Surface Functionalisation of Alumina Ceramic Foams with Organic Ligands

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Different anchoring groups have been studied with the aim of covalently binding organic linkers to the surface of alumina ceramic foams. The results suggested that a higher degree of functionalisation was achieved with a pyrogallol derivative - as compared to its catechol analogue - based on the XPS analysis

of the ceramic surface. The conjugation of organic ligands to the surface of these alumina materials was corroborated by DNP-MAS-NMR measurements.

Ceramic foam, functionalisation, XPS, DNP-MAS-NMR, biomaterials, pyrogallol moiety.

Introduction

While today's gold standard to treat bone defects is still autologous iliac crest bone grafting, the development of synthetic bone graft materials appears as an appealing alternative. Following the foaming procedure developed by Gonzenbach *et al.*,[1] highly porous alumina can be produced into desired shapes to fill, for example, bone defects and provide mechanical resistance to the graft. The pore size and interconnectivity of this new material can be controlled during the foaming process and can be tuned accordingly.[2] The foams feature a macrostructure with pores between 100-350 µm which promotes osteogenesis, cells and ions transport for generation of bone tissue and a microstructure with pores <20 µm that favours the neovascularisation and fiberblast ingrowth.[3] Nevertheless, in order to promote efficient tissue regeneration within the biomaterial, seeding of the scaffold with osteogenic cells[4] or osteoinductive growth factors[4,5] may be required. In this context, the biocompatibility of these new open-porous alumina ceramic scaffolds for human fetal osteoblasts has been demonstrated *in vitro*.[6] Another challenge associated with large bone substitutes is the necessity to develop a functional vascular system within the biomaterials. Several studies have shown that cell proliferation and mineralized tissue formation is often restricted to a zone of 120-250 µm from the scaffold surface.[7] Even when uniform initial seeding is achieved, the cells within the scaffold might either die or migrate toward the periphery of the scaffold to be exposed to higher levels of oxygen and nutrients unless a vascularised system is also present within the scaffold to ensure the cells needs.[8] Angiogenesis for the vascularisation of the new graft can be promoted by the presence of progenitor cells (*e.g.* human umbilical vein endothelial cells (HUVEC)[4b,7a,9] and/or bioactive molecules (*e.g.* vascular endothelial growth factor (VEGF))[4b,5,7a,9,10] that could be covalently attached to the ceramic through a small spacer

molecule.[11]

In the course of our studies for the development of open-porous alumina scaffolds as new potential bone substitutes,[12] we envisaged the chemical functionalisation of the alumina matrix by small organic ligands to promote both the formation of blood vessels and the adhesion of bone cell progenitors to the material. Among the simple chemical groups that have been proposed for adhesion on alumina, 1,2-di- and 1,2,3-trihydroxy benzene (catechol and pyrogallol) present efficient adsorption on the material surface through a process of ligand exchange.[13] In the present work, we demonstrate the stable functionalisation of open-porous alumina ceramic foams with organic ligands deriving from catechol and pyrogallol. Chemical modification of the material surface was monitored by X-ray Photoelectron Spectroscopy (XPS), a technique classically used for the surface characterisation of various materials including ceramics,[14] and was confirmed by DNP-MAS-NMR measurements.

Results and discussion

Preparation of organic ligands for the functionalization of open-porous alumina scaffolds

Our first challenge was to produce the proper organic ligands to bind to the ceramic foam in a stable and efficient manner. Two anchoring moieties were considered – catechol and pyrogallol – since they can complex the aluminium present in the inorganic matrix and act as adhesive functionalities.[13] The ligands **1** (catechol derivative) and **2** (pyrogallol derivative) were chosen as models while **3** was used as a negative control (Figure 1).

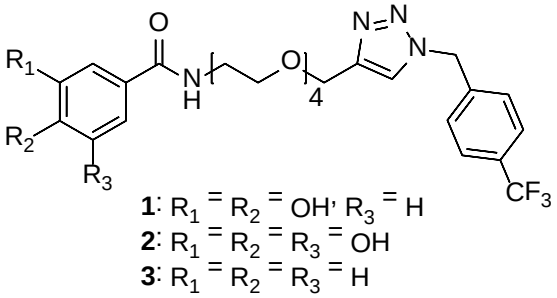
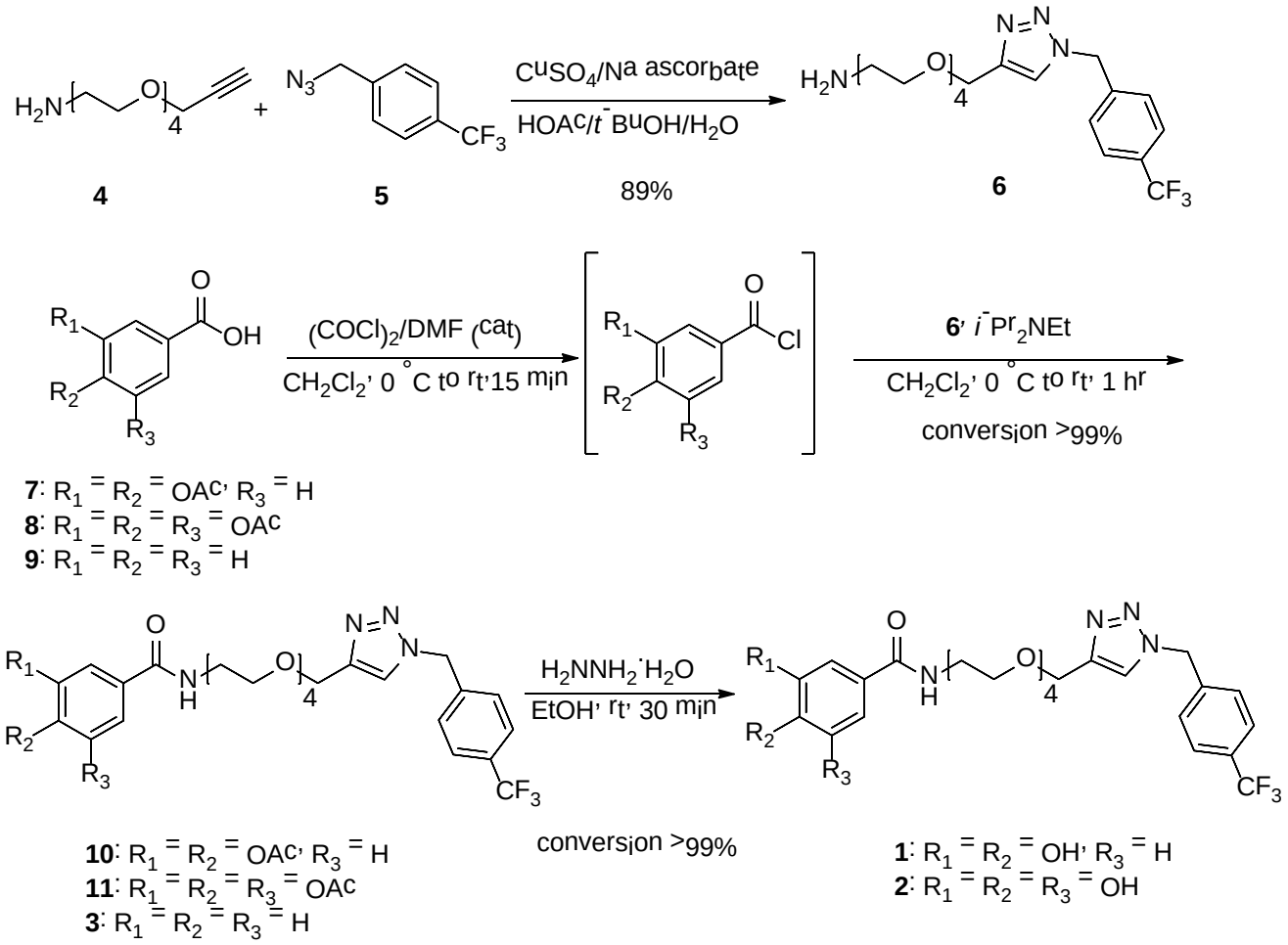


Figure 1. Linker **1** (catechol derivative), linker **2** (pyrogallol derivative) and **3** (negative control).

The synthesis of these molecules was carried out following the synthetic route shown in Scheme 1.

Copper catalyzed azide-alkyne cycloaddition between azide **4**[15] and alkyne **5**[16] gave the key intermediate **6** in 89% yield. After activation of the carboxylic acids **7**-**9** with oxalyl chloride/DMF (cat), the resulting acyl chlorides were coupled with amine **6** to deliver the corresponding amides in high yields. Final cleavage of the acetyl moieties in molecules **10**-**11** with hydrazine afforded the linkers **1** and **2** in 78 and 86% crude yields, respectively.

Scheme 1. Synthesis of linkers **1**-**3**.



4 **5** **6**

7: $R_1 = R_2 = OAc$, $R_3 = H$
8: $R_1 = R_2 = R_3 = OAc$
9: $R_1 = R_2 = R_3 = H$

10: $R_1 = R_2 = OAc$, $R_3 = H$
11: $R_1 = R_2 = R_3 = OAc$
3: $R_1 = R_2 = R_3 = H$

1: $R_1 = R_2 = OH$, $R_3 = H$
2: $R_1 = R_2 = R_3 = OH$

High-purity samples of **1**-**3** for conjugation studies were obtained after purification by reverse-phase HPLC.

Chemical modification of open-porous alumina ceramics and characterization of the functionalised materials

The ceramic foams at hand were produced according to a procedure published elsewhere.[2b] For this

study, we selected the ceramics presenting the composition and pore characteristics depicted in Table 1.

Table 1. Physical characteristics of ceramics **S1**, **S2** and **S3**. † Reference 4. ‡ Determined by XPS.

Ceramic	**S1**	**S2**	**S3**
Average pore size (μm) †	170	170	460
Average pore opening size (μm)†	50	50	95
Porosity (vol %)†	76	76	86
Al/Ca ratio‡	7.4	33.4	8.4

The functionalisation of the ceramic **S1**-**S3** surface with the linkers **1**-**3** was performed with a 1 mM aqueous solution of the corresponding linker, at room temperature for 16 h (for experimental details, see supporting information). After removal of the excess non-bound ligands by thorough washings with water, the samples were dried under vacuum and subsequently analysed by XPS. The fluorine content was determined and considered as measurement of functionalisation. For each ceramic, four samples were studied: untreated ceramic (sample **A**), negative control – incubated with **3** – (sample **B**), ceramic functionalised with linker **1** (sample **C**) and ceramic functionalised with linker **2** (sample **D**).

According to the survey scan spectra of sample **A**-**S1** (Figure 2, a), the elements of C, Ca, O and Al were found, of which the elements Ca, O and Al arose from the components of the ceramic itself since ceramic foams are made of alumina and calcium aluminate. The peaks associated with N and F in samples **C**-**S1** and **D**-**S1** after the functionalisation process demonstrated the presence of the linkers **1** and **2** respectively on the ceramic surface (Figure 2, c and d). Furthermore, the peaks at 293 eV and 688.5 eV are characteristics of a C1s and F1s from a trifluoromethyl group, respectively, proving undoubtedly the presence of the linkers at the surface of the ceramic scaffolds. No significant difference was observed between the untreated ceramic (**A**-**S1**) and the negative control (**B**-**S1**), which highlighted that the catechol or pyrogallol moieties are essential for the binding to the alumina foams (Figure 2, a

and b).[17] Similar observations were made for the **S2**-**S3** (data not shown).

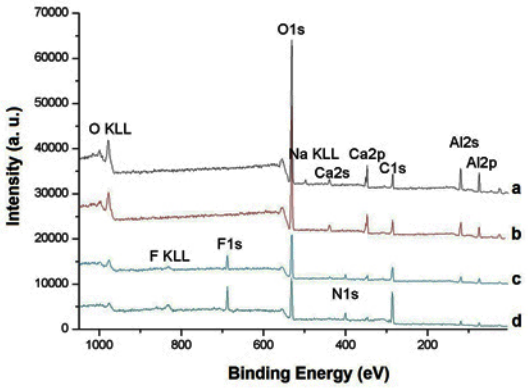


Figure 2. XPS wide survey spectra. (a) untreated ceramic **A**-**S1**, (b) negative control **B**-**S1**, (c) linker **1**-functionalised ceramic foam **C**-**S1**, (d) linker **2**-functionalised ceramic foam **D**-**S1**. Note the presence of F and N peaks in samples **C**-**S1** and **D**-**S1** and lack of them in sample **B**-**S1** (negative control).

Although XPS is widely used as unique technique for characterisation of surfaces, we decided to confirm the results using the cutting-edge analytical technique DNP-MAS-NMR.[18] For this experiment we used 3,4,5-trihydroxybenzamide (**12**) as a model compound for gallate derived organic ligands. Even with a relatively low functionalisation degree, the DNP enhanced ^{13}C-CP/MAS NMR spectrum of the functionalised alumina (Figure 3) clearly shows the signals from the organic component **12**, in agreement with the XPS results (for experimental details, see supporting information). The signal enhancement reached by DNP (ε_{DNP} = *ca.* 10) allows to obtain good signal/noise ratio and clear spectra, which was not possible using the standard ^{13}C-CP/MAS NMR.

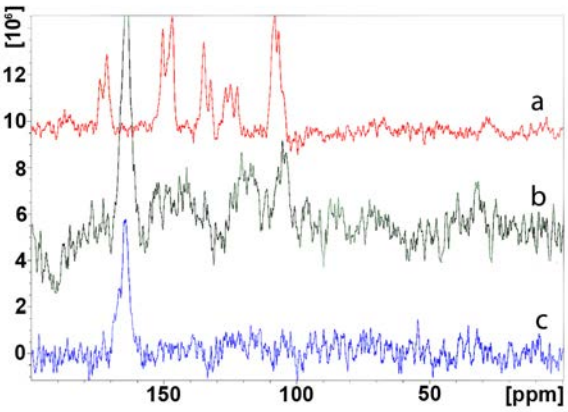


Figure 3. DNP-MAS-^{13}C NMR measurements. (a) pure 3,4,5-trihydroxybenzamide, (b) alumina functionalized with 3,4,5-trihydroxybenzamide, (c) untreated alumina

Influence of the composition and microstructure of the ceramic foams on the chemical functionalisation

XPS data of the untreated ceramics showed that **S2** has a much higher Al content, as compared to the two other ceramic foams (**S1** and **S3**), which was in agreement with the preparation method[1] (Table 2, entries 1-3). This higher Al content did not lead to an increased degree of functionalisation since at the same pore size, the F/Al ratio was lower in **S2** than in **S1** (entry 8 *vs* 7; entry 11 *vs* 10). This could be explained by the fact that the number of available Al sites may be limited by cluttering (an excess of linker was confirmed after incubation by analysis of the supernatant).

Table 2. Al/Ca and F/Al ratios determined by XPS

entry	ceramic	linker	Al/Ca	F/Al
1	**S1**	ø	7.4 ± 2.3	-
2	**S2**	ø	33.4 ± 10.5	-
3	**S3**	ø	8.4 ± 2.6	-
4	**S1**	**3**	5.5 ± 1.7	-
5	**S2**	**3**	43.2 ± 13.6	-
6	**S3**	**3**	9.5 ± 3.0	-
7	**S1**	**1**	7.5 ± 2.4	0.4 ± 0.1
8	**S2**	**1**	50.5 ± 15.9	0.2 ± 0.1
9	**S3**	**1**	9.6 ± 3.0	0.3 ± 0.1
10	**S1**	**2**	7.9 ± 2.5	1.1 ± 0.3
11	**S2**	**2**	27.7 ± 8.7	0.5 ± 0.1
12	**S3**	**2**	9.6 ± 3.0	0.7 ± 0.2

Bigger pores induced smaller specific surface available for functionalisation and, at the same chemical

composition of the ceramic, the F/Al ratio found was lower in **S3** than in **S1** (entry 7 *vs* 9; 10 *vs* 12). Finally, the higher degree of functionalisation with linker **2** indicated that the pyrogallol moiety is more efficient for the complexation of aluminium contained in the ceramic matrices than the catechol moiety (entry 7 *vs* 10; 8 *vs* 11; 9 *vs* 12).

Conclusions

The incubation of open-porous alumina scaffolds with organic ligands containing pyrogallol or catechol functionalities allowed their stable anchoring to the surface of the material. XPS analysis of the functionalised ceramic foams indicated than the pyrogallol moiety offers the highest degree of conjugation to the inorganic matrix. The chemical modification of the alumina matrices was confirmed by DNP-MAS-NMR measurements. Interestingly, the resulting binding showed to be stable after thorough washings with water (see protocol in supporting information). Furthermore, the conditions for the functionalisation are bio-compatible which should allow further conjugation of the ceramic foams to living cells.

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Supporting Information Available. Detailed protocols for synthesis and analytical data of compounds **1**, **2**, **3**, **6**, **10** and **11**. Protocol for the functionalisation of ceramic foams with **1** and **2**. Experimental details for DNP-MAS-NMR analyses. This material is available free of charge *via* the Internet at http://pubs.acs.org.

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