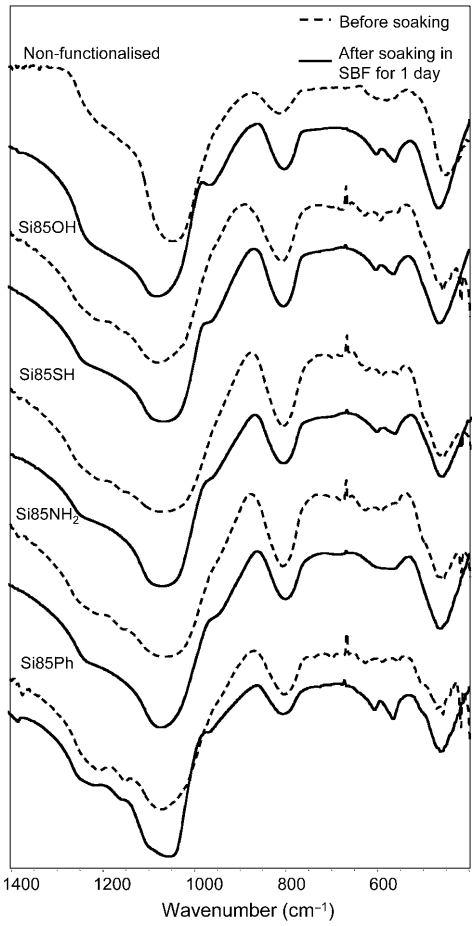


Figure 5. FTIR spectra of as-synthesised and ipriflavone-loaded functionalised mesoporous bioactive glasses before and after one day of immersion in simulated body fluid.

same ionic composition as human plasma[11] and it is widely accepted as an adequate test of the in vitro bioactivity of biomaterials for bone-restoring purposes.[12] The bioactive behaviour can be tested through the formation of an apatite-like phase at the surface of the materials. This new phase is very similar to the mineral component of bone and teeth and is easily followed by the crystalline phosphates revealed in FTIR spectroscopy.[13] Before soaking, characteristic bands of Si-O-Si bonds, organic and amorphous phosphate groups absorption bands are observed (see above). After one day of immersion in SBF, the spectra of all the materials except for $Si85NH_2$ reveals that the amorphous phosphate band splits into a doublet at 560 and 600 cm^{-1}, which corresponds to crystalline phosphate. This doublet is not clearly distinguishable until 3 days of immersion in amino-functionalised samples, thereby suggesting that the process is slowed in these materials. Materials undergo a similar surface evolution until the end of the bioactive essay (see the Supporting Information).

The evolution of pH and calcium concentration of SBF as a function of soaking time of the different materials is displayed in Figure 6. The analysis of SBF as a function of time allows one to follow the first stages of the bioactive behaviour, which is in agreement with the theory developed by Hench.[14] Similar evolution is obtained in all the essays. Both calcium and pH levels increase in the first hours of experiment. This increase is delayed in essays carried out with $Si85NH_2$ relative to the rest of materials. After this initial rise, calcium concentrations decrease until the end of the essay. The pH levels also diminish slightly after the initial increase.

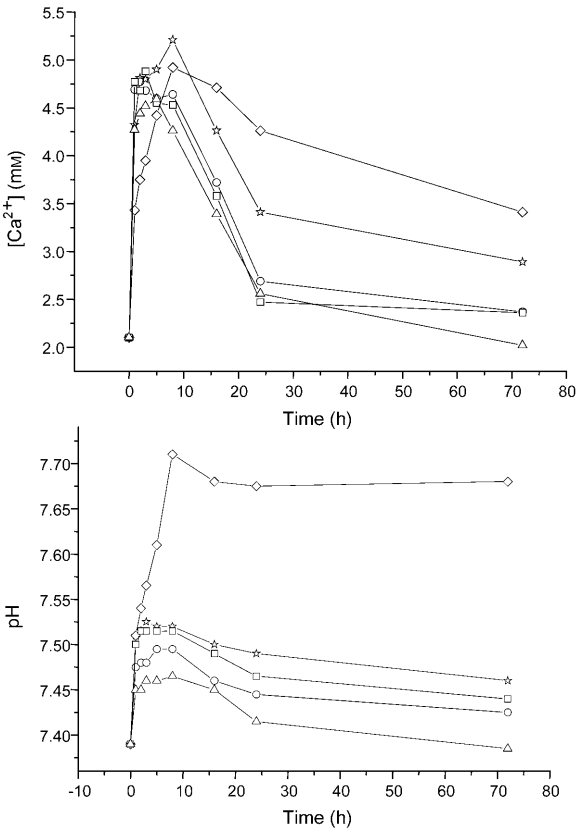


Figure 6. Evolution of calcium concentration and pH levels of simulated body fluid after different times of soaking of as-synthesised and functionalised materials (△ = non-functionalised, □ = phenyl, ○ = OH-propyl, ☆ = SH-propyl and ◇ = NH_2-functionalised).

One day after soaking in SBF, a new apatite phase is formed. This means that MBGs exhibit excellent in vitro bioactive behaviour, which can be attributed to: 1) chemical composition based on a SiO_2–CaO–P_2O_5 bioactive system, 2) outstanding surface area and porosity values and 3) a hy-

drophilic surface that facilitates ion exchange when soaked in a hydric environment. In addition, these materials exhibit added value due to the incorporation of ipriflavone. As demonstrated by the very low release rate, the low diffusion towards hydric media allows for long-term local administration. Consequently, the drug remains associated with the bioactive matrix and the release will be closely associated to the biodegradation of the MBGs. Moreover, the formation of an apatite layer on the surface of the materials in a biological environment would hinder the release of the drug,[7g] which reinforces the role of functionalised MBGs as long-term drug-delivery devices. The incorporation of hydrophobic substances onto hydrophilic MBGs can seriously affect the bioactive behaviour since it strongly depends on the ion exchange with the hydric environment. In this sense, both functionalisation and drug-loading methods have been developed to retain this bioactive performance.

As an example of this, calcium has shown to be an essential component for the obtainment of highly bioactive materials. Ca^{2+} immediately exchanges with H^+ from the medium when MBGs come into contact with an aqueous environment. As a result, all the chemical processes for functionalisation and drug loading detailed in the present work were carried out using non-polar solvents to keep the chemical composition of the materials. The process to achieve surface modification may not only retain the chemical nature of the material, but also both the textural and structural characteristics of the mesoporous glasses. The extensive characterisation carried out in the present work shows that the functionalisation process employed is not significantly modifying the mesoporous network and the very fast bioactivity kinetics of MBGs are maintained.

According to the results from the in vitro bioactivity tests, surface functionalisation of MBGs does not considerably affect the bioactive performance of these materials. All functionalised and ipriflavone-loaded glasses, excepting $Si85NH_2$, show a similar surface evolution to that observed in MBGs: fast formation of an amorphous phosphate phase that crystallises after one day of essay. In the case of amino-functionalised mesoporous glasses, the process seems to be slowed down due to the presence of positive charges from functionalising groups on the surface of the material, which may make the exchange of calcium and protons between the glass and SBF more difficult. In any case, at the end of the experiment, after three days of soaking in SBF, all materials developed a new layer of crystalline phosphate over their surface, as revealed by FTIR analyses (see Figure S1 in the Supporting Information). These bioactive kinetics are faster than the one of conventional sol–gel glasses and are in concordance with previous bioactivity investigations carried out with functionalised MBGs.[4f] For non-functionalised MBGs, this outstanding bioactive behaviour is explained as a result of the high reactive surface area shown by these materials. Although chemical functionalisation and drug loading lead to a decrease in the surface area of the materials, textural parameters shown by ipriflavone-loaded functionalised MBG are high enough to keep an excellent bioactive kinetics. Moreover, calcium concentration and pH evolution of SBF during the essay follow the trend expected of the bioactive process (i.e., an initial increase due to Ca^{2+}–H^+ exchange between material and medium followed by a decrease as a result of calcium phosphate crystallisation). This fact suggests that the functionalisation degree and ipriflavone loading are not high enough to hinder the reactions that take part in the bioactive process, and it would ensure long-term drug dispensibility when implanted.

Conclusion

Mesoporous bioactive glasses have been functionalised to incorporate ipriflavone, a highly hydrophobic anti-osteoporotic drug. For the first time, it is shown that by tailoring the hydrophobicity of the surface with chemical groups, it is possible to attach a drug to the surface of MBG to achieve long-term drug delivery. Moreover, functionalised MBGs show outstanding bioactive behaviour despite the presence of hydrophobic groups on their surface.

The possibility of incorporating a bone-resorption inhibitor added to the bioactive behaviour of mesoporous glasses enhances the potential of these materials as promising components in bone-tissue regeneration.

Experimental Section

A mesoporous bioactive glass (MBG) of composition $SiO_2/CaO/P_2O_5$ (85:10:5 % mol) was synthesised following an evaporation-induced self-assembly (EISA) method.[15] This material was denoted as Si85. A post-synthetic grafting procedure was used to perform the functionalisation of the surface of the MBG with different chemical groups. Phenyl, hydroxypropyl, aminopropyl and mercaptopropyl groups were attached to MBG. The resulting materials were denoted as Si85Ph, Si85OH, $Si85NH_2$ and Si85SH. respectively. For purposes of comparison, butyl (Si85C4)- and cloropropyl (Si85Cl)-functionalised materials were also synthesised.

Tetraethyl ortosilicate (TEOS), triethylphosphate (TEP), calcium nitrate ($Ca(NO_3)_2{\cdot}4H_2O$) and ipriflavone (7-Isopropoxy-3-phenyl-4*H*-1-benzopyran-4-one) were purchased from Aldrich. Functionalisation agents phenyltriethoxysilane, (3-aminopropyl)triethoxysilane, (3-mercaptopropyl)-trimethoxysilane, (3-chloropropyl)triethoxysilane and *n*-butyltrimethoxysilane were purchased from ABCR. Pluronic P123 was obtained as a gift from BASF. All these chemicals as well as all those detailed in the present work were used without further purification.

Synthesis of the mesoporous bioactive glass: A highly ordered mesoporous glass with a composition $SiO_2/CaO/P_2O_5$ (85:10:5 % mol) was synthesised following an EISA method described before.[4a] Briefly, Pluronic P123 (2 g) was dissolved in ethanol (30 g) with 0.5 N HCl (0.5 mL). Afterwards, TEOS (3.7 g), TEP (0.34 g) and calcium nitrate ($Ca(NO_3)_2{\cdot}4H_2O$) (0.49 g) were added under continuous stirring in 3 h intervals. Sols were cast in Petri dishes at room temperature over 7 d, when homogenous transparent membranes were obtained. Dried gels were calcined at 700 °C for 3 h to obtain the final glass powder.

Functionalisation of the materials: MBG was functionalised following a post-synthetic grafting method. According to the surface area of Si85, the amount of silanol groups was estimated to be 3.6 mmol of SiOH per gram of mesoporous material.[16] To ensure an excess amount of functionalising agent, the SiOH/functionalising agent molar relationship was

fixed to be 1:1 during the functionalisation process. Si85 (1 g) was degassed overnight at 110 °C and then suspended in toluene (20 mL). The corresponding amount of functionalisation agent was diluted in toluene (10 mL), which was added dropwise to the suspension of Si85 under continuous stirring. Solution was kept stirring overnight at 60 °C under reflux conditions. All the process was carried out under a nitrogen atmosphere. The suspension was filtered and gently washed with trichloromethane and dried at 70 °C.

Si85OH was obtained by further modification of Si85Cl following a method detailed elsewhere.[17] Briefly, Si85Cl (1 g) was degassed overnight at 110 °C. Afterwards, hydrated methanol (30 mL) was added and the resulting suspension was heated under continuous stirring at 70 °C during 2 h at reflux. The suspension was filtered, gently washed with ethanol and dried at 70 °C.

In vitro drug release essay: Ipriflavone was loaded into the MBG by an impregnation method from a highly concentrated solution of the drug, in an analogous way to that used for loading active principles into silica mesoporous materials.[6b] The different mesoporous glasses (400 mg) were added to a solution of ipriflavone (2 g) in anhydrous acetone (30 mL). This suspension was vigorously stirred over 24 h at room temperature while the evaporation of acetone was prevented. The material was gently washed with acetone after the drug loading and dried at 40 °C overnight. Due to the almost complete water insolubility of ipriflavone, a mixture of isopropanol/water (60:40 v/v), already used in previous ipriflavone delivery essays,[10] was chosen as release medium. The loaded material (100 mg) was immersed in the mixture isopropanol/water (10 mL) at 37 °C. Samples of 0.1 mL were taken at different time intervals and were replaced by preheated solution. Samples were analysed by HPLC at 273 nm.

The determination of the ipriflavone loading in the material was carried out by thermogravimetric (TG) analyses using a Seiko Thermobalance TG/DTA 320, as well as CHN elemental microanalyses.

Assessment of bioactive behaviour: Mesoporous material (100 mg) was soaked in filtered simulated body fluid (SBF; 20 mL) in polyethylene containers at 37 °C under sterile conditions. SBF has a composition and ionic concentrations similar to those of human plasma.[11] Samples were removed at given time intervals and the evolution of their surface was analysed by Fourier transform infrared (FTIR) spectroscopy. The evolution of calcium concentration and pH levels of SBF during the essay were also analysed using an Ilyte Na^+ K^+ Ca^{2+} pH system.

Characterisation: Powder X-ray diffraction (XRD) experiments were carried out with a Philips X'pert diffractometer equipped with $Cu_{K\alpha}$ radiation (wavelength 1.5406 Å). XRD patterns were collected in the 2θ ranges between 0.8 and 6° with a step size of 0.02° and a counting time of 5 s.

Textural properties of the materials were determined by nitrogen sorption porosimetry by using a Micromeritics ASAP 2020 device. To perform the N_2 measurements, samples were previously degassed under vacuum for 24 h at 70 °C. The surface area was determined using the Brunauer–Emmett–Teller (BET) method. The pore-size distribution between 0.5 and 40 nm was determined from the desorption branch of the isotherm by means of the Barret–Joyner–Halenda (BJH) method.

FTIR analyses of the glass were carried out using a Nicolet Magma IR 550 spectrometer with KBr as sample inert medium.

HPLC measurements were performed using a Waters Alliance automatic analysis system (Waters Co. Massachusetts, USA) composed of a model #2695 separations module and a model #2996 photodiode array detector. The employed column was a Mediterranea Sea 18, 5 μm, 150×2.1 mm (Teknokroma). The mobile phase was a mixture of acetonitrile and 0.1% H_3PO_4 solution (80:20 v/v) delivered at 0.4 mL min^{-1} at 50 °C.

Acknowledgements

The financial supports of CICYT Spain, through research projects MAT2008-00736 and CAM S2009/MAT-172 are acknowledged. CIBER-BBN is an initiative funded by the VI National R&D&i Plan 2008–2011, Iniciativa Ingenio 2010, Consolider Program and CIBER Actions and financed by the Instituto de Salud Carlos III with assistance from the European Regional Development Fund. We thank Dr. F. Conde (CAI X-ray diffraction, Universidad Complutense) and CAI Microanálisis, Universidad Complutense for their valuable technical assistance. We also thank Dr. M. Manzano for help in HPLC analyses and fruitful discussion.

[1] L. L. Hench, J. Wilson, *Science* **1984**, *226*, 630–636.

[2] X. X. Yan, C. Z. Yu, X. F. Zhou, J. W. Tang, D. Y. Zhao, *Angew. Chem.* **2004**, *116*, 6106–6110; *Angew. Chem. Int. Ed.* **2004**, *43*, 5980–5984.

[3] a) C. T. Kresge, M. E. Leonowicz, W. J. Roth, J. C. Vartuli, J. S. Beck, *Nature* **1992**, *359*, 710–712; b) J. S. Beck, J. C. Vartuli, W. J. Roth, M. E. Leonowicz, C. T. Kresge, K. D. Schmitt, C. T. W. Chu, D. H. Olson, E. W. Sheppard, S. B. McCullen, J. B. Higgins, J. L. Schlenker, *J. Am. Chem. Soc.* **1992**, *114*, 10834–10843; c) D. Y. Zhao, J. L. Feng, Q. S. Huo, N. Melosh, G. H. Fredrickson, B. F. Chmelka, G. D. Stucky, *Science* **1998**, *279*, 548–552.

[4] a) A. López-Noriega, D. Arcos, I. Izquierdo-Barba, Y. Sakamoto, O. Terasaki, M. Vallet-Regi, *Chem. Mater.* **2006**, *18*, 3137–3144; b) Q. H. Shi, J. F. Wang, J. P. Zhang, J. Fan, G. D. Stucky, *Adv. Mater.* **2006**, *18*, 1038; c) B. Izquierdo Barba, D. Arcos, Y. Sakamoto, O. Terasaki, A. Lopez-Noriega, M. Vallet-Regi, *Chem. Mater.* **2008**, *20*, 3191–3198; d) X. X. Yan, X. H. Huang, C. Z. Yu, H. X. Deng, Y. Wang, Z. D. Zhang, S. Z. Qiao, G. Q. Lu, D. Y. Zhao, *Biomaterials* **2006**, *27*, 3396–3403; e) T. A. Ostomel, Q. H. Shi, C. K. Tsung, H. J. Liang, G. D. Stucky, *Small* **2006**, *2*, 1261–1265; f) J. Sun, Y. S. Li, L. Li, W. R. Zhao, J. H. Gao, M. L. Ruan, J. L. Shi, *J. Non-Cryst. Solids* **2008**, *354*, 3799–3805; g) H. S. Yun, S. E. Kim, Y. T. Hyun, *Solid State Sci.* **2008**, *10*, 1083–1092; h) H. S. Yun, S. E. Kim, Y. T. Hyeon, *Mater. Lett.* **2007**, *61*, 4569–4572.

[5] a) H. S. Yun, S. E. Kim, Y. T. Hyeon, *Chem. Commun.* **2007**, 2139–2141; b) H. S. Yun, S. E. Kim, Y. T. Hyun, S. J. Heo, J. W. Shin, *Chem. Mater.* **2007**, *19*, 6363–6366; c) X. Li, X. P. Wang, H. R. Chen, P. Jiang, X. P. Dong, J. L. Shi, *Chem. Mater.* **2007**, *19*, 4322–4326; d) X. Li, J. L. Shi, X. P. Dong, L. X. Zhang, H. Y. Zeng, *J. Biomed. Mater. Res. Part A* **2008**, *84*, 84–91; e) Y. F. Zhu, C. T. Wu, Y. Ramaswamy, E. Kockrick, P. Simon, S. Kaskel, H. Zrelqat, *Microporous Mesoporous Mater.* **2008**, *112*, 494–503; f) Y. F. Zhu, S. Kaskel, *Microporous Mesoporous Mater.* **2009**, *118*, 176–182.

[6] a) M. Vallet-Regi, A. Ramila, R. P. del Real, J. Perez-Pariente, *Chem. Mater.* **2001**, *13*, 308–311; b) M. Vallet-Regí, F. Balas, D. Arcos, *Angew. Chem.* **2007**, *119*, 7692–7703; *Angew. Chem. Int. Ed.* **2007**, *46*, 7548–7558; c) A. L. Doadrio, E. M. B. Sousa, J. C. Doadrio, J. Perez-Pariente, I. Izquierdo-Barba, M. Vallet-Regí, *J. Controlled Release* **2004**, *97*, 125–132.

[7] a) Y. F. Zhao, S. C. J. Loo, Y. Z. Chen, F. Y. C. Boey, J. Ma, *J. Biomed. Mater. Res. Part A* **2008**, *85*, 1032–1042; b) W. Xia, J. Chang, *J. Controlled Release* **2006**, *110*, 522–530; c) D. Arcos, A. Lopez-Noriega, E. Ruiz-Hernandez, O. Terasaki, M. Vallet-Regi, *Chem. Mater.* **2009**, *21*, 1000–1009; d) Y. Fan, P. P. Yang, S. S. Huang, J. H. Jiang, H. Z. Lian, J. Lin, *J. Phys. Chem. C* **2009**, *113*, 7826–7830; e) X. Li, X. P. Wang, L. X. Zhang, H. R. Chen, J. L. Shi, *J. Biomed. Mater. Res. B* **2009**, *89*, 148–154; f) S. B. Wang, *Microporous Mesoporous Mater.* **2009**, *117*, 1–9; g) L. Z. Zhao, X. X. Yan, X. F. Zhou, L. Zhou, H. N. Wang, H. W. Tang, C. Z. Yu, *Microporous Mesoporous Mater.* **2008**, *109*, 210–215.

[8] a) M. Yagi, Y. Ono, T. Minegishi, T. Uchiyama, K. Tanaka, Y. Mizumura, S. Sato, K. Ito, *J. Health Sci.* **2007**, *53*, 435–442; b) S. Benvenuti, M. Petilli, U. Frediani, A. Tanini, G. Fiorelli, S. Bianchi, P. A. Bernabei, C. Albanese, M. L. Brandi, *Biochem. Biophys. Res. Commun.* **1994**, *201*, 1084–1089; c) S. H. Kim, M. G. Lee, *Life Sci.*

2002, *70*, 1299–1315; d) D. Agnusdei, L. Bufalino, *Calcif. Tissue Int.* **1997**, *61*, S23–S27.
[9] a) A. J. Di Pasqua, K. K. Sharma, Y.-L. Shi, B. B. Toms, W. Ouellette, J. C. Dabrowiak, T. Asefa, *J. Inorg. Biochem.* **2008**, *102*, 1416–1423; b) Z. Tao, B. B. Toms, J. Goodisman, T. Asefa, *Chem. Res. Toxicol.* **2009**, *22*, 1869–1880; c) R. Kumar, I. Roy, T. Y. Hulchanskyy, L. N. Goswami, A. C. Bonoiu, E. J. Bergey, K. M. Tramposch, A. Maitra, P. N. Prasad, *ACS NANO* **2008**, *2*, 449–456; d) P. Horcajada, A. Rámila, G. Ferey, M. Vallet-Regí, *Solid State Sci.* **2006**, *8*, 1243–1249.
[10] a) G. Khang, J. M. Rhee, J. K. Jeong, J. S. Lee, M. S. Kim, S. H. Cho, H. B. Lee, *Macromol. Res.* **2003**, *11*, 207–223; b) P. A. Tarantili, H. Koumoulos, *Eur. Polym. J.* **2008**, *44*, 444–452.
[11] T. Kokubo, H. Kushitani, S. Sakka, T. Kitsugi, T. Yamamuro, *J. Biomed. Mater. Res.* **1990**, *24*, 721–734.
[12] T. Kokubo, H. Takadama, *Biomaterials* **2006**, *27*, 2907–2915.
[13] L. D. Warren, A. E. Clark, L. L. Hench, *J. Biomed. Mater. Res.* **1989**, *23*, 201–209.
[14] L. L. Hench. , O. Andersson in *Bioactive Glasses. An Introduction to Bioceramics* (Eds.: L. L. Hench, J. Wilson), Elsevier, New York, **1995**, p. 477.
[15] C. J. Brinker, Y. F. Lu, A. Sellinger, H. Y. Fan, *Adv. Mater.* **1999**, *11*, 579.
[16] L. T. Zhuravlev, *Langmuir* **1987**, *3*, 316–318.
[17] C. Venkatesan, A. P. Singh, *Catal. Lett.* **2003**, *88*, 193–197.

Received: January 19, 2010
Published online: July 26, 2010

