

Multi-Analyte Sensitive Fluorophore Cross-Linked Nanofibers Based Antimicrobial Surface Enabling Bacterial Detection and Their Usage as Crystal Growth Template

Zeynep Dikmen* and Vural Bütün

In situ preparation of bifunctional thiazolo[5,4-d]thiazole (TTz)-based fluorophore cross-linked nanofibers is successfully performed. These multifunctional nanofibers are used as both crystal growth template and antibacterial substrate that makes bacterial detection possible simultaneously. These highly fluorescent nanofibers act as optical sensors toward many analytes. The acid, base, and metal cation-sensitive nature of the 2,5-bis(4-hydroxyphenyl)thiazolo[5,4-d]thiazole (HPhTT) dye makes the prepared PVA nanofibers sensitive against these chemicals. Metal nanoparticles (MNPs) or metal oxide crystal formation occurs on the surface of nanofibers without any reducing agent. The strong interaction between metal cations and fluorophores results in crystal formation on the surface, which is named for the first time as “complexation-triggered crystallization.” These dye cross-linked and metal-interacted nanofibers exhibit antibacterial effect against *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), *P. aeruginosa* (ATCC 27853), and *E. faecalis* (ATCC 25922) strains. Also, the emission wavelength change of nanofibers in bacterial contamination makes it possible to use smart nanofiber films for optical bacteria detection. These novel materials pave the way for new applications such as smart wound bands, optical sensors, and one-step preparation of surface-enhanced Raman spectroscopy substrate.

1. Introduction

Nanofibers (NFs) are intriguing materials due to their high porosity, specific surface, and excellent mechanical properties and thus NFs have found use in drug release, sensors, tissue engineering scaffolds, filtration, metal ion adsorption, and battery separators for environmental applications.^[1]

The functionalization of NFs with metal oxides or metal nanoparticles makes them great candidates for a wide variety of practical applications thanks to their improved mechanical strength, ductility, and electrical conductivity.^[2–4] These metal nanoparticle-decorated films and NFs have been generally utilized to perform the detection of various chemicals via surface-enhanced Raman spectroscopy (SERS) as also reported in our previous studies.^[3,5,6]

The sensor design using photoluminescent NFs is also an emerging approach because of the fast, cheap, and sensitive nature of photoluminescent sensors. Such light-emitting electrospun NFs get attention as they have the potential to be used in optical sensors, and photonic applications such as miniature light sources,

detectors, and waveguides.^[7] Photoluminescent NFs can be produced by two approaches. The most common one is based on the use of transparent or optically inert polymers that can be doped with luminescent materials (inorganic quantum dots, organic chromophores, polymers, and bio-chromophores),^[8–13] and the other one is the usage of self-luminous conjugated polymers.^[14,15] These materials have variability in solubility, rheological and optical properties, uniform morphology, and fluorescence properties. A successful synthesis of fluorescent NFs by doping with CdSe@ZnS quantum dots and improvement of their thermal, and optical properties was reported.^[16] However, the biomedical applications of such NFs are limited in practical use because of their heavy metal release over time. Instead of heavy metal containing quantum dots, carbon dot (CD) doped photoluminescent NF was used for drug release.^[17] Photoluminescent dye-modified NFs are also promising candidates for practical applications thanks to their biocompatibility. Benzimidazole-containing aramid NFs enable naked-eye detection of heavy metal ions with recyclable and high detection efficiency.^[18] Selective and sensitive mercury sensors made of rhodamine-fullerene

Z. Dikmen
Department of Biomedical Engineering
Faculty of Engineering
Eskisehir Osmangazi University
Eskisehir 26040, Turkey
E-mail: zeryilmaz@ogu.edu.tr

V. Bütün
Department of Chemistry
Faculty of Science
Eskisehir Osmangazi University
Eskisehir 26040, Turkey

 The ORCID identification number(s) for the author(s) of this article can be found under <https://doi.org/10.1002/mame.202300226>

© 2023 The Authors. Macromolecular Materials and Engineering published by Wiley-VCH GmbH. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

DOI: 10.1002/mame.202300226

doped polyurethane NFs,^[19] arsenic sensors,^[20] rapid detection of ethanol,^[21] and optical detection of trifluoroacetic acid^[22] are also reported as sensing applications of photoluminescent NFs. Such photoluminescent NFs are used in photonic devices,^[23] optical sensors for various materials such as; nerve gas,^[24] explosive molecules,^[25] inorganic metal cations, and biological structures^[26–29] Furthermore, such fluorescent NFs are being explored for their emerging applications in bioimaging, design of bacteria-sensitive smart band-aid and pH sensitivity.^[30,31]

pH change in the case of infected wounds in tissues or skin is a well-known phenomenon.^[32,33] The pH change is associated with the increased amount of microorganisms, and the critical colonization threshold is decisive for wound treatment.^[34] The critical colonization threshold is generally considered to be the point where clinical intervention is required. It is essential to treat and diagnose the wound before it reaches this stage.^[34] Optical detection of bacterial growth via photoluminescent sensors in the near-infrared region by using functionalized single-walled carbon nanotubes is reported.^[35] Because infectious diseases are a significant cause of morbidity and mortality worldwide, wearable sensors for bacterial growth detection are crucial. Fluorescence imaging, as the highly sensitive, simple, and rapid method, has been recently reported as a talented way for bacteria detection^[36–39]

Herein, cost-effective, easy-to-produce, and multi-functional smart NFs were successfully prepared. These photoluminescent NFs exhibit unique properties thanks to the multi-analyte sensitive HPhTT fluorophore and its dynamic covalent chemistry (DCC) with PVA polymer.^[6,40–42] The strong interaction of metal cations with HPhTT and modified PVA enables the growth of metal nanoparticles on the surface. Such metal nanoparticle formation on the NFs without any reducing agent and additives facilitated by the molecular interactions and dynamic covalent chemistry explained with a new phenomenon named “complexation-triggered crystallization”. HPhTT cross-linked and metal nanoparticle decorated NFs exhibited antibacterial effects both on Gram-negative and positive strains (i.e., *E. coli* (ATCC 25 922), *S. aureus* (ATCC 25 923), *P. aeruginosa* (ATCC 27 853), and *E. faecalis* (ATCC 25 922)). Moreover, highly photoluminescent HPhTT cross-linked NFs were sensitive towards bacterial strains, acid–base/pH change, and metal cations. The analytes are detected optically via emission wavelength shift.

2. Experimental Section

2.1. Materials

All the following reagents were obtained from Sigma–Aldrich: Trifluoroacetic acid (TFA, 99%), sodium hydroxide (NaOH, 98%, anhydrous, pellets), gold (III) chloride hydrate ($\text{HAuCl}_4 \cdot x\text{H}_2\text{O}$, 99.999% trace metals basis), dithioamide (97%), potassium tetrachloroplatinate (II) (K_2PtCl_4 , 99.99% base metals), silver nitrate (AgNO_3 , $\geq 99.0\%$), poly(vinyl alcohol) (PVA, 89 000–98 000, 99%) +hydrolyzed), cadmium chloride (CdCl_2 , $\geq 99.0\%$), lead (II) acetate trihydrate ($(\text{Pb}(\text{CH}_3\text{CO}_2)_2 \cdot 3\text{H}_2\text{O})$, $\geq 99.0\%$). Poly(vinyl alcohol) (PVA, 89 000–98 000, 98% + hydrolyzed) was purchased from Alfa Aesar. Cobalt (II) chloride (CoCl_2), pyridine (ACS Reag.), and cyclohexanone (ACS Reag.) were purchased from

Merck and used as received. 4-Hydroxy benzaldehyde (98%) from Abcr, ammonia solution (NH_4OH , 25%) from VWR Chemicals, and hydrochloric acid (HCl , 37%) from Honeywell were purchased.

2.2. Characterization

Scanning electron microscopy (SEM) analysis was performed with a Hitachi Regulus 8230 at 1–10 kV acceleration voltages. Samples were coated with Au-Pd before analysis with Leica EM ACE600. A powder diffractometer (Empyrean, Panalytical) with $\text{CuK}\alpha$ radiation scanned at 2θ angles between 10° and 90° was used for X-ray diffraction (XRD) measurements. ^1H NMR spectroscopy experiments were performed with a Jeol ECZ 500R spectrometer at 80°C . Confocal images were captured with a Zeiss LSM 800 microscope.

2.3. Preparation of HPhTT Cross-Linked Fluorescent Nanofibers

10% PVA (M_n : 135 000) solution was prepared by dissolving 10 g of PVA in 90 mL of water at 95°C by stirring for 6 h. HPhTT solution (10 mM) was prepared by dissolving 0.0326 g HPhTT in 0.1 M 10 mL NaOH solution. Nanofiber pre-solutions were prepared by adding 1 mL HPhTT, 3 mL distilled water, and 100 μL CTAB solution (1 g/2 mL in distilled water) into 5 mL PVA solution (10%), respectively. 0.5 mL HPhTT solution was used to prepare blue emissive NFs. NFs were prepared at a distance of 10 cm at 29 kV and 17 kV voltages. The resulting NFs were characterized by SEM and ATR-IR studies.

2.4. Metal Nanoparticle Decorated Nanofiber Preparation

Gold nanoparticles (AuNPs), silver nanoparticles (AgNPs), and lead oxide (PbOx) crystals decorated NFs were prepared by immersing the NFs into 10 mM metal salt solutions (i.e., $\text{KAuCl}_4 \cdot 3\text{H}_2\text{O}$, AgNO_3 , $\text{Pb}(\text{OAc})_2$) in distilled water without any additional chemicals.

2.5. Optic Sensitivity Studies of Fluorescent Nanofibers

2.5.1. Metal Cations

NFs prepared with 10 mM HPhTT (in 0.1 M NaOH) were used to determine the sensitivity of the fibers against metal cations. The NF films were cut into 1 cm^2 pieces for further immersion process. 10 mM $\text{HAuCl}_4 \cdot x\text{H}_2\text{O}$ (0.170 g, 0.5 mmol), K_2PtCl_4 (0.21 g, 0.5 mmol), AgNO_3 (0.085 g, 5 mmol), $\text{Pb}(\text{CH}_3\text{COO})_2$ (0.190 g, 5 mmol), CoCl_2 (0.065 g, 5 mmol), CdCl_2 (0.092 g, 5 mmol) solutions were prepared by dissolving the related salts in 50 mL of distilled water. The NFs were immersed into 5 mL metal salt solutions for 10 min and dried at room temperature for optical measurements. Spectroscopic and spectrofluorometric analyses were performed to detect the change in the optical properties of these NFs due to their interaction with metals. Changes in surface morphology and possible nanoparticle formations were determined by SEM and XRD analysis.

2.5.2. Acid and Base

Since the HPhTT fluorophore is acid and base-sensitive, the optical responses of the obtained NFs to NH_3 and TFA solutions were examined by immersing the NFs into acid and base solutions prepared at different concentrations in the range of $0.2\ \mu\text{M}$ – $2.0\ \text{M}$ to observe the limitation of the optical sensitivities. The NFs prepared with $10\ \text{mM}$ HPhTT (in $0.1\ \text{M}$ NaOH) were put in these solutions for $10\ \text{s}$, and their fluorescence spectra were recorded. TFA-sensitive films were cut into 1 – $2\ \text{cm}^2$ sizes and thrown directly into TFA solutions of different concentrations. The resulting film did not need to be activated with TFA before NH_3 sensitivity studies.

2.5.3. Antimicrobial Activity and Bacterial Detection

E. coli (ATCC 25 922), *S. aureus* (ATCC 25 9), *P. aeruginosa* (ATCC 27 853), and *E. faecalis* (ATCC 25 922) strains were used for antimicrobial activity studies and bacterial sensing. The antimicrobial activities of fiber films were investigated in-vitro against the strains mentioned above using a disk diffusion method.^[13] Briefly, the cultured bacterial stocks ($3 \times 10^6\ \text{CFU mL}^{-1}$) were swabbed on an agar plate surface, and the NF films were placed on agar for further incubation for $24\ \text{h}$ at $37\ ^\circ\text{C}$. After $24\ \text{h}$ incubation, zone of inhibition (ZOI) values were measured. *E. coli*, *S. aureus*, *P. aeruginosa*, and *E. faecalis* microorganisms were cultured by incubating at $37\ ^\circ\text{C}$ in Mueller-Hinton Broth solution to perform naked-eye bacterial sensing under UV-light excitation. The cultured microorganisms were adjusted to $3 \times 10^6\ \text{CFU mL}^{-1}$ in Mueller-Hinton Broth solution for further biofilm studies. The bacterial stocks were swabbed on MHB – agar plate surface and incubated for 18 – $24\ \text{h}$ at $37\ ^\circ\text{C}$ temperature. After $24\ \text{h}$ incubation, fluorescent NF films were placed onto the biofilms, and photoluminescence response was observed under UV light illumination, and digital images were recorded.

3. Results and Discussion

HPhTT fluorophore renowned for its distinct characteristics such as excellent cross-linking ability, non-release into the environment, and remarkable interactions with metal cations has been used to prepare fluorescent NFs.^[6] However, NF synthesis by cross-linking of the electrospinning precursor is a challenging route because of the increasing viscosity of the precursor in time. We demonstrated in situ cross-linking of PVA NFs by using the HPhTT cross-linker as a highly photoluminescent fluorophore. Furthermore, these photoluminescent NFs act as a crystal growth template by facilitating AgNPs, AuNPs, and PbO crystal formation via dynamic covalent chemistry on the NF surfaces.

Various strategies for preparing metal nanoparticle decorated NFs are based on the addition of synthesized nanoparticles to the polymer precursor before electrospinning,^[43] reduction of metal cations embedded into NFs by heat and reductive agent treatment,^[44] or assembly of the metal nanoparticles on the surface of NFs^[45–47] after electrospinning. We improved a new approach to prepare reductant and stabilizer-free metal/metal oxide nanoparticle decorated NFs via complexation-triggered crystallization. Since, this crystallization process is post-modification

of NFs by in-situ nanoparticle formation, NFs and the anchor sites required for crystallization—dye in the concept—need to be cross-linked or chemically bounded. Herein, cross-linking of the NF precursor was performed with dye precursor in an alkali medium before the electrospinning process, so the fibers were obtained at a high-potential ($29\ \text{kV}$). SEM and confocal micrographs of the prepared fibers are given in Figure 1a–c, respectively.

While the thickness of the fibers obtained at $29\ \text{kV}$ was in the range of 70 – $250\ \text{nm}$, their overall diameter was determined to be $\approx 200\ \text{nm}$. The production of such NFs prepared from a viscous precursor without any additives results in a very limited amount of collected fiber because of the increasing viscosity of the polymer solution in time, and thus flow rate changes during the process, and spraying may not occur in time, as shown in Figure S1 (Supporting Information). Even if NaCl was added to increase the conductivity, fiber production in a large amount was not achieved (see Figure S2, Supporting Information). However, a high amount of NFs were obtained by using hexadecyl trimethylammonium bromide (CTAB) surfactant at $17\ \text{kV}$ potential and $10\ \text{cm}$ distance. NF preparation in a large amount and lower potential was achieved via CTAB surfactant addition, which facilitates fiber formation by decreasing surface tension. Thus, fiber films with an average diameter of 300 – $400\ \text{nm}$ were prepared, as shown in Figure 2a.

These NFs were used to prepare nanoparticle (NP) decorated NF preparation by immersing $1\ \text{cm}^2$ NF films in metal salt solutions. It has been observed that the NF film colors, which are opaque yellow, immediately become transparent yellow (like films) in the solution medium. Although the NFs did not dissolve, their fiber forms were not protected in the solution. SEM images of deformed NFs, which turned into films after treatment with metal cation solutions, are given in Figure S3 (Supporting Information). These prepared NF films were kept at $100\ ^\circ\text{C}$ for $24\ \text{h}$ to prevent deformation. So the fiber form was preserved by increasing the interactions between PVA–HPhTT and the chains of PVA via thermal curing. After thermal curing, 1 – $2\ \text{cm}^2$ of NF films were cut and immersed into metal salt solutions.

The changes in the surface morphology and possible metal nanoparticle formations on the NF films after treatment with metal salt solutions for two hours were determined by SEM analysis and EDS mapping (Figure S4, Supporting Information). SEM images of Au^{3+} treated NFs are given in Figure 2b. The 150 – $200\ \text{nm}$ -sized crystalline AuNP formation was observed on the surface of the NFs. The fact that most of the surfaces of NFs are coated makes it possible to use these AuNP–NF composites for various applications. The gold metal nanoparticle formation capability of NFs is much higher than the HPhTT cross-linked films when considering the AuNP-coated films that is reported in our previous study.^[6]

The increased AuNPs formation on the NF surface can be attributed to the larger surface area of the NFs. This leads to increased concentration of the HPhTT molecules assembled close to the surface resulting in improved chelate formation by interacting with metal cations easily. Similarly, AgNP formation is observed as a result of the treatment of these NF films with Ag^+ (Figure 2c). It is seen that the resulting AgNPs are much smaller when compared to AuNPs. 10 – $60\ \text{nm}$ AgNP was obtained without any reducing and stabilizing agents by using these NF films.

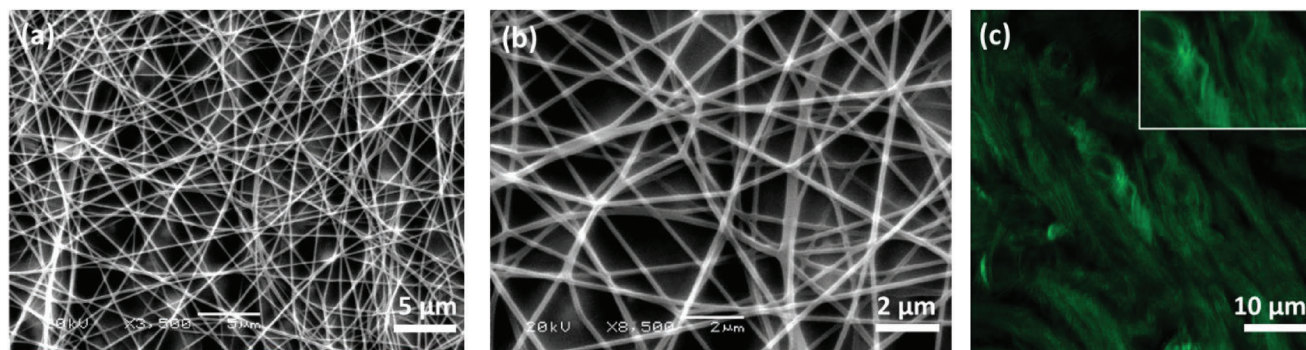


Figure 1. SEM images (a,b) and confocal microscopy image (c) of NFs prepared at 29 kV potential.

It can be argued that AgNP nucleation is much slower than AuNP nucleation on the surface of the NFs.

A small number of nanoparticles were observed after treatment with 0.01 M Pb^{2+} (Figure S5, Supporting Information). Afterward, the NF film was treated with a more concentrated (0.1 M) Pb^{2+} solution for further crystallization maintained from the seeds formed on the surface of the NFs. Thereby, rose-thorn-like PbO_x crystal formation on the NFs surfaces was observed as given in SEM images (Figure 2d). Hexagonal PbO_x crystals as platelet on the hydrogel films were reported in our previous study and similar crystal formations were also formed on these NFs.^[6] The crystal growth on the NFs begins from the interior of the NFs and moves to the surface as in the hydrogel films. But the crystals formed on the NFs, 100–150 nm in size, are much smaller than the crystals formed on the film.

Cubic NaCl crystals also occurred in situ during the preparation of NFs in which NaCl is added to increase conductivity (Figure S2, Supporting Information). This proves that HPhTT fluorophore is a trigger for crystal formation. All these particle formations can be explained by “Complexation Triggered Crystallization (CTC)” phenomena as illustrated in Figure 3a. The molecules with active functional groups that can form chelates with metal cations trigger the reduction of the metal cations into nanoparticles depending on the conditions of the medium. Seed formation occurs on these active sites when these chelates are in an electron-rich medium supplied by chemical species in the environment. The crystal growth is the following step after seed formation which is dependent on the concentration of the metal cations in the medium. If the medium is concentrated enough, the crystal growth continues until the sizes are big enough to get unstable on the surface. We observed this phenomenon in our previous study with gold nanoparticle formation on polymer microparticles composed of poly(methacryloxy quinoline) for the first time.^[48] This phenomenon is also reported in our previous studies with different molecules that can interact with metal cations.^[48–50]

The deformations of the Pb^{2+} treated NFs may occur due to the interaction of metal cations with the fluorophores located in different fiber chains and crystal growth process.^[51] Such deformation like sticking of the NFs into each other is seen in the SEM images of the Pb^{2+} , Pt^{2+} , and Cd^{2+} treated NFs (Figures S5–S8, Supporting Information). Any crystal formation was not observed after the treatment of NF films with Pt^{2+} , Cd^{2+} as shown in the SEM image (Figure S6–S8, Supporting Information).

Metal cation interaction of the polymer chain is depicted in Figure 3a–c as to be one chain interaction with one metal cation. However, one metal cation can interact with more polymer chains and the crystal growth on the anchor points affect the fiber form.

Metal particle/NF composites, which can form crystals and nanoparticles by interacting very well with the metal cations, were characterized by XRD analysis. The XRD pattern given in Figure 3d shows that the AuNCs are in face-centered cubic (fcc) lattice structure with the reflections of Au [1 1 1], [2 0 0], [2 2 0], [3 1 1], and [2 2 2] at 38°, 44°, 65°, 77°, and 82°, respectively. In the XRD pattern of the AgNP-NF sample, AgNP-related peaks are also observed with very low intensity.

The XRD patterns of the films treated with Pt^{2+} , Co^{2+} , and Cd^{2+} (Figure 3f–i) were not signed as they were very low-intensity peaks. Metal cation interaction with these fibers also increases the crystallinity of PVA, as can be seen in the XRD patterns of the metal cation-treated PVA NF films (Figure 4). The crystalline solid characteristic reflections of PVA at $2\theta = 19.88^\circ$ and 20.18° are the reflections of (1 0 1) and (1 0 $\bar{1}$) monoclinic unit cell. The sharper (1 0 1) and (1 0 $\bar{1}$) PVA peaks after Cd^{2+} and Pb^{2+} cation treatment was observed.^[52] Even if PVA crystallization blocked by ferric ions has been reported before, the presence of HPhTT molecules with rigid and planar backbone and its interactions with polymer chains or metal cations reversed the situation in our case and the crystallization degree of PVA is increased.^[53]

HPhTT is a unique fluorophore that can produce films with tunable emission wavelengths and a high quantum yield of up to 39.22% in the solid phase.^[6] So, these HPhTT cross-linked NFs are good candidates for optical sensing of different materials. Acid, base, metal cation, and bacterial detection might be promising applications for many engineering areas. Emission wavelengths of these films after HCl and NaOH treatment are given in Figure 3j–k. The film exhibited a 45 nm blue shift after immersing the film in an acid solution while this acid-treated film exhibited a 59 nm red shift when they are treated with base. Wavelength shift is highly dependent on the increasing acid or base concentration. This concentration dependency facilitates usage of the smart films as optical sensors.

The optical properties of these NFs were investigated by spectrofluorometric methods. Wavelength shift and the intensity of the NFs after acid, base, and metal cation treatment were observed as shown in Figure 4a–e. In addition, a 50 nm blue shift in the emission was observed with TFA-treated NFs (Figure 4a).

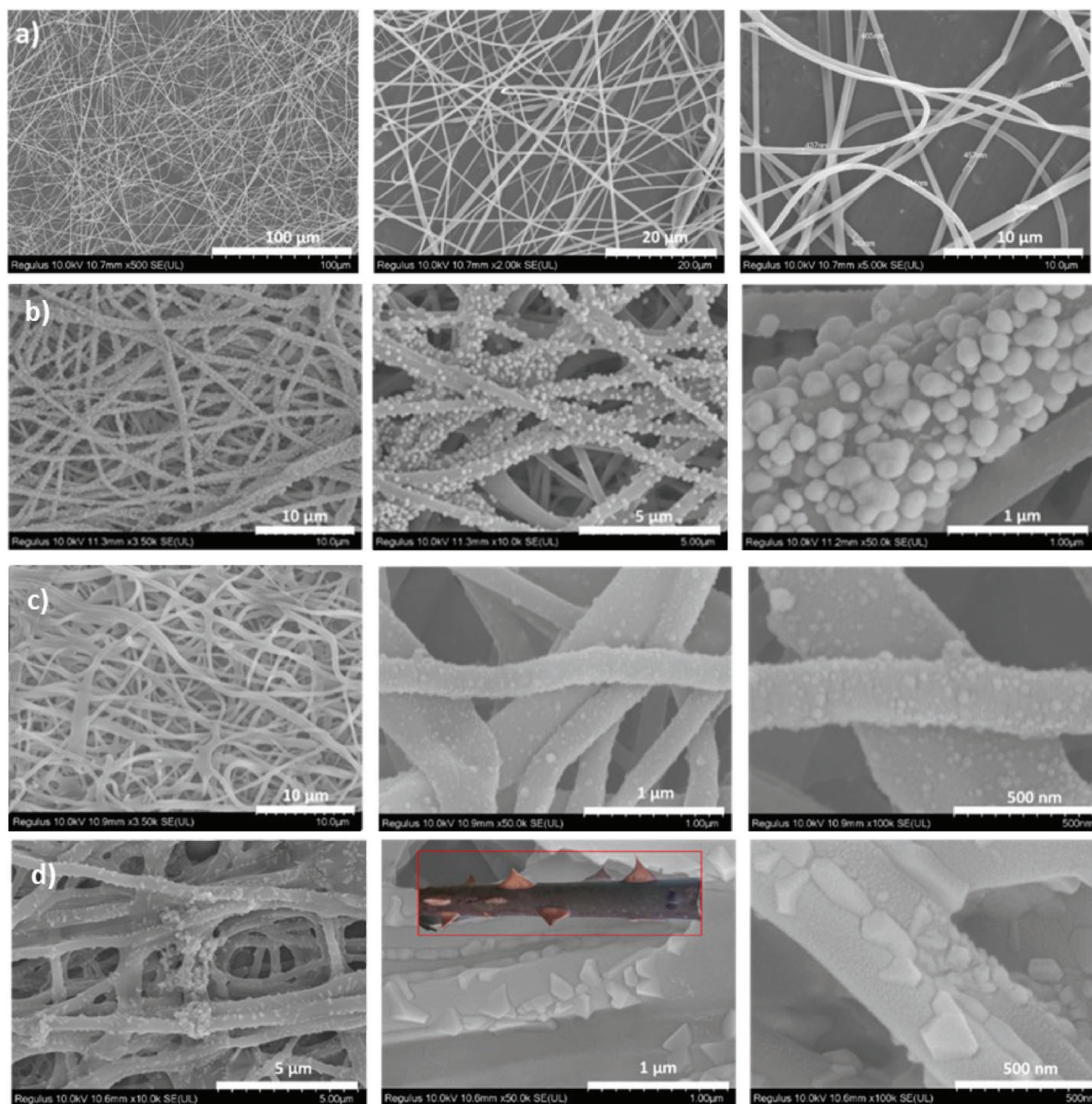


Figure 2. SEM images of HPhTT cross-linked NFs (NF3) (a), AuNP (b), AgNP (c) decorated NFs, and rose thorn-like PbO_x crystal formation on the NF surface (d).

17 nm blue-shift in the emission wavelength was observed as a result of direct treatment with NH₃, as expected (Figure 4b). When TFA-treated NFs were treated with NH₃, a red-shift of 50 nm in the emission wavelength was observed with increasing NH₃ concentration (Figure 4c). The NFs exhibited reversible optical response as wavelength shift when treated with acid or base. The lowest naked eye detectable concentration of NH₃ is observed as 200 nM while it is 20 μ M with TFA. Digital photos of NFs after interaction with acid, base, and cations under daylight and UV-light excitation are given in Figure 4e–g.

The optical properties of NFs after interacting with metal cations were investigated via color change observation under UV-light excitation and spectrofluorometric measurements as shown in Figure 4. Treatment with Pt²⁺ and Ag⁺ resulted in a decrease in fluorescence intensity without emission wavelength shift. The fluorescence emission of the Ag⁺ treated NF film was almost quenched as Au³⁺ treated films. On the other hand, the films treated with Pb²⁺, Co²⁺, Cd²⁺ showed an increase in fluorescence intensities and a blue-shift in the emission wavelength. The optical properties of NFs after interacting with metals were quite

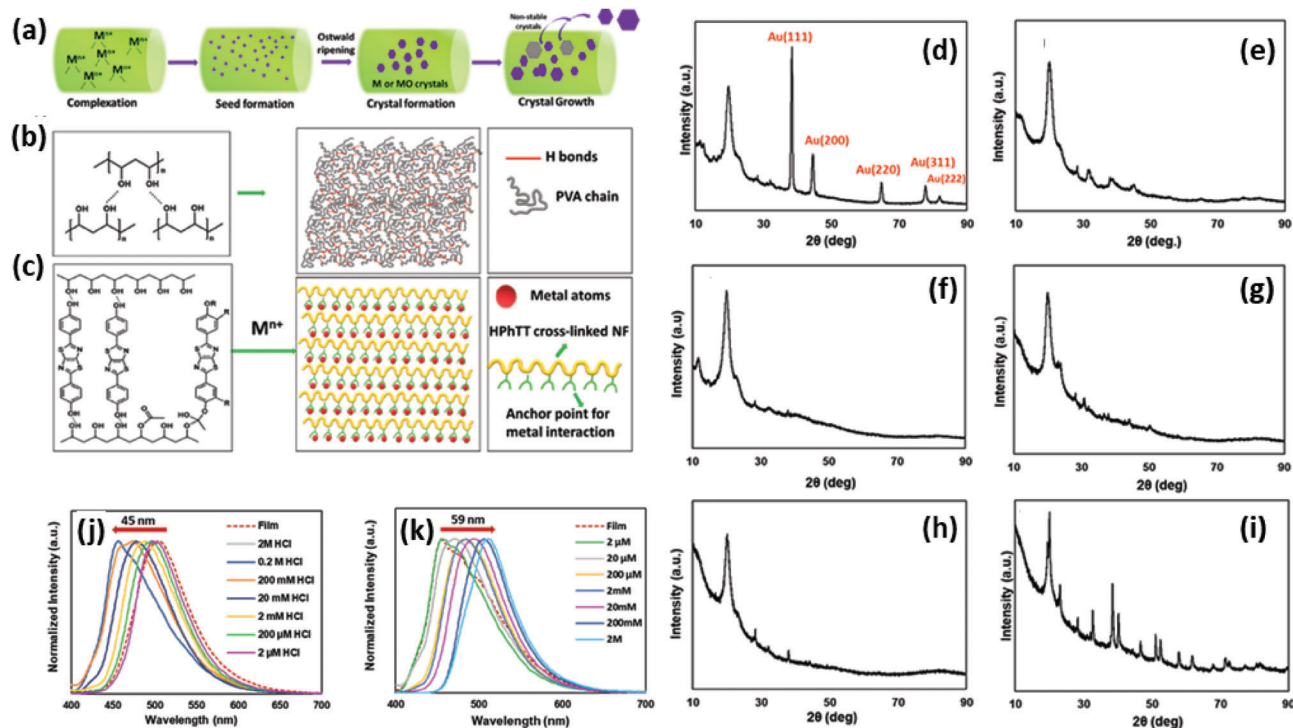


Figure 3. Illustration of metal (M) or metal oxide (MO) crystal formation mechanism via CTC on the NF surface (a), organization of PVA NF chains triggered by; hydrogen bonds (b), HPhTT fluorophore and metal cations (c). XRD patterns of Au³⁺ (d), Ag⁺ (e), Pt²⁺ (f), Cd²⁺ (g), Co²⁺ (h), and Pb²⁺ (i) treated NF films. Emission wavelength shifts of the films after; HCl treatment (j), NaOH treatment (k).

different from films. Treatment with Pt²⁺ and Ag⁺ resulted in a decrease in fluorescence intensity without emission wavelength shift. In fact, the fluorescence emission of the Ag⁺ treated NF film was quenched, as Au³⁺ treated films. However, the white color of the Au³⁺ treated NF films turned brown when it was wet. An increase in the fluorescence intensities accompanied by a blue-shift in the emission wavelength was observed with the Pb²⁺-, Co²⁺-, and Cd²⁺-treated films (Figure 4-d).

3.1. Antibacterial Activity and Bacterial Detection

These smart fibers were used as antimicrobial surfaces for *E. coli* (ATCC 25 922), *S. aureus* (ATCC 25 923), *P. aeruginosa* (ATCC 27 853), and *E. faecalis* (ATCC 25 922) strains by investigating their effectiveness via disk diffusion method. The strains were swabbed on the MHB agar following test samples placement on it. After 24 h incubation, a clear bacteria-free zone, named the

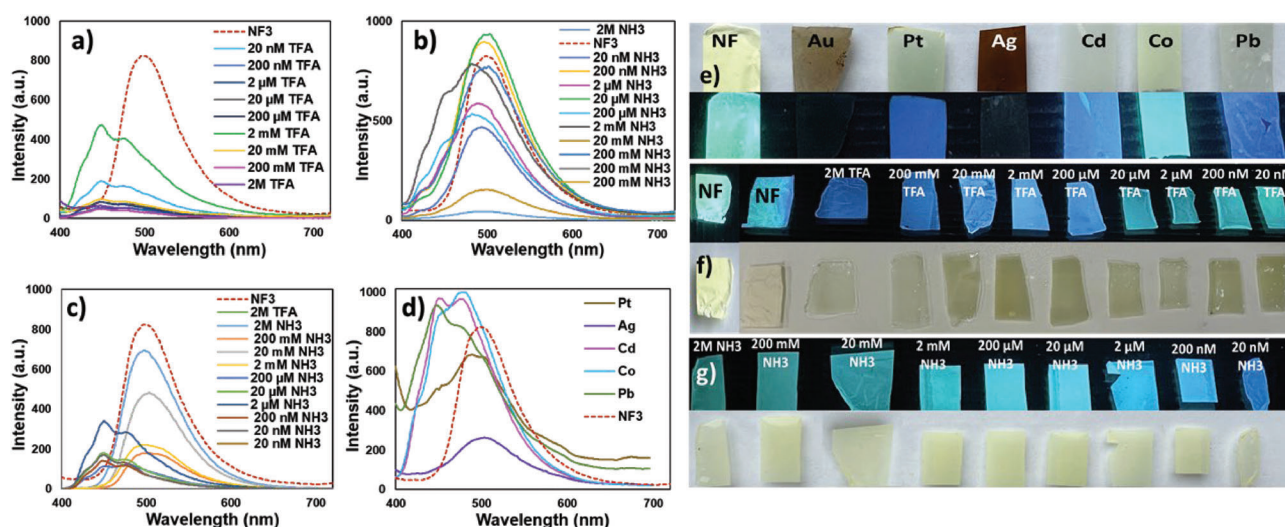


Figure 4. Fluorescent spectra of the NF3 NF films after; TFA treatment (a), NH₃ treatment (b), NH₃ treatment after TFA activation (c), and metal cation treatment (d). Digital images of the NF films under daylight and UV-light after treatment with metal cations (e), TFA (f), and NH₃ after TFA activation (g).

Table 1. Zone of inhibition diameter of the test samples.

Samples	<i>E. coli</i>	<i>S. aureus</i>	<i>E. faecalis</i>	<i>P. aeruginosa</i>
HPhTT-NF	11	6.4	9.4	7
Au	17	12	22.0	18
Ag	14	16	16.8	20
Pt	11	10	15.4	14

zone of inhibition (ZOI), was measured from the samples exhibiting antibacterial activity as given in **Table 1**.

PVA NFs without any additive indicated no zone of inhibition as reported before.^[54–56] HPhTT cross-linked NFs had an antibacterial effect against all strains but a higher effect was observed against *E. coli* strain as to be 11 mm ZOI radius. After Au³⁺ and Ag⁺ metal cations treatment that results in nanoparticle formation on the surfaces of NFs, the antimicrobial effect of NFs increased as expected.^[57–60] The effectiveness of NFs did not increase after Pt⁴⁺ treatment for *E. coli* strain. The NFs were not too effective against *S. aureus* with a 6.4 mm ZOI radius while the metal cation treated samples effect were much higher with 12, 16, and 10 mm ZOI radii for Au³⁺, Ag⁺, and Pt⁴⁺ treated sam-

ples, respectively. Au³⁺ and Pt⁴⁺ treated NFs exhibited the highest antibacterial effect against *E. faecalis* while Ag⁺ treated NFs were also effective with a 16.8 mm ZOI radius. The NFs after metal cation treatment exhibited a higher effect against *P. aeruginosa* strain while HPhTT cross-linked NF had a low effect on the strain with a 7 mm ZOI radius. The highest effect against *P. aeruginosa* was observed with Ag⁺-treated NFs.

Optical detection of the strains under UV-light was performed with biofilms formed on the MHB agar surface. Fluorescent NF films were placed onto the strains incubated for 24 h at 37 °C on MHB—agar plate surface that resulted in biofilm formation. Photoluminescence response was observed under UV light illumination right after the placement of the films, and digital images were recorded as shown in **Figure 5**.

The emission color of the NFs can be adjusted from blue to green via increasing HPhTT cross-linker concentration and base concentration, as we reported before by preparing fluorescent films of the HPhTT.^[6] The NFs numbered 2 in Figure 5 are produced by using a lower concentration of HPhTT (5 mM).

The blue emission color of these NF films changed to green after interaction with *E. coli* and *S. aureus* strains. Even though, the emission wavelength changed slightly after interaction with *E. faecalis* and *P. aeruginosa* strains but the emission intensities also

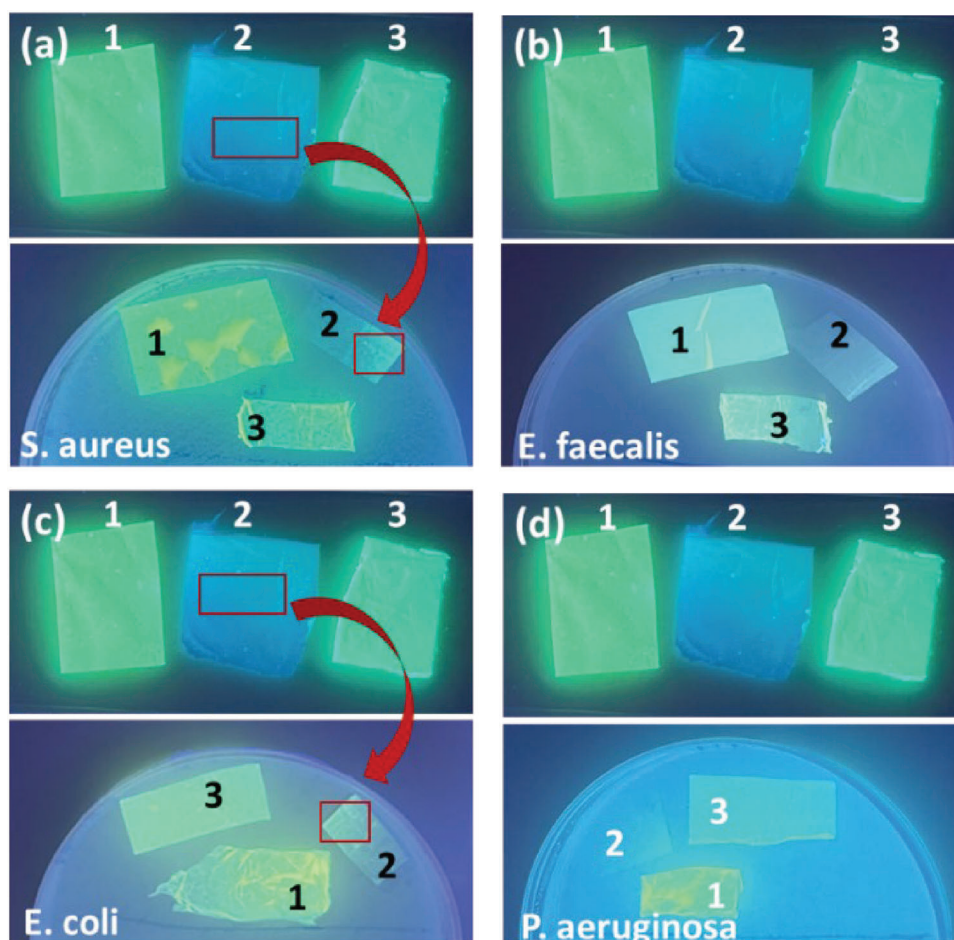


Figure 5. Digital images of HPhTT cross-linked NFs under UV-light excitation before and after treatment with biofilms of *S. aureus* (a), *E. faecalis* (b), *E. coli* (c), *P. aeruginosa* (d).

decreased. These films acted as antibacterial surfaces enabling simultaneous optical detection of bacterial growth. The materials with these unique properties pave the way for usage in various applications.

4. Conclusion

In this study, we proposed a novel crystal growth mechanism on the fluorophore cross-linked nanofibers named “Complexation Triggered Crystallization (CTC)” phenomenon. The process of crystal formation on the substrate is initiated with complexation between electron-rich fluorophores and metal cations. The progress step was succeeded by the reduction of metal cations via the acquisition of electrons from the surrounding environment and dynamic covalent chemistry. Subsequently, crystal growth occurred via Ostwald ripening. Thus, one-step and additive-free preparation of metal nanoparticles on the NF surfaces resulting from the CTC phenomena makes the NFs multi-functional materials enabling usage as a catalyst, antimicrobial dressing, and easy-to-prepare SERS substrates. Moreover, we demonstrated the usage of in-situ fluorophore cross-linked NF as an optical sensor for various analytes such as metal cations, acids, bases, and strains. Also, HPhTT cross-linked NFs and metal nanoparticle decorated NFs exhibit significant antibacterial properties. These smart NF films also make optical detection of bacterial growth possible. The effect of wound monitoring based on bacterial growth can be increased by early detection of bacteria. The NF sensor shows great advantages that can be adopted on point-of-care diagnostic tools. These novel hybrid fibers are encouraging for many sensing and biomedical applications.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

This work was supported by the Scientific and Technological Research Council of Turkey (TUBITAK, GN: 119Z519). The authors are also thankful to Dr. Bükay Yenice Gürsu for valuable guidance about biological studies.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

antimicrobial fibers, bacterial detection, complexation, crystallization, dynamic covalent chemistry, fluorescent nanofibers, optic sensor, thiazolo[5,4-d]thiazole

Received: June 26, 2023
Revised: August 8, 2023
Published online:

- [1] C.-T. Hsieh, C.-W. Lou, Y.-J. Pan, C.-L. Huang, J.-H. Lin, Z.-I. Lin, Y.-S. Chen, K.-C. Chiang, *Fibers Polym.* **2016**, *17*, 1217.
- [2] M. Fan, G. F. S. Andrade, A. G. Brolo, *Anal. Chim. Acta* **2011**, *693*, 7.
- [3] C. Chen, Y. Tang, B. Vlahovic, F. Yan, *Nanoscale Res. Lett.* **2017**, *12*, 451.
- [4] M. M. Demir, M. A. Gulgun, Y. Z. Menciloglu, B. Erman, S. S. Abramchuk, E. E. Makhaeva, A. R. Khokhlov, V. G. Matveeva, M. G. Sulman, *Macromolecules* **2004**, *37*, 1787.
- [5] Z. Dikmen, G. Dikmen, V. Bütün, *J. Photochem Photobiol A Chem* **2023**, *445*, 115074.
- [6] Z. Dikmen, V. Bütün, *Eur. Polym. J.* **2021**, *159*, 110759.
- [7] A. Camposeo, M. Moffa, L. Persano, in *Electrospinning for High Performance Sensors*, (Eds: A. Macagnano, E. Zampetti, E. Kny), Springer International Publishing, Cham **2015**, p. 129.
- [8] J. Yang, P. Li, B. Zhao, K. Pan, J. Deng, *Nanoscale Adv.* **2020**, *2*, 1301.
- [9] X. Hu, Y. Xu, J. Wang, J. Ma, L. Wang, W. Jiang, *Chem. Eng. J.* **2023**, *451*, 139031.
- [10] Ashoka, A. H., A. S. Klymchenko, *ACS Appl. Mater. Interfaces* **2021**, *13*, 28889.
- [11] M. Zhang, S. Zhao, Z. Qin, Y. Lv, H. Zhu, B. Zhao, K. Pan, *J. Mater. Chem. C* **2023**, *11*, 8446.
- [12] X. Zhai, R. Chen, W. Shen, *TrAC, Trends Anal. Chem.* **2022**, *146*, 116502.
- [13] F.-H. Zhang, R.-X. Jiang, W. Cao, B. Du, D.-Y. Cao, Z.-J. Ding, Z.-J. Li, *RSC Adv.* **2019**, *9*, 12585.
- [14] A. Alvarez, J. M. Costa-Fernández, R. Pereiro, A. Sanz-Medel, A. Salinas-Castillo, *TrAC, Trends Anal. Chem.* **2011**, *30*, 1513.
- [15] H. Xia, T. Chen, C. Hu, K. Xie, *Polymers* **2018**, *10*, 1086.
- [16] E. Atabey, S. Wei, X. Zhang, H. Gu, X. Yan, Y. Huang, L. Shao, Q. He, J. Zhu, L. Sun, A. S. Kucknoor, A. Wang, Z. Guo, *J. Compos. Mater.* **2013**, *47*, 3175.
- [17] Y. Zhai, X. Bai, J. Zhu, X. Sun, G. Pan, B. Dong, L. Xu, W. Xu, S. Zhang, H. Song, *J. Mater. Chem. B* **2018**, *6*, 3579.
- [18] Z. Cheng, Z. Bai, Y. Dai, L. Luo, X. Liu, *Analyst* **2018**, *143*, 5225.
- [19] K. S. Min, R. Manivannan, Y.-A. Son, *Sens Actuators B Chem* **2018**, *261*, 545.
- [20] A. Saikia, N. Karak, *Mater. Today Commun.* **2018**, *14*, 82.
- [21] N. L. T. Nguyen, S. H. Baek, Z. A. Akbar, S.-Y. Jang, S. Ha, J. P. Park, T. J. Park, *BioChip J.* **2018**, *12*, 240.
- [22] Y. Shan, S. Li, D. Luo, R. Wang, F. Wu, C. Zhong, L. Zhu, *New J. Chem.* **2018**, *42*, 2089.
- [23] H. Y. Xia, T. Chen, C. Hu, K. Xie, *Polymers* **2018**, *10*, 1086.
- [24] L. Chen, H. Oh, D. Wu, M. H. Kim, J. Yoon, *Chem. Commun.* **2018**, *54*, 2276.
- [25] B. Zu, Y. Guo, X. Dou, *Nanoscale* **2013**, *5*, 10693.
- [26] Terra, I. A. A., R. C. Sanfelice, G. T. Valente, D. S. Correa, *J. Appl. Polym. Sci.* **2018**, *135*.
- [27] S. Trupp, M. Alberti, T. Carofiglio, E. Lubian, H. Lehmann, R. Heuermann, E. Yacoub-George, K. Bock, G. J. Mohr, *Sens Actuators B Chem* **2010**, *150*, 206.
- [28] H. Wang, D. Wang, Z. Peng, W. Tang, N. Li, F. Liu, *Chem. Commun.* **2013**, *49*, 5568.
- [29] H. Wang, W. Tang, H. Wei, Y. Zhao, S. Hu, Y. Guan, W. Pan, B. Xia, N. Li, F. Liu, *J. Mater. Chem. B* **2015**, *3*, 3541.
- [30] P. Pal, B. Das, P. Dadhich, A. Achar, S. Dhara, *J. Mater. Chem. B* **2017**, *5*, 6645.
- [31] J. Zhou, D. Yao, Z. Qian, S. Hou, L. Li, A. T. A. Jenkins, Y. Fan, *Biomaterials* **2018**, *161*, 11.
- [32] E. M. Jones, C. A. Cochrane, S. L. Percival, *Adv Wound Care (New Rochelle)* **2015**, *4*, 431.
- [33] F. Rippke, E. Berardesca, T. M. Weber, *Curr. Probl. Dermatol.* **2018**, *54*, 87.

- [34] N. T. Thet, D. R. Alves, J. E. Bean, S. Booth, J. Nzakizwanayo, A. E. Young, B. V. Jones, A. T. Jenkins, *ACS Appl. Mater. Interfaces* **2016**, *8*, 14909.
- [35] R. Nißler, O. Bader, M. Dohmen, S. G. Walter, C. Noll, G. Selvaggo, U. Groß, S. Kruss, *Nat. Commun.* **2020**, *11*, 5995.
- [36] J. Tang, B. Chu, J. Wang, B. Song, Y. Su, H. Wang, Y. He, *Nat. Commun.* **2019**, *10*, 4057.
- [37] R. S. Dacosta, I. Kulbatski, L. Lindvere-Teene, D. Starr, K. Blackmore, J. I. Silver, J. Opoku, Y. C. Wu, P. J. Medeiros, W. Xu, L. Xu, B. C. Wilson, C. Rosen, R. Linden, *PLoS One* **2015**, *10*, e0116623.
- [38] H. J. Chung, T. Reiner, G. Budin, C. Min, M. Liong, D. Issadore, H. Lee, R. Weissleder, *ACS Nano* **2011**, *5*, 8834.
- [39] Z. Zhao, R. Yan, X. Yi, J. Li, J. Rao, Z. Guo, Y. Yang, W. Li, Y.-Q. Li, C. Chen, *ACS Nano* **2017**, *11*, 4428.
- [40] Z. Dikmen, O. Turhan, A. Özbal, V. Bütün, *Spectrochim. Acta, Part A* **2022**, *279*, 121452.
- [41] Z. Dikmen, O. Turhan, M. Yaman, V. Bütün, *J. Photochem. Photobiol. A Chem.* **2021**, *419*, 113456.
- [42] U. Olgun, Z. Dikmen, H. Çetin, F. Arican, M. Gülfen, *J. Mol. Struct.* **2022**, *1250*, 131816.
- [43] G.-M. Kim, A. Wutzler, H.-J. Radusch, G. H. Michler, P. Simon, R. A. Sperling, W. J. Parak, *Chem. Mater.* **2005**, *17*, 4949.
- [44] A. N. Severyukhina, J. Zhu, Y. Xu, Y. Qin, D. Jiang, *ACS Appl. Mater. Interfaces* **2015**, *7*, 15466.
- [45] T. Yang, J. Ma, S. J. Zhen, C. Z. Huang, *ACS Appl. Mater. Interfaces* **2016**, *8*, 14802.
- [46] L.-B. Zhong, J. Yin, Y.-M. Zheng, Q. Liu, X.-X. Cheng, F.-H. Luo, *Anal. Chem.* **2014**, *86*, 6262.
- [47] Z. Liu, Z. Yan, L. Jia, P. Song, L. Mei, L. Bai, Y. Liu, *Appl. Surf. Sci.* **2017**, *403*, 29.
- [48] Z. Dikmen, R. Javanifar, V. Bütün, *Eur. Polym. J.* **2020**, *129*, 109623.
- [49] Z. Dikmen, H. Ünver, V. Bütün, *Catal. Lett.* **2021**, *151*, 3656.
- [50] Z. Dikmen, M. Isik, O. Turhan, M. Akbari, C. Tuncer, R. Javanifar, V. Bütün, *Eur. Polym. J.* **2022**, *175*, 111391.
- [51] T. Wang, N. Zhang, W. Bai, Y. Bao, *Polym. Chem.* **2020**, *11*, 3095.
- [52] S. Gupta, A. K. Pramanik, A. Kailath, T. Mishra, A. Guha, S. Nayar, A. Sinha, *Colloids Surf., B* **2009**, *74*, 186.
- [53] Y. Su, Y. Wu, M. Liu, Y. Qing, J. Zhou, Y. Wu, *Materials* **2020**, *13*, 1412, **13**.
- [54] E. Muthusankar, S. Vignesh Kumar, N. Rajagopalan, D. Ragupathy, *J. Bionanosci.* **2018**, *8*, 1008.
- [55] S. Suganthi, S. Vignesh, J. Kalyana Sundar, V. Raj, *Appl. Water Sci.* **2020**, *10*, 100.
- [56] C.-W. Lou, M.-C. Lin, C.-H. Huang, M.-F. Lai, B.-C. Shiu, J.-H. Lin, *Polymers* **2022**, *14*, 1054.
- [57] Q. Zhang, Y. Wang, W. Zhang, M. E. Hickey, Z. Lin, Q. Tu, J. Wang, *Colloids Surf., B* **2019**, *184*, 110506.
- [58] M. E. El-Naggar, E. S. Shalaby, A. H. Abd-Al-Aleem, M. A. Abu-Saied, A. M. Youssef, *J. Mater. Res. Technol.* **2021**, *15*, 3447.
- [59] K. Kalwar, M. Shen, *Nanotechnol. Rev.* **2019**, *8*, 246.
- [60] S. Pal, R. Nisi, M. Stoppa, A. Licciulli, *ACS Omega* **2017**, *2*, 3632.