RESEARCH ARTICLE



Electrospun composite nanofibers of deoxyribonucleic acid and polylactic acid for skincare applications

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Abstract

The development of useful biomaterials has resulted in significant advances in various fields of science and technology. The demand for new biomaterial designs and manufacturing techniques continues to grow, with the goal of building a sustainable society. In this study, two types of DNA-cationic surfactant complexes were synthesized using commercially available deoxyribonucleic acid from herring sperm DNA (hsDNA, <50 bp) and deoxyribonucleic acid from salmon testes DNA (stDNA, \sim 2000 bp). The DNA-surfactant complexes were blended with a polylactic acid (PLA) biopolymer and electrospun to obtain nanofibers, and then copper nanoparticles were synthesized on nanofibrous webs. Scanning electron microscopic images showed that all nanofibers possessed uniform morphology. Interestingly, different diameters were observed depending on the base pairs in the DNA complex. Transmission electron microscopy showed uniform growth of copper nanoparticles on the nanofibers. Fourier-transform infrared spectroscopy spectra confirmed the uniform blending of both types of DNA complexes in PLA. Both stDNA- and hsDNA-derived nanofibers showed greater biocompatibility than native PLA nanofibers. Furthermore, they exerted significant antibacterial activity in the presence of copper nanoparticles. This study demonstrates that DNA is a potentially useful material to generate electrospun nanofibrous webs for use in biomedical sciences and technologies.

KEYWORDS

antibacterial, biomaterial, DNA, electrospinning, polylactic acid (PLA)

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1 | INTRODUCTION

Deoxyribonucleic acid (DNA) is a polynucleotide biopolymer that encodes the genetic information specifying the biological development and functions of living organisms.¹ Since the seminal report of Nadrian Seemann in 1980s,² DNA has received attention among researchers working in the field of nanotechnology. DNA nanotechnology is currently used across a wide range of research fields, from material science to biotechnology.³⁻⁵ Based on its programmability to generate various higher order structures, DNA has been actively exploited for biomedical applications as well as nanofabrication.⁶⁻¹³ In addition, the complexation of DNA with cationic surfactant has been used to enhance its utility. Sotzing and coworkers constructed white-luminescent DNA-based nanofibers using DNA and cetvltrimethylammonium chloride surfactant.¹⁴ They suggested that the use of this DNA-surfactant complex could be useful for photoelectronic applications. Recently, Maleckis and Dzenis reported on the electrospinning of continuous DNA nanofibers and showed that they could be produced with significant mechanical properties.¹⁵

Electrospinning is a versatile method that creates nano-sized fibers and facilitates the construction of polymer arrangements for various purposes.¹⁶⁻²³ Electrospun nanofibers have incredible potential for various applications, especially in biomedical sciences, including wound dressings,²⁴⁻²⁶ tissue engineering, bio-implants,²⁷ biosensors, water decontamination,²⁸ and supercapacitors.²⁹ Polymers for nanofiber formation can be classified as natural or synthetic based on their origin. Manufactured polymers can be mixed or blended to achieve specific properties such as mechanical strength, spinnability, and viscosity.³⁰ Commonly used polymers used in the fabrication of electrospun nanofibers are polyethylene oxide (PEO). polyvinyl alcohol (PVA), polycaprolactone (PCL) and its copolymers, polyvinylpyrrolidone (PVP), polyacrylonitrile (PAN), and polylactic acid (PLA). All of these materials have been endorsed and recognized by the US Food and Drug Administration for tissue design applications.³¹ PLA has noteworthy properties, including high modulus, high mechanical strength, and decent translucence. These features make PLA suitable for the fabrication of nanofibers used in drug delivery and tissue engineering. In non-biomedical applications, PLA has been used to produce disinfectant materials for the protection of packed items.³²

Herein, we report on our creation of electrospun DNA composite nanofibers using DNA-cationic surfactant complexes and PLA, with intended use in biomedical applications. We describe these nanofibers and evaluate their antibacterial activity in the presence of copper nanoparticles.

2 | EXPERIMENTAL WORK

2.1 | Materials

Dodecyl trimethyl ammonium chloride (DTAC) was purchased from TCI Chemicals and used without further purification. Herring sperm DNA (hsDNA, <50 bp) and salmon testes DNA (stDNA, \sim 2000 bp) were bought from Sigma-Aldrich Inc. The viscous polymer PLA, with

2.2 | Method

Two different types of DNA-cationic surfactant complexes were prepared using commercially available hsDNA and stDNA.^{2,3} A water-based solution of DNA (1% for hsDNA and 0.5% for stDNA, w/w) was used for the preparation of DNA-cationic surfactant complexes. An aqueous solution of DTAC (1%, w/w) was prepared and added dropwise to the above DNA solution at ambient temperature. After mixing, the prepared solution was stirred at room temperature for approximately 12 h. The subsequent precipitates were washed away with distilled water and dehydrated overnight in a vacuum oven at 60°C. The complex of hsDNA was obtained as pale-yellow solids, while the complex of stDNA was a mixture of white powder and some fibrils. The DNA complexes were mixed with PLA biopolymer and electrospun to obtain nano-fibers, followed by synthesis of copper nanoparticles on the nanofibers.

An electrospinning solution of DNA:PLA was prepared with a weight ratio of 20:80 in chloroform:DMF (9:1, w/w), and stirred for 24 h (until a completely homogenous solution was obtained). The electrospinning was performed at a high voltage of 22 kV, and the distance from tip to collector (TCD) was kept at 20 cm with a flow rate of 0.8 mL/h achieved by a syringe pump. A 21-gauge (~0.6 mm) syringe needle was connected to the positive terminal of the high-voltage supply. The nanofibers were collected on the ground electrode (collector). Obtained nanofibers were then characterized to evaluate morphological, chemical, and antibacterial properties. Table 1 shows sample details.

2.3 | Nanofiber characterization

To characterize the morphology of fabricated nanofibers consisting of PLA, hsDNA, stDNA, PLA-Cu, hsDNA-Cu, or stDNA-Cu, surface observation was performed with a scanning electron microscope (SEM; JSM-6010LA, JEOL, Japan). The nanofibers were coated with platinum to make them electrically conductive. The average fiber diameter was calculated based on 50 randomly selected fibers in SEM images using Image-J software. Nanofiber composition was

TABLE	1	Sample	concentrations	and	codes.
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Serial no.	Weight proportion	Sample codes
S1	DNA: PLA: 20 wt %, 80 wt %	PLA
S2		hsDNA
S3		stDNA
S4		PLA-Cu
S5		stDNA-Cu
S6		hsDNA-Cu



qualitatively investigated using an energy dispersive X-ray analyzer (EDS; JSM-6010LA, JEOL, Japan). The existence of copper nanoparticles inside the fabricated nanofibers was confirmed by transmission electron microscopy (TEM). Fourier transform infrared spectroscopy (FTIR) using an IRPrestige-21 (Shimadzu Co, Japan) was used to study the structures and possible interactions of the fabricated nanofibers. Wavelengths from 500 to 4000 cm⁻¹ were used in ATR total reflectance measurement mode, whereas those from 400 to 4000 cm⁻¹ were used for DNA complexes. The crystal structures of all fabricated nanofibers were investigated using x-ray diffraction (XRD) spectra at 25°C using the Rota flex RT300 mA XRD apparatus (Rigaku, Osaka, Japan) with an angle between $5 \le 2\theta \le 80^\circ$, and all experiments were performed with nickel-filtered Cu Ka radiation. The antibacterial properties of the nanofibers were evaluated with Escherichia coli (BUU25113, Gram negative) and Bacillus subtilis (168, Gram positive) using the disk diffusion method (AATCC 147-1998). E. coli and B. subtilis were maintained in agitation culture for 24 h. Solutions containing cultured bacteria were diluted with phosphate buffer. Fifteen milliliters of agar medium was added to Petri dishes, and the mixture was stirred and dried. A sterilized disk (diameter: 13 mm) was prepared from each sample. Samples were placed on agar medium and cultured at 37°C for 24 h. Then, Equation (1) was used to calculate the inhibition of proliferation (inhibition area I) based on the diameter of the transparent blocking circle (C) and the sample diameter (D)

$$I = \frac{C - D}{2},\tag{1}$$

The antibacterial activity of PLA nanofibers, DNA nanofibers, and DNA-Cu nanofibers was evaluated by measuring OD_{600} , as described in our previous study.³³ Native PLA nanofibers were used

as a negative control, while Cu nanoparticles were used as a positive control.

A lactate dehydrogenase (LDH) assay using fibroblast cells (NIH3T3) was used to evaluate cell proliferation, following the procedures of previous study.³⁴ The NIH3T3 cells were cultured and incubated in a 5% CO₂ environment at 37°C for 30 min. Round disks made of nanofibrous mats were cut to a diameter of 6 mm and sterilized by immersion in 70% ethanol for 30 min. After sterilization, the scaffold nanofibers were washed three times in PBS for 5 min and then plated on 96-well plates containing NIH3T3 cells. Cells and nanofibrous mats were incubated at 37°C with 5% CO₂ in a humidified incubator for 24 h. Based on the reported protocols, an LDH assay kit was used to measure cell proliferation at 490 nm using a microplate reader.³⁴ Cell proliferation was measured at intervals of 1, 3, 5, and 7 days.

Cell viability was calculated using Equation (2). All experiments were performed three times.

$$Cell viability (\%) = \frac{Abs_{sample}}{Abs_{control}} \times 100.$$
(2)

Here, "Abs $_{\rm control}$ " represents a reference well that does not include a sample.

2.4 | Statistical analysis statement.

Origin pro software (version 9.1) was used for statistical analysis (standard deviation, mean, etc.). All graphs were also plotted using the same software. Statistical significance based on the unpaired *t*-test was calculated using the data of at least five samples.



FIGURE 1 SEM images of PLA, PLA-Cu, hsDNA, hsDNA-Cu, stDNA, and stDNA-Cu nanofibers.

RESULTS 3

3.1 Morphological properties

Figure 1 shows scanning electron microscopy (SEM) images of nanofibers prepared from PLA, PLA-Cu, hsDNA, hsDNA-Cu, stDNA, or stDNA-Cu. Figure 1A shows that native PLA nanofibers had a smooth morphology, with no beads seen in the nanofiber composite. However, the fiber diameter and surface morphology changed with Cu loading. SEM images showed that the DNA-fabricated nanofibers had a uniform morphology. Introduction of copper nanoparticles induced morphological changes similar to those seen in PLA nanofibers. Interestingly, the fiber diameter varied depending on the DNA source, with an average diameter of 300 nm.

3.2 **EDS** analysis

Analysis using SEM showed that nanofiber morphology changed after loading with copper nanoparticles. Energy-dispersive x-ray

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spectroscopy (EDS) analysis confirmed the presence of copper nanoparticles on the prepared nanofibers, with an intrinsic Cu peak at 0.95 keV. As shown in Figure 2, a clear Cu peak was observed in all nanofibers generated from PLA and DNA. This indicates that copper nanoparticles can be worn on the nanofibers fabricated with stDNA and hsDNA.

Fourier transform infrared spectroscopy 3.3

To analyze the functional groups of different nanofibers, FTIR was performed using a Jasco spectrometer, model 6300. Figure 3 shows the FTIR spectra of nanofibers prepared from native PLA, PLA-Cu, hsDNA, hsDNA-Cu, stDNA, and stDNA-Cu nanofibers, while Table 2 presents respective functional groups. The stretching frequencies of PLA polymer were 1752 cm^{-1} for C=O, 1081 cm^{-1} for stretching C-O, 1452 cm⁻¹ for bending C–H, and 2928 cm⁻¹ for stretching C–H. Nanofibers fabricated with DNA and copper loading showed similar absorption peaks as the original PLA nanofibers. The FTIR spectra indicated the uniform mixing of both types of DNA complexes in PLA.



FIGURE 2 EDS analysis of PLA-Cu, hsDNA-Cu, and stDNA-Cu nanofibers.

3.4 | X-ray diffraction (XRD)

The x-ray diffraction pattern of PLA and DNA/PLA nanofibers exhibited broader peaks around 12°-24°, indicating an amorphous structure. The XRD pattern of PLA-Cu, hsDNA-Cu, and stDNA-Cu (Figure 4) shows crystalline peaks at 20 of 17.5°, 17.6°, and 17.2°, corresponding to basal spacings of 11.18°, 10.26°, and 8.92°, correspondingly. The peaks shifting from lower to higher angle directs to the decrease in the interlayer spacing because the blend composites have an ordered structure. Since PLA was the primary component in the prepared nanofibers, the crystalline peak position was almost similar to that in the original PLA nanofibers. This blend exhibited increased interlayer spacing, with the d-spacing change from 11.49° (pure PLA) to 10.79° at the diffraction peak of 18.28°. The increased interlayer distances of PLA/DNA nanofibers indicates that this mixture has more amorphous regions and that the addition of copper nanoparticles creates an ordered structure, making crystallization easier. The changes in d-spacing may result from variations between samples in the distribution of crystalline and amorphous regions.



FIGURE 3 FTIR spectra of PLA, PLA-Cu, hsDNA, hsDNA-Cu, stDNA, and stDNA-Cu nanofibers.

3.5 | Transmission electron microscopy

The TEM images of PLA-Cu, hsDNA-Cu, and stDNA-Cu nanofibers are shown in Figure 5. Copper nanoparticles were incorporated into the nanofibers of PLA or DNA blend, and they are dispersed on the nanofiber composite. Thus, the results of EDS and TEM analysis confirmed the presence of copper nanoparticles on nanofiber surfaces. Although the concentration of copper nanoparticles seemed relatively lower, it was sufficient to induce antibacterial activity, as described below.

3.6 | Thermogravimetric analysis

Thermogravimetric analysis (TGA) was performed in a Perkin-Elmer (Waltham, MA) TGA7 thermogravimetric analyzer. In this study, a 5-10 mg sample was heated at 30-600°C at a rate of 10°C min⁻¹, and nitrogen was used as a purge gas at a flow rate of 20 mL min⁻¹. From 25 to 150°C, all the tested samples exhibited similar tendencies (Figure 6).³⁵⁻³⁷ The onset temperatures of PLA-Cu nanofibers (210°C) and of stDNA and hsDNA nanofibers (267°C, 296°C) were higher than that of the original PLA nanofibers, while those of stDNA-Cu and hsDNA-Cu composite nanofibers were lower. The decreased



FIGURE 4 XRD spectra of PLA, PLA-Cu, hsDNA, hsDNA-Cu, stDNA, and stDNA-Cu nanofibers.

TABLE 2	FTIR peak distributions	representing functiona	I groups of PLA	, hsDNA, and stDNA nanofiber	rs.
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No	Samples	Stretching C—O (cm $^{-1}$)	Bending C—H (cm $^{-1}$)	Stretching C=O (cm $^{-1}$)	Stretching C—H (cm $^{-1}$)
1	PLA	1081	1452	1752	2928
2	PLA-Cu	1083	1457	1752	2927
3	hsDNA	1084	1452	1751	2919
4	hsDNA-Cu	1086	1453	1753	2920
5	stDNA	1084	1451	1752	2949
6	stDNA-Cu	1085	1454	1752	2946





FIGURE 5 TEM analysis of PLA-Cu, hsDNA-Cu, and stDNA-Cu nanofibers.



FIGURE 6 Thermal degradation study of PLA, PLA-Cu, hsDNA, hsDNA-Cu, stDNA, and stDNA-Cu nanofibers.

thermal stability of DNA-Cu nanofibers might be due to their amorphous nature. PLA-Cu composite nanofibers were stable up to 250°C and exhibited even degradation up to 326°C. The degradation temperatures of stDNA and hsDNA were 304°C and 369°C, respectively. Overall, the thermal stability of PLA and DNA nanofibers is satisfactory for practical applications.

3.7 | X-ray photoelectron spectroscopy measurements

Figure 7 shows X-ray photoelectron spectroscopy (XPS) spectra of all fabricated nanofibers. Native PLA nanofibers showed C-1 and O-1 peaks at binding energies of 282.6 and 538 eV, respectively. Gauss distribution fitting was used to evaluate the C-1 peak, and it could be further divided into three obvious peaks with binding energies of 282.5, 284.6, and 286.8 eV. In addition, XPS analysis showed that the copper nanoparticle spectra consisted of peaks for Cu2p1/2 and Cu2p3/2, with corresponding binding energies of 929.3 eV and 948.1 eV. These

results indicate the presence of copper nanoparticles on the surface of PLA- and DNA-fabricated nanofibers.³⁸ The peak intensities of oxygen and carbon in native PLA- and DNA-fabricated nanofibers were smaller than those in Cu-coated nanofibers.

3.8 | Water contact angle

An arch shape forms when a water drop is placed on the surface of a material. Generally, the water contact angle or wetting angle is defined as the angle between the line tangent to the edge of the drop of the water and the surface. PLA is hydrophobic polymer, and nanofibrous mats of PLA therefore had an average water contact angle of 96°. As shown in Figure 8, water contact angles of both types of DNA-fabricated nanofibers reflected hydrophobic characteristics. The water contact angles for hsDNA and stDNA composite nanofibers were 102° and 99°, respectively. It is generally considered that growing nanoparticles on the surface of a hydrophobic substrate is quite challenging. In this study, a slight decrease in the water contact angle was observed in all samples after application of the copper nanoparticle coating.

3.9 | Antibacterial activity testing

Antibacterial activity testing was carried out using the disc diffusion method. All samples coated with copper nanoparticles were cut into round discs with a 13-mm diameter, and were placed on agar plates with *E. coli* (Gram negative) and *B. subtilis* (Gram positive). Figure 9A shows that all samples had excellent antibacterial activity against both species.

The antibacterial activity of DNA composite nanofibers with and without the presence of copper was also analyzed by measuring the optical density (OD) of bacterial cells (*E. coli* as a Gram-negative bacteria) spread on nanofibrous mats. The mats were incubated in liquid broth medium for 24 h. Copper nanoparticles were used as a positive control because they have known antibacterial activity, while native PLA nanofibers were used as a negative control. Optical density (OD) was measured at 600 nm. As shown in Figure 9B, for PLA nanofibers the bacterial survival rate was higher than for hsDNA and stDNA nanofibers. Interestingly,



FIGURE 7 XPS spectra (Narrow, C-1s, and O-1s) of pure PLA, PLA-Cu, hsDNA, hsDNA-Cu, stDNA, and stDNA-Cu nanofibers and (Cu-2p) of PLA, PLA-Cu, hsDNA, hsDNA-Cu, stDNA, and stDNA-Cu nanofibers.



FIGURE 8 Water contact angles for PLA, hsDNA, stDNA, PLA-Cu, hsDNA-Cu, and stDNA-Cu nanofibers (n = 5).



FIGURE 9 (A) Zone of inhibition against Gram-negative and Gram-positive bacteria: antibacterial activity of PLA-Cu, hsDNA-Cu, and stDNA-Cu nanofibrous mats, and (B) OD₆₀₀ of DNA composite nanofibers with and without Cu. The values and error bars represent the mean ± standard deviations of at least five samples, with statistical significance calculated using the unpaired t-test (***p < 0.005).



Cell proliferation of samples after 1, 3, 5, and 7 days. FIGURF 10 The values and error bars represent the mean ± standard deviations of at least five samples, with statistical significance calculated using the unpaired *t*-test (****p* < 0.005).

both hsDNA and stDNA nanofibers exhibited slight resistance against the bacteria. The addition of copper nanoparticles to PLAand DNA-fabricated nanofibers reduced the bacterial survival rate by more than 90% in all cases.

3.10 Cell compatibility test

A LDH assay was performed to assess cell proliferation. The results of cell viability measurements are shown in Figure 10. NIH3T3 cells were cultured with composite nanofibers for 1-7 days, and cell compatibility was measured by LDH assay. The cell viability of wells tested without a

sample was used to define the control value of 1000. According to ISO 10993-5, cell viability over 80% is nontoxic, 60%-80% is weakly poisonous, 40%-60% is addictive, and below 40% is exceedingly toxic.

After day 1, the cell count with PLA nanofibers was 760, while in the case of PLA-Cu nanofibers it was 510. The slight toxicity of the PLA-Cu nanofibers can be explained by the presence of Cu nanoparticles on nanofiber surfaces. Higher cell growth was observed with stDNA nanofibers, with a cell count of around 1680. The hsDNA nanofibers also yielded a relatively high cell count of around 1125. The toxicity of DNA-Cu-fabricated nanofibers was milder than that of PLA-Cu nanofibers. Cells were counted after incubation for 3, 5, and 7 days, and similar tendencies were maintained throughout the cell culture experiments. These results indicate that DNA-fabricated nanofibers are highly biocompatible and provide a suitable environment for cell growth.

DISCUSSION 4

This study is the first to report the synthesis of DNA- and PLA-blended electrospun nanofibers. Based on a previous study, we selected readily available fish DNA sources to synthesize DNA-cationic surfactant complexes and used PLA as a wellestablished nanofiber component.^{39,40} As previously stated, DNAcationic surfactant complexes⁴¹ have been used to create electrospun nanofibers for optoelectronic application,^{14,42} and DNA itself has been actively investigated for biomedical applications.43,44 However, there are no studies (in literature) on the use of DNA-cationic surfactant complexes to fabricate nanofibers for biological applications. Considering solubility (use of common solvent for PLA and DNA) and effective use of PLA in biomedical field, we found that the DNA-cationic surfactant complexes could be blended with PLA, and the prepared nanofibers had comparable features to pristine PLA nanofibers, including uniform morphology and similar hydrophobicity.

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We characterized our DNA-fabricated nanofibers using various analytical techniques. We obtained different fiber diameters depending on the DNA source. This suggests that we can modify morphological properties based on the length and sequence of the DNA. Theoretically, hsDNA having less base-pair units should have decreased the diameter of nanofibers. Less base-pair caused significant drop in electrospinning solution, however as there is no such literature available so we cannot claim this as a thumb rule. In our research, the nanofibers' diameter was larger in case of hsDNA as compared to that of for the stDNA. It might be because of better homogeneity of stDNA in PLA or swelling of hsDNA in PLA solution.

Infrared study (IR) showed that there was no peak shifting except from slight variation in the intensity of peaks. As PLA and DNA were dissolved in solvent by stirring and no catalyst or initiator was added, there was possibility of no chemical interaction. It was confirmed that both types of DNA were physically bonded with PLA, however the mixing was uniform. Water contact angle of copper nanoparticles coated nanofibers was slightly decreased which can be associated with surface roughness due to presence of copper nanoparticles. However, nanofibers were still in hydrophobic range.

Although, PLA is also biocompatible polymer however, we observed that in cell viability assays, both stDNA and hsDNA nanofibers showed better biocompatibility than the original PLA nanofibers. Cell growth was significantly increased from day 1 to day 7 in case of both types of DNA. Copper nanoparticles have been proven as effective antibacterial agents, to verify the utility of DNA nanofibers⁴⁵ we coated them with copper nanoparticles and evaluated their antibacterial activity. Although TEM analysis indicated that the copper nanoparticle coating on the surface of DNA nanofibers was less efficient compared with PLA nanofibers, this coating exerted significant antibacterial activity. Better antibacterial and biocompatibility properties of DNA blended PLA nanofibers may be considered as one of the important parameters for selection of DNA as useful biomaterial.

The present system is a work in progress, and further refinement and investigation should facilitate a wide range of practical applications of DNA.^{46,47} Electrospinning of DNA complexes is still a challenging task, which was one of the reasons to blend DNA complexes with PLA to get free-standing fibrous mats. As mentioned in literature that few researchers have been successful in converting DNA biopolymer in to nanofibers but no one has reported free-standing mats/ webs which can be used for practical applications. It is expected that with modifications in the methods for DNA, use of different solvents, changing the technique of nanofibers production, it will be possible to produce nanofibers of pristine DNA which may be replacement of several polymers which have been used for similar applications.

5 | CONCLUSION

In conclusion, we devised new types of DNA-based electrospun nanofibers by blending DNA-cationic surfactant complexes with PLA. Commercially available stDNA and hsDNA were readily exploited for this purpose. DNA-fabricated nanofibers were coated with copper nanoparticles to exert antibacterial activity. SEM images showed uniform morphology of nanofibers containing DNA, while copper peaks were clearly demonstrated by EDX analysis and XPS spectra. The chemical functional groups of the prepared nanofibers were analyzed by FTIR, which confirmed that electrospinning solution was uniformly mixed. In a cell compatibility study, DNA nanofibers afforded superior biocompatibility to native PLA nanofibers. In addition, they showed significant antibacterial activity when coated with copper nanoparticles. Further research is ongoing to improve the properties of DNAbased nanofibers. We hope that the present study inspires many researchers to develop versatile DNA-based biomaterials and thereby facilitate the establishment of a sustainable society.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest for this research.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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