Intrinsic fluorescence from cellulose nanofibers and nanoparticles at cell friendly wavelengths

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ABSTRACT

Nanocellulose has emerged as a promising material for many biomedical applications. Depending on the source of the nanocellulose and the chemical treatment, the resulting optical properties can vary significantly. In particular, autofluorescence can exist in various wavelength ranges. Except for chlorophyll related autofluorescence, all other studies have reported intrinsic fluorescence in the blue to yellow range. However, this range coincides with the autofluorescence range of cells. Here, we report on cell friendly red fluorescence of nanocellulose fibers and nanocellulose fiber-derived nanoparticles. Photostability, spectral features, and lifetimes of fibers and nanoparticles are investigated. Subsequently, those nanostructures were successfully integrated into cells, and photostable bright in vitro emission was recorded and analyzed. Our work paves the way to further optical studies of nanocellulose and its related nanostructures, which may find uses in future biomedical applications.

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I. INTRODUCTION

Nanocellulose, a biopolymer extracted from native cellulose, has attracted much attention due to its remarkable physical and chemical properties including excellent mechanical properties, tailorable surface chemistry, and interesting optical properties.1,2 On the basis of these unique properties, nanocellulose has been used for a wide range of applications, from optoelectronics to medical devices.3–5

In particular, nanocellulose is an emerging biomaterial for many medical applications including tissue bioscaffolds for cellular culture, drug excipient and drug delivery, immobilization and recognition of enzyme/protein as well as blood vessel and soft tissue substitutes, skin and bone tissue repair materials, and antimicrobial materials.1,4–6 Three different types of nanocellulose, cellulose nanocrystals (CNCs), cellulose nanofibrils (CNFs), and bacterial cellulose (BC), are used for biomedical applications.4 It is generally accepted that BC has excellent biological properties including good biocompatibility and low toxicity. On the other hand, these biological properties are still questionable for other nanoparticles (NPs) of cellulose, such as CNCs and CNFs. The toxicity of these nanocellulose is reported to depend on particle size, surface chemistry, and process purity,1 with different studies reporting various results due to the range of sample fabrication and additional processing. Even though there is no definitive answer on the biological properties of CNCs and CNFs, they have already found applications in areas of tissue engineering5 and wound dressing.6

The options for chemical and material processing of nanocellulose are extremely versatile, opening up a wide range of possibilities in terms of structural and functional properties of this nanomaterial. Additional functionalities were also achieved with the incorporation of NPs into nanocellulose substrates that enabled production of novel nanocomposites for a wide range of applications. For example, liquid crystal self-assembly of semiconductor quantum dots into cellulose nanocrystal-templated silica was developed and green-yellow emission was observed.7 Another example where gold NPs/bacterial cellulose nanocomposites were prepared and
evaluated for their applicability as a surface-enhanced Raman scattering substrate was recently reported.\textsuperscript{9} Indeed, fluorescent labelling of nanocellulose with a variety of fluorescent nanomaterials is of emerging interest for applications in bioimaging and sensing.\textsuperscript{9-11}

The intrinsic fluorescence from nanocellulose was also reported. The autofluorescence was attributed to lignin (wood samples),\textsuperscript{12} chlorophyll (wood samples),\textsuperscript{13} or proteins (tunicate/bacterial samples). There were also reports of nonfluorescent compounds found in wood that turned into flavonoid fluorophores after soaking in alkaline water.\textsuperscript{14} Confocal and Raman microscopy were used to confirm that the fluorescent species were unique for each cellulose source and demonstrated that such methods could be useful for monitoring purity during CNC/CNF processing.\textsuperscript{15}

Except for chlorophyll related autofluorescence reported in Ref. 13, all other studies report intrinsic fluorescence in the blue to yellow range. However, these wavelengths are not suitable for in vitro applications because of cells’ autofluorescence caused by the presence of components such as collagen. They typically absorb light in the range of 300–500 nm and fluoresce at a 400–550 nm range.\textsuperscript{16} Therefore, it is crucial for bioapplications for a fluorescent marker to absorb light at wavelengths longer than 500 nm and to emit light at wavelengths longer than 600 nm.

Here, we report on intrinsic fluorescence of CNFs at cell friendly wavelengths. We investigate their emission properties: intensities, spectral features, and lifetimes of both CNFs and CNF derived NPs (CNPs). We also demonstrate that these CNFs are traceable by their own photostable fluorescence after their uptake by human skin keratinocyte and prostate cancer cells.

II. METHODS

A. CNF and CNP preparation

Dried soft-wood pulp [commercial northern bleached softwood kraft [NIST reference material 8495 (Ampulski 2001)]] was used as the starting material to prepare cellulose nanofiber (CNF) and CNP suspensions. The dry pulp was first disintegrated and then subjected to ball milling using the Spex 8000M shaker mill. The CNFs were prepared using the ratio of beads to pulp of 40:1, the balls with a diameter of 0.3 mm, and milling for 1.5 h. The CNPs were prepared using the ratio of beads to pulp of 150:1, the balls with a diameter of 0.8–1 mm, and milling for 2 h. The nanocellulose has been suspended in water only. The resulting CNF and CNP suspensions were stored at 4 °C in cold storage. Other detailed information on milling could be referred to Ref. 17.

B. SEM imaging

Scanning electron microscopy (SEM) was used to investigate the particle morphology using a Philips XL30 Field Emission Scanning Electron Microscope (FESEM) equipped with an energy dispersive X-ray detector (EDS, Oxford Instruments). A high efficiency (HE-SE2) detector was used for collecting secondary electrons. Around 2 µl of the synthesized sample solution was transferred to a silicon wafer and allowed to air dry. The sample was then sputter coated with 5–6 nm iridium, and high resolution visualisation was carried out at EHT 5.0 kV. For each SEM image, the diameters of all the fibers that could be recognized in the image were measured using the Image-pro Plus image analysis software. This was performed manually by drawing across the fiber in the image and then measuring the distance as fiber diameter. More details were described in the previous work.\textsuperscript{17}

C. Confocal imaging

Confocal fluorescence scanning was performed using a customized confocal microscope. A 532 nm frequency doubled Nd:YAG continuous wave laser was used for sample illumination at a power of 200 µW through a 100×, 0.95 NA objective. The excitation power used for CNFs had to be reduced to 100 µW due to high saturation level of agglomerated features.

The samples on silicon (Si) and cover glass substrates were mounted on a computer controlled stage facing the objective perpendicularly and equipped with an xyz closed loop positioner with 100 µm travel in each direction. The in-plane optical resolution was approximately 300 nm. The fluorescence from the samples was collected with an avalanche photodetector (APD) in the red to near infrared wavelength range. The emission was filtered by using a 560 nm long pass filter to remove the incident beam before being detected.

The photoluminescence signal from the confocal microscope was coupled into a spectrometer and detected by using a CCD camera (Acton SpectraPro 2300i with Pixis100 camera). Spectra were background corrected by subtracting the signal accumulated 1 µm from the individual defect.

The in-solution spectroscopy was performed to obtain the photoluminescence signal from CNPs and CNFs at an excitation wavelength of 450 nm. A supercontinuum Fianium laser was set at an excitation wavelength of 450 ± 10 nm and a power of 100 µW. A 450 ± 25 nm bandpass filter was employed to restrict the wavelength around the 450 nm range. The photoluminescence collected was filtered by using a longpass 500 ± 25 nm filter in order to avoid the back reflection.

D. Wide field fluorescence imaging

Wide field imaging was performed with a commercial fluorescence microscope (Nikon, Eclipse Ti-U), using a 532 nm Verdi laser operating with a typical power density of 30 W mm\textsuperscript{2} in a temperature controlled environment of 37 °C. A 5× beam expander was used to expand the excitation beam to 10 mm diameter before focusing the excitation light onto the back aperture of the 100×, 1.45 NA (Nikon) oil immersion objective through a dichroic mirror (Semrock-Di02-R561–25 × 36). A focusing lens (f = 300 mm) focused the excitation light onto the back aperture of the objective creating a uniform wide-field illumination. The laser power P = 140 mW was spread over 80 × 80 µm\textsuperscript{2} area, and the wide-field fluorescence image was detected with an sCMOS camera (Andor, Neo). The setup is also equipped with the bright field microscope that
was first used to find a particular area of interest on the sample. The laser was then turned on to image the fluorescence scan for the particular area. Fluorescence imaging of the NPs was performed several microns above the surface of the coverglass where the fluorescent NPs came into focus. Care was taken to ensure the focal point did not exceed the average height of the cultured cells to make sure that only the NPs inside the cells are being imaged. Imaging was performed at 37 °C by using a temperature controlled environmental chamber around the microscope. In our confocal systems, we do not tag our nanomaterials. Quite the opposite, we rely on intrinsic fluorescence from nanoparticles.

E. Lifetime measurements

Fluorescence lifetimes were measured in a confocal microscope similar to that described in Sec. II C, adapted to include a pulsed laser source and a time resolved photon counting module (Fast ComTec MCS6A). The pulsed green excitation source was constructed from a 520 nm pigtailed laser diode (Thorlabs LP520-SF15) and a bias-T circuit (Thorlabs LDM91P) that allows fast current modulation. A pulse generator (SpinCore PulseBlasterESR-PRO 500 MHz) was used to modulate the diode current and create 50-ns laser pulses with a measured fall time of ≈0.5 ns and a repetition rate of 2 MHz. Fluorescence decay traces were obtained by time-tagging the photon arrivals following the end of each pulse, averaging the signal over a total integration time of about 10 s. The decay traces were then fitted to a single exponential decay to extract the fluorescence lifetime. For each sample (CNPs and CNFs), about 20 fluorescent spots were measured in order to build up statistics.

F. Cell culture

Human skin keratinocyte (HaCaT) cells and human prostate cancer (PC-3) cells were maintained in the Roswell Park Memorial Institute 1640 (RPMI 1640) media (Life Technologies) supplemented with 10% foetal bovine serum (Invitrogen) and 1% penicillin-streptomycin (Life Technologies). The cells were grown at 37 °C with 5% CO₂ and 95% humidified air.

For in vitro wide field imaging, the cells were grown in a chambered coverglass for 24 h and treated with 10% (v/v) CNPs and CNFs for 12 h. The cells were then washed in phosphate buffered saline (PBS), and live cells were imaged.

G. Cytotoxicity test

Cytotoxicity was assessed by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay. Cells were grown in 96 well plates for 24 h at a seeding density of 1 × 10⁴ cells/well. This was followed by treatment with increasing concentrations of CNPs and CNFs ranging from 0% to 10% (v/v) in growth media for 24 h. 10 µl of MTT solution (5 mg/ml in PBS) was added to each well and incubated for 4 h at 37 °C. The media were removed, and 100 µl of acidified isopropanol was added to each well to dissolve the formazan crystals. The plates were shaken for 30 s, and optical density was recorded at 570 nm using a microplate reader.

III. RESULTS

Structural characterization results of CNFs, using SEM, are shown in Sec. III A. Fluorescence properties of both CNPs and CNFs, investigated using confocal microscopy, are presented in Sec. III B, including the spectral features and lifetimes of both samples. The findings of wide field imaging of cell cultured CNFs and CNPs in human skin keratinocyte cells and prostate cancer cells are given in Sec. III C. Mobility of the NPs and fibers inside cells is compared in the same section. Cytotoxicity analysis results of CNFs and CNPs are provided in Sec. III D.

A. Structural characterization

The CNFs and CNPs are imaged using SEM as shown in Fig. 1. An accelerating voltage of 5 kV is used to collect images at high magnifications of 5000× to 15 000×. In all samples, variation of shapes and sizes can be observed. In Fig. 1(a), a bundle of fibers with a diameter of hundreds of nanometers can be observed. The sub-micron features are CNPs that are by-products of the soft ball-milling fabrication process. They can be easily removed or controlled. Figure 1(b) displays the larger individual CNPs in white and agglomerated smaller NPs in gray. The fibers exhibited a narrow distribution size ranging from 730 ± 20 to 930 ± 40 nm with 95% of fiber diameters within 200 nm measured using the Image-Pro Plus image analysis software. The sizes of the CNPs refer to the average values of the largest distances through their centres of any irregularly shaped particles. The nanoparticles showed a broader distribution ranging from 200 ± 5 nm to 830 ± 40 nm. This is common not only for a ball milling but also for many methods of nanoparticle synthesis.¹⁷
B. Confocal microscopy

We investigate intrinsic fluorescence properties of both NPs (CNPs) and fibers (CNFs) deposited on the cover glass. The samples were optically characterised using a home-built scanning confocal microscope described in Sec. II C. Figures 2(a) and 2(b) show the $50 \times 50 \, \mu m^2$ confocal images of the CNPs and CNFs samples, respectively. The background fluorescence, denoted by light blue, is low, of the order of 3 kcounts/s, enabling clear identification of CNP and CNF fluorescence. Both images display bright red fluorescence with a maximum of the order of $10^6$ counts/s. The scans show smaller NPs with individual particles as well as the larger agglomerated ones.

To examine the detailed fluorescence characteristics of the individual features, zoomed images were recorded for both samples. Figures 3(a) and 3(b) show scanning confocal maps of $5 \times 5 \, \mu m^2$ regions on the both samples. Figure 3(a) displays bright round NPs, and in Fig. 3(b), the fiber shaped is notable. Note that the confocal images are representative of the fluorophores inside the structures and not the fiber morphology compared to the SEM images.

The individual CNPs and CNFs provide photostable bright counts as shown by the count trace of Fig. 4(a) for CNPs and Fig. 4(b) for CNFs. The general trend of background subtracted emission counts both for brightest nanostructures exhibits an initial decreasing trend with the stabilization of counts appearing after few minutes. A broader band is recorded for CNPs with counts range from $10^4$ to $10^6$. CNFs exhibit a smaller range of counts, mainly around $10^5$ with some initial photobleaching effect evident in Fig. 4(b). The emission counts are a function of excitation power and are expected to increase with an increase in excitation power. However, photobleaching may occur when using higher power. Photostability of fluorescing features is an important characteristic requirement for long-term bioimaging. In addition, we choose to use lower excitation power in order to ensure non-invasive imaging consistent with our in vitro measurement described in Sec. III C.

Red fluorescence is attributed to numerous types of NPs when illuminated with a green laser, such as nanodiamonds and metal oxide NPs. However, each type of NPs has their characteristic emission band and characteristic spectral features. Therefore, fluorescence emission spectra of CNPs and CNFs are investigated. The spectra of both CNPs and CNFs were recorded as described in Sec. II C and presented in Figs. 5(a) and 5(b), respectively. The spectral characteristics were also investigated to analyze any possible differences in the emission properties for the two samples.

As explained in introduction, it is crucial for bioapplications for a fluorescent probe to absorb light at wavelengths longer than 500 nm and to emit light at wavelengths longer than 600 nm to avoid cells’ autofluorescence. For that reason, a 532 nm CW laser was used for sample illumination.
Both samples exhibited broad fluorescence between 580 and 900 nm with no recorded difference in any characteristic spectral features. Both samples reveal peaks around 640 nm and characteristic features at 585, 610, and 630 nm. The side-band of CNFs extends from 625 to 800 nm, while that of CNPs ranges from 625 to 900 nm due to higher excitation power used. The excitation power used for CNFs had to be reduced to 100 µW due to the high saturation level of agglomerated features. Note that emission was filtered by using a 560 nm long pass filter to remove the incident beam (532 nm) not to be reflected back in the detector and therefore the cutoff at 560 nm is artificial.

When 450 nm CW laser excitation is used, an additional peak at 535 nm is appearing for both samples, see Fig. 5(b). Fluorescence for both samples is extended between 500 and 800 nm, consistent with previously reported studies, with no recorded difference in any characteristic spectral features for CNPs and CNFs. For both samples, the same power of 100 µW is used and therefore the stronger intensity response is recorded for larger CNF structures compared to smaller CNPs.

In addition to spectral features, the fluorescence lifetime is another intrinsic property of an emitter in a given medium. The lifetime measurements were performed for both CNPs and CNFs as described in Sec. II E. The measurements reveal that CNP lifetimes range from 1.1 to 2.4 ns, with the mean value 1.4 ns; CNF lifetimes range from 1.5 to 2.2 ns, with the mean value 1.8 ns. Therefore, we can conclude that there is no substantial difference in lifetimes for those two samples, consistent with findings for other fluorescence features, such as count rates and spectral properties. Moreover, the relatively short fluorescence lifetime makes these NPs particularly suitable for fluorescence lifetime microscopy. Future studies will address physical principle that causes red fluorescence.

### C. In vitro wide field imaging

Previously, mainly yellow and orange intrinsic fluorescence were reported for NC. As explained in the Introduction, for bioimaging, it is crucial that probe's fluorescence is not obstructed by cell's autofluorescence, which usually appears in the blue and green band. Additionally, in this way, any possible changes in the properties and dynamics of the sample, induced by monitoring, are avoided. Two types of cells, a normal human skin keratinocyte cell line and human prostate cancer cell line, were chosen and prepared as described in Sec. II F. In Fig. 6, bright field and wide field images for cells only are shown.

Both keratinocyte and cancer cells show almost immediate photobleaching and stabilize at a reference point for the background count rate of approximately 1.5–2 kcounts/s, after 30–40 s. The low level of autofluorescence and low count rate are essential for NPs to be detected and their dynamics observed.

In this section, we investigate if fluorescent properties are retained when the nanostructures are uptaken by cells. Fluorescence tracking of nanostructures in cells was performed using wide field imaging described in Sec. II D.
Both types of cells, without cellulose, exhibit autofluorescence that show almost immediate photobleaching behavior consistent with our previous reports. As pointed out in Sec. II D, care was taken to ensure that only the nanostructures inside the cells are being imaged. Figure 7(a) shows the bright field microscopic image of the CNPs cultured inside skin cells. The fluorescence of the cultured CNPs illuminates the whole cell is shown in Fig. 7(b).

Figures 7(c) and 7(d) show the bright field and fluorescence images, respectively, of the CNFs cultured inside skin cells. The bright field and the wide field fluorescence images do not always correspond to each other because only cells that contained NPs and are in the focal plane will appear in the wide field fluorescence images. The fluorescence images clearly indicate bright NPs inside cells. The figure shows that the emission rate collected from NPs inside the cells ranges from 40–90 kcounts/s indicating their suitability for in vitro experiments. The average stable count rates of CNFs and CNPs that are 10–45 times greater than the count rate of the untreated cells ensure that the nanostructures are detectable and traceable.

In addition to normal cells, we investigate fluorescence properties of nanostructures uptaken by cancer cells. Cancer cells were chosen for two reasons. First, it is known that cancer cells are more sensitive than normal cells to DNA damage response. Second, on many occasions, it has been reported that NPs can be selectively toxic toward cancer cells. Figures 8(a) and 8(b) show bright field and fluorescence images of CNFs cultured inside prostate cancer cells.

Both CNPs and CNFs in cancer cells is similar to the uptake of these nanostructures in normal cells. Bright field and fluorescence images in Fig. 8 indicate no substantial difference in fluorescence to fluorescence of the images shown in Fig. 7 for normal cells. Both cell lines (Figs. 7(a) and 7(c) and Figs. 8(a) and 8(c)) show normal polygonal morphology and no unusual intracellular granulation, which indicates the cells are functioning normally and not undergoing stress as a result of exposure to the nanostructure. Cell viability studies were performed to confirm this and are shown in Sec. III D below.

Count traces for CNFs and CNPs inside cancer cells were recorded for extended time intervals. Note that those counts are averaged over counts of all nanostructures uptaken by cells as the incident beam is spread over 80 × 80 µm².

Both nanostructures exhibit extremely photostable counts, as shown in Fig. 9. There is no significant drop in the counts as recorded for nanostructures on their own. Furthermore, it appears that a cell optical environment even further stabilizes the emission rate. The stable count rate may be due to the power density being lower, 22 µW/µm², in these cell experiments which is lower power measurement compared to 100 and 200 µW in confocal imaging described in Sec. II C and results presented in Sec. III B. Additionally, confocal and wide field imaging were performed under different optical conditions. It has been shown that the emission rate of a radiating dipole within a nanoparticle is crucially dependent on its surrounding refractive index environment. The refractive index of cells (wide field imaging) is similar to the
refractive index of water $n = 1.332$ (at $\lambda = 532$ nm). In confocal imaging, the sample is deposited on a cover glass ($n = 1.515$) in air with the refractive index of $n = 1.0$.

Next, we observe nanostructures' motion inside the human skin keratinocyte cells and record their trajectories. After uptaken by the cells, the cellulose particles and fibers...
FIG. 9. Emission counts of (a) CNFs and (b) CNPs, cultured in cancer cells, as a function of time. Excitation power was 140 mW at the back of the objective. This provides an average power of 22 µW/µm² on the sample’s surface.

exhibit the free Brownian motion and a linearly increasing mean squared displacement as a function of time. This provides a constant value of the diffusion coefficient, indicating that the CNFs and CNPs are not immobilized or trapped in the cell membranes. Following the video processing of the fluorescence images and tracking of nanostructures’ position, the diffusion coefficients (D) have been calculated for 18 CNPs and 21 CNFs, using the mean square displacements $\langle r^2 \rangle = 4Dt$, as reported in our previous studies. The diffusion coefficient for each of the nanostructures has been calculated for a time interval of $t = 10$ s with the time step between consecutive measurements is 0.08 s. A histogram plot of diffusion coefficients of CNFs and CNPs cultured in human skin keratinocyte cells is shown in Fig. 10.

For CNF fibers in the cells, the intracellular mobility values range from $(4.6 \pm 1.4) \times 10^{-3}$ µm²/s to $(72.5 \pm 3.5) \times 10^{-3}$ µm²/s. For CNP nanoparticles cultured in cells, the mobility ranges from $(2.2 \pm 0.3) \times 10^{-3}$ µm²/s to $(70.0 \pm 3.5) \times 10^{-3}$ µm²/s.

According to the literature, low diffusion coefficients, less than $10^{-2}$ µm²/s, are expected for nanoparticles with diameters greater than 30 nm in cells. For example, gold NPs of similar size range showed mobilities of the order of $0.1 \times 10^{-3}$ to $0.7 \times 10^{-2}$ µm²/s, and selenium NPs with 50 nm diameters are reported to give D values ranging from $0.1 \times 10^{-2}$ to $2.2 \times 10^{-2}$ µm²/s, and nanodiamonds with an average diameter of 45 nm have been reported to provide an average diffusion coefficient of $0.3 \times 10^{-2}$ µm²/s inside cells. Therefore, intracellular mobility for cellulose nanostructures exists in the range of values reported for other NPs in the similar size range.

Additional data and analysis are available in the expanded version of this article.

D. Cytotoxicity analysis

Optical observation of cells treated with nanocellulose investigated in Sec. III C has shown normal cellular morphology [Figs. 7(a) and 8(a)]. To further investigate the cytotoxicity, we performed MTT assay for 24 h, as described in Sec. II G. MTT assay also provides information on mitochondrial respiration, and these results suggest that the cellular energy capacity is not affected as a result of exposure to the CNPs and CNFs after 24 h. Acute toxicity provides information on the effects on cells following administration of a substance or nanoparticle within a 24 h period and has similarly been used to establish the adverse effects of NPs in an in vitro scenario.

The results in Fig. 11 indicate that there was no significant toxicity to both mammalian cells in the presence of CNPs and CNFs, except for 10% v/v of CNPs, which showed negligible toxicity (~75% cell viability) to the HaCaT cells. Similar cytotoxicity studies performed on other normal and cancer cell lines, including PC-3 cells, have shown no toxicity which suggests the benign nature of these nanostructures, despite the differences in their morphology and size. This indicates that both CNPs and CNFs may be used for fluorescence imaging of normal and cancer cells.

Figure 11 also shows an increase in cell viability above 100% with the lower concentrations of the NPs. In MTT assay, % viability is calculated with respect to the untreated cells, assuming 100% of the cells are alive in the untreated sample. An increase in viability can occur if the cells treated with NPs show an increase in cell number more than the untreated cells. Experimental error when seeding the cells can also result in slightly higher cell numbers in some wells, which will result in higher absorbance readings in those wells. Since these
results are an average of three replicates with low standard deviation (error bars in Fig. 11), it indicates that at low concentrations, the cellulose NPs might have a stimulatory effect on cell growth through a process known as hormesis. This further suggests that no adverse effects are observed on the cells as a result of acute exposure to the cellulose NPs. However, the authors acknowledge that long term/chronic toxicity and the pharmacokinetics of these NPs will need to be tested for future biomedical applications.

IV. CONCLUSIONS

In this manuscript, we have reported and analyzed the intrinsic fluorescence from nanocellulose fibers and fibers derived NPs. Room temperature photostable broad red fluorescence, between 600 and 800 nm, from those nanostructures has been recorded and analyzed. A distinct fluorescence in the visible to near infrared range has been recorded with no difference in any characteristic spectral features between fibers and NPs. Relatively short lifetimes, between 1 and 2 ns, have been measured for both samples. Subsequently, both types of nanostructures have been taken up by human keratinocyte HaCaT cells and human prostate cancer cells. Fluorescence tracking in cells has been performed using wide field fluorescence imaging. Not only that fluorescence has been photostable in vitro but also that nanostructures have appeared to be mobile inside the cells. Both optical imaging and toxicity analysis have indicated that fibers and particles do not appear to affect the cellular viability. This infers that the nanocellulose structures can be tracked and imaged in cells, without tags or dyes, that are known to chemically modify the biological environment under study. In this way, there are two essential advantages. One is that there is no induced change of the chemistry or dynamics of these nanoparticles. Second, we point out that their fluorescence differs from the cells’ auto-fluorescence. Hence, this non-invasive optical imaging capability based on cellulose autofluorescence at cell friendly wavelengths is a new powerful tool to be used in studying and testing of these nanostructures for future biomedical applications.

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