A WAY TO IMMOBILIZATION OF ANTIMICROBIAL PEPTIDENISIN ON POLYDIMETHYLSILOXANE SURFACE

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ABSTRACT

Medical devices associated infections due to microbial attachment and biofilm formation are with a high impact on human health and huge socioeconomic costs. Increasing resistance to traditional antibiotics and multidrug treatments are already recognized as one of the top-most serious threats to human health. This rises an argent need in the development of new antimicrobial agents, materials and strategies for improved protection of medical devices against infections. Immobilization of antimicrobial peptides onto the material surface is one of them. Many medical devices are fabricated by chemically inert polymers, like polydimethylsiloxane (PDMS), polystyrene, polyethylene, etc. to which surfaces is difficult the chemical bonding of biomolecules.

The aim of this investigation is to demonstrate the ability of plasma-based Ar^+ beam (PBAIB) to initiate antimicrobial peptides immobilization onto the chemically inert PDMS surface, using the bacteriocin nisin as an example. Earlier developed by us multi-step procedure was utilized that makes possible three types bonding of antimicrobial peptides: i) linker - free, at the first step, just after the PBAIB treatment; ii) via vinyl monomer linker, at the second step after grafting of vinyl monomer and iii) via flexible spacer after coupling of di-NH_2PEG on vinyl monomer grafted surface. A parallel plate reactor, equipped with a serial capacitance, was employed to ensure arise of an ion flow inside the plasma volume, directed toward the treated sample. The changes in the chemical composition of the PDMS surface were studied at every step of the modification procedure and the successful immobilisation of nisin via flexible spacer (di-NH_2-PEG5000) was proved by XPS analysis.

This multi-step procedure to biofunctionalization of strong hydrophobic chemically inert polymer surfaces has a potential to be used whenever need arises to control antimicrobial activity of PDMS or other chemically inert polymeric materials and medical devices fabricated by them.

Keywords: PDMS, surface activation, plasma-based Ar^+ beam (PBAIB), antimicrobial peptides, nisin, immobilization, linker-free, via vinyl monomer, via flexible spacer.

INTRODUCTION

Medical devices associated infections due to microbial attachment and biofilm formation are with a high impact on human health and huge socioeconomic costs. For example, prosthetic devices associated biofilm infections affect more than 4.1 million patients per year, with a total cost of the treated complications of around 7 billion Euros in Europe [1, 2]. Providing environment for the most stable microbial live, the medical biofilms cause extensive antibiotic resistance, morbidity, mortality, and substantial economic loss. Living in a biofilm bacterial species, have great viability and require 500 - 5000 times higher antibiotic doses to be eradicated in a comparison

with the planktonic organisms [3].

The increasing spread of infectious diseases caused by pathogenic bacteria as well as the increasing resistance to the traditional antibiotics and multidrug treatments are already recognized as one of the top-most serious threats to human health [4, 5]. The protection of medical devices against attachment of pathogenic species and biofilm formation requires innovative approaches development and new antimicrobial agents use. The development of mitigating strategies includes a redesign of the material surfaces by deposition of antimicrobial coatings, incorporation of metal/metal oxide nanoparticles [6, 7], graphene and its derivatives [8, 9], as well as of natural biologically active substances from marine and terrestrial biota, etc., everyone with own advantages and disadvantages [6, 10]. Synthetic and natural antimicrobial peptides (AMPs) that demonstrate a broad-spectrum antimicrobial activity with a high specificity and low toxicity are accepted now as one of the candidates for overcoming the antibiotic resistance [6, 10].

Numerous medical devices, made by synthetic polymers, whose surface easily attracts biofilm forming pathogenic microorganisms, play an essential role in the transmission of infections. Because of its relatively good biocompatibility, elasticity, stability in water media, transparency, etc. the cross-linked polydimethylsiloxane (PDMS), known as silicon rubber, is one of the most often used polymeric materials for the fabrication of medical devices: microchips, biosensors, urinary catheters and stents, and many others. Immobilization of AMPs onto the polymer surface is one of the possible ways to add antimicrobial performance but the chemical inertness of the PDMS makes impossible the chemical immobilization of any biologically active molecule without preliminary activation.

Cold plasma was often used to activate polymer surfaces (including that of PDMS) by generation of free radicals. However, the plasma-activated surfaces undergo "surface reconstruction" that leads to a significant restoration of the former properties [11 - 15]. Grafting of suitable vinyl monomers like acrylic acid (AA), hydroxyethyl methacrylate, (HEMA), etc. is required to achieve a durable modification [14 - 16]. On the other hand, ion-beam without followed grafting is known as another way to improve some properties of PDMS and other materials [17, 18]. Plasma based Ar^+ beam (PBAIB), performed in RF low-pressure glow discharge combines some advantages of both: ion-beam and plasma treatment, namely the durability of the modifying effect of the ion-beam with the simplicity of the plasma equipment as compared to the ion-beam equipment. In a former investigation, the ability of PBAIB was demonstrated for development of modified PDMS surfaces with improved interactions with eukaryotic cells [19 - 21].

The aim of this investigation is to demonstrate the ability of the same PBAIB to initiate AMPs immobilization onto the PDMS surface using the bacteriocin nisin as an example. The material surface, created in this way, would combine improved interactions with eukaryotic cells and antimicrobial activity.

Nisin is a polycyclic peptide whit broad-spectrum antibacterial activity [22, 23], that has been utilized for linker-free covalent immobilization onto polystyrene surface (using atmospheric pressure plasma induced grafting, based on its ability to participate in radical interactions) [24], into a cross-linked poly(vinyl alcohol) matrix [25], as well as for effective adsorption on the polystyrene surface (using hydrophobin HGFI) [26]. Microbe killing polymeric films made by melt compounding and compression of nisin peptide assemblies with polyethylene were reported that significantly reduce the number of bacteria, viruses, and fungi. These innovative biologically active polymeric films can potentially be applied for medical device wrapping, food packaging, and agriculture applications [27]. One of the main challenges when using nisin in polymer composites or when it was adsorbed on the polymer surfaces is its easy release. Therefore, covalent bonding is preferable [28, 29]. No report was found about a covalent immobilization of nisin on a cross-linked PDMS surface. In a former investigation, we demonstrated that PBAIB treatment of PDMS leads to its surface activation by a formation of free radicals and peroxides allowing AA grafting, amino-PEGs coupling and collagen immobilization [19]. The nisin ability to participate in radical reactions as well as its terminal carboxyl and amine groups allow its covalent immobilization on three ways: linker-free, at the first step (just after PBAIB treatment); via vinyl monomer linker, at the second step and via flexible spacer at the final step [21, 24]. Therefore, nisin was selected as the peptide for an illustration of the PBAIB ability to initiate antimicrobial peptides immobilization.

EXPERIMENTAL

Samples preparation

Glass plates (10x10x2 mm) were spin-coated (at 3500 r.min^{-1}) with a primer consisting of ethyltriacetoxysilane (50 wt. % toluene solution) and a catalyst (3 wt. % dibutyltindilaurate) to provide good adhesion of the PDMS coating to the glass surface. The primed dry glass plates were then spin-covered with platinum silicone elastomer A-RTV-1556 (Factor II, Incorporated, Lakeside, USA) under the same conditions. Prior to testing, the samples were stored under ambient room conditions for at least 30 days to be completely cross-linked. The thickness of the dry coatings was of 200 - 220 μm as measured by a stereo microscope Leica MZ16 FA (Leica, Wechsler, Germany).

PDMS surface modification

Our strategy to antimicrobial peptide immobilization on the chemically inert PDMS surface is schematically presented in Fig. 1. It includes surface activation by PBAIB treatment, leading to peroxides and radicals formation, playing active centers for linker-free bonding of AMP or to grafting of AA. The last one could act as linker for chemical bonding of antimicrobial peptides containing terminal amino groups. Furthermore, the grafted carboxyl groups could be utilized in the coupling of di-NH_2-PEG acting as a flexible spacer for following immobilization of antimicrobial peptides with terminal carboxyl groups. As an example, we present immobilization of the antimicrobial peptide nisin (HANDARY s.a., Belgium) by peptide synthesis reaction, using its terminal carboxyl groups for interaction with the amino groups of di-NH_2PEG5000, coupled to the PDMS surface.

Plasma-based Ar^+ beam treatment (PBAIB)

PBAIB treatment of the samples was performed in RF (13.56 MHz) low-pressure (200 mTorr) glow discharge at 1200 W (surface density of the discharge power 1.1 W cm^{-2}) for 1 min, using the plasma chamber, equipped with a serial capacitance, presented in Fig 2. The procedure was in detail described in [19].

Acrylic acid (AA) grafting

AA grafting was performed on PDMS coated samples, treated by PBAIB and kept in air for 10 min. Then they were immersed in water solution (50v/50v) of AA (Merk) at 60°C for 6 h. The grafted samples were taken out of the flasks, Soxlet extracted with ethanol for 24 h, and rinsed three times with deionized water to remove any adsorbed homopolymers. The density of the grafted AA could be varied by varying the time of keeping in the AA solution [19].

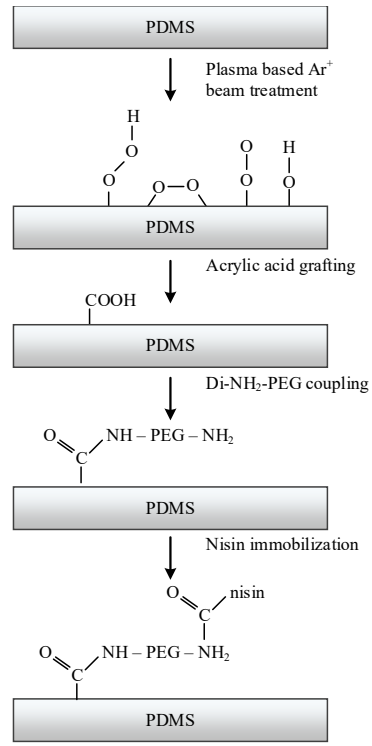

Fig. 1. Scheme of antimicrobial peptides immobilization, initiated by plasma based Ar^+ beam treatment (PBAIB).

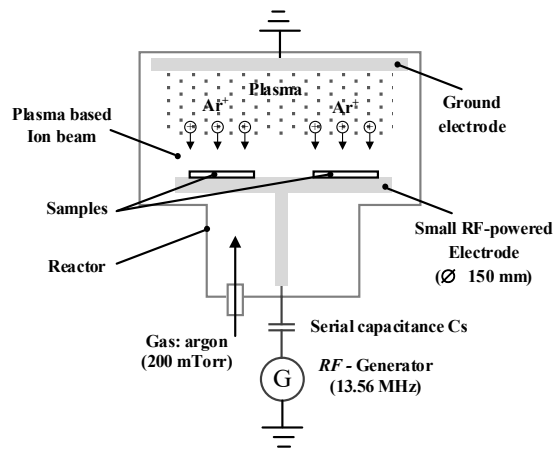

Fig. 2. Parallel plate single-wafer reactor equipped with a serial capacitance to obtain an ion flow in the plasma volume.

Di-NH_2-PEG coupling

AA-grafted PDMS samples were reacted with di-NH_2-PEG5000, bearing two terminal NH_2-groups, in a known way: they were placed in a flask with 10 mL commercial buffer solution (pH 5) containing 140 mg of (N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide) and the closed flask was kept for 1 h at 4°C for activation of the carboxyl groups [30]. Then 40 mL of 0.1 mM di-NH_2-PEG5000 solution (pH 5) was added, and the reaction was performed at 4°C for 24 h under gentle stirring. At the end of the reaction, the di-NH_2-PEG5000-couplet samples were washed three times with distilled water.

Antimicrobial peptide immobilization

A known peptide synthesis reaction was used for the immobilization of the antimicrobial peptide nisin (HANDARY s.a., Belgium) (1 mg in 1 mL citric acid) on the AA-grafted and PEG spacer-couplet PDMS samples [30]. The amine groups available on the modified surface were utilized for the immobilization of the nisin by formation of amide bonds thorough the mediation of EDC. Di-NH_2-PEG5000 modified PDMS samples were placed in a flask with 10 mL commercial buffer solution (pH 5) containing 500 μL nisin; then 100 mg of EDC (Fluka Chemie GmbH, Buchs, Switzerland) were added. The immobilization reaction was performed at 4°C for 24 h under mild stirring and the nisin-grafted samples were three times washed with distilled water.

Surface characterization

X-ray Photoelectron Spectroscopy (XPS) analysis was carried out with ESCALAB II MK VG Scientific spectrometer. The excitation X-ray source was AlKα (excitation energy: 1486.6 eV). Complete spectral scans as well as detailed records of the main peaks were made at 10^{-8} Pa. The binding energy scale was fixed by assigning E_b = 285 eV to C1: C-C or CH C1s peak. The carbon chemical shifts for different oxygen containing groups are:

C2 from hydroxyl, hydro peroxide, ether, alkyl or sulfate ester, ΔE_b = 1.5 eV;

C3: > C = O from carbonyl or amide, ΔE_b = 3.0 eV;

C4: - COO - from carboxyl or the corresponding ester, ΔE_b = 4.2 eV.

The area of the different peaks was computed graphically and corrected by using Scofield's relative cross sections [31].

In order to support the XPS analysis, the equilibrium water contact angle (WCA) of modified surfaces was measured by Easy Drop instrument (Krüss, Germany) [32].

RESULTS AND DISCUSSION

Antimicrobial activity is necessary to avoid non-desirable medical devices associated infections due to a biofilm formation by pathogenic microbial species. AMPs immobilization onto the material surface is one of the possible ways to improvement the antimicrobial performance. The covalent bonding of antimicrobial peptides requires preliminary activation of the chemically inert PDMS surface. Here we present a multistep procedure for nisin immobilization and creation of surface combining improved interactions with eukaryotic cells and antimicrobial performance. The procedure starts with PBAIB treatment to create active centers for a graft copolymerization of vinyl monomer (in this case AA) followed by a flexible spacer (in this case di-NH_2-PEG5000) coupling for immobilization of antimicrobial peptide (as an example nisin). Thus, the antimicrobial peptide nisin could be covalently immobilized onto the PDMS surface on three ways: i) linker-free to the PBAIB activated polymer surface, as it was described in [24]; ii) or via AA linker, as it was described in [19]; iii) or via di-NH_2-PEG as flexible spacer, coupled to the AA grafted PDMS surface.

The conformational freedom of the immobilized biomolecules is very important for their biological activity saving. Therefore, as an example, here is presented the immobilization of nisin via the flexible spacer di-NH_2-PEG5000 using one of its amino groups for bonding to the carboxyl groups of the AA grafted surface and the other one for peptide synthesis reaction with the carboxyl groups of the nisin.

Plasma based Ar^+ beam (PBAIB) treated PDMS surface

Creation of ion flow in the plasma volume of the used in this investigation plasma reactor (Fig. 2) supposes an ability for adding the durable modifying effect of the ion beam to the activation effect of the plasma. Fig. 3 presents schematically the process of plasma-based Ar^+ beam (PBAIB) treatment and expected chemical alterations of the PDMS surface.

During the treatment in argon plasma [33] many

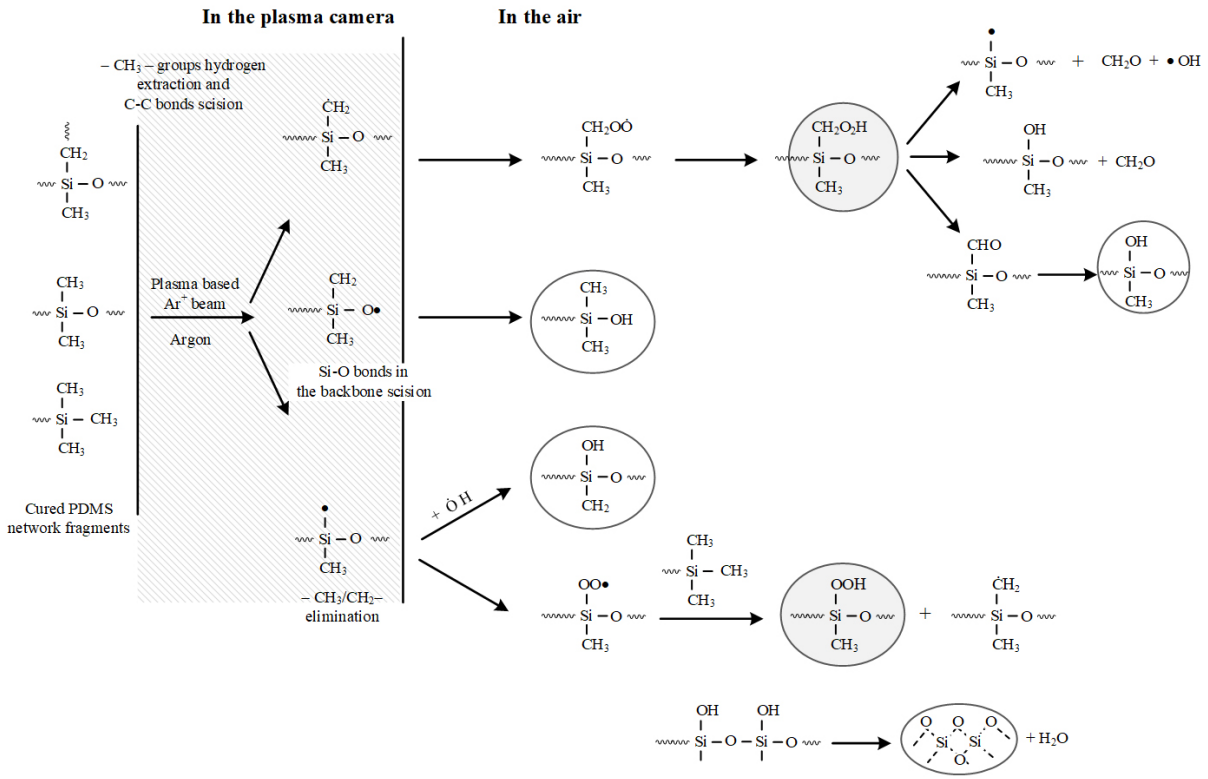

Fig. 3. Reaction scheme of the PDMS surface modification by plasma-based Ar^+ beam (PBAIB) treatment.

radicals could be formed on the PDMS surface trough: a brake of - CH_2 - CH_2 - cross-links and extraction of hydrogen atom from CH_3-groups; - Si - O - backbone chain scission and - CH_3/CH_2 - groups elimination as it is shown in Fig 3. A part of them re-combines during the treatment and form inactive species or new radicals. Large part of the existing radicals quickly oxidase in contact with the air and turn in corresponding peroxides or hydro peroxides (Fig. 3, the fragments in the grey circles), which are relatively stable at room temperature. However, they easily destruct under heating to form radicals that could be utilized as active centers for initiation of free radical graft polymerization of vinyl monomers, such as acrylic acid is. The amount of the active centers could be controlled by variation of the discharge power and the duration of the treatment, as it was demonstrated in a former investigation [19].

In air, the (PBAIB) treated PDMS surfaces undergo deep chemical alterations associated with the appearance of significant amount oxygen containing groups, including hydroxyl (Fig. 3, the fragments in the circles) and silanol groups. Surface silanol groups could be formed by hydrogen extraction, followed by a reaction of methylene radicals and air oxygen and reorganization (Fig. 3). Other possibility for silanol groups generation is the backbone chain scission. The water elimination from neighbouring silanol groups results in surface Si-O-Si bonds formation, as it is shown in Fig. 4 (the fragment in ellipse). The silanol groups can form a stable partially mineralized surface layer that is a base of the durable PDMS surface hydrophilization, the last one being a pre-requisite of improved interactions with eukaryotic cells [19, 34].

PBAIB treatment effectively alters the chemistry of the cross-linked PDMS surface, as confirmed by XPS analysis. Fig 4. presents C1s (a), O1s (b) and Si2p (c) peaks of PDMS: non-treated (curves 1) and PBAIB treated at 1200 W for 1 min (curves 2).

The supposed possible chemical alterations of the PBAIB treated PDMS are confirmed by the changes in the intensity, position and wade of C1s (Fig. 4a), O1s (Fig. 4b) and Si2p (Fig. 4c) pecks. A comparison of C1s, O1s and Si2p pecks of PBAIB treated PDMS (Fig. 4, curves 2) with the corresponding C1s, O1s and Si2p peaks of the non-treated samples (Fig. 4, curves 1) demonstrates the following: the intensity of the C1s peak

(Fig. 4a) decreases simultaneously with increasing of O1s (Fig. 4b) and Si2p (Fig. 4c) pecks. This indicates a significant decrease of the carbon amount, accompanied with increasing of Si and O content on the treated surface, clearly expressed by the elemental analysis data, presented in Table 1.

Column 2 in Table 1 clearly demonstrates the sharp carbon content decrease - from 49.9 at. % for the non-treated PDMS down to 18.2 at. % for the PBAIB treated one. This could be due to a formation of a modified surface layer because of chemical extraction including mainly oxidation of the carbon, some of them accompanied with deliberation of volatile compounds. The varying of the discharge power and durability of the treatment allows a control over the amount of radical (active centers) formation and the surface hydrophilization, the last one improving the interactions with the eukaryotic cells, as it was shown in a former investigation [19].

The active centers on the PDMS surface, formed after PBAIB treatment, could be utilized for linker-free covalent immobilization of nisin, based on its ability to participate in radical interactions, as it was shown for atmospheric pressure plasma induced, covalent bonding to polystyrene surface [24].

PBAIB treatment leads to a formation of a partially mineralized surface layer, like that obtained after conventional Ar^+ beam treatment as proved by XPS analysis. PBAIB treatment turns the strong hydrophobic surface of the non-modified PDMS (WCA 103.4°, Table 1) into hydrophilic one (WCA 61.3°, Table 1), the hydrophilicity depending on the discharge power and treatment duration and thus improving initial interactions with eukaryotic cells, all in details discussed in [19].

AA grafted surface

It is known that the plasma treatment of polymers, including PDMS leads to surface activation due to arise of radicals, including such that turns in peroxides

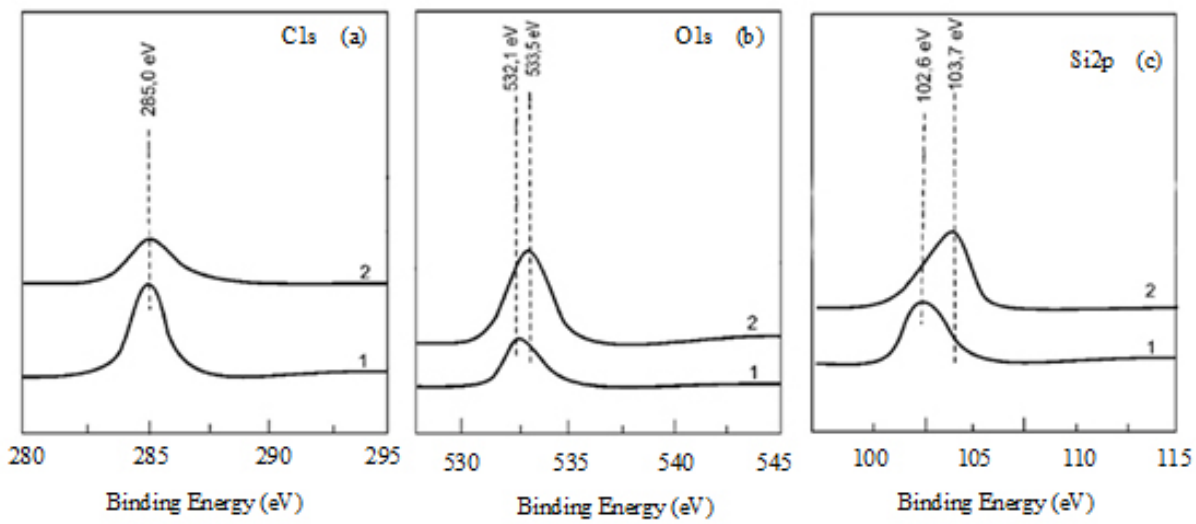

Fig 4. C1s (a), O1s (b) and Si2p (c) peaks of PDMS: non-treated (curves 1) and plasma-based Ar^+ (PBAIB) treated at 1200 W for 1 min (curves 2).

Table 1. Water contact angle, WCA and elemental composition (at. %) of non-modified and modifies PDMS surfaces as derived from XPS analysis.

PDMS Sample	WCA (°)	C, at. %	O, at. %	Si, at. %	N, at. %	S, at. %	Si/C	Si/O	Si/N
non-modified	103.4	49.9	24.3	25.7	-	-	0.51	1.05	
PBAIB treated	61.3	18.1	50.0	29.8	-	-	1.65	0.60	
AA grafted	74.0	33.0	39.6	27.4	-	-	0.83	0.69	
di-NH_2-PEG5000 bonded	16.9	55.2	25.8	13.6	5.4	-	0.24	0.53	2.52
nisin immobilized	49.6	55.0	28.7	7.3	8.3	0.7	0.13	0.25	0.89

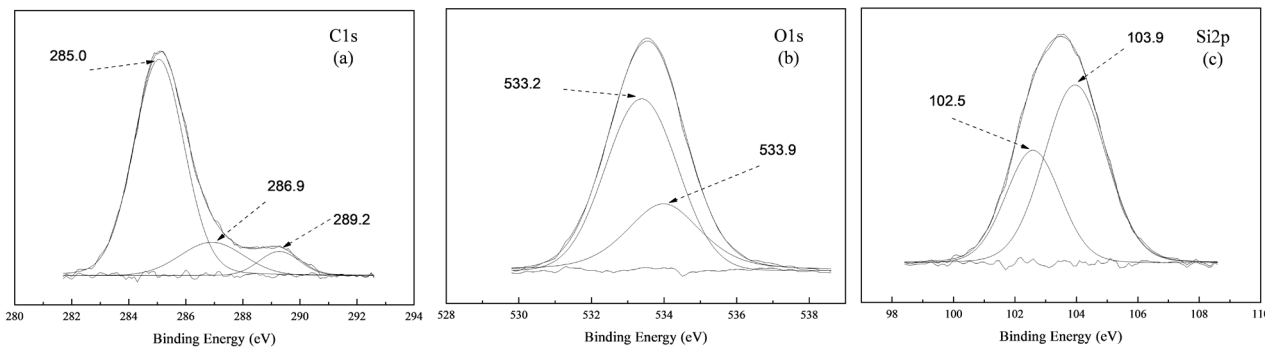

Fig. 5. C1s (a), O1s (b) and Si2p (c) deconvoluted picks of AA grafted (for 6 hours) PDMS, pre-activated by plasma-based Ar^+ beam (PBAIB) treatment at discharge power of 1200 W for 1 min.

[35 - 37]. The last ones are used for initiation of graft copolymerization with vinyl monomers like AA, HEMA, etc. [14]. PBAIB treatment also leads to formation of similar radicals (Fig. 3), opening a way to further chemical alterations of the surface. In Fig 5. are presented deconvoluted C1s (a), O1s (b) and Si2p (c) picks of AA grafted (for 6 h) PDMS, pre-activated by PBAIB treatment at discharge power of 1200 W for 1 min.

The shape of the C1s peak (Fig. 5a) clearly demonstrates that the AA grafting is successful: on the main carbon C1s peak, centred at E_b = 285.0 ± 0.4 eV ($-CH_2/CH_3$) appears shoulder at about E_b = 289.0 ± 0.4 eV, corresponding to O=C-O accompanied with decrease of silica content on the surface (Table 1) [38]. The amount of the grafted - COOH groups was of 6.4 nmol mm^{-2} (Toluidine O test, [39]). The AA grafting leads to alteration of the topography and the hydrophilic/ hydrophobic balance (Table 1) on the surface and thus to improved interactions with eukaryotic cells, as it was found in our former investigation [19].

Nisin immobilized surfaces

One of the main challenges when using nisin as antimicrobial agent at polymeric materials is its easy release from the polymer composites and composite coatings. Therefore, its covalent bonding is preferable [29, 40]. Nisin is a polycyclic, 34-amino-acid polypeptide with amino and carboxyl end groups, and five internal ring structures involving disulfide bridges. It is a small protein antibiotic that contains dehydro residues (dehydroalanine [DHA] and dehydrobutryine [DHB] and thioether cross-linkages (lanthionine and B-methyllanthionine) that are introduced by posttranslational modifications of ordinary amino acids (serine, threonine, and cysteine), as it is evident in Fig. 6 [41, 42].

Reports about a covalent bonding of nisin to the surface of a cross-linked PDMS were not found. However, its ability to participate in radical reactions, as well as the terminal carboxyl and amine groups, depicted in Fig 6. allow its covalent immobilization to the activated and AA grafted PDMS surface [24]. The use of a flexible spacer was preferred to be saved the natural biological activity of the nisin. One of the NH_2-groups of di-NH_2-PEG5000 were used for its bonding to the AA-grafted PDMS surface and the other one - to the carboxyl group of the nisin (Fig. 1) by peptide synthesis reaction. The chemical composition of the di-NH_2-PEG5000 bonded and nisin immobilized PDMS surface is presented in Table 1, Fig. 7 and Fig. 8, respectively.

The ester carbon in C1s pick centred at 286.5 eV (C-O) is significantly higher for di-NH_2-PEG5000 coupled (Fig. 7a) as compared to the AA grafted PDMS surface (Fig. 5a). This is strong evidence for the presence of a bonded di-NH_2-PEG5000 on the surface, because the increase of the C1s ester component could be due to a presence of $-CH_2CH_2O-$ of the di-NH_2-PEG 5000. The covalent bonding of di-NH_2-PEG5000 to the AA grafted PDMS surface confirms by the detection also of a significant nitrogen amount after its bonding (Fig. 7 and Table 1). The detailed N1s pick, presented in Fig. 7d demonstrate two components centred at about 400.5 ± 0.4 eV and 402.9 ± 0.4 eV which could originate from amino and amide groups [20, 30]. In our case, they originate respectively: one from the non-reacted amino group of the di-NH_2-PEG5000 and the second one - from amide bonded nitrogen, resulting from the interaction of the surface - COOH groups of the AA grafted PDMS and the other free NH_2 group of the di-NH_2-PEG5000.

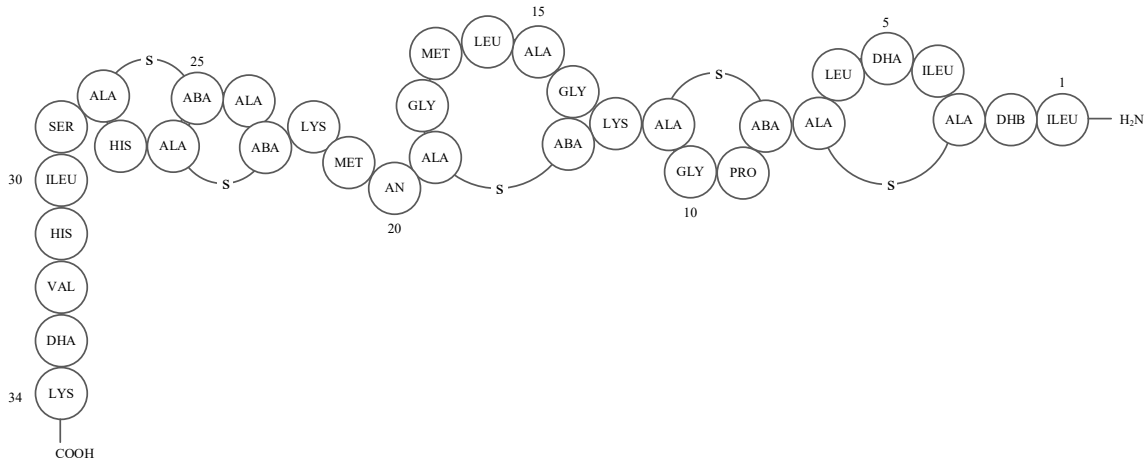

Fig. 6. Structure of nisin molecule elucidated in 1971.

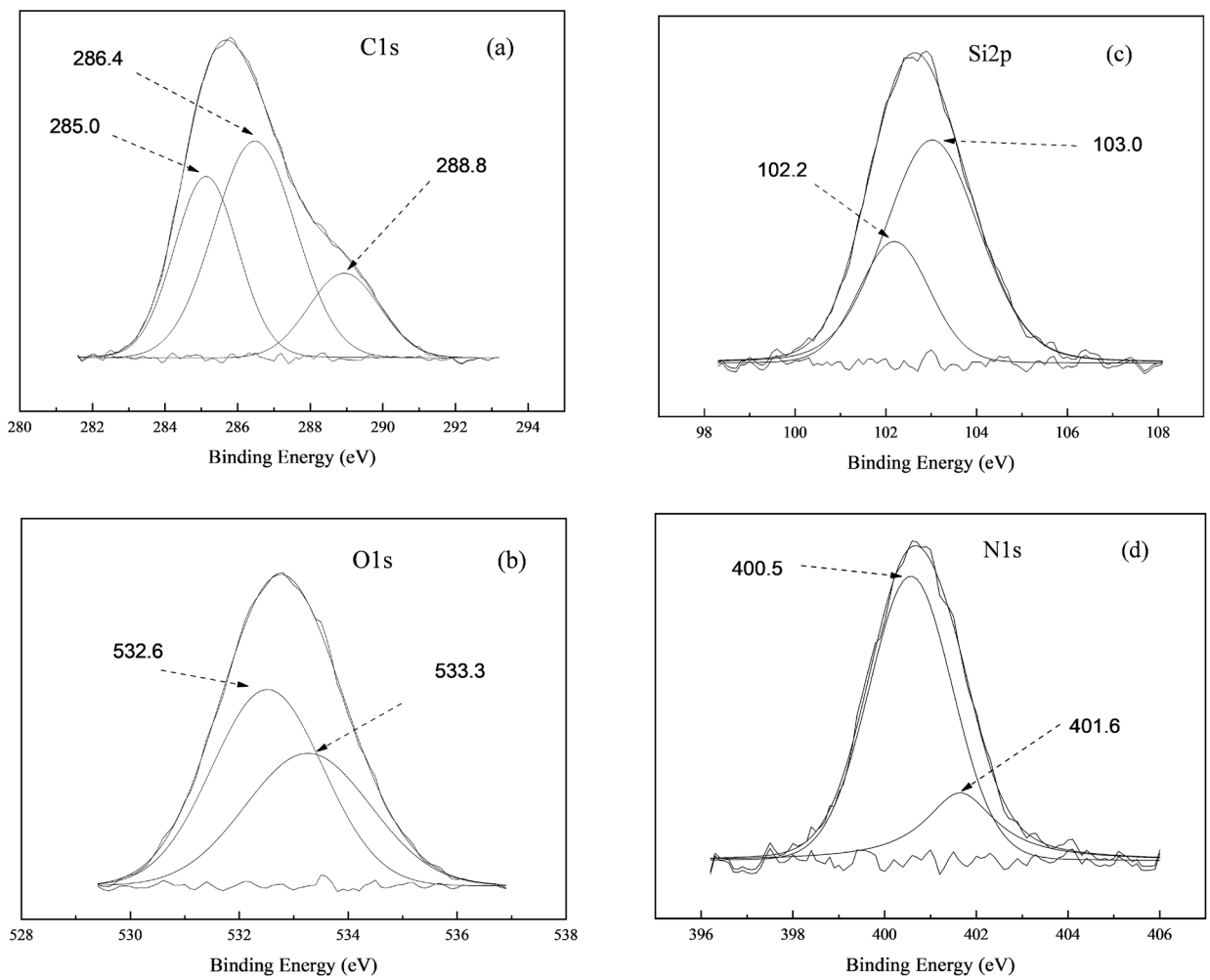

Fig. 7. Deconvoluted C1s (a), O1s (b), Si2p (c) and N1s (d) spectra of di-NH_2-PEG5000 coupled AA grafted PDMS pre-activated by plasma-based Ar^+ beam (PBAIB) at 1200 W for 1 min.

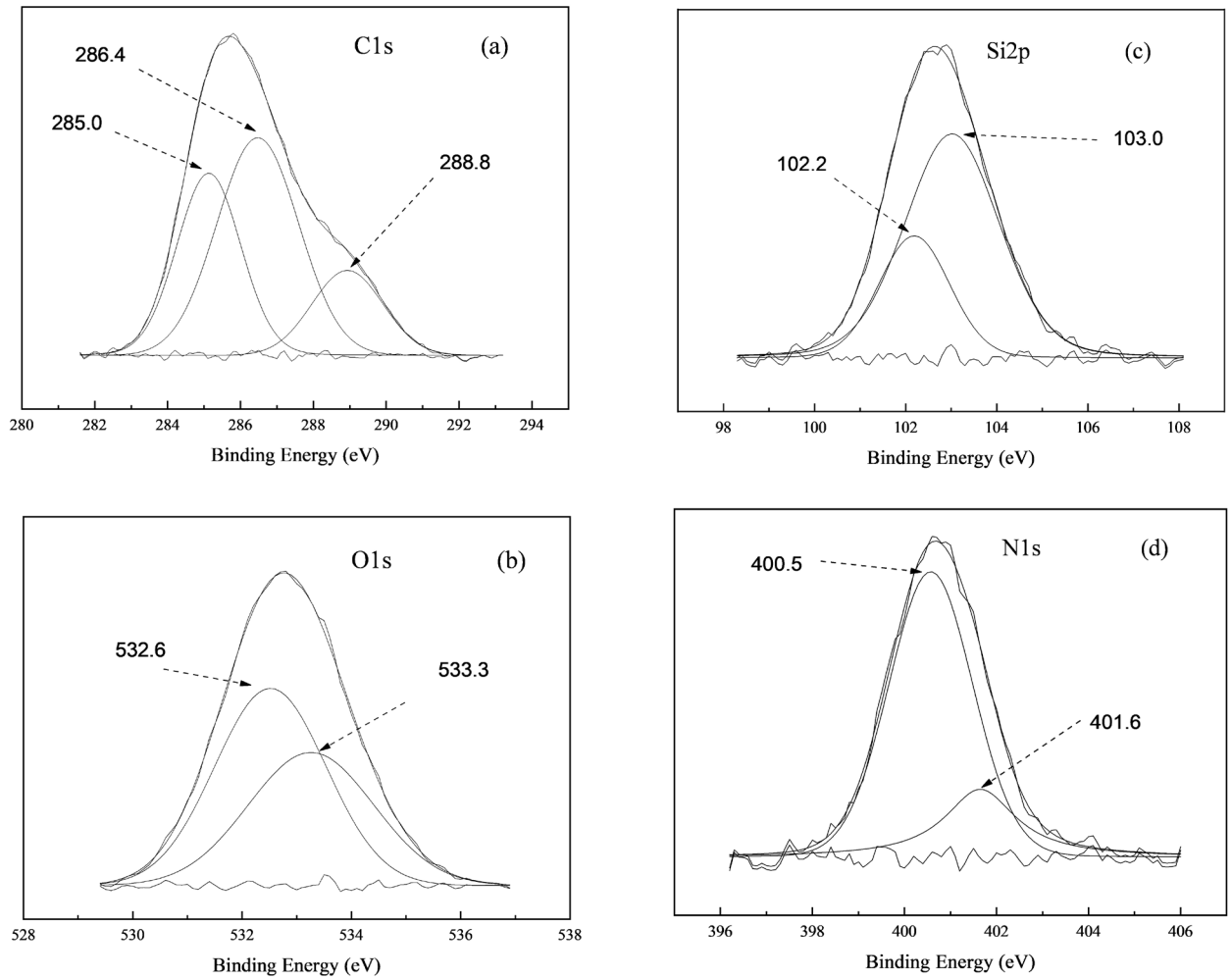

Fig. 8. Deconvoluted C1s (a), O1s (b), Si2p (c) and N1s (d) spectra of nisin immobilised, di-NH_2PEG5000 coupled and AA grafted PDMS pre-activated by PBAIB treatment at 1200 W for 1 min.

The immobilization of nisin to the covalent bonded flexible di-NH_2-PEG5000 spacer is the last step of the selected by as strategy to add antimicrobial activity of the PDMS surface. One indication for successful nisin immobilization is the presence of small sulfur amount on the nisin-immobilized sample (Table 1) originating from the disulfide bridges of the nisin structure (Fig. 6). A comparison of the N1s picks of the di-NH_2-PEG5000 bonded PDMS surface before (Fig. 7d) and after immobilization of nisin (Fig. 8d) demonstrates significant alteration of their shape and intensity. Significant alterations are observed in the C1s picks of di-NH_2-PEG5000 bonded PDMS surface before (Fig. 7a) and after immobilization of nisin (Fig. 8a). The intensity of the C1s component centred at 289.0 ± 0.4 eV (O - C = O) also undergoes significant alteration after the immobilization of the nisin. The altered chemical compound of the di-NH_2-PEG5000 bonded PDMS surface due to the caring out a carbodiimide reaction with the nisin confirms by the comparison of the data in the last two rows of Table 1. Sulfur appears which is missing on di-NH_2-PEG5000 bonded surface; nitrogen and oxygen content are increased; the silica content as well as the Si/C, Si/O and Si/N ratios are significantly reduced. Correspondingly, the hydrophilic/hydrophobic balance is altered. Both, the surface chemical composition and hydrophilic/hydrophobic balance (Table 1) alteration could be accepted as indication for a successful immobilization of the nisin on the PDMS surface. The chemistry of the nisin immobilized PDMS surface is very complicated and therefore, other information from the XPS analysis is difficult to be obtained.

CONCLUSIONS

It was demonstrated that the plasma-based Ar^+ beam (PBAIB) initiates multi-step procedure to antimicrobial peptides immobilization onto the strong hydrophobic, chemically inert PDMS surface. This procedure opens a way to three types bonding of antimicrobial peptides: linker – free, at its first step, just after the PBAIB treatment; via vinyl monomer linker, at the second step and via flexible spacer at the last step. Nisin immobilisation via flexible spacer, di-NH_2-PEG5000 was successfully performed on preliminary activated (PBAIB treated) and acrylic acid grafted PDMS, as proved by XPS analysis. Hydrophylized PDMS surfaces were created at every stage. This multi-step procedure to biofunctionalization of strong hydrophobic chemically inert polymer surfaces has a potential to be used whenever need arises to control antimicrobial activity of PDMS or other chemically inert polymeric materials and medical devices fabricated by them.

Acknowledgments

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Authors' contributions*: T.G.V.: conceptualization.; D.N.G.: graphical design; P.D.D.: plasma equipment; T.G.V., D.H.G., P.D.D.: experimental work; T.G.V writing; T.G.V.: project administration; T.G.V.: funding acquisition. All authors have read and agreed to the published version of the manuscript*.

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