Dendrimer-Modified Solid Supports: Nanostructured Materials with Potential Drug Allergy Diagnostic Applications

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Abstract: Complex functional materials consisting of bioactive molecules immobilized on solid supports present potential applications in biosensoring. Advances in the fabrication of these surface materials are of growing interest for antibody-based diagnosis. This work exploits dendrimers as versatile nanostructures for templating sensor surfaces and the critical role of the immobilization protocol in the solid supports cellulose and zeolites, of organic and inorganic composition respectively. The fabrication and characterization, including the degree of functionalization and reproducibility, of different nanostructured materials are described. To validate the approach, the fabricated supports were further used as a solid phase for developing a radioimmunoassay to detect immunoglobulin E (IgE) specific to penicillin, the antibody involved in immediate allergy responses to this drug. The dendrimer-modified supports provide assays with significantly enhanced sensitivity, as well as increase the availability of biomolecules for specific interaction and minimize nonspecific adsorptions through appropriate functionalization protocols in each case. The manufacturing methodology involved the use of a long, flexible hydrophilic spacer in the cellulose materials, and a higher surface density of the immobilized dendrimers in the zeolite crystals. The ability of hybrid zeolite materials in such biosensing applications was evaluated for the first time. The assays were validated in human serum samples from patients allergic to penicillin and from non-allergic controls. The specificity and improved sensitivity of the dendrimer-modified supports make these strategies versatile for different bioactive molecules and could have significant implications for the quantification of a wide range of specific IgE antibodies and other biomolecules of diagnostic interest.

Keywords: Allergy, IgE antibody, biosensor, dendrimer, penicillin, spacer, surface support, zeolite.

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INTRODUCTION

The modification of different surface supports with nanostructures is an interesting way to achieve novel functional materials. In addition, the possibility of immobilizing biomolecules to the substrates is attracting substantial research efforts because of their potential biosensor applications [1-7].

Emergent functional materials for solid-phase assays require improving the flexibility, reproducibility, analytical selectivity and sensitivity of existing choices. The behavior of protein adsorption to material surfaces is also of critical importance in biosensors, since nonspecific protein-surface interactions are often the cause of low sensitivity or selectivity limitations [8, 9]. Advances in hybrid materials that combine the high functionality of surfaces with a defined nanosize and controlled peripheral multivalence of dendrimers allow the construction of well-defined building blocks, like a scaffold with reactive chemical functionalities to couple the desired biomolecules over a solid phase. In this context, dendrimers have been chemically attached onto substrates to exhibit biofunctional surfaces for applications involving biomolecular recognition, like drug delivery or biosensors [1, 2, 10-13]. In these latter materials, the ligands used for the dendrimer peripheral decoration determine the specificity of the sensor. In addition, multivalency and the defined molecular structure of the dendrimers increase ligand surface density and provide uniform ligand distribution, which may improve the sensitivity and reproducibility of the assay. Moreover, dendrimers could play an important role in minimizing protein adsorption behavior to the surface.

In the field of bioassay improvement, immunoassays are a good choice to focus on, since immunoglobulins are the most specific protein in molecular recognition processes, and they can be used for the diagnosis of allergy. One well-established solid-phase immunoassay is the radio-allergo-sorbent test (RAST), a blood test used to determine what a person is allergic to based on the quantification of IgE that interact specifically with suspected or known allergens [14]. To diagnose drug allergy, the RAST technique employs solid supports covalently attached to carrier macromolecules, which are further conjugated to multiple units of the drug hapten [15]. Of special relevance in these materials is the chemical structure of the drug haptens, on which the selectivity of the test depends. However, the nature of the carrier molecule, the solid phase and the chemical structure of the covalent linkers between them may also play sig- nificant roles in the preparation of biocompatible materials and the modulation of the recognition process. Regarding solid supports, cellulose is a biocompatible, hydrophilic and non-toxic polymer, with a high density of free hydroxyl groups [16, 17]. These proper- ties make cellulose an adequate solid substrate in biosensor applica- tions; indeed, it is the solid support conventionally employed in materials for RAST. For the carrier, human serum albumin (HSA) and poly-L-lysine (PLL) have traditionally been used, although nowadays PLL is the most common.

/3-lactams are the most common cause of IgE-mediated allergy, affecting up to 10% of patients treated with penicillins [18]. The benzylpenicilloyl group, the open form of the /3-lactam in ben- zylpenicillin resulting from conjugation with proteins, is the major determinant responsible for the allergic response to benzylpenicil- lin. Therefore, these chemical structures are included in diagnostic tests to capture selectively IgE directed to benzylpenicillin. To identify penicillin IgE-mediated allergy, RAST has been employed as a complementary diagnostic method to in vivo tests, skin testing and the drug provocation test. Among the various diagnostic meth- ods, in vitro tests are preferred to the in vivo test [19], as this latter is more invasive and can present a risk. Since in vitro tests to detect specific IgE antibodies to these □-lactams are not as sensitive as skin testing they need to be improved to enable better diagnosis of allergic patients.

To address this challenge, dendrimers have been used as carrier emulators in RAST materials, due to their well-defined symmetrical structures and their potential as multivalent protein-like materials for biotechnological applications [20, 21]. Nanoconjugates consist- ing of PolyAMinoAMide (PAMAM) dendrimers decorated at the periphery with the penicillin hapten, benzylpenicilloyl (BPO), mimic recognition with natural hapten–protein conjugates [22]. In fact, IgE specific to benzylpenicillin can be determined by RAST using BPO-PAMAM dendrimer conjugates coupled to cellulose surface disks activated with either cyanogen bromide [23] or ha- loalkanoyl halide reagents [24]. These chemical methodologies can be used to reproducibly prepare standardized materials with a defi- nite amount of attached dendrimers. Though the use of these nano- conjugates improved the reproducibility of conventional immuno- assays, the defined size, shape and large number of surface func- tional dendrimer groups could be better exploited in solid supports for this diagnostic application. Indeed, in solution, lower generation dendrimers are generally open and floppy structures, whereas higher generations become robust, less deformable and have more spherical structures [25]. However, the dendrimers tend to flatten on the surface [26, 27], and as a consequence, many of their end groups near the surface remain inaccessible and might not be rec- ognized by the specific IgE antibodies, which may influence the sensitivity and could even lead to false negative results.

We employed two different solid supports to fabricate a variety of modified surfaces displaying chemically attached hapten drug- dendrimer conjugates, (shown in Scheme 1), and studied their solid- phase use in biosensor applications. In particular, we focused on the activation of cellulose surfaces with reagents that act as a spacer, providing a well-defined distance between the nanoconjugate and the surface, in order to improve the sensitivity of the technique. Hydrophilic spacers have been used as linkers to increase the hy- drophilicity and flexibility of compounds at surfaces [28], and to distance the immobilized substrate from the surface of a solid sup- port [29-31], which can reduce steric interferences between the substrates leading to higher recognition efficiency with the specific biomolecule to detect. This paper reports two effective strategies for covalent immobilization of penicilloylated dendrimers onto cellulose surfaces via a flexible bifunctional oligoethylengycol linker. The density of the hapten-dendrimer attached to the resulting solid supports was analyzed and compared to that of those prepared with direct covalent binding. We also addressed the fabrication of nanoconjugate-functionalized supports using microporous material of zeolitic nature, which has not yet been used in these antibody- based diagnostic applications, although their functionalization with biomolecules and antibodies has already been reported.[32, 33] Zeolite L is a biocompatible, aluminosilicate which is optically transparent, size tunable and crystalline and can be interfaced with biological systems [34, 35]. In sensor applications, the presence of a flat substrate compared with the fibrous and rough surface of cellu- lose may allow a much larger surface area for the adsorption of the proteins. The molecular recognition and quantification of IgE anti- bodies specific to benzylpenicillin obtained with all the materials was evaluated by using sera from tolerant subjects and patients allergic to benzylpenicillin.

MATERIALS AND METHODS

Synthesis of Spacer I

(1-(p-Benzylchloro)-13-(p-chlorobenzoyl)-1,4,7,10,13-pentaoxa- tridecane)

A round-bottom flask containing 300 mg (0.71 mmol) of de- symmetrized diaryl-TEG 1 [36] was placed in a cold bath and an excess of thionyl chloride (1.5 mL) was added dropwise to the mix- ture. The reaction was left to reach room temperature and refluxed for two hours. After reaching room temperature, the mixture was concentrated to dryness to provide a yellow oil corresponding to the desired product Spacer I (316 mg, 97%). 1H-NMR (400 MHz, CDCl3) □ ppm:

3.63-3.72 (8 H, m, TEG), 3.80-3.90 (4 H, m, TEG), 4.10 (4 H, m, TEG), 4.53 (2 H, s, Ph-CH2), 6.86 (2 H, d, J = 8.6 Hz, H-3, H-5), 6.95 (2 H, d, J = 9.0 Hz, H-3′, H-5′), 7.27 (2 H, d, J = 8.6 Hz, H-2′, H-6′), 8.04 (2 H, d, J = 9.0 Hz, H-2, H-6). 13C-NMR (100 MHz, CDCl3) ppm: 46.2, 67.4, 67.8, 69.3, 69.6, 70.6, 70.8, 70.8, 114.7, 114.7, 125.5, 129.8, 129.9, 133.9, 158.8, 164.6, 167.1. EI-MS m/z (%): 458 (M+ + 2, 7), 456 (M+, 11), 422 (39), 421 (44), 420 (93), 193 (32), 183 (100), 155 (38), 120 (37), 107 (48), 89 (38).

Synthesis of Spacer II
(1-(p-Benzylchloro)-13-(N-(trimethoxisililpropyl)-p-benzoyl- amide)-1,4,7,10,13-pentaoxatridecane)
A solution under inert atmosphere containing APTMS (128 mg, 0.7 mmol) and freshly distilled TEA (98 mL) in anhydrous CH2Cl2 (3 mL) was added dropwise to a solution of Spacer I (320 mg, 0.7 mmol) in anhydrous CH2Cl2 (1 mL). The mixture was stirred for one hour at room-temperature. The crude was concentrated to dry- ness. Then, anhydrous THF (2 mL) was added to the mixture to precipitate the formed triethylamonium chloride salt. The precipitate was filtered off and the resulting solution was concentrated to isolate the desired compound as a colorless oil with quantitative yield. 1H-NMR (400 MHz, CDCl3) ppm: 0.68 (2 H, t, J = 7.9 Hz, CH2-Si), 1.71 (2 H, m, CH2-CH2-Si), 3.41 (2 H, m, CH2NH), 3.54 (9 H, s, 3(OCH3)), 3.66 (8 H, m, TEG), 3.83 (4 H, m, TEG), 4.09 (4 H, m, TEG), 4.52 (2 H, s, Ph- CH2), 6.85 (2 H, d, J = 8.7 Hz, H-3, H-5), 6.89 (2 H, d, J = 8.7 Hz, H-3′, H-5′), 7.26 (2 H, d, J = 8.7 Hz, H-2′, H-6′), 7.68 (2 H, d, J = 8.7 Hz, H-2, H-6). 13C-NMR (100 MHz, CDCl3) ppm: 6.5, 22.7, 42.1, 42.6, 50.6, 67.4, 67.5, 69.5, 69.6, 70.6, 70.7, 114.2, 114.7, 127.1, 128.5, 129.8, 130.0, 158.8, 161.1, 166.9.

Preparation and Preactivation of Cellulose Disks
Cellulose disks of 6 mm diameter, with an average weight of
2.1 mg, were prepared from Whatman 54 filter paper. The disks were immersed in a 20% solution of trifluoracetic acid (TFA) in CH2Cl2 and shaken for 5 minutes. The acid solution was carefully decanted from the disks. The disks were then washed by adding then filtering off CH2Cl2 (x7). The disks were subsequently dried under high vacuum for 24h.

Preparation of C-Spacer I Disks
Groups of around 50 preactivated disks (weighting approxi- mately 0.1 g) were placed into vials under argon atmosphere. The vials were sealed with a septum, charged with dry THF (3 mL), DMAP (12 mg, 0.1 mmol) and distilled TEA (0.3 mL, 2.13 mmol) and then placed in a cold bath. After this, a solution of Spacer I (884 mg, 1.9 mmol) in dry THF (2 mL) was added dropwise to each vial containing disks. The mixtures were left to react under shaking for 24 h. The solution was subsequently removed and the disks were washed with CHCl3 (x4).
Preparation of C-APS Disks
Around 400 preactivated disks (approximately 0.8 g) were placed in a flask containing a solution of 25% APTMS in EtOH (95%). The suspension was shacked for 4 hours. After that, the reactive solution was carefully decanted from the cellulose disks. The disks were subsequently washed by adding then decanting 50 mL portions of MeOH (x3) and acetone (x3) (5 minute in each bath). Finally, the disks were introduced in the oven at 100ºC for 10 min.
Preparation of C-Spacer II Disks
Groups of around 50 C-APS disks were placed into vials under argon atmosphere. The vials were sealed with a septum, charged with anhydrous CH2Cl2 (2.5 mL) and distilled TEA (0.1 mL, 0.7 mmol) and then placed in a cold bath. After that a solution of Spacer I (272 mg, 0.6 mmol) in anhydrous CH2Cl2 (5 mL) was added dropwise to each vial containing disks. The mixtures were left to react under shaking for 24 h. The solution was subsequently removed and the disks were washed with CH2Cl2 (x4).

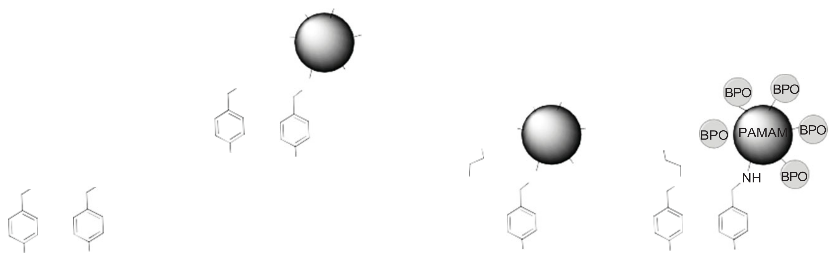
Activation Dendrimer Attachment Surf ace Deactivation Drug Conjugation

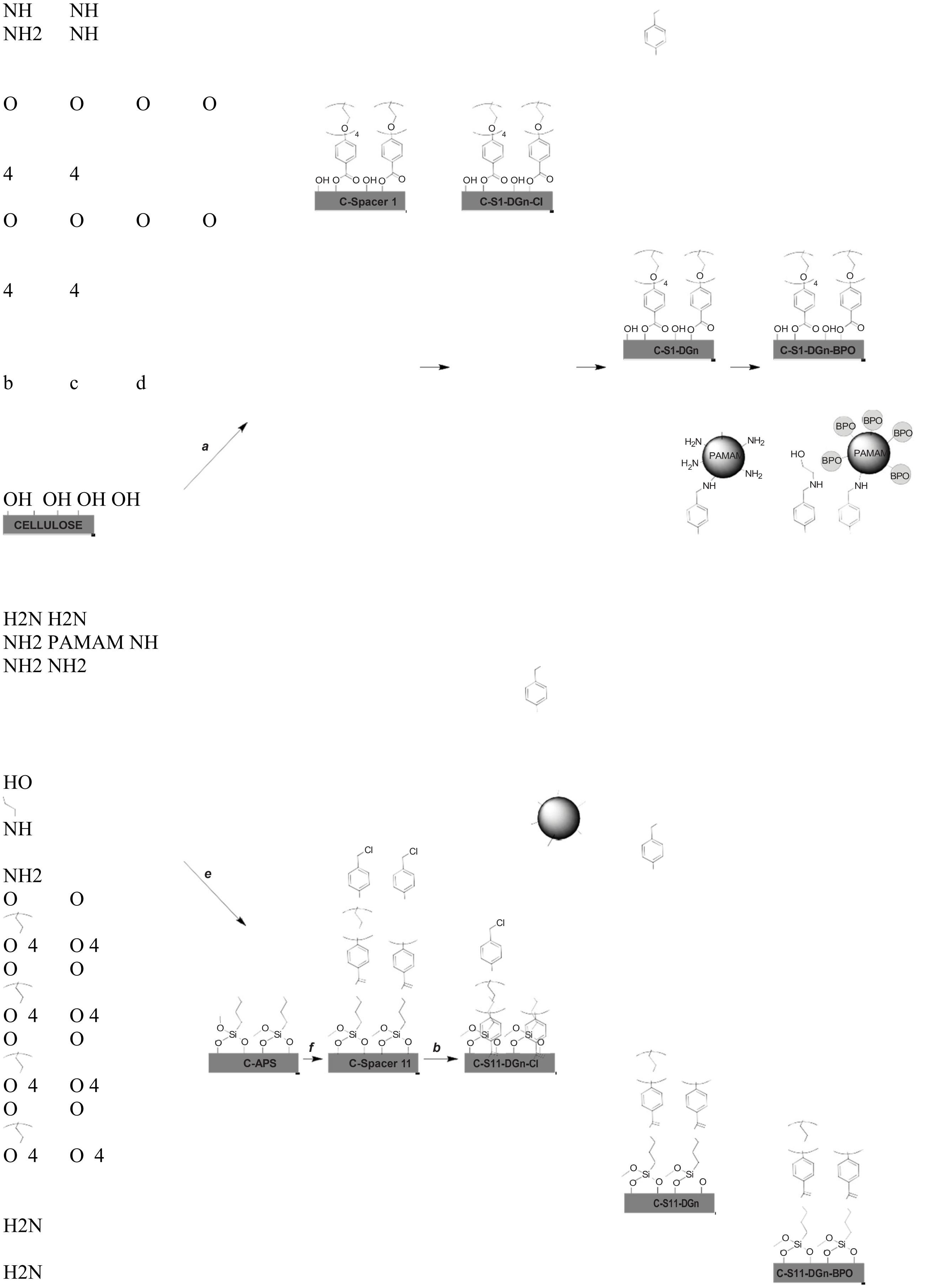
H2N NH2
H2N PAMAM NH2

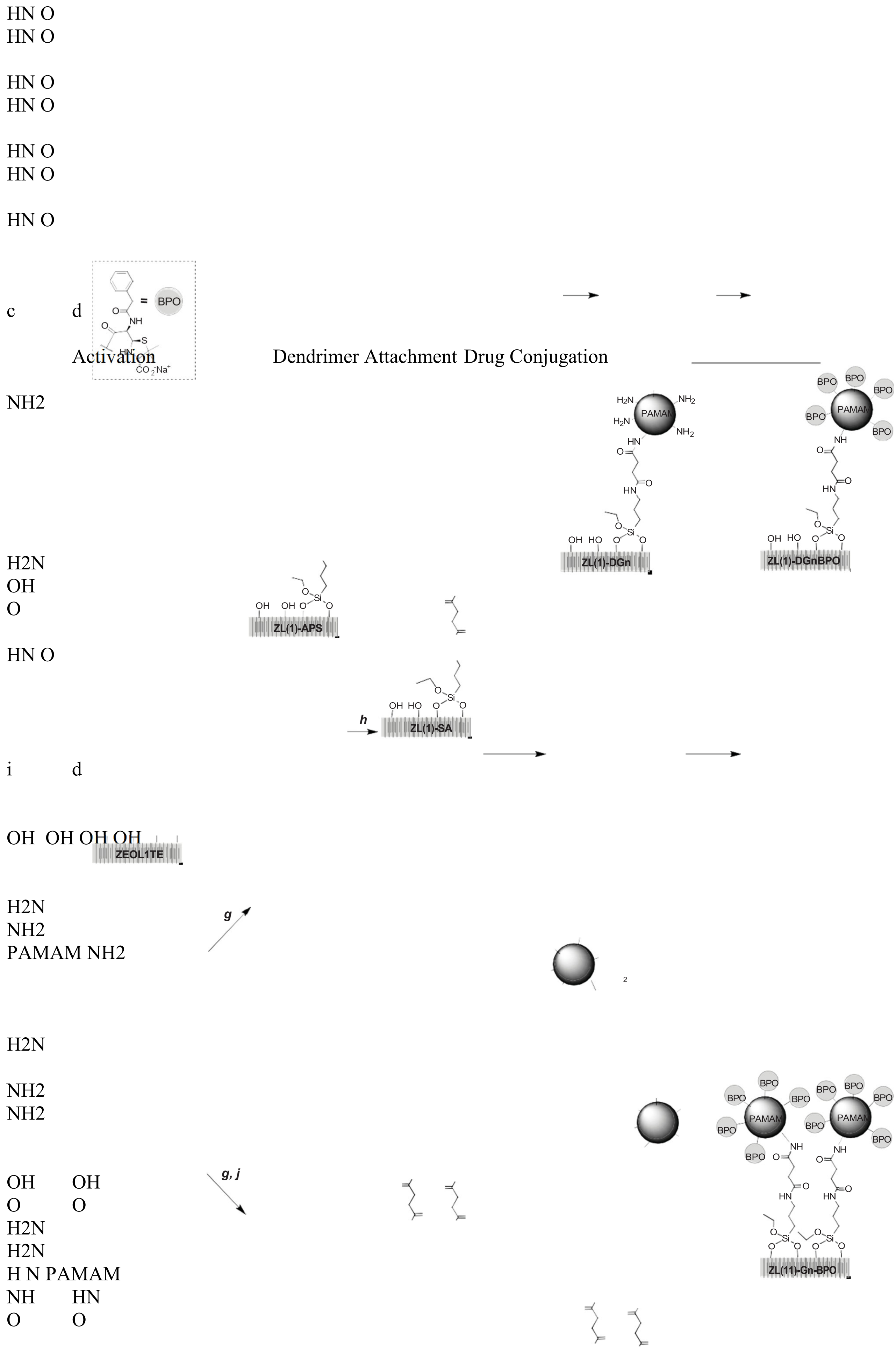
H2N NH2
HO H2N PAMAM NH2 HO

CI CI

CI NH
NH2




NH NH
NH2 NH
O O O O
4 4
O O O O
4 4
b c d
OH OH OH OH
CELLULOSE
a
C-Spacer 1
C-S1-DGn-Cl
C-S1-DGn
C-S1-DGn-BPO
PAMAM
BPO
H2N H2N
NH2 PAMAM NH
NH2 NH2
HO
NH
NH2
O O
O 4 O 4
O O
O 4 O 4
O O
O 4 O 4
O O
O 4 O 4
H2N
H2N
HN O
e
f
b
Cl
Si
C-APS
C-Spacer 11
C-S11-DGn-Cl
C-S11-DGn
C-S11-DGn-BPO


HN O
HN O
HN O
HN O
HN O
HN O
HN O
c
d
= BPO
NH
S
HN
CO2Na+
Activation
Dendrimer Attachment
Drug Conjugation
NH2
H2N
PAMAM
ZL(1)-DGn
BPO
ZL(1)-DGnBPO
H2N
OH
O
HN O
OH
Si
ZL(1)-APS
h
HO
ZL(1)-SA
i
d
OH OH OH OH
ZEOL1TE
H2N
NH2
PAMAM NH2
g
2
H2N
NH2
NH2
OH
OH
O
O
H2N
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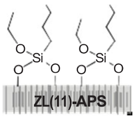
H N PAMAM
NH
HN
O
O
g, j
ZL(11)-Gn-BPO

NH2

H2N

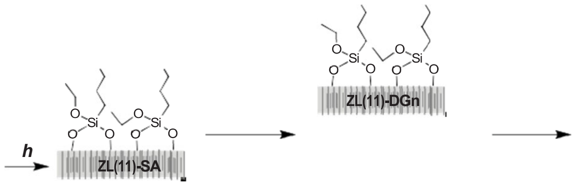


H2N

HN O HN O

HN O HN O

i d



Scheme 1. Top: Modification of the cellulose surfaces through two different activation methodologies followed by attachment of nanoconjugates. (a) Spacer I, dimethylaminopyridine (DMAP), triethylamine (TEA), tetrahydrofurane (THF); (b) PAMAM Gn (n = 2,4), dimethylformamide (DMF)/0.1M NaHCO3 (1:1);
(c) Ethanolamine, NaHCO3 0.1 M; (d) Benzylpenicillin, Na2CO3/NaHCO3 0.05M, pH = 10.2, 4ºC; (e) 3-(aminopropyl)-trimethoxysilane (APTMS), EtOH 95%; (f) Spacer I, TEA, CH2Cl2. Bottom: Activation of zeolite-L surfaces and subsequent attachment of nanoconjugates: (g) 3-(aminopropyl)-triethoxysilane (APTES), toluene, 50ºC; (h) Succinic anhydride, DMF; (i) PAMAM G2, 1-(3-(dimethylamino)propyl)-3-ethyl-carbodiimide hydrochloride (EDCI), N-hydroxysuccinimide (NHS), DMF ; (j) APTES, toluene, 110ºC.

Attachment of Dendrimers to Activated Cellulose: C-SI-DGn- Cl and C-SII-DGn-Cl

This was carried out by the previously published procedure [23] using series of 50 disks (C-Spacer I or disks C-Spacer II) with 2 mL of 10 mg/mL solution of PAMAM (Gn, n = 2, 4) in a mixture of DMF/0.1 M NaHCO3 (1:1). Those disks to be subjected to the ninhydrin test were washed with water, filtered off, and dried; all others were ready for subsequent reaction.

Deactivating Unreacted Activated Chloride Groups: C-SI-DGn and C-SII-DGn

This was carried out by the previously published procedure for neutralization of activated groups [23], reacting groups of 50 disks with 50 mM ethanolamine in 0.1M NaHCO3 for 1 h. The disks to be used as blanks in the RAST (carrier without hapten) were stored in 0.05 M Na2CO3/NaHCO3 buffer at pH 10.2 at 5 °C until analysis, whereas all others were ready for subsequent reaction.

Binding of Penicillin G to Dendrimeric Cellulose: C-SI-DGn- BPO and C-SII-DGn-BPO

Groups of 50 disks were treated with a freshly made solution of penicillin G in an aqueous buffer at pH 10.2 as previously described procedure [23]. The disks were stored in phosphate buffer saline (PBS) at 4 °C for later use (RAST assay or ninhydrin test).

Preparation of Zeolites

The disc-shaped zeolite L crystals were prepared according to the procedure described in reference [37]. This zeolite L has a cylindrical shape and a hexagonal symmetry, with a height of 120 nm and a diameter of 800 nm (coin shaped).

Amino Functionalization of Zeolite L Crystals: ZL(I)-APS

A suspension of unmodified zeolite L crystals (100 mg) in toluene (30 mL) was sonicated for 10 min and APTES (100 µL) was added. The suspension was sonicated for further 1 h at 50 °C. After that, the zeolites were centrifuged and the supernatant was removed. Then the zeolites were washed with toluene (x2) and ethanol (x3) and dried in the oven at 70 °C for > 5 h.

Extra Amino Functionalization of Zeolite L Crystals: ZL(II)- APS

A suspension of unmodified zeolite L crystals (100 mg) in toluene (30 mL) was sonicated for 10 min and after adding APTES (100 µL) it was sonicated for further 1 h at 50 °C. Then more APTES (100 µL) was added to the suspension and it was stirred at 110 °C overnight. Finally the suspension was centrifuged and the supernatant removed. Then the zeolites were washed with toluene (x2) and ethanol (x3) and dried in the oven at 70 °C for > 5 h.

Carboxy Functionalization of Zeolite L Crystals: ZL(I)-SA and ZL(II)-SA

ZL-APS zeolites (80 mg) were suspended in DMF (2 mL) and sonicated for 10 min. A solution of succinic anhydride (450 mg) in DMF (3 mL) was added to the suspension and sonicated for 10 min. Two drops of TEA were added and stirred overnight at room tem- perature. Then the suspension was centrifuged and the supernatant removed. Finally the zeolites were washed with DMF (x4) and with ethanol (x3) and dried in the oven at 70 °C for > 5 h.

Attachment of Dendrimers to Zeolites: ZL(I)-DGn and ZL(II)- DGn (n=2)

ZL-SA zeolites (70 mg), EDCI (24 mg), NHS (28 mg) and DMF (1 mL) were mixed and sonicated for 10 min. A solution of PAMAM G2 dendrimer (12 mg) in DMF (1 mL) was added to the suspension, sonicated for 10 min and stirred overnight at room tem- perature. Then the suspension was centrifuged and the supernatant removed. The zeolites were washed with DMF (x4) and ethanol (x3) and dried in the oven at 70 °C for > 5 h.

Binding of Penicillin G to Dendrimeric Zeolites: ZL(I)- DGnBPO and ZL(II)-DGnBPO (n=2)

ZL-DG2 zeolites (40 mg) were suspended in 0.05 M Na2CO3/NaHCO3 aqueous buffer at pH 10.2 (1 mL) and sonicated for 10 min. 1 mL of a freshly solution of penicillin G (10 mg/mL) in 0.05 M Na2CO3/NaHCO3 aqueous buffer at pH 10.2 was added over the suspension, sonicated for 10 min at 0 °C and stirred for seven days at 4 °C. 10 mg of penicillin G was added at approxi- mately 24 h intervals and sonicated for 10 min at 0 °C after each addition. Then the suspension was centrifuged and the supernatant removed. The zeolites were washed with PBS buffer (x2) and bidis- tilled water (x3) and dried in desiccators under vacuum for 12 h and stored at room temperature.

Quantification of Free Primary Amine Groups Bonded to the Solid Phase

Ninhydrin stock solutions were freshly prepared.

Cellulose Disk Supports

The estimation of the number of amino groups on the supports was carried out by using the previously described ninhydrin proce- dure for cellulose disks [23]. Around ten cellulose disks of each kind were analyzed (Supplementary Material) and the average val- ues were calculated (Table 1).

Zeolite Supports

The zeolite measurement procedures were adapted from litera- ture methodologies [38]. The test solution was prepared dissolving ninhydrin (300 mg) and hydrindantin (45 mg) in water-free DMSO (11.25 mL). Afterwards 4 M Lithium acetate buffer pH 5.3 (3.75 mL) was added and the solution was flushed with nitrogen for 5 min. The ninhydrin solution was stored at 4 °C in the dark. To 1 mL aqueous sample solution, 1 mL ninhydrin solution was added and the solution was heated at 100 °C for 15 min. Afterwards samples were cooled in an ice bath and 5 mL of a 50 % EtOH/water solution added. The reference sample consisted of 1 mL blank and 1 mL ninhydrin solution. A calibration was performed with APTES in a concentration range of 0.010 –0.100 µmol mL-1. The measurements were carried out at 1 mg of each sample in duplicate, resulting in reproducible values (Table 2).

Immunochemical Studies

Selection of Patients

Patients with an immediate (IgE-mediated) allergic reaction to benzypenicillin were included. The diagnostic procedure was basi- cally as described in the European Network of Drug Allergy (ENDA) protocol, based on skin testing, in vitro tests ImmunoCAP system, and a drug provocation test if necessary [39].

Subjects with confirmed good tolerance to penicillins and nega- tive skin test and ImmunoCAP to penicillin derivatives were in- cluded as tolerant controls.

Table 1. Average Obtained in the Quantification of the Number of Amine Groups and, Therefore, Dendrimers Attached to the Cellulose Disks

	Generation n (Amine Groups)	µmol of Amine Groups/ Gram of Cellulose		µmol of BPO/ Gram of Cellulose	µmol of Dendrimer/ Gram of Cellulose
Without Spacer		C-DGn	C-DGnBPO		
	2 (16)	5.3	0.4	4.9	0.354
	4 (64)	3.4	0.3	3.1	0.053
Spacer I		C-SI-DGnCl	C-SI-DGn-BPO		
	2 (16)	16.7	4.5	12.2	1.111

	4 (64)	16.3	2.0	14.3	0.259
Spacer II		C-SII-DGnCl	C-SII-DGn-BPO		
	2 (16)	51.3	1.4	49.9	3.41
	4 (64)	51.9	1.9	50.0	0.824

Table 2. Z Potential Measurements and Quantification of Amine Groups of Zeolite Materials

Zeolite	Z Potencial (mV)	µmol Amine / g zeolite
ZL-OH	-41	---
ZL(I)-APS	-11	60
ZL(I)-SA	-42	10
ZL(I)-DG2	-3	50
ZL(I)-DG2BPO	3	35
ZL(II)-APS	-8	135
ZL(II)-SA	-40	20
ZL(I)-DG2	45	170
ZL(II)-DG2BPO	-2	70

The study was conducted according to the Declaration of Hel- sinki principles and was approved by the Ethics Committee of Car- los Haya hospital. All subjects, patients and controls, included in the study were informed orally about the study and signed the cor- responding informed consent.

Specific IgE Antibody Determination by Radioallergosorbent Test (RAST)

RAST to benzylpenicillin using the new nanostructured mate- rial solid phases (disks or zeolite-L) was performed as described elsewhere [40]. Briefly, 30 µL of the patient's sera was incubated with the different solid phases, involving either a modified cellulose disk or 0.1 mg of modified zeolite L. After washing, radiolabeled anti-IgE antibody (kindly provided by ALK-Abello Laboratory, Madrid, Spain) was added and the mixture incubated overnight. Washing phases for disk solid supports were removed by aspiration and for zeolite supports by decantation after centrifugation. The radioactivity of the solid phases was measured in a gamma counter. Results were calculated as a percentage of the maximum (% RAST). Samples were considered positive if they were higher than 2.5% of label uptake.

RESULTS AND DISCUSSIONS

Two different materials were employed as solid phases, cellu- lose and zeolites L. For both, the methodology involved chemical surface reactions for the modification of the surface monolayer. This method offers the possibility of applying a large variety of different organic reaction pathways on surfaces, which allows the introduction of a wide range of terminal end groups on well-defined monolayers.

Cellulose Solid-Phase

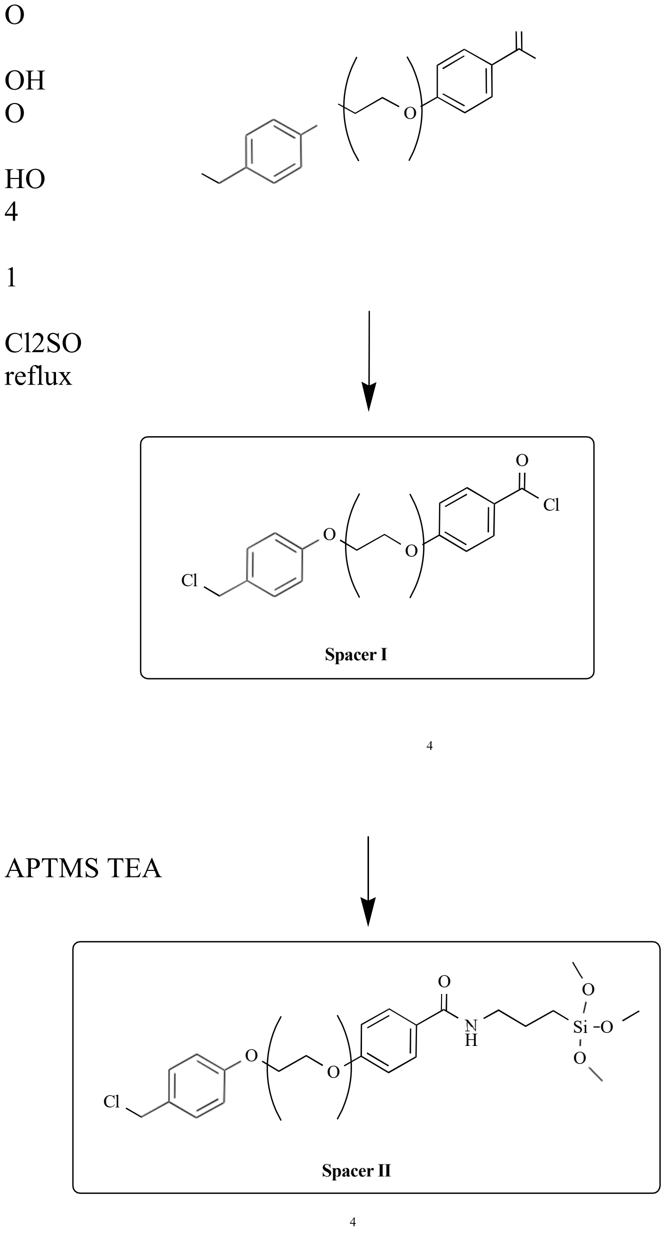
Although the direct immobilization of benzylpenicilloylated- dendrimer conjugates onto cellulose surfaces has already been de- scribed and validated with human sera to detect IgE specific to penicillin [23, 24], the proximity of the support may prevent the interaction of antibodies with some hapten units on the conjugate (those placed in close contact with the support surface). Therefore, a proper immobilization of the conjugate may be critical for the successful detection of the antibodies [31]. The use of a spacer linker may provide less steric hindrance to conjugation and retain almost full accessibility to the interaction against the antibody.

Spacer Design

Spacer I (Scheme 2) was designed considering the following:

(1) a linker with a long flexible structure allows enough distance between the nanoconjugate and the surface to avoid steric hindrance interactions, both in the covalent conjugation process (during mate- rial fabrication) and in antibody recognition (during biosensor ap- plication), (2) a hydrophilic polyethylenglycol (PEG) spacer re- duces nonspecific binding with the surface and holds the nanocon- jugate suspended in aqueous solution away from the support, preventing it from flattening onto the surface, (3) a bifunctionality bearing chloride and acylchloride groups at opposite chain termini facilitates surface conjugation.

The synthetic approach starts from commercially available TEG with a previously described effective methodology in which the desymmetrized diaryl-TEG 1 is obtained through a Cannizaro reac- tion of the symmetric precursor bis-aryloxylaldehyde [36, 41]. Treatment of 1 with an excess of thionyl chloride led to the chlori- nation of the two reactive functional groups to provide Spacer I. A further functionalization of Spacer I with APTMS provided a longer linker (Spacer II) with quantitative yields. This product was isolated and characterized by NMR, but became easily unstable through polymerizations. To solve this inconvenience, in the prepa- ration of final surfaces, instead of synthesizing the molecule Spacer II, Spacer I was reacted with a preactivated aminopropylsilane cellulose surface, as described in the following section.



Scheme 2. Synthesis of Spacer I and Spacer II.

Synthetic Methodology to Dendritic Cellulose Solid-Phases

The cellulose surfaces were activated using the two different spacer linker reagents and then covalently bound to hapten-carrier conjugates following (Scheme 1 top). The activation in the first methodology involved the immobilization of Spacer I through the haloalkanoyl functionality with the hydroxyl groups in the cellulose to yield C-Spacer I disks. Thus, the spacer was attached to the cellulose through an ester bond whereas the other end of the spacer molecule, the chloride functionality, was able to form an amine linkage with the PAMAM dendrimers. The second procedure in- volved the silanization of the cellulose support with APTMS through ether linkage. This silane coupling reagent allows the homogeneous functionalization of the support and maximizes the density of the functional groups introduced to yield C-APS sur- faces. In the first step of this coupling reaction, the silane agent is smoothly hydrolyzed to silanol by the water on the cellulose sur- face. In the second step, the absorbed silanol partially reacts with the surface hydroxyl group on the cellulose to form an ether bond. Since some parts of the silanol condense among themselves and produce a polymeric silanol, both the monomeric and the polymeric structures having the ether bond may exist [42]. However, a long time for the silanization reaction leads to non-homogeneus deposition of silica on the surfaces [13]. Bearing in mind these aspects for the silanization, both the reaction time and the reaction media were optimized. The influence of the organic solvent and water on the modification of the cellulose solid phase with APTMS was studied under various conditions and the highest homogeneous aminosilanization of the cellulose was obtained using a mixture of ethanol/water (95/5) as solvent and 4 hours of reaction time. The amino functionalized surface was then reacted with Spacer I, under reaction conditions optimized previously with the synthesis of Spacer

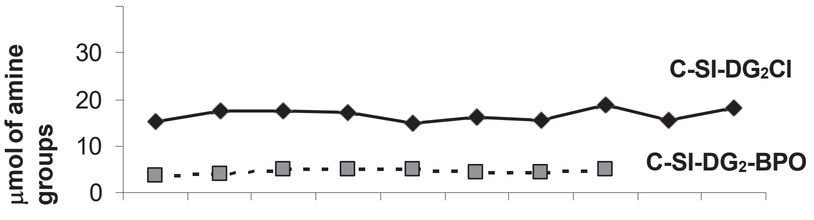
II. The resulting cellulose surface C-Spacer II displays the chloride functionality, and as in C-Spacer I surfaces, forms amine linkage with the PAMAM dendrimers of generations 2 and 4, with 16 and 64 amine end groups, respectively. This procedure leads to the formation of a chemically activated dendritic cellulose support (C- SI-DGnCl and C-SII-DGnCl, n = 2,4). The unreacted activated hydroxyl groups were neutralized by reaction with 2-aminoethanol to obtain C-SI-DGn and C-SII-DGn which can be used for the covalent immobilization of the desired biomolecules, such as hapten drugs. Thus, these dendronized surfaces allowed the covalent immobilization of benzylpenicillin, providing BPO-PAMAM conjugates on cellulose disks.

Characterization of the Dendritic Cellulose Surfaces

The ninhydrin method enabled the quantification of primary amine groups on the resulting cellulose surfaces. Tests were conducted on a series of 10 disks of cellulose surfaces containing amino groups (from APTMS reagent or PAMAM dendrimers) and after the amine derivatization in order to study the degree of reaction. As can be seen in Figs. (1 and 2), disks of the same type resulted in a very similar degree of functionalization, which indicates the homogeneity of the cellulose surfaces obtained within each type of methodology and the reproducibility of the functionalization methods. The averages of these data are shown in (Table 1) and are compared with dendritic surfaces when using conventional activated cyanogen bromide cellulose surfaces [23] in which the dendrimers are not spaced from the surface. These data also enabled us to determine the number of dendrimers attached on cellulose disks, and the amount of amines that reacts in each step. The high efficiency of the reactions is reflected by the elevated coverage with amine groups after reaction with APTMS Fig. (2A) and dendrimers Fig. (1 and Fig. 2B,C), and the few amine groups remaining in the surface support after reaction with the spacer Fig. (2A) and with benzylpenicillin Fig. (1 and Fig. 2B,C).

Regarding the addition of dendrimers to cellulose surfaces, the ninhydrin quantification results showed different amounts of amine groups bound to solid phases (expressed as µmols of amines per gram of cellulose, Table 1). For G2 and G4 they were 5.3 and 3.4 µmol g-1, respectively, using cyanogen bromide activated surfaces (C-DGn); around 16 µmol g-1 with Spacer I surfaces (C-SI-DGn) for both generations; and around 51 µmol g-1 with Spacer II surfaces (C-SII-DGn) for both generations. From these data it can be deduced that the use of the spaced linker, compared to a short direct isocyanate bond, increases significantly the number of dendrimers attached to the surfaces Fig. (3A), and therefore also the amount of peripheral amine groups present on the cellulose surfaces available to immobilize the bioligands Fig. (3B). This was to be expected due to the fact that when the dendrimers are directly bound to the solid support the surface is essentially blocked, especially considering their tendency to flatten on the surface, and therefore, even though anchoring points are available, there is no space for more dendrimers to reach them. For that reason, the separation from the support surface and the flexibility that the dendrimer could adopt due to the use of the spacer significantly increase the amount of anchored dendrimers. It can also be observed that the use of the silane coupling agent results in a highly homogeneous aminosilylation of the cellulose support which, coupled with the use of the spacer, is translated into an increased dendritic density on the cellulose surfaces.

40



40
30
1 2 3 4 5 6 7 8 9 10
Disks
20 C-SI-DG4Cl
10
0 C-SI-DG4-BPO
1 2 3 4 5 6 7 8 9 10 Disks

µmol of amine groups

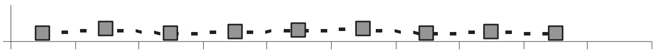
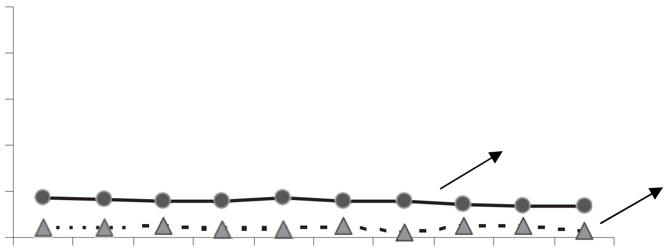


Fig. (1). Determination of total amount of primary amine groups bound to cellulose disks with the ninhydrin method. Data obtained from ten different samples of dendritic cellulose disks, before (◆) and after (■) reaction with benzylpenicillin, with generations A) 2 and B) 4.

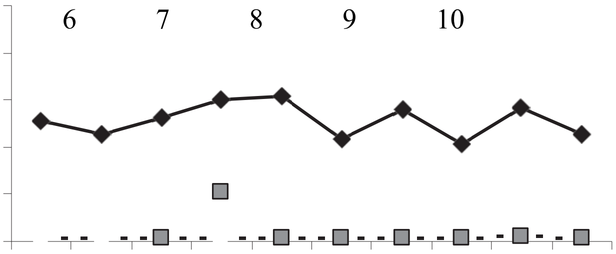
100
80
60
40
20
0

µmol of amine groups

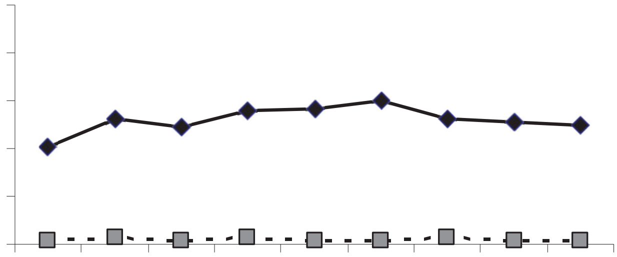


C-APS
C-Spacer II
B)
100
80
1 2 3 4 5 6 7 8 9 10
Disks
60 C-SII-DG2
40
20 C-SII-DG2-BPO
0 ■ ■

µmol of amine groups µmol of amine groups



100
80
60
40
1 2 3 4 5 6 7 8 9 10



C-SII-DG4

Disks
20 C-SII-DG4-BPO
01 2 3 4 5 6 7 8 9
Disks

Fig. (2). Determination of total amount of primary amine groups bound to cellulose disks with the ninhydrin method. Data obtained from ten different samples of cellulose disks of: A) activated with APTMS, before (●) and after (▲) reaction with Spacer I; and dendritized, before (◆) and after (■) reaction with benzylpenicillin, with generations B) 2 and C) 4.

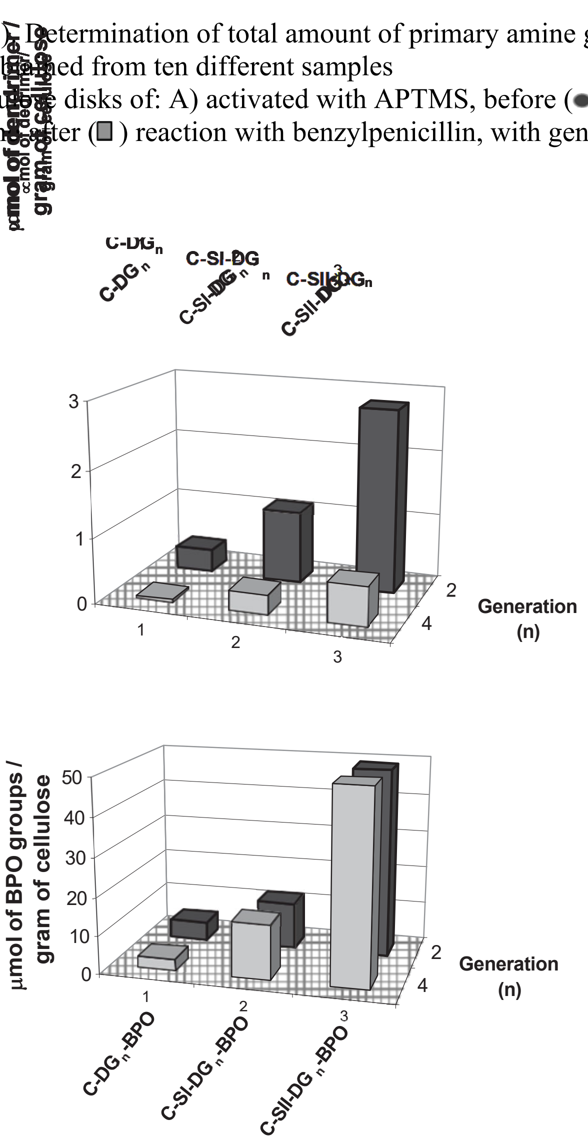


Fig. (3). Results obtained from the determination of free primary amino groups with three different activated disks and two different generation dendrimers, expressed in A) amount of dendrimers and B) amount of BPO groups, per gram of cellulose.

Concerning the generation used, when dendrimers are directly attached over the cellulose surfaces (C-DGn-BPO) the number of immobilized penicillins with G2 is around two-fold higher than with G4. However, on surfaces activated with the spacers (C-SI- DGn and C-SII-DGn), the amount of penicillin ligands does not depend on the dendrimer generation. This can also be attributed to the fact that dendrimers flatten on the surface when directly attached onto the solid support.

Validation of Dendritic Cellulose Surfaces As Solid Phases in RAST In Vitro Assay

The resulting surfaces were validated for specific IgE recognition by RAST using sera from β-lactam-allergic patients and controls who tolerated this drug Fig. (4). As can be seen in Fig. (4), the different disks produced positive RAST results in high specific IgE sera from patients (patients 1-4) allergic to benzylpenicillin. The percentage of RAST was high (between 10-20%) in these four patients, with no differences in the three types of disks and two generations. The use of spacers compared to short linkers makes no difference in the diagnosis of patients with a high IgE level.

To evaluate the ability of the spacers to improve the sensitivity of the assay, sera were mainly selected from those patients who being diagnosed as allergic to benzylpenicillin, although with a low IgE level, did not reach positive RAST values when using cyanogen bromide activated cellulose surfaces (patients 5-10) and the different RAST results were later compared. Patients 5 and 8 were tested with only second generation materials because of the finite amount of sera available. In both cases the use of the spacer involved an increased percentage of RAST, reaching (patient 5) or not reaching

(patient 8) positive values. This pattern of higher detection of IgE with the use of the spacer was observed with the other low IgE sera, especially with Spacer I and generation two. In fact, C-SI-DG2- BPO disks enabled cases 5, 6, 7, 9 and 10 to be correctly diagnosed as allergic to benzylpenicillin. The percentage of RAST obtained with all the controls (patients 11-13) gave negative values and showed practically no differences between the different materials. Thus, the specificity reached with these disks was very high, as deduced from the results obtained from the controls.

In the characterization of the disks, Spacers I and II units were shown to improve the accessibility of support-bound molecules for subsequent reactions, but their accessibility on support assays seems to be increased to a higher degree with Spacer I compared to Spacer II, when the concentration of specific IgE antibodies in the sera is low. This may be due to a higher functionalization density in surfaces with Spacer II, which may block the positions of benzylpenicillin haptens close to the other conjugates linked with the same Spacer II, resulting in steric interferences between them, and preventing access to the specific IgE interaction. The less dense cellulose surfaces, although still highly functionalized with Spacer I, provide greater flexibility, allowing the immobilized nanoconjugates to move into position to establish the IgE-specific interaction. In addition, this seems to be favored with the low generations (G2) compared to the higher generations (G4).

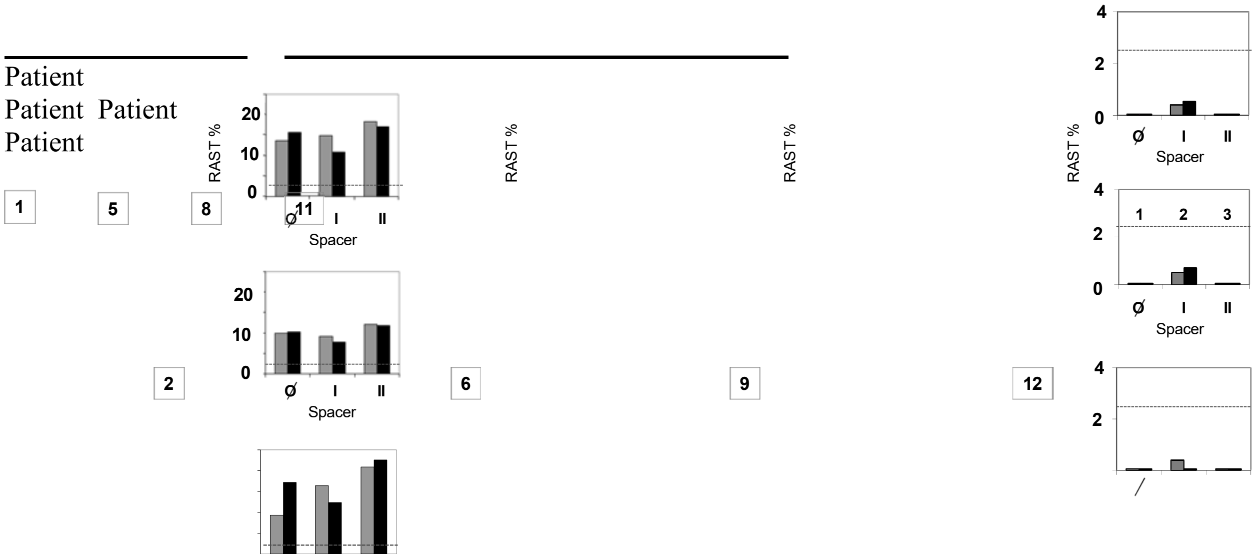
Zeolite Solid Phase

The covalent grafting of organic compounds such as PAMAM dendrimers on a silica surface requires the introduction of electrophilic reactive groups over the solid phase. The flat and highly functionalized surface may allow a high coverage with the artificial dendrimeric antigens. In addition, the use of an adequate linker which also acts as a spacer may allow a major availability of the peripheral functional groups of dendrimers to react and also to be recognized by biomolecules. Our approach to attaching the dendrimers over the zeolites consists of a grafting to methodology by previous activation of the surface with a linker displaying the appropriate functionality (Scheme 1 bottom). As can be seen in (Scheme 1), every other reaction in the preparation of the dendritic zeolites involved amine functionalization, and thus the strategy alternates the amine group introduction with their derivatization. Given that the functionalization is expected to modify the charge of the zeolite surface [10] and the presence of free amine groups, the derivatization of zeolite materials was preliminary monitored by measuring the Z potential and quantification of amine groups with the ninhydrin test (Table 2).

Dendritic Zeolite Solid-Phases: ZL(I)-DG2BPO

Reactive amine functional groups were introduced on the zeolite surface by reaction with APTES reagent for one hour at 50ºC to yield ZL(I)-APS. The Z potential increased significantly from unmodified zeolite ZL-OH (-41 mV) to amino functionalized zeolite ZL(I)-APS (-11 mV). This is in agreement with the ninhydrin test, which indicates the introduction of 60 µmol of amine groups per gram of zeolite L. These amine moieties were reactive with succinic anhydride to obtain the carboxylic acid terminated surfaces ZL(I)- SA. The decreased Z potential value (-41 mV) coupled to the remaining amount of amine groups (10 µmol g-1) reflects the efficiency of the reaction. The further reaction of these carboxyl functionalized surfaces with PAMAM dendrimers (G2) via previous carboxyl group activation with carbodiimide reagent provided amide linkages and therefore the dendrimeric zeolite surfaces ZL(I)- DG2. The dendritic functionalization introduces amine groups to the surface, according to the ninhydrin test (50 µmol g-1), which also modifies the charge of the solid support in accordance with the Z potential (-3 mV). The last step involved the covalent immobilization of penicillin hapten drug to the material through the peripheral decoration of dendrimers. The modification of Z potential val-

High Specific IgE Sera Low Specific IgE Sera Control Sera



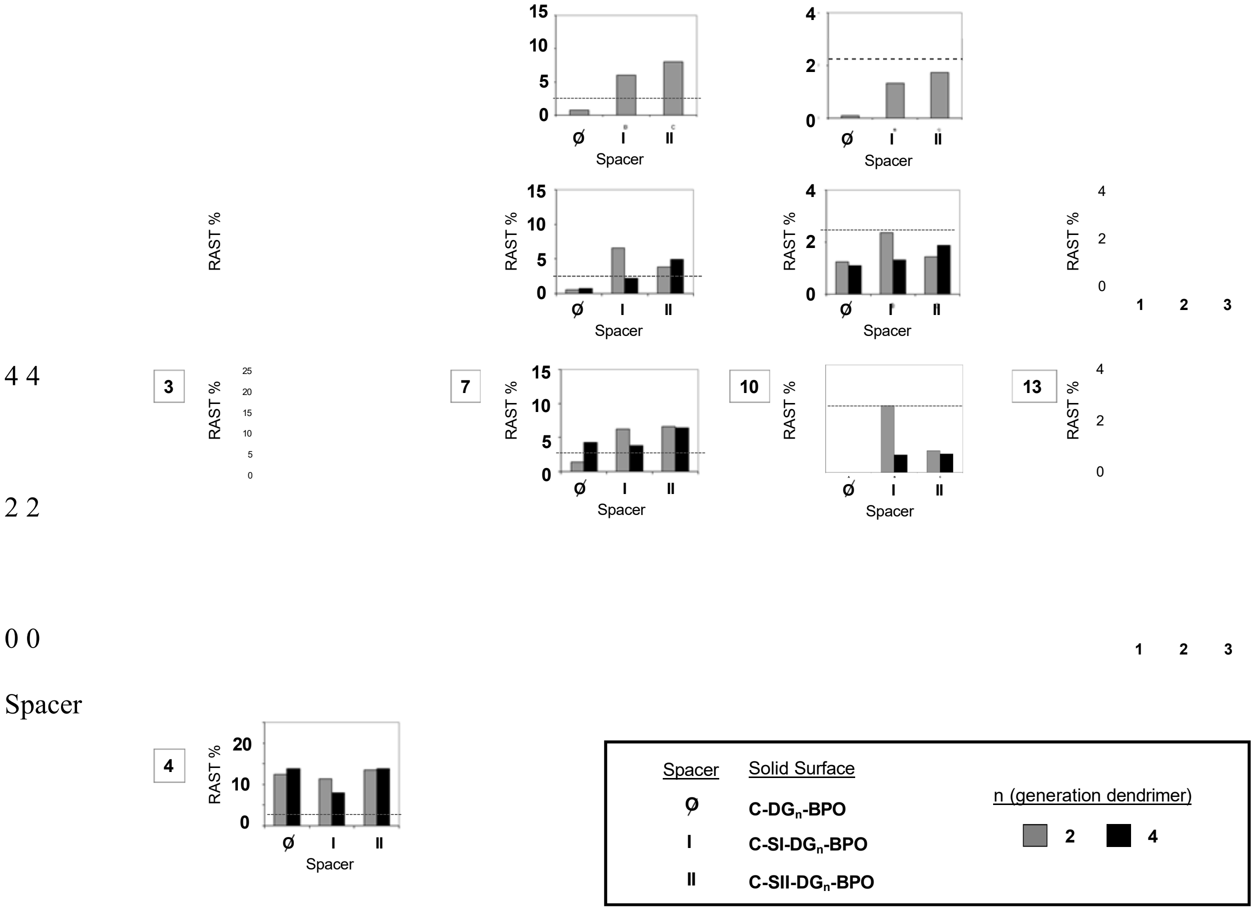
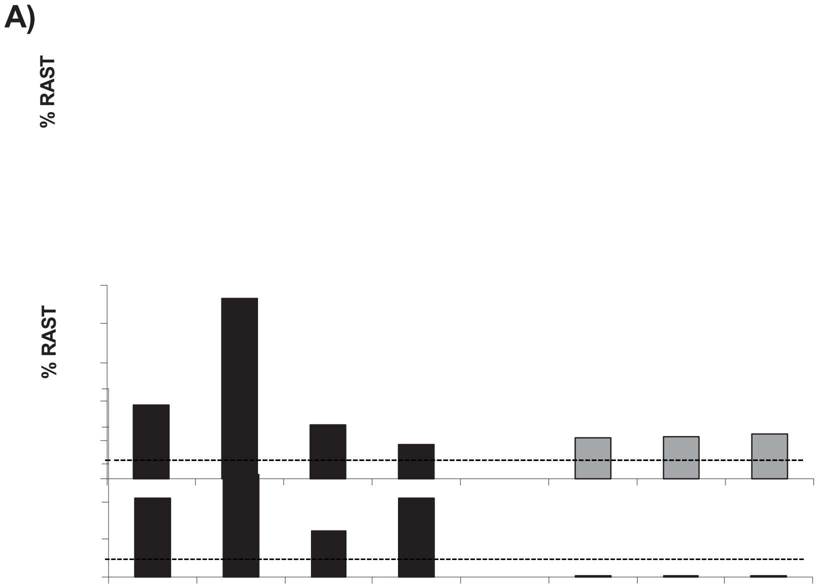


Fig. (4). RAST values (as RAST %, y-axis) for patients' sera as a function of both the linker (□, I, II) and generation number (2, 4) used to prepare the solid surface supports (x-axis). Sera with high and low specific IgE levels, and controls were used. Samples were considered positive if they were higher than 2.5% of label uptake. RAST analyses were carried out in duplicate, showing reproducible values and the average value is represented.

ues and the reduction of free amine groups corroborate the penicilloylation of the dendrimeric zeolitic solid supports. Since the detection of antibody depends on the substrates and on the orientation of the recognition sites on the surface [32], in order to address our research efforts in the adequate direction, we first evaluated how the developed functionalization influences the specific molecular recognition. Thus, these prepared hybrid zeolite materials ZL(I)-DG2BPO were clinically tested by RAST using sera from penicillin-allergic patients and controls who tolerated this drug. As can be observed in Fig. (5A), the dendrimeric zeolite sup- port produced positive RAST results in three sera from patients allergic to benzylpenicillin. Nevertheless, the supports ZL(I)- DG2BPO evaluated using sera from controls resulted in a positive percentage of RAST, above 2.5%, which would mean that these sera are positive (allergic). These false positive data are not satis- factory clinical results and are probably due to nonspecific interac- tions of antibodies with the zeolite surface. To overcome this prob- lem, we prepared new zeolite surfaces with a higher dendritic cov- erage to block the adsorption positions on the zeolite surface, as explained in the following section.

Dendritic Zeolite Solid-Phases: ZL(II)-DG2BPO

To increase the functionalization coverage of zeolites we forced the reaction conditions with APTES by using double the amount of reagent, a higher temperature and a longer reaction time. The pro- cedure worked and the resulting ZL(II)-APS surfaces incorporate a larger amount of amino surface groups compared with ZL(I)-APS (135 compared with 60 µmol g-1). The next steps involving the succinic anhydride reaction, dendrimerization and penicillin func- tionalization were carried out as previously described for ZL(I)- DG2BPO. The Z potential measurements and the quantification of amine groups described in (Table 2) are in agreement with a more elevated functionalization of the supports. The anhydride reaction yielded carboxyl functionalized supports ZL(II)-SA with 20 μmol g-1 remaining amine groups according to the ninhydrin test. The following coupling of dendrimers was analyzed by FT-IR, which showed the presence and amplification of the amide stretch at 1650 cm-1, and other characteristic bands of PAMAM, with the addition of dendrimers to the surfaces, which was not seen for carboxyl ZL(II)-SA Fig. (6). The dendrimer functionalization extensively increased the number of amines to 170 μmol g-1 and the Z potential from -40 to +45 m V. It is worth mentioning that a higher function- alization density was definitely reached since both of these values in ZL(II)-DG2 are considerably superior to those obtained with ZL(I)-DG2. Finally, the further decrease of amine groups and Z potential in ZL(II)-DG2BPO indicates that the haptenization step takes place (Table 2). X-ray photoelectron spectroscopy (XPS) was further employed to verify the functionalization of the developed zeolites Figs. (S1- S8 in Supplementary Material). The C 1s spectrum of zeolites ZL(I/II)-APS consists of a main peak at around 285.0 eV, as ex- pected for a hydrocarbon chain containing C–C/C–H bonds, with an overlapping contribution of two peaks with binding energies of 286.0 and 286.4 eV attributed to C–N and C–O bonds, respectively Figs. (S1-S8). In the following modified zeolites ZL(I/II)-SA the intensity of the 285 eV peak decreased due to a higher contribution of the overlapping peak at 288 eV corresponding to O–C=O and N– C=O, showing the surface amidation (Table 3). The contribution of this peak increased in the successive zeolites ZL(I/II)-DG2 and ZL(I/II)-DG2BPO, which is in agreement with the polyamide con- tent of the anchored dendrimers. The N 1s signals for all the fabri- cated zeolite materials have two clearly separated components. The first peak at around 399.5 eV is assigned to free amine groups and amide bonds since aliphatic amines are generally seen at a binding



25
20
15
10
5
0

25
20
15
10
5
0

Patient 14

Patient 14

Patient 15

Patient 15
Patient 16
Patient 16
Patient 17
Patient 17
Control Patient 18
Control Patient 18
Control Patient 19
Control Patient 19
Control Patient 20
Control Patient 20

Fig. (5). RAST values obtained by evaluation of 4 sera previously assessed by ImmunoCAP system from BPO-allergic patients and 3 sera from tolerant con- trols with the different BPO-dendrimerized zeolites A) ZL(I)-DG2BPO and B)

ZL(II)-DG2BPO. RAST analyses were carried out in duplicate, showing repro- ducible values and the average value is represented.

Table 3. Surface Chemical Composition of the Different Modified Zeolite L Materials, As Deduced from the Deconvolution of Both the C1s and N1s Signals

	C1s Spectrum Peaks			N1s Spectrum Peaks	
	C1 (C–C/C–H)	C2 (C–N/C–O)	C3 (O–C=O/N–C=O)	N1 (R–NH2/N–C=O)	N2 (R–+NH3)
Energy (eV)	285.0	286.0- 286.4	288.0	399.5	401.4
ZL(I)-APS	75.0	19.8	5.2	71.4	28.6
ZL(I)-SA	61.1	20.9	18.0	66.9	33.1
ZL(I)-DG2	58.4	18.9	22.6	85.7	14.3
ZL(I)-DG2BPO	40.3	34.5	25.2	86.4	13.6
ZL(II)-APS	64.8	29.0	6.2	72.0	28.0
ZL(II)-SA	63.2	19.1	17.7	81.5	18.5
ZL(I)-DG2	62.5	16.7	20.8	91.7	8.3
ZL(II)-DG2BPO	63.2	15.2	21.7	93.6	6.4

energy of 399.2 eV whereas amides are around 399.8 eV. The sec- ond peak, at 401.4 eV, has been assigned to protonated amines [43, 44]. The high contribution of 399.5 eV in the dendrimer functional- ized zeolites ZL(I/II)-DG2 and ZL(I/II)-DG2BPO can be ex- plained by the higher content of amide groups belonging to the attached PAMAM dendrimers (Table 3).

Fig. (7) shows the ratio of carbon/silicon and nitrogen/silicon, which was analyzed by the relative atomic concentration of C 1s/Si 2p and N 1s/Si 2p. Thus, the functionalization of the zeolite materi- als was also shown by a higher content of nitrogen and carbon after each synthetic step involved in the zeolite fabrication procedures Fig. (7). The C/Si and N/Si ratios increase significantly on surfaces ZL(I/II)-DG2 and ZL(I/II)-DG2BPO due to the dendrimer content. Moreover, these results indicate a higher functionalization in zeo- lites II (ZL(II)-DG2BPO) compared with zeolites I, which is also consistent with Z potential and ninhydrin data.

To evaluate whether a larger dendrimeric functionalization of the zeolite surface makes these materials useful for the detection of specific IgE antibodies, ZL(II)-DG2BPO materials were assessed for specific IgE recognition by RAST using the same three control sera. The percentage of RAST obtained with these controls gave values below 0.2%, which correctly indicates that these patients are not allergic to penicillin Fig. (5B). The subsequent validation of these materials involved a RAST test with sera from patients aller- gic to penicillin and resulted in positive RAST percentages in all three sera. These results indicate that the optimization of surfaces with a more dense dendrimeric coverage of zeolite surfaces is able to minimize the nonspecific interaction obtained in controls with ZL(I)-DG2BPO. Thus ZL(II)-DG2BPO materials avoid false negative and diagnose allergic patients appropriately.

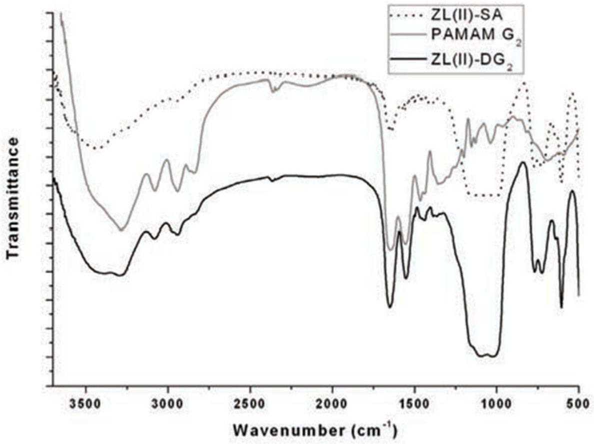


Fig. (6). FT-IR spectra of the carboxyl zeolite ZL(II)-SA, PAMAM-G2 and their resulting dendrimer functionalized zeolite ZL(II)-DG2.

bond to the surface, which are more robust toward hydrolysis than the ester bonds in Spacer I. After modification of the surfaces with both Spacer I and Spacer II, the remaining corresponding chloro- benzyl group should be more reactive and stable in the reaction with the amine groups of dendrimers than the isocyanurate bond. Moreover, the solid phases including Spacer II, treated previously with APTMS, allowed the preparation of more highly dense den- drimeric surfaces. Both protocols, involving the use of Spacer I and II, provide reproducible materials and increase significantly the density of conjugates on the surface obtained with direct binding. This is attributed to the less steric hindrance and greater flexibility provided by the spacers. Concerning clinical evaluation, the RAST results suggest that the use of the spacer improves the sensitivity of the diagnostic tests for allergic reactions to /3-lactams, presenting a higher percentage of positivity with Spacer I and G2. We conclude that the spacer provides a more available conjugate for IgE molecu- lar recognition and can avoid false negative results in those patients with low but nevertheless positive IgE levels.

Two different series of hapten-dendrimer functionalized zeolite L were successfully fabricated and characterized. The strategy al- lows chemical control, obtaining different functionalization degrees of the surface silica. The methodology involved an activation of the surface by consecutive reactions with APTES and succinic anhy- dride, a further dendrimer attachment through amide linkage and
80 the subsequent penicilloylation. The inorganic composition in these
75 hybrid materials makes them more hydrophobic, which signifi-
65 cantly influences the nonspecific protein adsorption interactions.
55 The degree of dendrimer functionalization on zeolite surfaces plays
50 an important role in the specificity of the RAST assay, since highly
40 dense dendrimeric functionalized surfaces are able to minimize
30 nonspecific interactions obtained with the less functionalized sur-
faces. Thus, the most covered dendritic zeolite surfaces display the appropriate functionality to optimize specific IgE recognition and develop a clinically useful diagnostic test.

50 In summary, different dendrimer-modified solid supports, cellu-
45 lose and zeolites L, were appropriately fabricated and validated as
35 solid phases in RIA. These nanostructured materials contain peni-
25 cillin-dendrimer conjugates as hapten-carrier conjugates which can
20 interact with specific IgE antibodies through molecular recognition,
10 thus meeting all the requirements needed in biosensors to detect
0 penicillin allergy. The specificity and improved sensitivity of the dendrimer-modified solid supports make these strategies versatile for different bioactive molecules and has significant implications for the quantification of a wide range of specific IgE antibodies and other biomolecules of diagnostic interest. Cellulose disks have con-

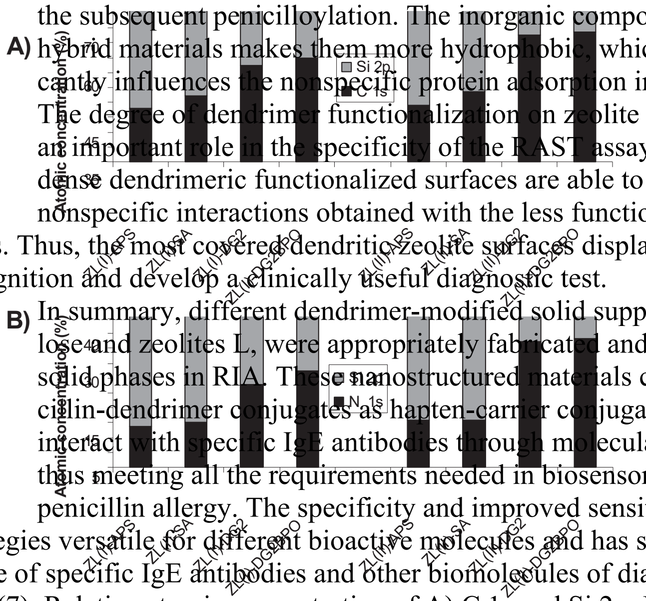


Fig. (7). Relative atomic concentration of A) C 1s and Si 2p; B) and N 1s and Si 2p.

CONCLUSIONS

We successfully addressed the novel use of zeolites L material in a radioimmunoassay (RIA) diagnostic application and showed a significant improvement in sensitivity of cellulose solid phases. Two major problems found with these solid phases were (a) unspe- cific interactions in zeolite L materials and (b) low and irreproduci- ble functionalization density in cellulose materials. With a fixed number of functional groups on the surface and a well-defined structure, dendrimer-based surfaces manufactured with the proper protocol improved on both counts in the present study.

Two new different immobilization protocols were successfully developed for cellulose surfaces. Derived benzylchloro-TEG- chlorobenzoyl chains proved to be more efficient linkers than cya- nogen bromide for attaching hapten-dendrimer conjugates onto cellulose surfaces. When using Spacer I, the resulting ester linkage with the surface should be more stable and robust than the iso- cyanurate bond. Spacer II is only a little bit longer (5 atoms) than Spacer I, but Spacer II also includes an amide bond and silane

ventionally been the preferred solid support in these applications, mainly because of their easy handling for routine hospital assays, which with the described spacer protocol also present the advantage of improving the sensitivity of the assay. On the other hand, the flat and transparent feature of zeolite L allows a more complete charac- terization of the substrates not possible on the fibrous and rough surface of cellulose. Moreover, the larger surface area of zeolite L compared to cellulose results in a higher functionalization with haptens, which is in agreement with the higher adsorption of spe- cific immunoglobulins, with only 0.1 mg of substrate necessary to diagnose whether a serum sample does or does not belong to an allergic patient.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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SUPPLEMENTARY MATERIALS

Supplementary material is available on the publishers web site along with the published article.

ABBREVIATIONS

APTMS = 3-(Aminopropyl)-Trimethoxysilane APTES = 3-(Aminopropyl)-Triethoxysilane BPO = Benzylpenicilloyl
IgE = Immunoglobuline E DMAP = Dimethylaminopyridine DMF = Dimethylformamide
EDCI = 1-(3-(Dimethylamino)propyl)-3-ethyl-
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NHS = N-Hydroxysuccinimide PAMAM = PoliAminoAmine
PBS = Phosphate Buffer Saline
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PEG = Polyethylenglycol
PLL = Poli-L-Lysine
RAST = Radio-Allergo-Sorbent-Test RIA = Rasioimmunoassay
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TEA = Triethylamine
TEG = Tetraethylenglycol
THF = Tetrahydrofurane

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TFA = Trifluoracetic Acid
XPS = X-Ray Photoelectron Spectroscopy

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