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Nanodiamond–drug conjugates for coating xenogenic heart valve prostheses

Maria G. Chernysheva, Tianyi Shen, Ivan S. Chaschin, Gennadii A. Badun, Viktor G. Vasil'ev, Ivan V. Mikheev and Natalia P. Bakuleva

S1. Experimental details

Collagen matrices of bovine pericardium pretreated with glutaraldehyde were prepared and kindly provided by A. N. Bakulev National Medical Research Centre of Cardiovascular Surgery (Moscow, Russia). The matrices were used as 1×1 cm^2 matrices for *in vivo* trials and as 1×7 cm^2 matrices cut in two directions regarding the fibrils of the collagen matrices for stress-strain characteristics.

Nanodiamond suspension, SDND, was a product of PlasmaChem GmbH (grade SDND^{TM}). It was diluted with water purified with Milli-Q, Millipore system. Amikacin sulfate powder was purchased from Apexbio Technology. Levofloxacin and chitosan (mol wt 190 kDa) were products of Sigma-Aldrich. Tritium-labeled nanodiamonds, amikacin, levofloxacin and chitosan were obtained through the tritium thermal activation method.[S1–S3]

Coating preparation

Complexes of nanodiamonds, SDND, with amikacin and levofloxacin were obtained according to the procedure described previously[S3] under the conditions of maximum drug uptake when 1 mg of nanodiamonds were mixed with 6 g l^{-1} drug. Then nanodiamond–drug composites were applied to the surface of the collagen matrices of the bovine pericardium, pretreated with glutaraldehyde.[S4] To this end, a piece of collagen matrices was placed in a suspension and stirred for 5–7 hours; the suspension was then stored at 4 °C overnight. Then the matrices were pulled out of the suspension and placed in a 0.9% sodium chloride solution. In this form, the samples were stored at 4 °C. A layer of chitosan was applied to the surface of the matrices from a solution in carbonic acid under the conditions of super-critical carbon dioxide.[S1,S5]

Mechanical strength characteristics of the coated pericardium

The mechanical properties of the coated pericardium matrices were measured on 7×1 cm^2 matrices pieces, which were cut in two orthogonal directions from the pericardial sac in areas with similar thicknesses. A universal 5 kN load cell LLOYD Instruments LR5R Plus constant cross-head speed of 50 mm min^{-1}, and an effective gauge length of 20 mm were used for recorded stress-strain curves.

Radiotracer determination of coating composition

Collagen matrices were decomposed in boiling concentrated nitric acid. The solution was centrifuged, and the radioactivity over the nanodiamond and the nanodiamond was directly measured using liquid scintillation counting. The GoldStar scintillation liquid was used to measure radioactivity, designed to measure solutions with low pH. The amount of nanodiamonds on the surface of the collagen matrices was determined based on the data obtained.

In vivo stability of nanodiamond–drug–chitosan coatings

Complexes of the following compositions: (1) [^{3}H]nanodiamond–levofloxacin–chitosan, nanodiamond–[^{3}H]levofloxacin–chitosan and nanodiamond–levofloxacin–[^{3}H]chitosan; (2) [^{3}H]nanodiamond–amikacin–chitosan, nanodiamond–[^{3}H]amikacin–chitosan and nanodiamond–amikacin–[^{3}H]chitosan were prepared according to the procedure described above. 1×1 cm^2 samples of collagen matrices was coated by labeled complexes (6 collagen samples per complex) and then subcutaneously implanted into rats of the Wistar line. After implantation, the animals were kept under normal vivarium conditions for four months. The diet included vitamin D3 to absorb calcium better. Four months after implantation, samples were extracted from animals under anesthesia and washed with saline to remove formula elements of animal blood. Then the samples were dried using the heating process to a constant mass, then dissolved in 0.2 ml of concentrated nitric acid followed by dilution in 1.5 ml of water and centrifugation to separate the nanodiamond phase. The radioactivity of the solution and the nanodiamond sediment was measured using liquid scintillation spectrometry. The composition of the coatings after exploitation by animals was determined based on measured radioactivity and the specific radioactivity of the labeled component. Since calcium deposits on prostheses are one of the most important problems when using them, the nitric acid solution was analyzed using the ICP-AES method for the presence of metals, particularly calcium.[S6] ANOVA analysis was performed using Statistica10 software.

Inductively coupled plasma atomic emission spectroscopy (ICP-AES)

ICP-AES have been used for the elemental analysis as an Agilent 720 spectrometer (Agilent, Australia). The 10-fold diluted sample was diluted by deionized water. A series of ten calibration standards in the 0.1–100 ppm (mg l^{-1}) range were prepared from single-element CRM (Certified Reference Materials, Inorganic Ventures, USA). ICP-AES instruments have set up the parameters such as RF-power, gas flow rate, *etc.*, according to the optimization experiments using 5 ppm Mn and Ca stock-solution. The wavelengths have been chosen according to ISO 11885:2007. Spiked analysis (spike recovery test) has been conducted for the trueness estimation. A 20 ppm Sc solution has been used as an internal standard with online premixing to analyze the solution (not more than 5%) to increase the reproducibility of the results. Quality control has been conducted by (1) analyzing two replicates solution and (2) 10 ppm 29 Element ICP Calibration/Quality Control Standard measurements (Inorganic Ventures, USA).

SEM imaging

The target-oriented approach was utilized to optimize the analytic measurements.[S7] Before measurements, the samples of collagen matrices were mounted on a 25 mm aluminum specimen stub, fixed by conductive carbon tape, and coated with a 10 nm film of Au. The observations were conducted using a Hitachi SU8000 field-emission scanning electron microscope (FE-SEM). Images were acquired in the secondary electron mode at 4 kV accelerating voltage.

S2. Mechanical characteristics of coated matrices

Stress–strain behavior of collagen matrices coated with nanodiamond–drug–chitosan, nanodiamond–chitosan, and chitosan was analyzed. In these experiments collagen matrices without any coating was used as a control. Bovine pericardium demonstrates different mechanical characteristics in two major orthogonal directions that are determined by orientation of fibril growth.[S1,S8,S9] Therefore, the stress-strain behavior has been analyzed in two directions regarding the fibrils of collagen matrices. The results are summarized in Table S2.

Table S2. Mechanical and strength characteristics of collagen matrices with coatings

Coating composition	Young's module, MPa		Rupture strain, %		Ultimate tensile strength, MPa	
	axial direction	transverse direction	axial direction	transverse direction	axial direction	transverse direction
control	52±8	43±17	53.1±9.8	46.6±3.0	11.41±0.35	8.87±1.73
chitosan	60±15	37±22	50.7±4.9	47.8±13.3	11.75±1.29	8.8±3.1
nanodiamond-chitosan	48±15	40±13	46.2±1.7	44.2±6.2	9.84±2.20	8.75±5.00
nanodiamond-levofloxacin-chitosan	49±13	44±8	44.1±4.9	45.1±5.9	9.16±4.25	8.37±3.04
nanodiamond-amikacin-chitosan	48±26	38±17	51.4±8.9	52.4±5.4	9.74±1.91	8.28±5.62

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

It has been shown that SDND nanodiamonds do not have such a strong effect on the mechanical strength characteristics of collagen matrices as was previously observed for nanodiamonds supplied by sellers in the form of powders.[S10]

Moreover, the coated material containing SDND nanodiamonds not only retains the mechanical and strength characteristics, but its surface also has an antibiotic that retains its antimicrobial properties during adsorption.[S3]

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