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Surface Functionalization of Alumina Ceramic Foams with Organic Ligands

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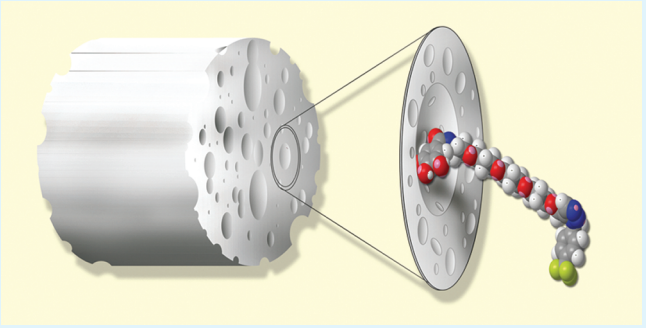
Supporting Information

ABSTRACT: 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 functionalization 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.



KEYWORDS: *ceramic foam, functionalization, XPS, DNP-MAS NMR, biomaterials, pyrogallol moiety*

INTRODUCTION

Although 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 and 350 μm which promotes osteogenesis, cells and ions transport for generation of bone tissue and a microstructure with pores <20 μm that favors the neovascularisation and fibroblast ingrowth.[3] Nevertheless, 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 functionalization 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]

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In the present work, we demonstrate the stable functionalization 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 characterization of various materials including ceramics,[14] and was confirmed by dynamic nuclear polarization under magic angle spinning (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 − because they can complex the aluminum present in the inorganic matrix and act as adhesive functionalities.[13] The ligands **1** (catechol derivative) and **2** (pyrogallol derivative) were chosen as models, whereas **3** was used as a negative control (Figure 1).

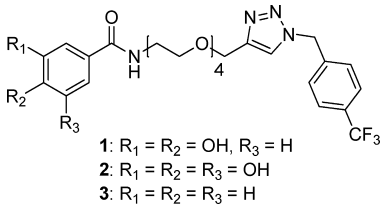


Figure 1. Linker **1** (catechol derivative), linker **2** (pyrogallol derivative), and **3** (negative control).

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.

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 Functionalized 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

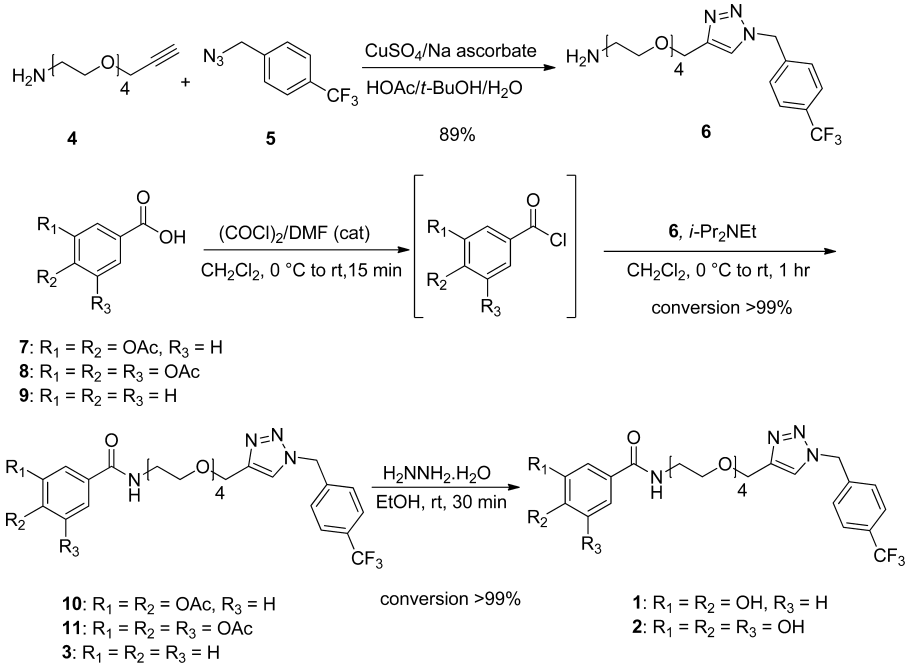
	S1	S2	S3
average pore size (μm)[a]	170	170	460
average pore opening size (μm)[a]	50	50	95
porosity (vol %)[a]	76	76	86
Al/Ca ratio[b]	7.4	33.4	8.4

[a]Reference 4. [b]Determined by XPS.

The functionalization 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 the Supporting Information). After removal of the excess nonbound ligands by thorough washings with water, the samples were dried under vacuum and subsequently analyzed by XPS. The fluorine content was determined and considered as measurement of functionalization. For each ceramic, four samples were studied: untreated ceramic (sample **A**), negative control − incubated with **3** − (sample **B**), ceramic functionalized with linker **1** (sample **C**) and ceramic functionalized 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

Scheme 1. Synthesis of Linkers 1−3



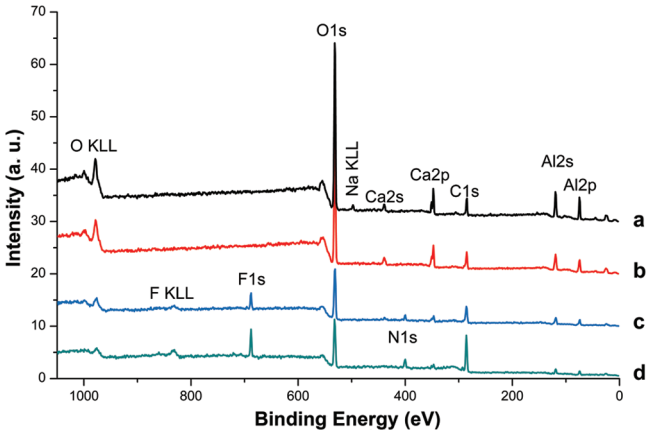


Figure 2. XPS wide survey spectra. (a) untreated ceramic **A-S1**, (b) negative control **B-S1**, (c) linker **1**-functionalized ceramic foam **C-S1**, (d) linker **2**-functionalized ceramic foam **D-S1**. Note the presence of F and N peaks in samples **C-S1** and **D-S1** and lack of them in sample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

ceramic itself because 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 functionalization process demonstrated the presence of the linkers **1** and **2** respectively on the ceramic surface (Figure 2c, d). Furthermore, the peaks at 293 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 2a, b).[17] Similar observations were made for the **S2-S3** (data not shown).

Although XPS is widely used as unique technique for characterization 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 functionalization degree, the DNP enhanced ^{13}C−CP/MAS NMR spectrum of the functionalized alumina (Figure 3) clearly shows the signals from the organic

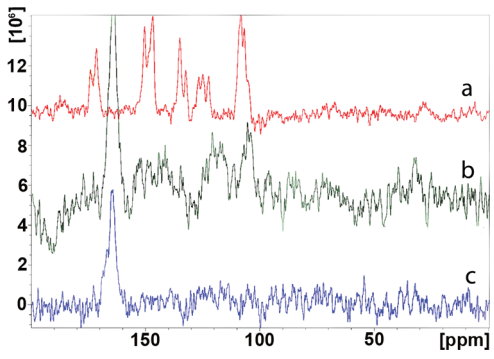


Figure 3. DNP-MAS−^{13}C NMR measurements. (a) pure 3,4,5-trihydroxybenzamide, (b) alumina functionalized with 3,4,5-trihydroxybenzamide, (c) untreated alumina.

component **12**, in agreement with the XPS results (for experimental details, see the 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.

Influence of the Composition and Microstructure of the Ceramic Foams on the Chemical Functionalization. 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

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

lead to an increased degree of functionalization 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).

Bigger pores induced smaller specific surface available for functionalization 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 functionalization with linker **2** indicated that the pyrogallol moiety is more efficient for the complexation of aluminum 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 functionalized 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 the Supporting Information). Furthermore, the conditions for the functionalization are biocompatible which should allow further conjugation of the ceramic foams to living cells.

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■ ASSOCIATED CONTENT

Supporting Information

Detailed protocols for synthesis and analytical data of compounds **1**, **2**, **3**, **6**, **10** and **11**. Protocol for the functionalization 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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Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) (a) Gonzenbach, U. T.; Studart, A. R.; Steinlin, D.; Tervoort, E.; Gauckler, L. J. *J. Am. Ceram. Soc.* **2007**, *90*, 3407−3414. (b) Gonzenbach, U. T.; Studart, A. R.; Tervoort, E.; Gauckler, L. J. *Angew. Chem., Int. Ed.* **2006**, *45*, 3526−3530. (c) Gonzenbach, U. T.; Studart, A. R.; Tervoort, E.; Gauckler, L. J *Langmuir* **2007**, *23*, 1025−1032. (d) Gonzenbach, U. T.; Studart, A. R.; Tervoort, E.; Gauckler, L. J. *Langmuir* **2006**, *22*, 10983−10988.

(2) Krauss Juillerat, F.; Gonzenbach, U. T.; Studart, A. R.; Gauckler, L. J. *Mater. Lett.* **2010**, *64*, 1468−1470. (b) Krauss Juillerat, F.; Gonzenbach, U. T.; Elser, P.; Studart, A. R.; Gauckler, L. J. *J. Am. Ceram. Soc.* **2011**, *94*, 77−83.

(3) (a) Yang, S.; Leong, K. F.; Du, Z.; Chua, C. K. *Tissue Eng.* **2001**, *7*, 679 andreferences therein. (b) Woodard, J. R.; Hilldore, A. J.; Lan, S. K.; Park, C. J.; Morgan, A. W.; Eurell, J. A. C.; Clark, S. G.; Wheeler, M. B.; Jamison, R. D.; Wagoner Johnson, A. J. *Biomaterials* **2007**, *28*, 45−54.

(4) (a) Meijer, G. J.; de Bruijn, J. D.; Koole, R.; van Blitterswijk, C. A. *PLOS Med.* **2007**, *4*, e9. (b) Ren, L.-L.; Ma, D. −Y.; Feng, X.; Mao, T.-Q.; Liu, Y.-P.; Ding, Y. *Med. Hypotheses* **2008**, *71*, 737−740.

(5) Huang, Y.-C.; Kaigler, D.; Rice, K. G.; Krebsbach, P. H.; Mooney, D. *J. Bone Miner. Res.* **2005**, *20*, 848−857.

(6) Krauss Juillerat, F.; Scaletta, C.; Applegate, L. A.; Borcard, F.; Comas, H.; Gaucker, L.; Gerber-Lemaire, S.; Juillerat-Jeanneret, L.; Gonzenbach, U. T. submitted.

(7) (a) Cassell, O. C. S.; Hofer, S. O. P.; Morrison, W. A.; Knight, K. R. *Brit. J. Plast. Surgery* **2002**, *55*, 603−610. (b) Sikavitsas, V. I.; Bancroft, G. N.; Mikos, A. G. *J. Biomed. Mater. Res.* **2002**, *62*, 136−148. (c) Shea, D. L.; Wang, D.; Franceschi, R. T.; Mooney, D. J. *Tissue Eng.* **2000**, *6*, 605−617. (d) Jain, R. K.; Au, P.; Tam, J.; Duda, D. G.; Fukumura, D. *Nat. Biotechnol.* **2005**, *23*, 821−823.

(8) (a) Ishaug, S. L.; Crane, G. M.; Miller, M. J.; Yasko, A. W.; Yaszemski, M. J.; Mikos, A. G. J. *Biomed. Mater. Res.* **1997**, *36*, 17−28. (b) Sednermir-Urkmez, A.; Jamison, R. D. *Biomaterials* **2007**, *28*, 45−54.

(9) (a) Hofmann, A.; Ritz, U.; Verrier, S.; Eglin, D.; Alini, M.; Fuchs, S.; Kirkpatrick, C. J.; Rommens, P. M. *Biomaterials* **2008**, *29*, 4217−4226.

(10) Jain, R. K.; Au, P.; Tam, J.; Duda, D. G.; Fukumura, D. *Nat. Biotechnol.* **2005**, *23*, 821−823.

(11) Hildebrand, H. F.; Blanchemain, N.; Mayer, G.; Zhang, Y. M.; Melnyk, O.; Morcellet, M.; Martel, B. *Key Eng. Mater.* **2005**, 288−289.

(12) For the chemical functionalization of other types of alumina scaffolds, see for example: (a) Gupta, S.; Ramamurthy, P. C.; Madras, G. *Ind. Eng. Chem. Res.* **2011**, *50*, 6585−6593. (b) Mahmoud, M. E.; Hafez, O. F.; Osman, M. M.; Yakout, A. A.; Alrefaay, A. *J. Hazard. Mater.* **2010**, *176*, 906−912. (c) Gomathi, A.; Hoseini, S. J.; Rao, C. N. R. *J. Mater. Chem.* **2009**, *19*, 988−995. (d) Hao, J.; Han, M.-J.; Meng, X. *J. Hazard. Mater.* **2009**, *167*, 1215−1221. (e) Atwater, J. E.; Akse, J. R. *J. Membr. Sci.* **2007**, *301*, 76−84. (f) Goldstein, C. S.; Weiss, K. D.; Drago, R. S. *J. Am. Chem. Soc.* **1987**, *109*, 758−61.

(13) (a) McBride, M. B.; Wesselink, L. G. *Environ. Sci. Technol.* **1988**, *22*, 703−708. (b) Laucournet, R.; Pagnoux, R. L.; Chartier, T.; Baumard, J. F. *J. Eur. Ceram. Soc.* **2001**, 869−878. (c) Tulevski, G. S.; Miao, Q.; Fukuto, M.; Abram, R.; Ocko, B.; Pindak, R.; Steigerwald, M. L.; Kagan, C. R.; Nuckolls, C. *J. Am. Chem. Soc.* **2004**, *126*, 15048−15050. (d) Chirdon, W. M.; O'Brien, W. J.; Robertson, R. E. *J. Biomed. Mater. Res.* **2002**, *66*, 532−538. (e) Hidber, P. C.; Graule, T. J.; Gauckler, L. J. *J. Eur. Ceram. Soc.* **1997**, *17*, 239−249. (f) Borah, J. M.; Sarma, J.; Mahiuddin, S. *Colloids Surf.: Physicochem. Eng. Aspects* **2011**, *387*, 50−56.

(14) (a) Zeng, H.; Lacefield, W. R. *Biomaterials* **2000**, *21*, 23−30. (b) Feddes, B.; Vredenberg, A. M.; Wolke, J. G. C.; Jansen, J. A. *Sudf. Interface Anal.* **2003**, *35*, 287−293. (c) Sufiyima, O.; Murakami, K.; Kaneko, S. *J. Eur. Ceram. Soc.* **2004**, *24*, 1157−1160. (d) Ingall, M. D. K.; Honeyman, C. H.; Mercure, J. V.; Bianconi, P. A.; Kunz, R. R. *J. Am. Chem. Soc.* **1999**, *121*, 3607−3613. (e) Boyer, C.; Bulmus, V.; Priyanto, P.; Teoh, W. Y.; Amalc, R.; Davis, T. P. *J. Mater. Chem.* **2009**, *19*, 111−123. (f) Mapkar, J. A.; Iyer, G.; Coleman, M. R. *Appl. Surf. Sci.* **2009**, *255*, 4806−4813. (g) Bahadur, N. M.; Furusawa, T.; Sato, M.; Kurayama, F.; Siddiquey, I. A.; Suzuki, N. *J. Colloid Interface Sci.* **2011**, *355*, 312−320.

(15) Asano, K.; Matsubara, S. *Org. Lett.* **2010**, *12*, 4988−4991.

(16) Borcard, F.; Godinat, A.; Staedler, D.; Comas Blanco, H.; Dumont, A.-L.; Chapuis-Bernasconi, C.; Scaletta, C.; Applegate, L. A.; Krauss Juillerat, F.; Gonzenbach, U. T.; Gerber-Lemaire, S.; Juillerat-Jeanneret, L. *Bioconjugate Chem.* **2011**, *22*, 1422−1432.

(17) For calculated atomic concentrations corresponding to Figure 2, see Table 3 in the Supporting Information.

(18) (a) Lesage, A.; Lelli, M.; Gajan, D.; Caporini, M. A.; Vitzthum, V.; Miéville, P.; Alauzun, J.; Roussey, A.; Thieuleux, C.; Mehdi, A.; Bodenhausen, G.; Coperet, C.; Emsley, L. *J. Am. Chem. Soc.* **2010**, *132*, 15459−15461. (b) Lelli, M.; Gajan, D.; Lesage, A.; Caporini, M. A.; Vitzthum, V.; Miéville, P.; Héroguel, F.; Rascón, F.; Roussey, A.; Thieuleux, C.; Boualleg, M.; Veyre, L.; Bodenhausen, G.; Coperet, C.; Emsley, L. *J. Am. Chem. Soc.* **2011**, *133*, 2104−2107. (c) Vitzthum, V.; Miéville, P.; Carnevale, D.; Caporini, M. A.; Gajan, D.; Copéret, C.; Lelli, M.; Zagdoun, A.; Rossini, A.; Lesage, A.; Emsley, L.; Bodenhausen, G., *Chem. Commun.* **2011**, *48*, 1988−1990.