

Facile bacterial cellulose nanofibrillation for the development of plasmonic paper sensor

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Highlights:

- The development of inexpensive biodegradable paper-based plasmonic sensor from cheap bacterial cellulose source, *nata de coco*
- Nanofibrillation of bacterial cellulose through high pressure homogenization for surface nanopatterning
- Plasmonic papersheet sensor development integrating bacterial cellulose and silver nanoparticles
- Physical and textural improvements of bacterial cellulose structure facilitating effective localized surface plasmon effects
- Detection of Rhodamine 6G on the proposed bacterial cellulose-based plasmonic paper sensor

Abstract

In this present work, a plasmonic sensor is developed through an extremely cheap cellulose-based source, widely known as food product, *nata de coco* (NDC). Capturing its interesting features such as innate surface roughness from naturally grown cellulose during its fermentation period, the engineering and modulation of NDC fibril size and properties was attempted through high pressure homogenization (HPH) treatment to obtain highly dense nanofibrils. After the transformation into a thin papersheet form through casting process, the HPH treatment resulting nanobacterial cellulose (NBC) was compared with the normally agitated bacterial cellulose (BC) pulp and decorated with silver nanoparticles (AgNPs), namely plasmonic papers for further application as SERS substrate. Carried out in the measurement of rhodamine 6G (R6G) molecule, the plasmonic NBC papersheet demonstrated more prominent SERS signals than the plasmonic BC due to its high surface roughness and improved textural properties from nanofibrillation process favoring better adsorption of AgNPs and effective SERS hotspots generation. The plasmonic NBC obtained two-order higher estimated SERS enhancement factor (EF_{SERS}) over the plasmonic BC. Results denote that the proposed approach provides a new green-synthesis route towards the exploration of biodegradable sources integrated in an inexpensive and simple nanostructuring process for the production of flexible paper-based plasmonic sensor.

Keywords: *nata de coco*, nanofibrillation, bacterial cellulose (BC), plasmonic paper, SERS

1. Introduction

Surface-enhanced Raman Scattering effect has been an evolutionary discovery where intensive electromagnetic field confinement and scattering phenomena known as the plasmonic effects are generated in nanoscale roughened noble metallic nanoparticles (NPs) [1], [2]. Since its first discovery in 1974 [3], strategy towards creation of SERS substrate has been widely expanding not only using conventional lithography but also metallic electrodes roughening [4], nanopatterning through chemical strategy, such as by surface oxidation [5], surface manipulation with polymer nano-template [6] and development of biodegradable plasmonic substrates [7]. The varied fabrication methods have been directed towards the optimized SERS substrate properties such as higher roughness for spot-to-spot and structures reproducibility, high enhancement factor, durable shelf life, resistance to laser excitation and mechanical stress. The key for effective SERS signal enhancement is the nanogap interspacing arrangement within metallic nanoparticles position to produce localized surface plasmon resonance (LSPR) coinciding the excitation wavelength which must be as small as 2-20 nm, called as “hot spots” [8]. Hence, there is urgent need to obtain highly well-ordered arrays of metallic nanostructures with high aspect ratio for excellent reproducibility of SERS-signal intensity. Nevertheless, nanopatterning and nanolithography are somehow followed by laborious and pricey fabrication in clean room scale as well as complex and non-eco-friendly chemical process mainly conducted in wet benches. In fact, there is ever-increasing demand also for a simple and handy SERS substrate which is cost-effective for the use as portable and disposable screening substrate.

Among SERS-active substrate materials, polymeric biomaterials, such as bacterial cellulose (BC)-based material, has been a captivating alternative for natural nanopatterning of a substrate from BC nanofibrils for the production of low-cost biodegradable paper-based sensor such as in the form transparent substrate [9]. In building up effective SERS hotspots, BC is more beneficial over plant cellulose for high entrapment, density and dispersion of noble metal nanoparticles due to their native fibrils with the width less than 100 nm which concomitantly provide large surface area, outstanding wicking ability with great compatibility with other printing-based methods [10], [11]. BC is also noted as remarkable nanoparticle matrix due to its high-purity, strong mechanical properties such as high tensile strength which lowers the NPs aggregation as well as the presence of their active hydroxyl-rich surface for the ease of surface engineering and modifications [12]. Pulp of the cellulose fibers can be modified through nanofibrillation process using high-speed blender, ultrasonic irradiation [13], a high-shear homogenizer [14] or microfluidization [15] with a variety of pretreatments such as to flexibilize the fibers and eliminate the energy consumption [16]. Among the treatment in fibrillation, high-pressure homogenization (HPH) has been noted as an efficient technique in biomass refinery since it is simple and highly efficient with minimum use of organic solvents [17]. The principles of HPH covers mainly the fall off of pressure gradient, combination of high turbulence and shearing force under depressurization of highly compressed aqueous suspensions [18]. This techniques opens a way for modification of the morphology, topology and rheology of the paper substrate, entrapment of NPs onto it could be tuned as well for maximum optical properties. A variety of NPs have been incorporated with BC nanofiber based organic/inorganic hybrid nanocomposites through different of processes. Almasi *et al.*, 2019 reported CuO impregnated-BC structure by *in-situ* synthesis methods including sonochemical and precipitation methods [19]. An *in-situ* hydrothermal grown Au on BC nanofibers by the assistance of amidoxime groups was also successfully performed for

catalyst production by Chen *et al.*, 2015 [20]. A novel avenue can be built through nanofibrillation methods and optimized NPs immobilization for effective SERS hotspots creation.

One of potentially exploited biodegradable BC-based material is *nata de coco* (NDC), a very popular food product, typically prepared in the form of thick jelly, simply prepared by the fermentation of coconut juice, mostly using *Acetobacter xylinum* [21], [22]. In a tropical country like in Indonesia, The Philippines, Vietnam and other South East Asian countries, NDC is widely produced not only by high-scale industries but in home industry and marketed with a considerably cheap price and therefore, potentially exploited as a new-generation of greenly synthesized thin film material. NDC has been highly regarded potential for its favorable functionalities in many applications such as in the production of optical and transparent substrate, food packaging, tissue scaffolds, smart textiles or substrate coating since it has smaller diameter of nanofiber (less than 100 nm) [23] in comparison with most plant fibers with their microscale diameter and it is capable of absorbing water about 200 times higher than its initial mass [24]. These properties can be prospectively incorporated with NPs entrapment for SERS-active substrate architecture. From noble nanoparticle class, silver nanoparticles (AgNPs) have been greatly noticed as a robust material to prepare SERS substrate pertaining to their high plasmon oscillation damping due to interband transition in the visible light generating highly energetic hot electrons with low energy, so-called localized surface plasmon resonance (LSPR) [25]. In addition, AgNPs attractive properties are reflected by their unique size and shape-dependent electrical, chemical, biological, magnetic and optical properties for a wide range of applications [26]. The embedment of AgNPs in nanopaper transparent substrate can be straightforwardly achieved through many ways such as via immersion or mixing of both organic/inorganic hybrid prior to the paper casting process without the need of linking molecules or reducing agents.

In this study, we are the first to report the production of *nata de coco*-nanopaper with different mechanical fibrillation methods to be incorporated with AgNPs for hybrid SERS-active substrate. The plasmonic papersheet prepared from smaller fiber size through disintegration and high-pressure homogenization processes, later called as plasmonic nanobacterial cellulose (plasmonic NBC) papersheet, showed more prominent SERS characters than the plasmonic Bacterial Cellulose (plasmonic BC) papersheet prepared by normal agitation in high-speed blending due to more effective entrapment of AgNPs as a result of rougher structure and tinier fibrils leading to hotspot formation as well as shown with higher enhancement factor in the detection of Rhodamine 6G (R6G) molecules. The proposed hybrid material had been demonstrated to offer the realization of green technology for cost-effective and simple fabrication of SERS-active substrate alternating the conventional chemical-route, nanostructuring and nanolithography involving harsh chemicals, tedious process and expensive instrumentations. Moreover, the plasmonic paper provides the means of easy-to use, portable, flexible (for roll to roll or swabbing samples) and disposable sensing instrument.

2. Materials and Methods

2.1 Materials

Nata de coco (NDC) gel blocks were purchased from Cianjur (Indonesia). Ascorbic acid and tri-sodium citrate dihydrate, NaOH solution and AgNO₃ were purchased from Merck (Germany). Rhodamine 6G (R6G) was purchased from Sigma Aldrich (USA). The deionized water used in the entire experiments (resistivity at 25°C = 18.2 M Ω •cm) was provided by a MilliQ system.

2.2 Apparatus

The crushing of *NDC* gels was conducted using kitchen blender (Philips, The Netherlands), the oven for the casting process of BC and NBC papersheets was (brand, country). In NBC papersheet preparation, the disintegrator machine was from Psychotron (Japan) and the homogenizer was HJP 25001 from Sugino (Japan). The Particle Size Analyzer (PSA) was Zetasizer Nano ZS from Malvern (UK). Ultra violet (UV) absorption study was conducted using V-670 UV-Vis Spectroscopy from Jasco (Germany). Field Emission Scanning Electron Microscope (FE-SEM) (HITACHI S-4700, Japan) was utilized to capture the morphological characteristics of the *NDC*-based papers. Infrared (IR) absorption was analyzed by Fourier Transform Infrared (FTIR) Spectrometer Nicolet iS 5 Thermo Scientific (USA). Crystallinity analysis was performed with X-ray diffraction (XRD) D8 Advance with LYNXEYE XE-T detector from Bruker (USA). Surface roughness analysis was done with Innova Atomic Force Microscope (AFM) from Bruker (USA). SERS analysis was conducted using RAMaker system from Protrustech.Co, Ltd, (Taiwan), equipped with a charge-coupled device (CCD) camera monitoring set and coupled with Olympus (Japan) microscope body and Andor-Solis for imaging software from Oxford Instruments (UK).

2.3. Methods

2.3.1 Silver nanoparticles (AgNPs) synthesis

The AgNPs were prepared through reduction and stabilization process using ascorbic and citric acids. First, a mixture of 8 mL of 0.6 mM ascorbic acid in aqueous phase and 3 mM tri-sodium citrate dehydrate was fixed to pH 6.0 by the addition of 0.2 mol/L citric acid or 0.1 mol/L NaOH solution. Once the pH fixation accomplished, the solution was carried out for stirring process with a velocity of 900 rpm in a 30°C temperature setup followed by the addition of 0.08 mL of 0.1 M AgNO₃ solution until the color of the solution gradually changed from transparent to yellow and finally to turbid greyish color. The reaction was prolonged to stability for about 15 mins. The prepared liquid was ready to be used for the entire experimental stages.

2.3.2 *Nata de coco* (*NDC*)-based paper production

Initially, blocks of *NDC* gel prepared from coconut juice fermentation with *A. xylinum* were exposed in running water to achieve pH neutrality. The gel blocks were then brought to boiling temperature with 1% w/v NaOH in order to complete the removal of non-cellulosic components and reduction of bacterial cells. In the next stage, the gels were washed again to remove the remaining alkali compounds until pH 7 obtained. The gels were transformed into the form of slurry by an hour crushing process using mechanical blender and subsequently stored in 4°C refrigerator. For nanobacterial cellulose (NBC) papersheet preparation, a 2% w/w suspension of *NDC* was homogenized using a laboratory disintegrator with rotational speed of 1550 rpm for 30 seconds. The fragmented *NDC* was then homogenized using high-pressure homogenizer at 20 MPA for 15 cycles and the slurry was ready for paper casting. For the paper sheet production, a 200 mL of the slurry (BC or NBC) was degassed using a bell jar under a vacuum condition and casted on a teflon-based templating tray at 45°C for overnight. The thin paper-like substrates were subsequently peeled off and ready for the next steps.

The particle size distribution after 60 min ultrasonication was tested with a Malvern Zetasizer Nano ZS instrument (Malvern Instruments Limited, Worcestershire, UK). Samples of nanocellulose suspended in water were diluted and analyzed with a particle size analyzer using dynamic light scattering (DLS). Non Invasive Back Scattering technique was used with a 173°

detector angle using a HeNe 4 mW laser (633 nm). Measurements were repeated 3 times for each sample

2.3.3 SERS-active substrates preparation

The preparation of the SERS-active substrate was done via AgNPs spotting onto both BC and NBC papersheets through drop and dry methods. A 10 μL droplet of AgNPs solution was dropped onto the *NDC*-based papers and stood to dry for about 60 mins at room temperature. Furthermore, in SERS measurement, the analyte of R6G with the volume of 15 μL was dropped right onto the AgNPs spot to allow an even dispersion and uniform adsorption. The process flow of SERS substrate preparation is illustrated in Figure 1.

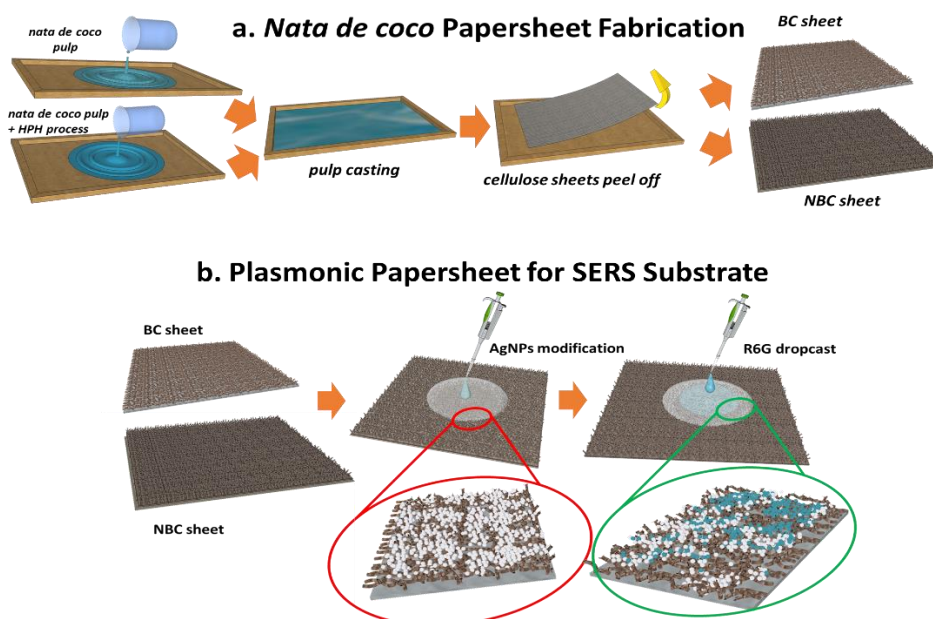


Figure 1a. Process flow of *nata de coco* (NDC) paper-sheet fabrication and b. plasmonic papersheet production for SERS substrate

2.3.4 Characterization of surface morphology, topology, absorption and SERS

To understand the morphology of the *NDC*-based SERS papers, surface observation was completed using FE-SEM with operation system set at 10.000 V accelerating voltage. The topology of the modified substrate was examined using AFM in tapping mode with scanning area of $1 \times 5 \mu\text{m}^2$. The UV absorption properties were studied using UV/vis spectrometer with a wavelength ranging from 200 to 600 nm, while the IR absorption spectra of the fabricated plasmonic papersheets was acquired using Fourier-Transform infrared (FTIR) spectrophotometer over a wavenumber from 4000 to 500 cm^{-1} . The crystallinity observation was carried out using XRD with $\text{Cu K}\alpha = 1.54 \text{ \AA}$, 0.020 degrees steps, and 164 time collections of 60 s per step.

The prepared substrates were carried out to measure the SERS intensity of the R6G Raman probe in a series of concentration ranging from 10^1 - 10^6 pM. A 20 μL of the R6G was placed and air-dried onto the fabricated plasmonic papersheets. SERS analysis was conducted using an excitation wavelength of 473 nm with the excitation and light collection performed with a $100\times$ objective lens (NA 0.5), 100 mW laser power, 2 seconds exposure time, scan coverage of $100 \mu\text{m} \times 100 \mu\text{m}$ area and three accumulation numbers for ten different locations. The data acquisition

and extraction of the baselines were performed using Raman software (Andor-Solis for Imaging, Oxford Instruments). The FDTD simulation for AgNP over the BC sheets was performed by 3D configuration setup by Lumerical software. The AgNP spheres were simulated with the diameter of 50 nm and the optical constant properties was obtained from Sultanova et al., 2009 [27]. The field source for the excitation was simulated using a Gaussian mode with a wavelength range from 400 to 700 nm in the full-width half maximum (FWHM). The mesh configuration is setup with a huge density mesh with a space less than 1 nm, particularly in the boundary region to obtain accurate profile of plasmonic field in the interface of nanoparticles. Finally, 2D monitoring was shown for the sake of simple mapping field of the simulated structures.

3. Results and Discussion

The study was initially conducted for particles size analysis using dynamic light scattering technology. Quantified in 1% w.t (volume fraction) of the aqueous NDC pulp, the NBC through HPH fibrillation technique had produced approximately fifty percent smaller particle size than the BC prepared via conventional grinding methods. The details can be seen in Figure 2a displaying the histogram distribution of the aqueous BC particles at 1194 nm while the NBC at around 617.4 nm. The particle sizes denote the hydrodynamic diameters of equivalent spheres and therefore, they are not the representation of actual physical dimension of the cellulose particles, however, valid for comparison aim [28]. In addition to smaller size, the NBC prepared by HPH system yielded relatively narrower particle size distribution than the BC reflecting more homogenous nano-sized cellulose production in HPH system. Figure 2b and c display the morphological properties of the BC and NBC nanosheets. Aiming the effective creation of SERS hotspots, the nanofibrillation successfully generated higher surface roughness as presented in the higher Rq value of the NBC papersheet with almost twice values than the BC papersheet. During the HPH the NBC sheets formed smaller fibril with more aggregate bundles as a consequence of the high surface hydroxyl group which strongly interact and resulted in agglomeration which ultimately contoured the surface.

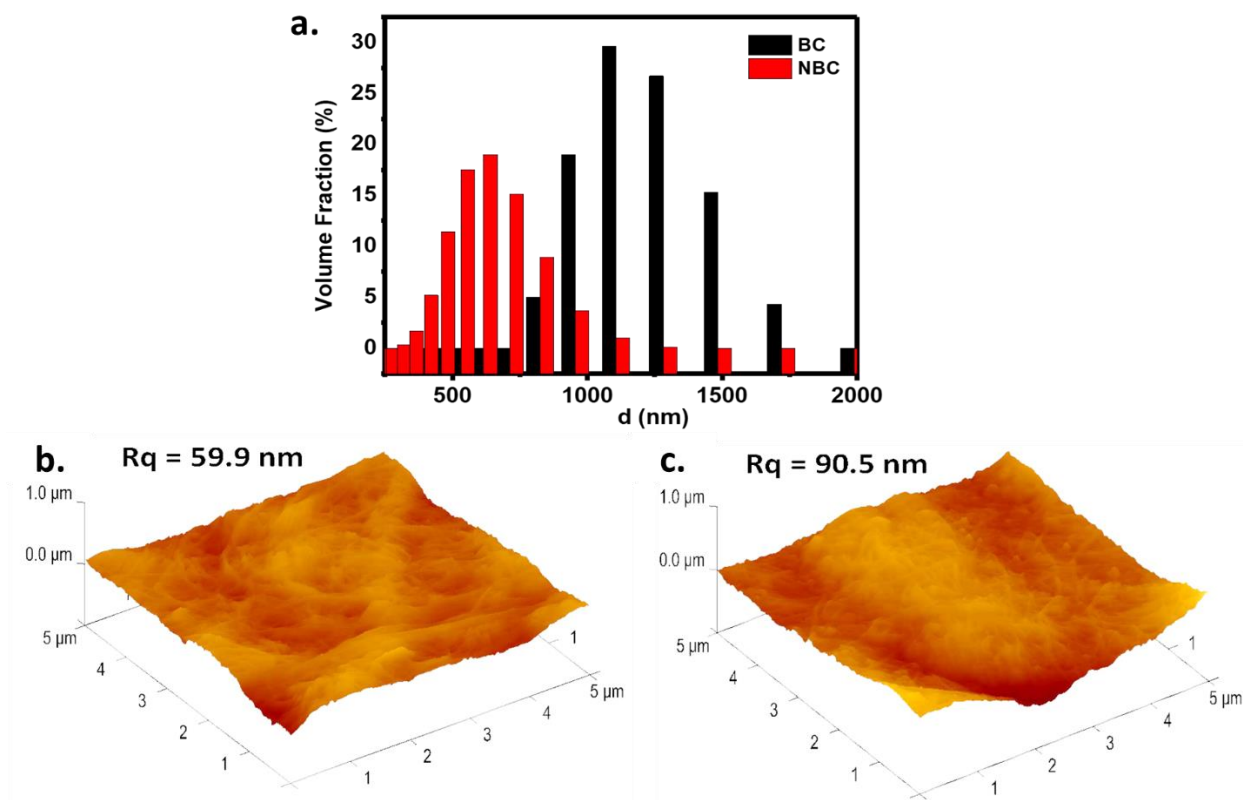


Figure 2a. Particle size distribution and three-dimensional structure showing surface roughness properties of c. BC and d. NBC papersheets

The SEM photographs in Figure 3 show that mechanical fibrillation of NDC slurry influences the topology of the produced papersheets. In Figure 3A, sheet structure of BC carried out in high-speed blending agitation demonstrates considerably larger and longer fibril networks with the tendency of generating much bigger holes on the surface. In a lower resolution observation, the fibrils clump and generated thicker root-like structure with the width of 300 to 500 nm. In contrast with mechanical fibrillation using disintegrator and high-pressure homogenizer (HPH), in Figure 3B, the sheet structure of NBC shows a lot of small pores onto the surface with mostly smaller tangled cellulose ribbons with the width of approximately less than 100 nm per single fiber. The finding is in a good agreement with Lee et al., 2009 who reported that the HPH of microcrystalline cellulose (MCC) resulted in the diameter of the cellulose fibrils majority ranging from 28-100 nm [29]. The pressure and cycles applied in HPH were known to be the major factor for optimum crushing of the fibrils as a result of coalescence and re-coalescence of the cellulose aqueous droplets [30] as well as found in other literature, that increasing both factors proportionally lowering the fibril size and restructuring the fibrous structure in the homogenous cellulose system [31]. Subsequently, in the plasmonic paper development, the drop-cast of AgNPs (particle size of ~ 50 nm) was successful to immobilize the NPs within the fibrillated papersheets. However, in BC papersheet, the AgNPs could not cover the whole substrate well along with the appearance of aggregated NPs as shown in Figure 3C. Whereas, with NBC papersheet structure, the high dispersion of AgNPs could be achieved leading to uniform coverage on the substrate

(Figure 3D) indicating that smaller fibrils facilitate more effective molecular entrapment. These findings are plausibly due to the higher surface area where nano-fibrillated cellulose may typically exhibit the order of 50–70m²/g area [32] along with the higher available surface hydroxyl groups to bind the Ag⁺ ion through chemical bonding which also serve as the seeding material for Ag reduction phenomenon [12].

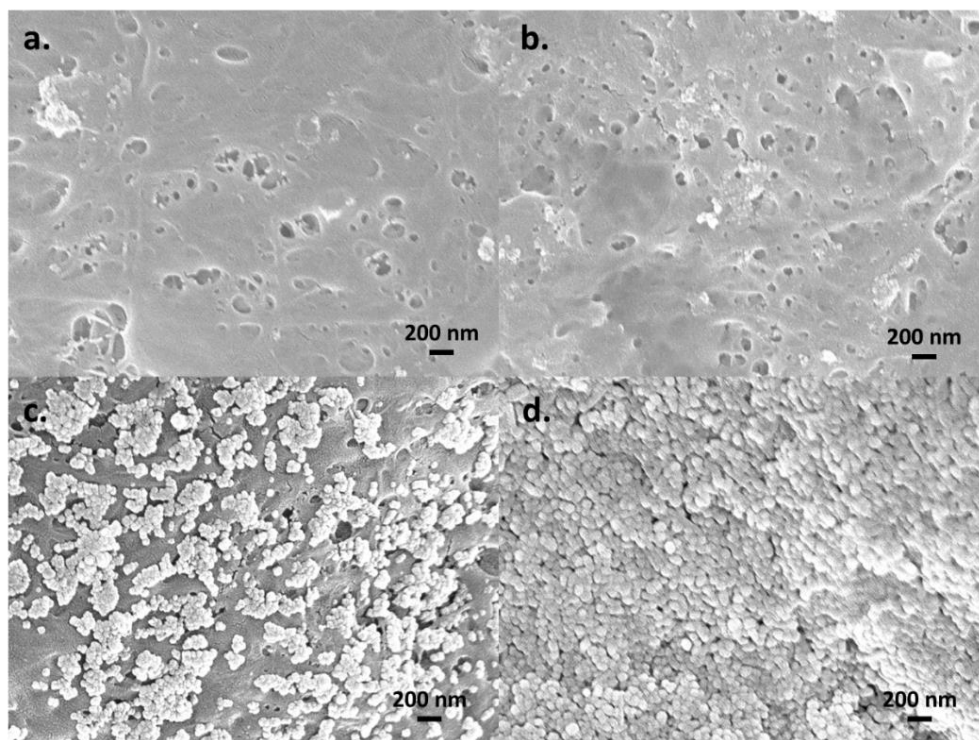


Figure 3. FE-SEM photographs of as prepared a. BC and b. NBC papersheets, and c. BC and d. NBC papersheets after AgNPs decoration (plasmonic papers)

Figure 4a shows the UV-visible spectra of the BC and NBC. The as prepared papersheets display relatively similar absorbance peak at around 255-260 nm, which is in a good agreement with the findings of Cazon *et al.*, 2019 reporting typically low UV-C transmittance peak of the pure BC fiber [33]. However, the HPH process produced higher transparency of the paper sheet which in turn lower the absorbance peak of the bacterial cellulose. This can be elucidated by the emergence of tinier fiber bundles from separated bigger fibrils triggering higher aspect ratio of fiber bundles in thinner fabricated sheet as more homogenization cycle applied [29]. Furthermore, the less pronounced absorbance peak of NBC papersheet can be explained by the shear thinning impact through HPH, implying the large flocculation and agglomerates dispersal by the shear forces [34] which finally resulted in more transparent substrate than BC papersheet. Accordingly, in Figure 4b, after modification, the characteristic peak of AgNPs was successfully recorded at around 420 nm confirming the embedment of the nanoparticles onto the papersheets. A more uniform coverage and higher density of AgNPs trapped in NBC fibril network was validated by a by about 4 times higher absorbance percentage than the AgNPs decorated BC papersheet. The entanglement of finer and thinner fibrils from mechanical HPH treatment in NBC papersheet

preparation triggers more effective penetration and diffusion of AgNPs due to 3D porous fibril insterspacing on the sheet surface.

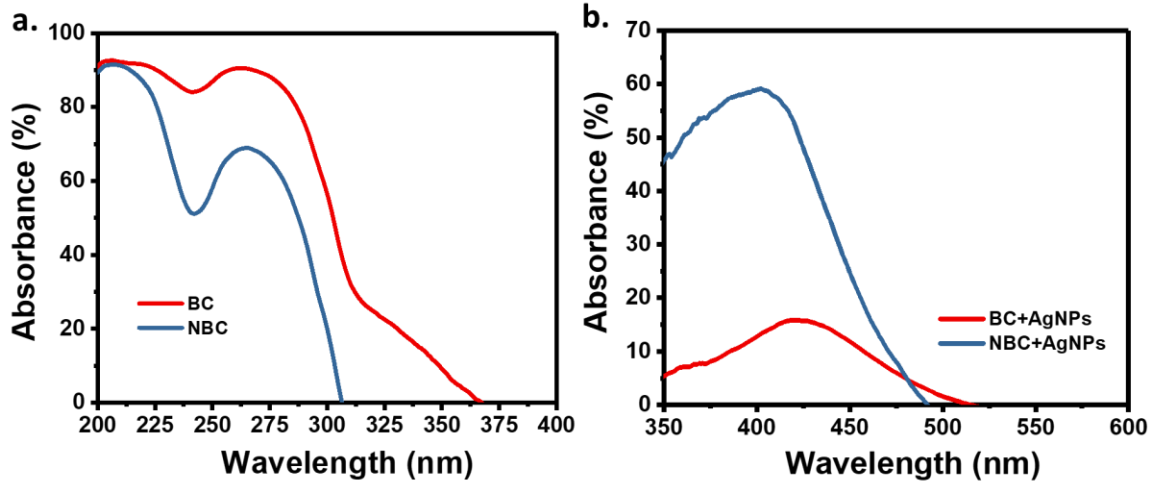


Figure 4. UV-vis absorption spectra of a. as prepared BC and NBC papersheets, and b. AgNPs-decorated BC and NBC papersheets (plasmonic papers)

The incorporation of the AgNPs onto the NDC-based sheet evaluated in the fourier transform infrared (FTIR) spectroscopy analysis is displayed in Figure 5a where in all modification approaches, the most dominant bacterial cellulose absorbance peaks appear at around 3342 cm^{-1} and 2895 cm^{-1} assigning OH stretch of cellulose type I and CH stretching of CH_2 groups, respectively, conspicuously observed. Additionally, some other bacterial cellulose typical ascribed peaks were visibly recorded at 1648 cm^{-1} , 1366 cm^{-1} , 1163 cm^{-1} and 1061 cm^{-1} assigning -CH-, C_O asymmetric stretching vibrations and pyranose ring skeleton vibrations, respectively [35]–[37]. This indicates that the backbone structure of the cellulose remained even after the NPs incorporation. In contrast, with immobilized AgNPs, the most dominant peaks show noticeable decrement of infrared absorbance indicating the AgNPs physical adsorption onto hydroxyl groups over both papersheets [36], [38]. In Figure 5b, in all conditions, the XRD pattern of the typical cellulose persistently appears at broad diffraction peaks at 14.8° (110) and 23.5° (200) assigning to the cellulose I α and I β allomorphs, respectively [39]. This represents that the HPH did not alter the native cellulose type I to other type of cellulose with its native interplanar spacing and thus, may facilitate well the entrapment of AgNPs especially in NBC, as displayed by the intense Ag peaks at around 37° (111), 44.8° (200), 64.7° (220) and 77° (311) reflections of the face-centered cubic metallic silver (JCPDS #76-1393), respectively. The degree of crystallinity of all samples was calculated by Seagal formula:

$$\text{CrI} = 100\% \times ((I_{002} - I_{\text{am}}) / I_{002})$$

where I_{002} is the major diffraction peak at $2\theta = 22.6^\circ$ and I_{am} is the intensity of the amorphous counterpart measured at $2\theta = 18^\circ$. Moreover, the average crystallite size of the nanocrystalline cellulose was estimated according to Scherrer's method as follows:

$$\langle L_{002} \rangle = k \lambda / \beta \cos \theta$$

where L_{002} is the average crystallite size, k is the shape factor (0.9), λ is the X-ray wavelength, β is full width half-maxima (FWHM) from the XRD peak in radians and θ is the Bragg angle corresponding to the intense (002) diffraction peak. The calculated values of crystallinity index, FWHM and the average crystallite size of all samples are presented in Table 1. Even though the HPH did not change the conformation of the cellulosic phase, this mechanical process was found to decrease the crystallinity index of the cellulose as seen in NBC papersheets before and after modification with AgNPs in comparison with that in BC papersheet group. The shearing and agitation during nanofibrillation with HPH process had caused crushing effects on the native cellulose crystals.

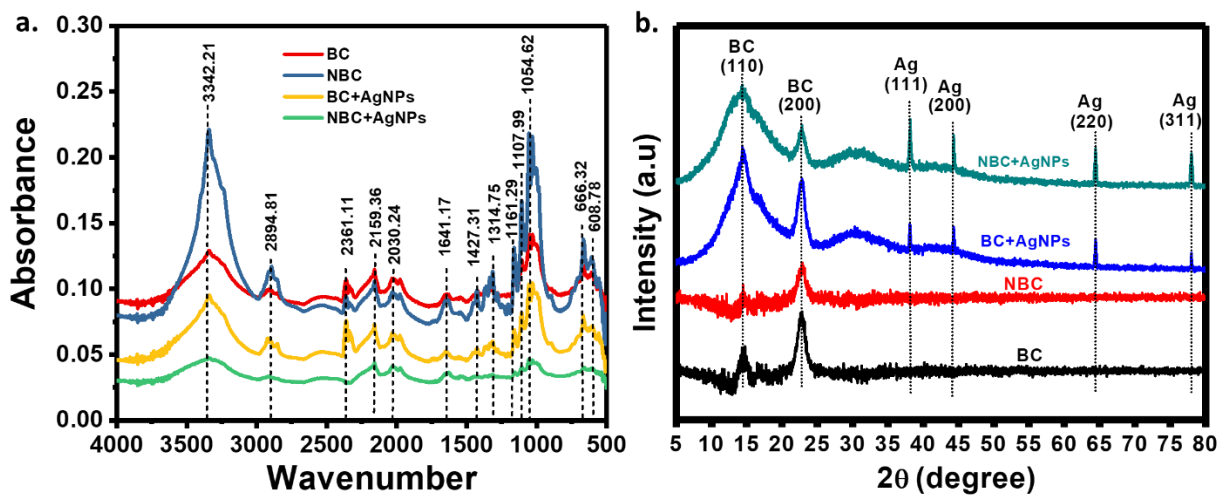


Figure 5. a. IR spectra and b. X-ray diffraction pattern of as prepared and plasmonic BC and NBC papersheets

Table 1. XRD physical properties of the fabricated papersheet SERS substrates

Sample	CrI(%)	FWHM	$\langle D_{av} \rangle$ (nm)
BC	71.31	1.38	5.73 ₍₂₀₀₎
NBC	43.32	1.43	5.87 ₍₂₀₀₎
BC+AgNPs (plasmonic BC)	62.17	1.20	6.40 ₍₂₀₀₎
NBC+AgNPs (plasmonic NBC)	33.41	1.46	5.46 ₍₂₀₀₎

Both plasmonic papersheets were then applied in SERS detection of a series of R6G molecules concentration due to its strong Raman resonance at molecular level when excited into its matching absorption band. Figure 6 represents better sensitivity of R6G molecules detection using the plasmonic NBC than the plasmonic BC. Principal peaks of R6G molecules were seen clearly at c.a. 1330 and 1574 cm^{-1} assigning for the in-plane vibration of C-H bonds. Other small peaks with the appearance at below 1300 cm^{-1} are attributed to the typical BC peaks [40]. The plasmonic NBC papersheet through HPH treatment clearly resulted in stronger SERS hotspots with

nanogaps of about 10 nm (Figure 3d), pertaining to high aspect ratio and high density of the tiny fiber bundles capturing more AgNPs and reinforcing localized surface plasmon resonance (LSPR) effects [41]. In such hotspots configuration, in regards with the photonics particles size and interstitial distance, the intraband transition was easier to occur when Ag is exposed to photons, yielding holes and super energetic hot electrons that can cross the Schottky energy barrier [42].

Additionally, the HPH process was noticed to maintain the textural properties of the NBC in which the firmness (in relation with water absorbance capacity) of the fibrils was not impacted by high pressure in the present setup in which in a good agreement with the report of Lin *et al.*, 2015 on BC aqueous suspension evaluation [31]. Hence, the immobilization of AgNPs and ultimately, the penetration of R6G molecules onto the plasmonic NBC papersheet could be facily performed. In R6G series screening, a typical Raman band at 1576 cm^{-1} attributed to strong aromatic carbon bond had shown the most conspicuous peak and was plotted in calibration curves where 10 different point positions were taken to ascertain the uniform distribution of the AgNPs on both plasmonic BC and NBC papersheet, as well as the low deviated signals presented in Figure 6c and d. With the dynamic range of 10^{-12} to 10^{-6} M of R6G, the NBC sheet with its high SERS hotspots has exhibited more than 50% higher sensitivity than the BC sheet and with the limitation of detection (LoD) of 92 fM as calculated based on the lowest concentration of the R6G producing the alteration in the Raman intensity signal equal to the three times the average background signals without the presence of R6G.

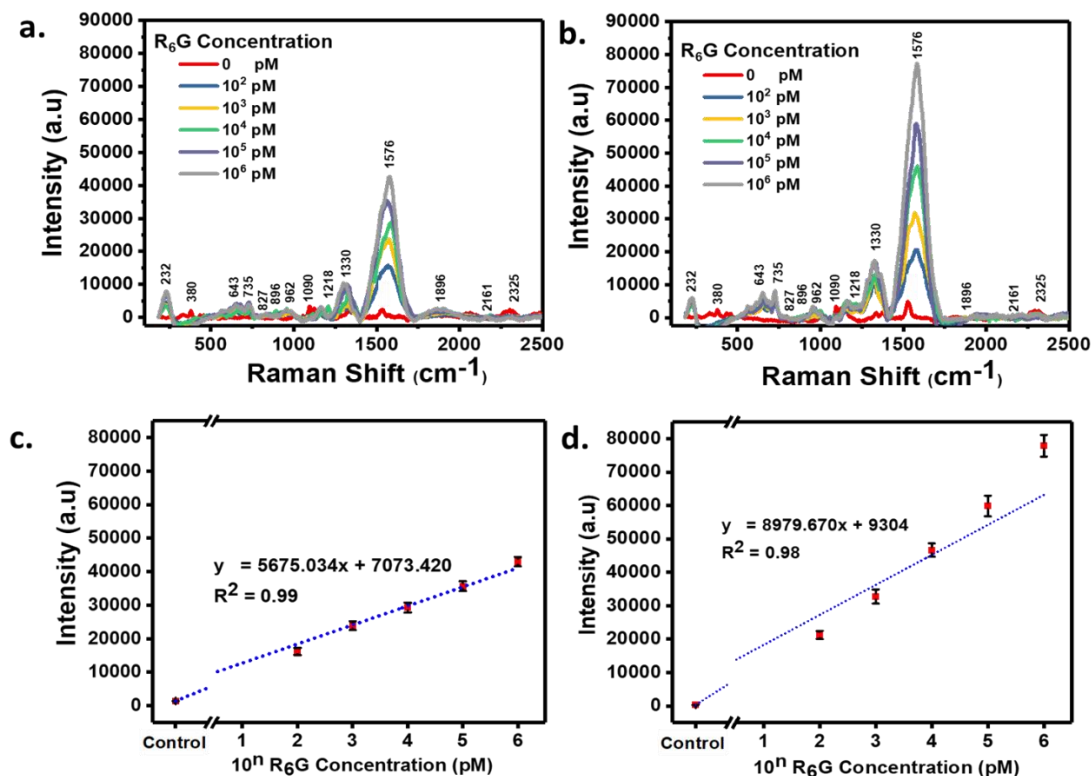


Figure 6. SERS spectra and calibration curves for a series of R6G dynamic range detection on a. and c. plasmonic BC and b. and d. plasmonic NBC papersheets for R6G detection

The intensity of the strongest R6G Raman peak at 1576 cm⁻¹ was later applied in the calculation of SERS enhancement factor (EF_{SERS}) with the formula below:

$$EF_{SERS} = \frac{I_{SERS}/N_{SERS}}{I_{R6G\text{ norm}}/N_{R6G\text{ norm}}}$$

where I_{SERS} and $I_{R6G\text{ norm}}$ refer to the SERS and normal Raman intensities of R6G molecules, whilst, N_{SERS} and $N_{R6G\text{ norm}}$ are the number in average of R6G molecules in the scattering volume (V) giving out both normal and Raman intensities [40], [43]. In Figure 7a and b, effective SERS hotspots from AgNPs entrapment within the cellulose fibrils was apparent in comparison with the as prepared BC and NBC papersheets with optimum spike in the plasmonic NBC which reflects favourable effects of HPH for molecular capture. In our calculation using the depth of field approach (Supp material, Table S1), the EF_{SERS} of the plasmonic NBC papersheet was 1.23 x 10⁵. This value showed effective enhancement by two orders as being compared to the EF_{SERS} of BC papersheet. This is in line with the findings of the morphological structure and SERS intensity as a consequence of the robust hotspots in plasmonic NBC papersheet resulted from HPH. To comprehend the impact of nanofibrillation in the construction of SERS active hotspots inducing electromagnetic (EM) field enhancement, we performed a finite difference time domain (FDTD) simulation as displayed in Figure 7c and d. The EM distribution of 50 nm size of AgNPs used in this study was simulated on a sparse mesh density and highly dense meshed mimicking the textural properties of the plasmonic BC and NBC papersheets, respectively. The closer interparticles distance arranged on the NBC with nearly 10 nm far exhibits greater EM field localization amplifying the oscillation of the electron clouds surrounding the AgNPs for optimum SERS enhancement factor than the BC sheet with sparser particle-to-particle distance. It was observed that the field enhancement amplitude exponentially decreased as the nanogap is farther. Additionally, it can be assumed that the EM distribution of each single AgNPs was contributed from AgNPs interfaces of cellulose and air concerning the normal photon direction to the AgNPs/nanosheet interfacial layer. The density of the particles were taken into important account where in our setup, approximately more than 100 particles were confined in a single laser spot of 78.5 μm², thus it the average signals produced from the nanogaps could be confirmed as uniform Raman signal from the nanosheet substrate. This simulation result is indicative to the potential niche and fitness of nanostructuring green-synthesized materials as an alternative for rigid solid state inorganic materials, such as silicon or glass, as excellent SERS paper substrate.

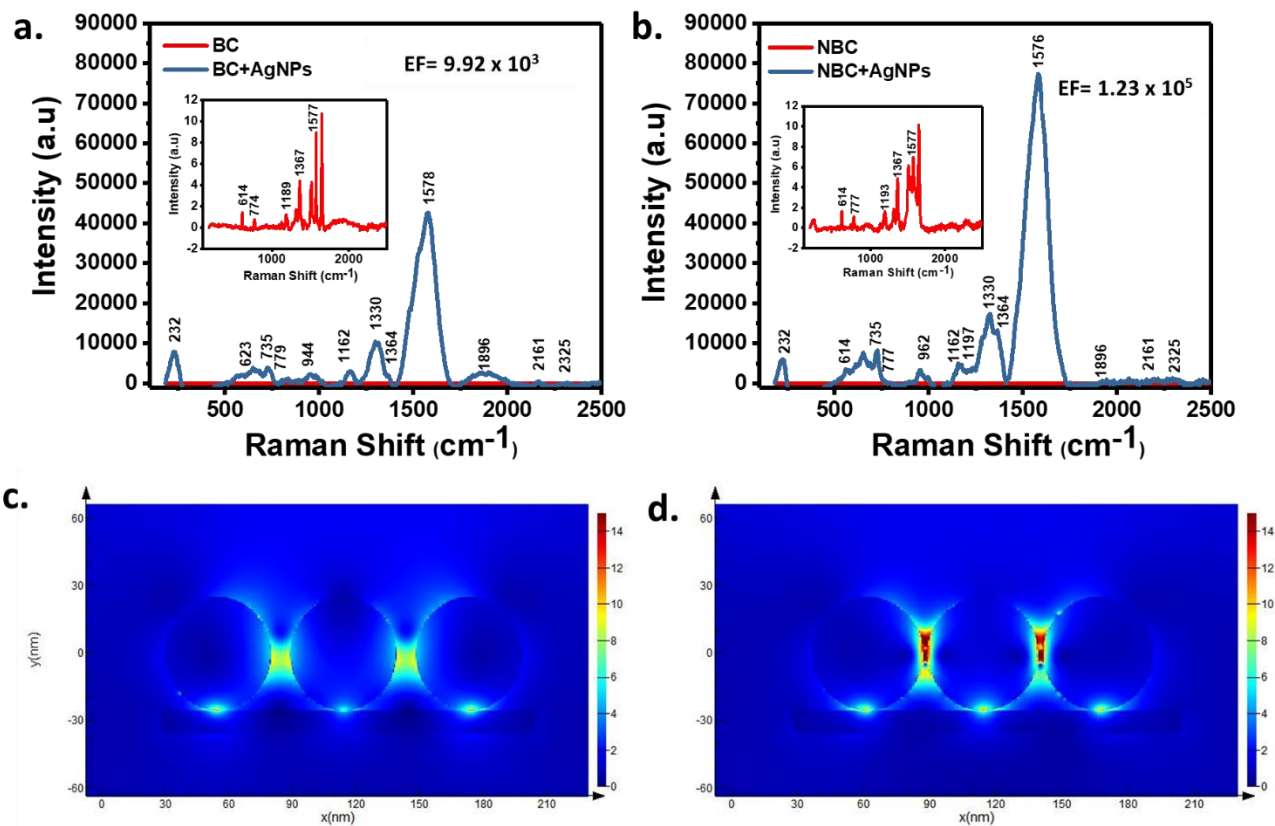


Figure 7. SERS signal comparison of a. BC and b. NBC papersheets with and without AgNPs decoration for 1 μM R6G measurement with respective normal Raman spectrum of 1 μM R6G depicted on each inset and two-dimensional FDTD simulation of EM field distribution on plasmonic a. BC and b. NBC papersheets

4. Conclusion

A simple and low-cost nanostructuring of natural and abundant polymer was realized to build up an enticing alternative for SERS paper substrate. Nanofibrillation involving high pressure homogenization of ubiquitous cellulose-based food products such as *nata-de-coco* yielding Nanobacterial Cellulose (NBC) papersheet effectively reinforced the SERS hotspots creation after the integration of AgNPs, due to morphological, textural, and crystallinity alteration of the cellulose. Immense reinforcement of SERS signal studied in R6G molecules detection was mainly resulted by the high surface roughness from dense and tangled nanocellulose ribbons by the mechanical pressure and cycles throughout the nanofibrillation process without changes of the fibril stiffness which ultimately provided more surface hydroxyl group and larger surface anchoring site for AgNPs. As compared to the naturally grown bacterial cellulose (BC), the NBC sheet effectively generated sub-nanometer interparticle spacing for a strong electromagnetic field localization favoring SERS enhancement as proven by more than 50% sensitivity enhancement in the screening of R6G as well as two-order higher enhancement factor. In addition, The NBC sheet SERS paper sensor has paved a novel path toward an easy in-situ nanostructuring which can be combined with a varieties of technique including dip coating, spraying, electrospinning towards highly ordered nanoparticles for more intense SERS enhancement.

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6. References

- [1] J. Xu, J. Xu, D. Wu, Y. Li, Z. Gao, and Y. Y. Song, “Plasmon-Triggered Hot-Spot Excitation on SERS Substrates for Bacterial Inactivation and in Situ Monitoring,” *ACS Appl. Mater. Interfaces*, vol. 1025, no. 30, pp. 25219–25227, 2018.
- [2] L. Chen *et al.*, “All-optical tunable plasmonic nano-aggregations for surface-enhanced Raman scattering,” *Nanoscale*, vol. 11, no. 28, pp. 13558–13566, 2019.
- [3] V. Stancovski and S. Badilescu, “In situ Raman spectroscopic-electrochemical studies of lithium-ion battery materials: A historical overview,” *J. Appl. Electrochem.*, vol. 44, no. 1, pp. 23–43, 2014.
- [4] W. Wang, Y. F. Huang, D. Y. Liu, F. F. Wang, Z. Q. Tian, and D. Zhan, “Electrochemically roughened gold microelectrode for surface-enhanced Raman spectroscopy,” *J. Electroanal. Chem.*, vol. 779, pp. 126–130, 2016.
- [5] J. V. Perales-Rondon, S. Hernandez, D. Martin-Yerga, P. Fanjul-Bolado, A. Heras, and A. Colina, “Electrochemical surface oxidation enhanced Raman scattering,” *Electrochim. Acta*, vol. 282, pp. 377–383, 2018.
- [6] A. Purwidyantri, C. H. Hsu, C. M. Yang, B. A. Prabowo, Y. C. Tian, and C. S. Lai, “Plasmonic nanomaterial structuring for SERS enhancement,” *RSC Adv.*, vol. 9, no. 9, pp. 4982–4992, 2019.
- [7] V. T. N. Linh *et al.*, “A facile low-cost paper-based SERS substrate for label-free molecular detection,” *Sensors Actuators, B Chem.*, vol. 291, no. April, pp. 369–377, 2019.
- [8] D. Ding, Q. Fan, K. Liu, and S. Ding, “Au/Ag alloy nanostructure with built-in hotspots fabricated by galvanic-replacement-assisted growth on AgI for surface-enhanced Raman spectroscopy,” *J. Alloys Compd.*, vol. 809, p. 151677, 2019.
- [9] S. S. Limei Tian, Qisheng Jiang, Keng-Ku Liu, Jingyi Luan, Rajesh R. Naik, “Bacterial Nanocellulose-Based Flexible Surface Enhanced Raman Scattering Substrate,” *Adv. Mater. Interfaces*, vol. 3, no. 15, p. 1600214, 2016.
- [10] S. P. Lin, I. Loira Calvar, J. M. Catchmark, J. R. Liu, A. Demirci, and K. C. Cheng, “Biosynthesis, production and applications of bacterial cellulose,” *Cellulose*. 2013.
- [11] H. S. Barud *et al.*, “Bacterial Cellulose,” in *Biomaterials from Nature for Advanced Devices and Therapies*, 2016.
- [12] S. Pal, R. Nisi, M. Stoppa, and A. Licciulli, “Silver-Functionalized Bacterial Cellulose as Antibacterial Membrane for Wound-Healing Applications,” *ACS Omega*, vol. 2, no. 7, pp. 3632–3639, 2017.
- [13] P. Paximada, E. A. Dimitrakopoulou, E. Tsouko, A. A. Koutinas, C. Fasseas, and I. G.

- Mandala, "Structural modification of bacterial cellulose fibrils under ultrasonic irradiation," *Carbohydr. Polym.*, vol. 150, pp. 5–12, 2016.
- [14] M. Mahardika, H. Abrial, A. Kasim, S. Arief, and M. Asrofi, "Production of nanocellulose from pineapple leaf fibers via high-shear homogenization and ultrasonication," *Fibers*, vol. 6, no. 2, 2018.
 - [15] H. Taheri and P. Samyn, "Effect of homogenization (microfluidization) process parameters in mechanical production of micro- and nanofibrillated cellulose on its rheological and morphological properties," *Cellulose*, vol. 3, no. 2, pp. 1221–1238, 2016.
 - [16] E. Espinosa, F. Rol, J. Bras, and A. Rodríguez, "Production of lignocellulose nanofibers from wheat straw by different fibrillation methods. Comparison of its viability in cardboard recycling process," *J. Clean. Prod.*, vol. 239, p. 118083, 2019.
 - [17] J. Li *et al.*, "Homogeneous isolation of nanocellulose from sugarcane bagasse by high pressure homogenization," *Carbohydr. Polym.*, vol. 90, no. 4, pp. 1609–1613, 2012.
 - [18] Z. Guangyin and Z. Youcai, "Harvest of Bioenergy From Sewage Sludge by Anaerobic Digestion," in *Pollution Control and Resource Recovery for Sewage Sludge*, Butterworth Heinemann, 2017, pp. 181–273.
 - [19] H. Almasi, L. Mehryar, and A. Ghadertaj, "Characterization of CuO-bacterial cellulose nanohybrids fabricated by in-situ and ex-situ impregnation methods," *Carbohydr. Polym.*, vol. 222, no. February, p. 114995, 2019.
 - [20] Meiyang Chen, H. Kang, Y. Gong, J. Guo, H. Zhang, and R. Liu, "Bacterial Cellulose Supported Gold Nanoparticles with Excellent Catalytic Properties," *ACS Appl. Mater. Interfaces*, vol. 7, no. 39, pp. 21717–21726, 2015.
 - [21] D. Onggo, I. Mulyani, F. J. Valverde-Muñoz, J. A. Real, and G. Molnar, "Bistable thermo-chromic and magnetic spin crossover microcrystals embedded in nata de coco bacterial cellulose biofilm," *Cellulose*, vol. 24, no. 5, pp. 2205–2213, 2017.
 - [22] M. Velásquez-Riaño and V. Bojacá, "Production of bacterial cellulose from alternative low-cost substrates," *Cellulose*, vol. 24, no. 7, pp. 2677–2698, 2017.
 - [23] K. Potivara and M. Phisalaphong, "Development and Characterization of Bacterial Cellulose Reinforced with Natural Rubber," *Mater. (Basel)*, vol. 12, no. 14, p. 2323, 2019.
 - [24] D. Klemm, D. Schumann, U. Udhardt, and S. Marsch, "Bacterial synthesized cellulose - Artificial blood vessels for microsurgery," *Prog. Polym. Sci.*, vol. 26, no. 9, pp. 1561–1603, 2001.
 - [25] M. Kang, M. Losego, E. Sachet, J. P. Maria, and S. Franzen, "Near-Infrared Optical Extinction of Indium Tin Oxide Structures Prepared by Nanosphere Lithography," *ACS Photonics*, vol. 3, no. 10, pp. 1993–1999, 2016.
 - [26] N. Pourreza, H. Golmohammadi, T. Naghdi, and H. Yousefi, "Green in-situ synthesized silver nanoparticles embedded in bacterial cellulose nanopaper as a bionanocomposite plasmonic sensor," *Biosens. Bioelectron.*, vol. 74, pp. 353–359, 2015.

- [27] N. Sultanova, S. Kasarova, and I. Nikolov, "Dispersion properties of optical polymers," *Acta Phys. Pol. A*, vol. 116, p. 585, 2009.
- [28] M. Voronova, N. Rubleva, N. Kochkina, A. Afineevskii, A. Zakharov, and O. Surov, "Preparation and characterization of polyvinylpyrrolidone/cellulose nanocrystals composites," *Nanomaterials*, vol. 8, no. 12, p. 1011, 2018.
- [29] S. Lee, S. Chun, I. Kang, and J. Park, "Journal of Industrial and Engineering Chemistry Preparation of cellulose nanofibrils by high-pressure homogenizer and cellulose-based composite films," *J. Ind. Eng. Chem.*, vol. 15, pp. 50–55, 2009.
- [30] Y. Wang *et al.*, "Homogeneous isolation of nanocellulose from eucalyptus pulp by high pressure homogenization," *Ind. Crops Prod.*, vol. 104, no. April, pp. 237–241, 2017.
- [31] D. Lin, R. Li, P. Lopez-Sanchez, and Z. Li, "Physical properties of bacterial cellulose aqueous suspensions treated by high pressure homogenizer," *Food Hydrocoll.*, vol. 44, pp. 435–442, 2015.
- [32] K. Missoum, M. N. Belgacem, and J. Bras, "Nanofibrillated cellulose surface modification: A review," *Materials (Basel)*, vol. 6, no. 5, pp. 1745–1766, 2013.
- [33] P. Cazón, M. Vázquez, and G. Velazquez, "Composite Films with UV-Barrier Properties Based on Bacterial Cellulose Combined with Chitosan and Poly(vinyl alcohol): Study of Puncture and Water Interaction Properties," *Biomacromolecules*, vol. 20, no. 5, pp. 2084–2095, 2019.
- [34] V. Calabrese *et al.*, "Surfactant controlled zwitterionic cellulose nanofibril dispersions," *Soft Matter*, vol. 14, no. 38, pp. 7793–7800, 2018.
- [35] R. T. A. Machado *et al.*, "Komagataeibacter rhaeticus as an alternative bacteria for cellulose production," *Carbohydr. Polym.*, vol. 152, pp. 841–849, 2016.
- [36] Y. Xie *et al.*, "The antibacterial stability of poly(dopamine) in-situ reduction and chelation nano-Ag based on bacterial cellulose network template," *Appl. Surf. Sci.*, vol. 491, pp. 383–394, 2019.
- [37] D. N. Phan *et al.*, "Synthesis and attachment of silver and copper nanoparticles on cellulose nanofibers and comparative antibacterial study," *Cellulose*, vol. 26, no. 11, pp. 6629–6640, 2019.
- [38] A. K. Bhardwaj *et al.*, "Power and time dependent microwave assisted fabrication of silver nanoparticles decorated cotton (SNDC) fibers for bacterial decontamination," *Front. Microbiol.*, vol. 8:330, 2017.
- [39] Z. Ling, S. Chen, X. Zhang, K. Takabe, and F. Xu, "Unraveling variations of crystalline cellulose induced by ionic liquid and their effects on enzymatic hydrolysis," *Sci. Rep.*, vol. 7, p. 10230, 2017.
- [40] A. Purwidyantri, I. El-Mekki, and C.-S. Lai, "Tunable Plasmonic SERS Hotspots on Au-Film over Nanosphere by Rapid Thermal Annealing," *IEEE Trans. Nanotechnol.*, vol. 16, no. 4, pp. 551–559, 2017.

- [41] A. K. Gupta *et al.*, “Au-spotted zinc oxide nano-hexagonrods structure for plasmon-photoluminescence sensor,” *Sensors Actuators, B Chem.*, vol. 290, no. February, pp. 100–109, 2019.
- [42] M. Valenti *et al.*, “Hot Carrier Generation and Extraction of Plasmonic Alloy Nanoparticles,” *ACS Photonics*, vol. 4, p. 1146–1152, 2017.
- [43] N. Mhlanga, T. Domfe, and A. Skepu, “Applied Surface Science Fabrication of surface enhanced Raman spectroscopy substrates on solid supports,” *Appl. Surf. Sci.*, vol. 476, no. August 2018, pp. 1108–1117, 2019.