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Nanodiamond–drug conjugates for coating xenogenic heart valve prostheses

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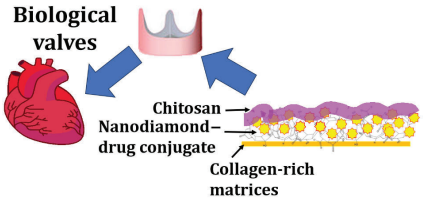
Multicomponent coatings of collagen-rich matrices of bovine pericardium were prepared from nanodiamonds and antibiotics, namely, amikacin, levofloxacin and chitosan. Preservation of the multilayers that consist of nanodiamonds, antimicrobial agents and chitosan on the material surface and preventing the calcification of the material were revealed.



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Mechanical and biological prosthetic valves are the most used in modern cardiac surgery.[1–3] The main characteristics of an 'ideal' valve prosthetic were listed in the review.[1] When using in transcatherer heart valve implants these parameters, along with the convenience for surgeons, total recovery after the collapse without any fatal damage and reasonable price for general people, should also include mechanical characteristics. Namely, it should not be too hard for the blood flow to open and, at the same time, not too soft – to avoid the blood backflowing. Finally, it has to eliminate calcific degeneration and blood coagulation. The calcific deposit is one of the challenges[4,5] that narrows the valve opening, reducing blood flow through the valve. In the cases of xenogenic heart valve prostheses drafted with glutaraldehyde, calcification occurs due to the attraction of host plasma calcium ions to glutaraldehyde groups.[5] Masking the negative groups of glutaraldehyde modified surface is one of the ways to reduce calcium deposits. To this end, chitosan is a promising material to solve the problem.[6,7]

Nanodiamonds are a suitable material for collagen-rich matrices modification.[8–10] If antimicrobial agents are used as surface modifiers, it is possible to suppress the development of the bacterial infections after implantation.[11,12] Therefore, a combined coating that includes a nanodiamond–drug composite and chitosan will be able to solve challenges related to mechanical characteristics, calcification and suppression of the pathogens development. In this work, the potential multicomponent nanodiamond-containing coatings for xenogenic heart valve prostheses were explored for the first time. Nanodiamond–drug conjugates comprising either amikacin or levofloxacin as an antimicrobial agent to enhance the self-antimicrobial properties of nanodiamonds were prepared by the adsorption technique (for details, see Online Supplementary Materials).[13] Selected conjugates were tailor-made to include either positive (amikacin) or negative (levofloxacin) drug molecules on nanodiamond surfaces thus illustrating the versatility of the proposed coating.

The stability of each component *in vivo* was controlled *via* the tritium label. Tritium-labeled nanodiamonds, amikacin, levofloxacin and chitosan were obtained through the tritium thermal activation method.[8,14] The compositions of the coatings were determined using tritium-labeled compounds. For this purpose, nanodiamond–drug–chitosan coatings were obtained with one tritium-labeled component: nanodiamonds, antibiotics or chitosan. After the decomposition of the matrices, the radioactivity of the nanodiamond sediment and the supernatant was measured, and the content of the tritium-labeled component was calculated based on its specific (mass) radioactivity. The compositions of coatings are summarized in Table 1.

SEM images indicate that nanodiamonds form sufficiently uniform coverage of collagen fibers (Figure 1).

Both initial and drug-modified nanodiamonds adsorb of collagen matrices of each size. In the case of levofloxacin, we observed the adhesion of fibers that can reduce the specific area of the collagen-rich matrices, that explains the lower nanodiamond coating of levofloxacin-containing material.

Chitosan adsorption does not affect the distribution of nanodiamonds over the surface. In other words, the nanodiamond layer remains stable under the conditions of a chitosan layer application. The presence and number of nanodiamonds on the surface of collagen-rich matrices do not alter the adsorption of chitosan under supercritical carbon dioxide conditions, which is

Table 1 Compositions of nanodiamond–drug–chitosan coatings[a] (mg of component per g of collagen matrices).

Entry	Nanodiamonds	Drug	Chitosan
1	not applicable	not applicable	12 ± 2
2	3.9 ± 0.8	not applicable	17 ± 5
3	1.8 ± 0.4	0.19 ± 0.02 (levofloxacin)	23 ± 6
4	3.3 ± 0.6	0.28 ± 0.02 (amikacin)	21 ± 4

[a] Results are the mean of three samples ± standard error of the mean.

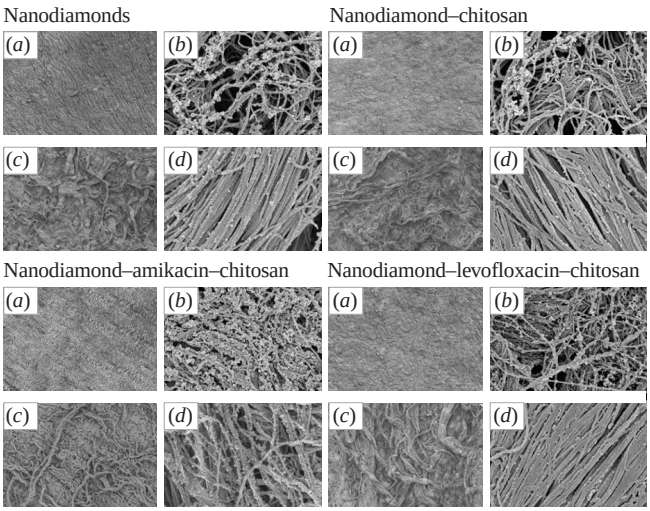

Figure 1 SEM images of collagen tissue coated with nanodiamonds, nanodiamond–chitosan, nanodiamond–amikacin–chitosan and nanodiamond–levofloxacin–chitosan: (*a*) smooth side (500 μm), (*b*) smooth side (2 μm), (*c*) 'fluffy' side (500 μm) and (*d*) 'fluffy' side (2 μm).

about 20 mg g^{-1} of matrices (Table 1, entries 2–4). In the case of free collagen-rich matrices chitosan coating is 12 ± 2 mg g^{-1} (entry 1). Nanodiamonds have a negative charge (zeta potential of an aqueous suspension is –30 mV); the adsorption of levofloxacin on their surface keeps the potential negative, whereas the adsorption of amikacin neutralizes the negative charge of the surface of nanodiamonds. It is safe to assume that the nanodiamond layer contributes to the increase in the surface available for chitosan adsorption, pushing the matrices fibers apart, beyond that the appearance of negative charge centers on the surface promotes the adsorption of chitosan from the solution.

It was noted that the quantity of nanodiamonds of positive zeta potential on the surface of the collagen-rich matrices turned out to be higher than the nanodiamonds that possess negative zeta potential. The difference in the adsorption ability on the surface of collagen matrices is probably due to some modification of the nanodiamonds surface by the manufacturer in order to stabilize them in an aqueous suspension or to the composition of the explosive mixture in the production of detonation nanodiamonds[15] supplied as a stable aqueous suspension. Modifying its surface with levofloxacin reduces adsorption on collagen-rich matrices, while zeta-potential of nanodiamond–drug conjugate remains negative.[13] In the cases of free nanodiamonds and nanodiamond–amikacin conjugate, the number of nanodiamonds on the collagen-rich matrices surface is almost the same. That suggests the similarity of the surface functional composition of nanodiamonds in both cases. Comparison of the FTIR spectra recorded for the above mentioned nanodiamonds indicates that the spectrum of nanodiamonds modified with amikacin is less different from the initial one then the spectrum of the nanodiamonds–levofloxacin complex.[13] To determine the potential of coatings to modify the surface of the prosthesis material, the mechanical characteristics

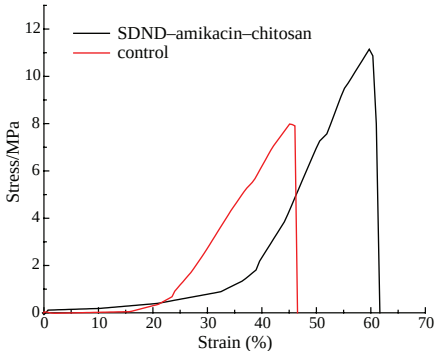

Figure 2 Stress–strain curve from uniaxial tensile loading of biological tissues of the bovine pericardium with nanodiamond–amikacin–chitosan coating and control sample.

of the collagen-rich matrices, as well as *in vivo* resistance and susceptibility of the material to calcification were determined. Stress-strain behavior (see Table S2 in Online Supplementary Materials) of bovine pericardium revealed that the largest increase in maximum deformation was observed for the samples containing amikacin coating. It is important to note that the J-shape of the stress–strain curve is preserved (Figure 2) when the surface of biological tissue is modified with nanodiamonds, that makes it similar to the human tissue.[16]

For an initial screening of the stability of nanodiamond containing coatings, collagen-rich matrices were tested *in vivo* in rats for their stability and susceptibility to calcinosis. Coatings of composition [^{3}H]nanodiamond–drug–chitosan, nanodiamond–[^{3}H]drug–chitosan and nanodiamond–drug–[^{3}H]chitosan were applied on the surface of collagen matrices to track a possible change in the composition of each component of the layer. After four months of subcutaneous exploitation collagen samples were extracted from animals, then dissolved in nitric acid and their composition was determined based on radioactivity data. The composition of the coatings before and after exploitation is summarized in Table 2.

Amikacin-containing layers showed higher retention on the surface of collagen-rich matrices in a living organism. Amikacin as a drug also showed good retention on the surface; a decrease in the chitosan layer is associated with the removal of layers that are poorly bound to the surface. This assumption is confirmed by the results obtained for the levofloxacin series when about 70% of chitosan was retained, probably due to electrostatic interactions. For matrices without a nanodiamond coating, the chitosan deposited from a solution under supercritical carbon dioxide conditions is about 10 mg per g of collagen-rich matrices.[17] It is noteworthy that in terms of antibacterial activity, amikacin applied to nanodiamonds has shown a better result than levofloxacin.[13] The layer of nanodiamonds with levofloxacin turned out to be less stable compared with the amikacin sample, where the layer was preserved for 60%. Therefore, our results revealed that the stability of the nanodiamond-containing coating was determined by the functional composition of the nanoparticle surface, which in turn depends on the antibiotic modifier of nanodiamonds.

Table 2 Composition of coating before and after 4-month exploitation in rats (mg of labeled component per g of collagen-rich matrices).[a]

Coating	Tritium labeled component content/mg g^{-1}	
	before exploitation	after exploitation
[^{3}H]nanodiamond–amikacin–chitosan	3.3 ± 0.6	2.1 ± 0.4
Nanodiamond–amikacin–[^{3}H]chitosan	23 ± 6	3.5 ± 0.4
Nanodiamond–[^{3}H]amikacin–chitosan	0.28 ± 0.02	0.22 ± 0.07
[^{3}H]nanodiamond–levofloxacin–chitosan	1.8 ± 0.4	0.21 ± 0.1
Nanodiamond–levofloxacin–[^{3}H]chitosan	21 ± 4	14.7 ± 0.7
Nanodiamond–[^{3}H]levofloxacin–chitosan	0.19 ± 0.02	0.06 ± 0.01

[a] Results are the mean of six samples ± standard error of the mean.

Table 3 Metal content in collagen-rich matrices after 4-month exploitation.

Coating	Metal content in matrices/mg g^{-1}				
	Ca	Fe	Zn	Na	K
Nanodiamond–amikacin–chitosan	1.58 ± 0.42	1.39 ± 0.59	0.11 ± 0.05	11.0 ± 6.9	11.33 ± 0.65
Nanodiamond–levofloxacin–chitosan	1.45 ± 0.48	0.89 ± 0.15	0.10 ± 0.08	16.0 ± 7.5	9.33 ± 3.46
No coating	11.3 ± 0.9	0.54 ± 0.05	0.07 ± 0.01	13 ± 3	9 ± 2

A key role of the chitosan layer in the complex coating is to prevent the calcification process of prostheses material. Metal content in collagen-rich matrices after their exploitation for each coating under the conditions of high calcium absorption was determined using ICP-AES (Table 3).

ANOVA analysis indicates that calcium content for nanodiamond–amikacin–chitosan and nanodiamond–levofloxacin–chitosan samples differs slightly, which emphasizes the role of chitosan in the process. The calcium content in the specimen without coating is almost an order of magnitude higher. Inasmuch as iron is the main impurity metal in the composition of nanodiamonds,[18] its concentration in nanodiamond-containing samples is significantly higher ($p < 0.05$) than in the control sample. The quantities of other metals (zinc, sodium and potassium) in the samples with and without coatings were comparable.

The observed *in vivo* stability of nanodiamond–drug conjugates (Table 3) is related to both the stability of the nanodiamond–drug complex and the ability of nanodiamonds to form strong aggregates, with an increase in the ionic strength of the solution or physiological media.[19] It was found that the presence of a drug molecule on the nanodiamond surface does not prevent the formation of a stable coating on both sides of the collagen-rich matrices. Note that the stress–strain characteristics of final material practically did not depend on the quantity of nanodiamonds on the material surface (see details in Online Supplementary Materials).

A chitosan layer was applied to the surface of collagen-rich matrices under the supercritical carbon dioxide conditions. This technique does not affect the organization of collagen fibrils as opposed to the chitosan adsorption from the solutions in acetic acid adversely affecting collagen-rich matrices mechanical characteristics.[20] The role of chitosan in the complex coating is to increase the resistance to calcification due to the masking of free aldehyde groups *via* their covalent binding to amino and hydroxyl groups in chitosan.[21] In the present study on the materials with nanodiamond coating, adsorption of chitosan was higher than in the cases of free collagen-rich matrices of bovine pericardium,[22] which can result from the increase of roughness and the surface of collagen-rich matrices available for chitosan coating. It was found that chitosan excess was weakly bound; it was removed from the surface during animal exploitation (Table 2). However, the residual quantity was enough to suppress calcification tenfold (Table 3).

In conclusion, the multicomponent coatings of the collagen-rich matrices of the heart valve prosthesis were prepared for the first time and analyzed from different angles. Application of such coatings allows one to create a material with the mechanical characteristics similar to those found in humans. *In vivo* studies revealed the preservation of the multilayers consisting of nanodiamonds, antimicrobial agents and chitosan on the material surface and prevention of calcification of the material. Work is underway to develop a multifunctional composite coating to increase the service life of xenogenic heart valve prostheses.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2024.01.031.

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