Nanocellulose as Novel Vaccine Adjuvant: Innate Immune Activation and Biocompatibility

Lingzhen Wang *

Western Reserve Academy, Hudson, OH, United States

* Corresponding Author Email: loretta.wang2025@gmail.com

Abstract. Aluminum remains the most widely accepted adjuvant to enhance the immunogenicity of vaccines. However, the clinical use of Aluminum is limited by its neurotoxicity and risk of immunoglobulin E production. Thus, alternative adjuvants with equivalent capability but higher biocompatibility are urgently needed. Among them, nanocellulose is a promising candidate for this purpose. In spite of their biodegradability, their physicochemical properties, including fibrillar nature, crystalline phase, and surface reactivity, affect their activation kinetics, and the relationship remains mostly unknown. Hence, I established a small library of nanocellulose materials using acid hydrolysis to obtain three CNCs with different sizes, aiming to investigate how the size of nanocellulose influences their biocompatibility and immunogenicity. I evaluated their proinflammatory effect on THP-1 cells, a monocyte cell line isolated from the peripheral blood of an acute monocytic leukemia patient, and J774, a murine macrophage cell. Then, I tested their cytotoxicity to both cells and compared their differential effects in IL-1β production. Finally, I correlated their proinflammatory adjuvant effects with their length. In conclusion, I found the production of IL-1β is CNC length-dependent but in a nonlinear relationship. By testing cell viability after stimulation with adjuvants, I found CNCs are a more biocompatible adjuvant than Alum, and the cytotoxicity of CNCs is also size-dependent. This study provides a preliminary demonstration of the optimal length range and crystallinity of CNCs that could induce the most adjuvant effects without posing cytotoxicity.

Keywords: vaccine adjuvant, nanocellulose, innate immunity, toxicity.

1. Introduction

1.1. Vaccination and adjuvant

Vaccination is one of the most prominent approaches to prevent infectious diseases. Over the past decades, vaccines have been expanded to treat non-infectious diseases such as cancer or neurodegeneration in order to satisfy public needs. Adjuvants are supporting components in vaccines that improve and prolong immune response during vaccination practices.(*1*) Until recently, just a few adjuvants were employed in vaccinations, with aluminum salts being the most widely utilized(*2*). Adjuvants boost proinflammatory effects by interacting with innate immune cells and eliciting the release of cytokines and chemokines, which leads to increased recruitment of immune cells and duration of immune response. The adjuvants facilitate in activation of pattern recognition receptors (PRRs) that are expressed by the recruited antigen-presenting cells (APCs)(*3*).

1.2. Aluminum adjuvant and its limitations

Aluminum remains the most widely used adjuvant in vaccines, because of its unparalleled ability to elicit immune response. However, it was demonstrated in recent studies to be a neurotoxin leading to neuronal apoptosis, motor abnormalities, and substantial cognitive deficits(*4, 5*). In addition to its neurotoxicity, aluminum also affects the homeostasis of the immune system. As reported by several preliminary studies, aluminum adjuvants may create major immunological difficulties in humans(*6*). In addition, aluminum in adjuvant form particularly poses a risk of autoimmune diseases, chronic brain inflammation, and associated neurological problems, and thus possibly results in broad negative health consequences(*7*). Aluminum impairs cognitive functions after entering human bodies, in order to avoid the undesired toxicity resulting from aluminum, alternative adjuvants are being actively explored(*5*).

1.3. Alternative adjuvant for aluminum

Accompanied by a growing body of research highlighting the adverse effects of alum adjuvants, such as autoimmunity and neurotoxicity, the need for the development of effective and safe vaccine adjuvants has become a pervasive yet underappreciated concern(*8*). In pursuit of more potent antigen-specific and innate responses, researchers have frequently investigated nanomaterials-based vaccine adjuvants. However, a significant limitation of most nano-vaccine adjuvants lies in their metal-based composition, which inherently exhibits cytotoxic properties, leading to their restricted use. The stable nature and poor biodegradability of these substances result in their accumulation within specific cells and organs, posing risks to human health(*9*). Consequently, the development of biocompatible vaccine adjuvants becomes imperative.

Biocompatible materials, such as cellulose, are gaining increasing popularity within the adjuvant markets due to their favorable attributes. Nanocellulose, in particular, poses minimal environmental and human health risks, along with distinct physiochemical characteristics that are of interest to various industries and businesses. Notably, the medical field has shown heightened interest in the technological advancement of nanocellulose Increased interest in the technological development of these materials in the medical disciplines of wound healing, dialysis membranes, or food additives is an example of this(*10*).

Though established studies have discussed the biodegradability of nanocellulose, nanocellulose performance in human cells and animal cells has not been widely researched. If nanocellulose is more biocompatible and possesses less cytotoxicity than common vaccine adjuvants such as alum and monosodium urate (MSU), its stimulation of adjuvant effect and the extent of innate immune response will be worth researching. This information can aid the development of novel immune-stimulating adjuvants with improved biocompatibility if nanocellulose can elicit an innate immune response while simultaneously being more nontoxic than alum and MSU adjuvants.

1.4. Nanocellulose and its physiological properties

Accompanied by the advancement of modern technology such as nanotechnologies and molecular biology, the research for discovering and inventing biocompatible adjuvants that pose fewer harms and potential risks than aluminum is ongoing. Nanocellulose rises to be one of the candidates.

Cellulose is the most prevalent carbohydrate in plants. It is a safe biomaterial for the human body because it is biocompatible and biodegradable. Nanocellulose is a one-of-a-kind material because of its intriguing intrinsic features, which include high mechanical strength, large surface area, unique optical and rheological properties, and ease of surface modification. Nanocelluloses are classified into three types: cellulose nanocrystals (CNC), cellulose nanofibrils (CNF), and bacterial cellulose (BC)(*11*). CNC is derived from native cellulose by removing amorphous areas and preserving the highly crystalline structure. CNF is made up of both individual and aggregated nanofibrils formed of alternating crystalline and amorphous cellulose domains, giving it a soft and lengthy chain shape. BC is often synthesized in a pure form by bacteria do does not require extensive processing to eliminate undesirable impurities.

1.5. Production and modification of CNC

CNCs are harvested from native cellulose by hydrolysis. Acid hydrolysis, cellulases, TEMPO (2,2,6,6-tetramethyl piperidine-1-oxyl)-mediated oxidation, and ionic liquids have all been used to create cellulosic nanoparticles(*11*). As Figure 1 shows, acid hydrolysis was used to eliminate the amorphous portions in the crystalline and those entangled with microfibrils, leaving the crystalline sections intact. During acid hydrolysis, the degree of polymerization (DP) of cellulose decreases rapidly until it stabilizes. The level-off degree of polymerization is the point at which depolymerization stabilizes (LODP)(*12*). The initial drop in DP is thought to be owing to the fast hydrolysis of amorphous areas caused by a fringed micelle-like structure of microfibrils, including amorphous and crystalline regions(*13*). Depending on the cellulose raw material and the preparation

time, the CNC's diameter and length will vary, and the hydrolysis time and the length are inversely related (*14*).

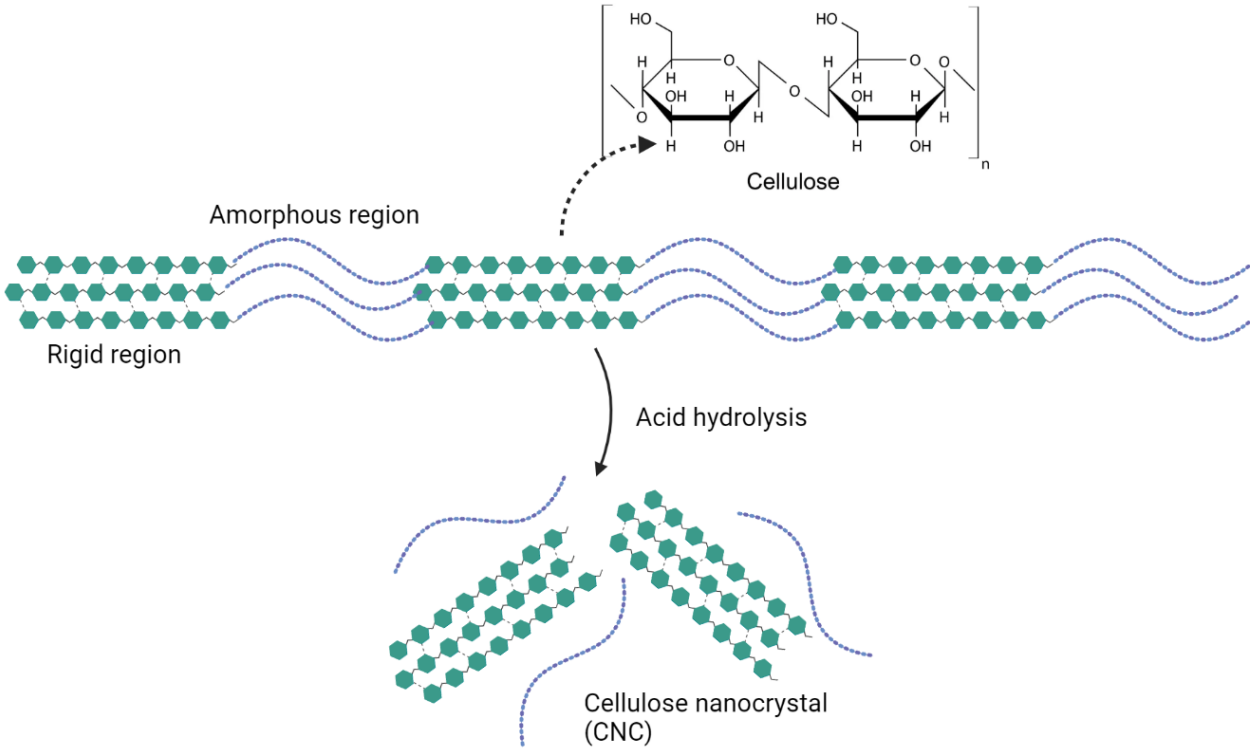

Figure 1. Details of the preparation of CNC by acid hydrolysis. Acid hydrolysis reduces the quantity of polymerization in raw nanocellulose while controlling the hydrolysis period to produce nanocelluloses with varying material lengths. This was done by removing the amorphous portion and interwoven microfibrils.

1.6. Nanocellulose as vaccine adjuvant

Once administered into the body, nanocellulose predominantly interacts with many innate immune system components, including neutrophils, monocytes, macrophages, dendritic cells, and natural killer cells(*2*). Nanocellulose has also been reported to induce adaptive immune responses(*15*). Nanocellulose activates innate immunity via the production of interleukin-1β (IL-1β)(*16*). Phagocytic uptake offers nanocellulose access to the lysosomal compartment, where close contact with reactive material surfaces might destabilize the lysosomal membrane, resulting in cathepsin B release and NLRP3 inflammasome formation (*17*). These intracellular processes can potentially cause pro-inflammatory and immunogenic consequences in the innate immune system. Finally, an activated inflammasome cleaves pro-caspase 1, releasing active caspase 1(*18*). The caspase-1 enzyme then converts pro-interleukin-1-β (IL-1β) to active form. These interleukins have a role in initiating inflammation and the immune response that protects against pathogens(*19*).

The design of the nanoparticle determines how it interacts with serum proteins and cell membrane receptors, which impacts cell uptake, gene expression, and toxicity. Various circumstances in which nanoparticles might interact with the cell surface membrane are depicted in Figure 2. The size of a nanomaterial within a specific geometric form determines how it interacts with serum proteins and cell membrane receptors, which in turn affects cell uptake, gene expression, and toxicity(*20*). The membrane-wrapping procedure is probably connected to the size-dependent absorption of nanoparticles. Compared to larger nanoparticles, small nanoparticles interact with receptors less(*21*).

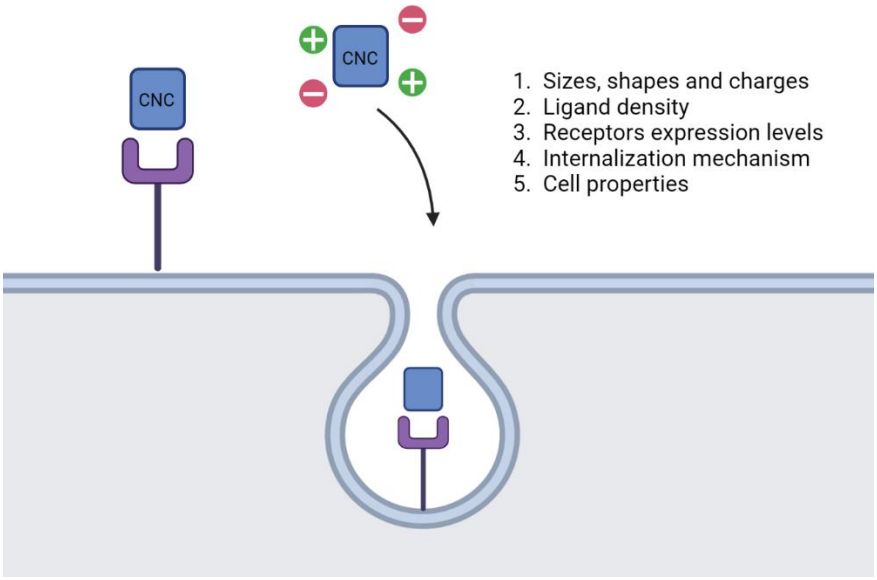

Figure 2. The impacts of physiochemical protein on toxicity, which include size, ligand density, receptor expression levels, internalization mechanism, and cell properties.

Our project aims to prove the adjuvant potential of a novel material – nanocellulose, which possesses health and cost advantages over the commercially used adjuvant aluminum. Moreover, I will experiment with nanocellulose with different sizes to testify which size has the best balance between cytotoxicity and stimulation of innate immune effects (IL-1β). Our project has four hypotheses.

Hypothesis 1: Comparing the innate immune response's stimulation (IL-1β production) of three CNCs and Alum adjuvant by ELISA, the adjuvant effects of nanocellulose improved by increasing lengths. For the in vitro study, I will use THP-1 cells and J774 cells.

Hypothesis 2: Comparing the cytotoxicity of Alum adjuvant and three synthesized CNCs in the 75-300 nm length scale with different concentrations using CCK8 Assay, nanocellulose is a safer alternative for vaccine adjuvant that poses less material toxicity. For the in vitro test, I will use THP-1 cells and J774 cells.

Hypothesis 3: Comparing the cytotoxicity of the three CNCs by using CCK8 assay, s2 possesses the highest cytotoxicity.

Hypothesis 4: Comprehensively analyze the impact of nanocellulose materials, nanocrystals (CNC), on their potential to induce pro-inflammatory and immune adjuvant effects by using ELISA to detect the IL-1β production of three synthesized CNCs in the 75–300 nm length scale derived by acid hydrolysis. Correlating their proinflammatory adjuvant effects with their length and aspect ratio, I can have a preliminary demonstration that 150 nm CNC induces adjuvant effects without posing cytotoxicity in macrophages.

2. Methods

2.1. Cell culture

THP-1 cells were cultured in RPMI 1640 supplemented with L-glutamine (MediaTech Cellgro cat# 10-041-CV or similar), 0.05 mM β-mercaptoethanol and 10 % heat-inactivated fetal bovine serum (PAA Laboratories cat # A15-204, lot # A20407-7003 or similar) and 1% Gibco™ Penicillin-Streptomycin (5,000 U/mL) (Cat# 15070063). The complete media was filtered (0.2μm) prior to use and stored at 4℃. To recover the THP-1 cells, the frozen contents were thawed in a 37℃ water bath upon arrival from ATCC. Subsequently, the cell suspensions were transferred to a 15 mL tube containing complete media (10 mL) and centrifuged at 300g for 5 min. The media supernatant was then discarded, and the cell pellet was resuspended in 3mL complete media. The supernatant was transferred to a T-75 cm^2 vented flask for culture (15 mL total volume) in a 37°C humidified

environment with 5% CO_2. The optimal cell concentration was maintained at $2 – 4 \times 10^5$ cells per mL. The media was changed approximately every 2 to 3 days, based on the cell density and growth rate, to ensure that the cell concentration did not exceed 1 × 106 cells per mL. J774 cells were cultured in DMEM supplemented with 1% Penicillin-Streptomycin and 10% heat-inactivated Fetal Bovine Serum. The complete RPMI1640 medium was stored at 4°C.

The initial steps remained consistent. To recover the J774 cells, the frozen contents were thawed at a 37°C water bath upon arrival from ATCC. Subsequently, the cell suspension was transferred to a 15mL tube and centrifuged at 300g for 5 minutes. After resuspending in the complete media, The media supernatant was transferred and cultured at 37°C.Similarly, the media was replenished based on the cell density and growth rate, ensuring that the cell concentration did not exceed 1 × 106 cells per mL. Given that J774 cells are adherent, a cell scraper was emplyed to suspend the cells for subculturing.

2.2. CNC preparation

Preparation of CNC stock solution began with weighing out the desired amount of CNC powder and adding DI water to make a 5 mg/mL stock suspension of CNC for exposure use.

2.3. CCK8 assay

For the CCK8 cytotoxicity assay, 100μ of cell suspension was dispensed into a 96-well plate, followed by pre-incubation of the plate for 24 hours in a humidified incubator at 37°C, 5% CO2. Then,various concentrations of the adjuvant samples were added into the culture media in the plate. The plate was then incubated for 24 hours in the incubator. Afterward, the CCK-8 well-plate was thawed in a water bath at 37°C and10 μL of CCK-8 solution was added to each well of the plate. Finally, The plate was incubated for 3 hours in the incubator and the absorbance at 450 nm was measured using a microplate reader.

2.4. ELISA of IL-1β production by CNC

The THP1 cells were resuspended in a complete RPMI medium containing 1 μg/mL PMA (PMA solution is 5000×, 5 mg/mL in DMSO), and then seeded in a 96-well plate at a cell density of 5×10^4 cells/well, 100 μL/well. The cells were incubated at a 37°C incubator overnight to allow for full cell adhesion.Following overnight incubation, the old medium was aspirated from each well and 100 μL of the corresponding CNC concentration/well was added. Simultaneously, Endotoxin(LPS) was added to the suspension with a working concentration of 10ng/mL. Asmall amount of LPS was co-cultured to induce pro-IL-1β production. Monosodium urate (MSU, Cat. #: tlrl-msu from Invivogen) was utilized as a positive control.

The ELISA for detecting lL-β production started with diluting the capture antibody to the working concentration (4 μg/mL) in PBS. I coated a 96-well microplate with 50 μL/well of the diluted capture antibody. Then I sealed the plate and incubated it overnight at room temperature, removed the liquid in each well, and washed the plate with Wash Buffer (200 μL each well) three times. After the last wash, I removed the remaining by aspirating. Blocking plates by adding 150 μL of Reagent Diluent to each well, then I incubated them at room temperature for a minimum of 1 hour. I repeated the aspiration/wash cycle. Then I added 50 μL of supernatants from cells treated with CNCs or standards (diluted in Reagent Diluent) to each well in the 96-well plate, then sealed the plates and incubated them for 2 hours at room temperature. I repeated the aspiration/wash and added 50 μL of the Detection Antibody (diluted in Reagent Diluent) to each well. I sealed the plate and incubated it for 2 hours at room temperature. Repeating the aspiration/wash as in step 2, I washed five times in total with incubation of 1 minute per wash. I added 50 μL of working dilution of Streptavidin-HRP (diluted in Reagent Diluent) to each well. Then, I sealed the plate and incubated it for 20 minutes at room temperature. I avoided placing the plate in direct light and repeated the aspiration/wash as in step 2 but with five washes in total with incubation of 1 minute per wash. I added 50 μL of Substrate Solution to each well and incubated it for 20 minutes at room temperature. Then I added 25 μL of Stop Solution

to each well and gently tapped the plate to ensure thorough mixing. At last, I determined the optical density of each well immediately, using a microplate reader (SpectroMax M5e, Molecular Devices Corp., CA) set.

3. Results

3.1. Characterization of CNC

To testify to the innate immune effects of different sizes of CNCs, I utilized acid hydrolysis to decrease the level of polymerization from raw nanocellulose and controlled hydrolysis time to obtain three nanocellulose with different material lengths. This was accomplished by subtracting the amorphous region in the crystalline area and intertwined microfibrils. Through variation hydrolysis time, three nanocellulsoe with lengths 150 nm (s1), 280 nm (s2), and 100 nm (s3) were synthesized. To justify the validity and effectiveness of acid hydrolysis customization, I measured their hydrodynamic sizes by dynamic light scattering (DLS). Dynamic Light Scattering produces the diameter of a sphere with the same translational diffusion speed as the non-spherical particle as the particle's hydrodynamic diameter. As shown in Figure 3 and Table 1, the hydrodynamic diameters of s1, s2, and s3 were determined to be 875.233 ±231.6854 nm, 1871.33 ±207.9621 nm, and 115.767 ±1.26842 nm, respectively, which disagreed with their actual longitudinal length. This resulted in the violation of the assumption of Stocks-Einstein equation that the colloid must be proximate to spheres for estimation (*11*).

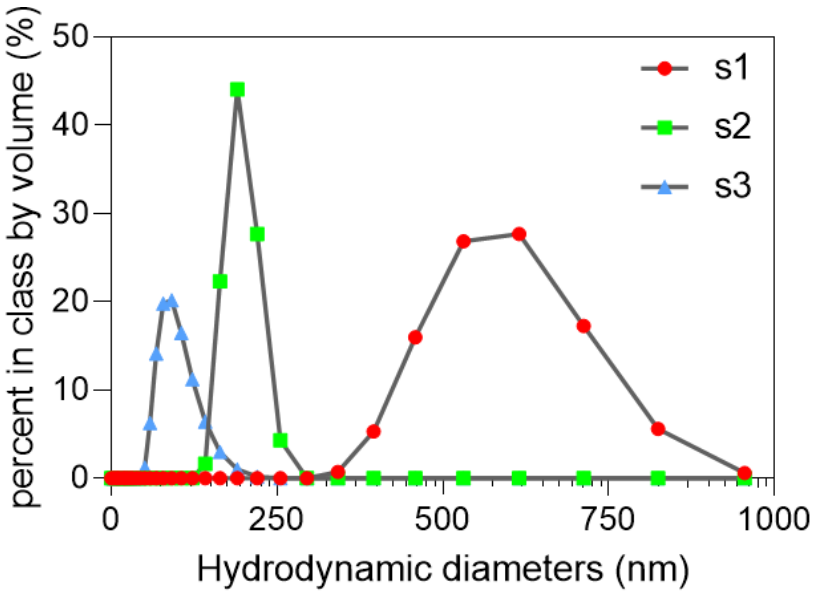

Figure 3. Characterization of CNC. Comparing the hydrodynamic sizes and the actual lengths of nanocellulose by referencing the table from established research, the physical property of the three samples are rectified. s1, s2, and s3 correspond to 150 nm, 280 nm, and 100 nm, respectively.

Table 1. Hydrodynamic diameters of CNCs

CNC	Length/nm	Hydrodynamic diameter/nm
s1	150	875.233 ±231.6854
s2	280	1871.33 ±207.9621
s3	100	115.767 ±1.26842

3.2. CNC-induced innate immune activation

After obtaining CNCs of different sizes, I wondered if they possess similar capabilities to activate the immune system. Therefore, I quantified the production of IL-1β in THP-1, a monocyte cell line isolated from the peripheral blood of an acute monocytic leukemia patient, and J774, a murine macrophage. IL-1β is a crucial modulator cytokine of the inflammatory response, important for the host's defense against infections and host response. IL-1β production has been an integral target for demonstrating innate immune response. ELISA was used to testify the adjuvant's IL-1β production. The optical density of each well was determined using a microplate reader 2 hours after adding the supernatants from cells treated with adjuvant samples. As Figure 4 and Figure 5 demonstrate, the production of IL-1β is concentration-dependent and size-dependent. Increasing doses of CNC elicit a stronger immune response, producing more IL-1β. The relationship between size and response is more complex as they are neither positively nor negatively related. In THP-1, at the low concentrations of 75 µg/mL and 150 µg/mL, s1 demonstrates the highest IL-1β production, followed by s2 and s3. When the concentration is raised to a higher level, 300 µg/mL, s3 demonstrates the highest IL-1β production, followed by s1 and s2. Among all the samples experimented with, Alum demonstrated the highest IL-1β production, followed by MSU. In J774 cells, at the low concentrations of 75 µg/mL, s3 demonstrates the highest IL-1β production, followed by s1 and s2. When the concentration is raised to a higher level, 300 µg/mL, s3 demonstrates the highest IL-1β production, followed by s1 and s2. Among all the samples experimented with, Alum demonstrated the highest IL-1β production, followed by MSU.

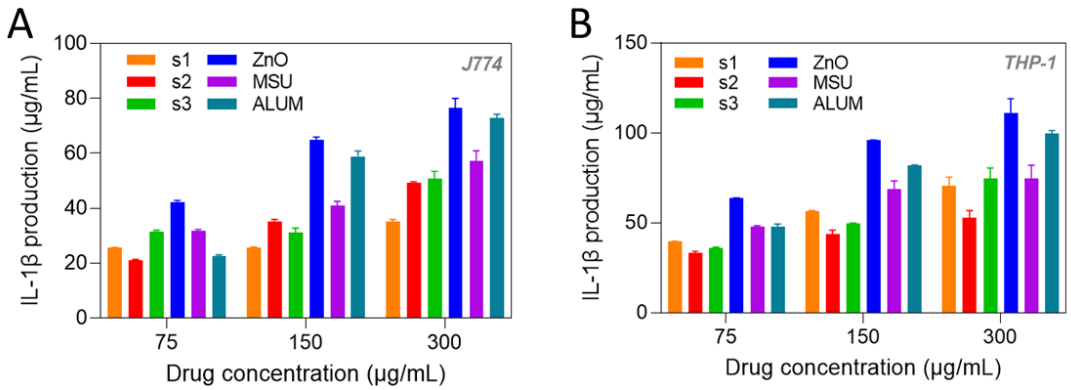

Figure 4. Production of IL-1β in J774 cells and THP-1 cells exposed to nanocelluloses, which was dose-dependent. During 24 hours, THP-1 cells were subjected to concentrations of 75, 150, and 300 ug/mL of each cellulose solution, alum, MSU, and ZnO. IL-1β production levels in the supernatants obtained by ELISA were assessed.

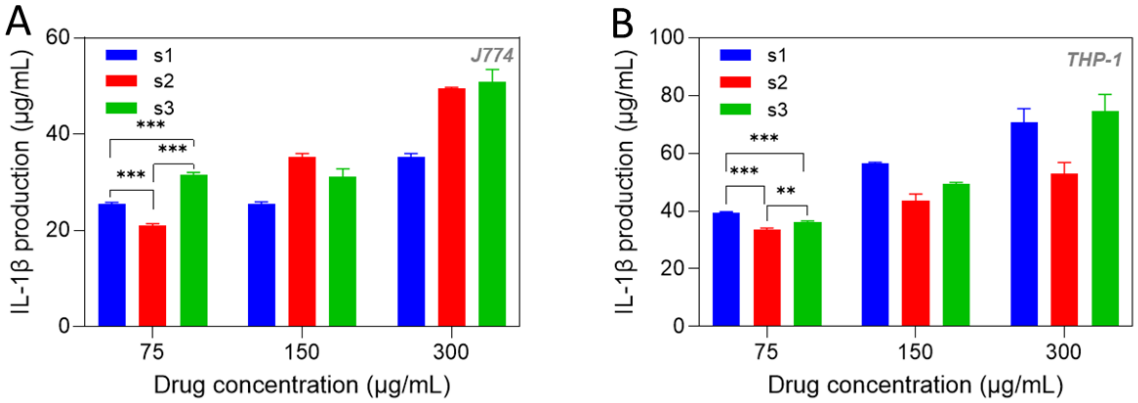

Figure 5. The production of IL-1β in response to CNCs is dependent on both concentration and size. Higher doses of CNCs result in greater IL-1β production. Two-tailed unpaired t-test P-values indicate statistical significance (*p< 0.05; **p < 0.01;***p < 0.001).

3.3. Cytotoxicity of CNC and comparison with other adjuvants

After confirming that CNC is able to induce proinflammation, I would like to evaluate the biocompatibility of our synthesized CNCs. Thus, I determined the adjuvants' cytotoxicity using CCK8 assay. After the incubation with nanocellulose, the microplate reader measured the absorbance of the well-plate, which was then normalized to cell viability. By calculating the cell viability by analyzing the standard deviation and wells which have the addition of cells without adjuvant samples, the trend of cytotoxicities for six samples, including the negative control, was determined. As shown in Figure 6, all three nanocellulose samples perform significantly higher cell viability, thus less cytotoxicity compared to nanoalum and MSU in all concentrations. The s2 sample presents the most negligible cytotoxicity in the cell suspension among the three CNCs. By observing the holistic trend of the graph, the cytotoxicity of all three CNCs is lower than alum and MSU in every concentration setting. Thus, the biocompatibility of nanocellulose was rectified in vitro conditions.

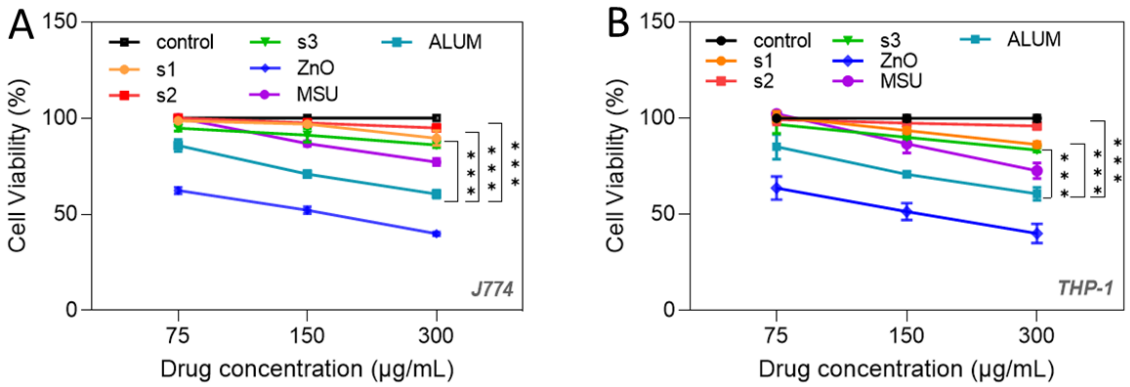

Figure 6. Cellular viability test in J774 cells (left) and THP-1 cells (right) exposed to three samples of CNCs (150 nm, 280 nm, and 100 nm), alum adjuvant, Monosodium urate adjuvant (MSU), and ZnO as a positive control. Statistical significance for CNCs vs. alum is indicated by asterisk(s). Two-tailed unpaired t-test p-values indicate statistical significance (*$p < 0.05$; **$p < 0.01$;***$p < 0.001$).

4. Conclusion

In summary, the production of IL-1β is CNC length-dependent but not in a linear relationship. By testing cell viability after the introduction of adjuvants, the 100 nm CNC induces the strongest effect, and CNC (75-300 nm) is a more biocompatible adjuvant compared with Alum, as confirmed by less cytotoxicity in CNCs measured by CCK8 assay. Cytotoxicity of CNC is both concentration- and size-dependent. Increased concentration can lead to higher cell mortality, and 100 nm possesses the highest cytotoxicity among the three CNCs. CNCs elicit an innate immune response, as proved by their IL-1β production. When concentrations do not pose obvious cytotoxicity to J774 cells, 280 nm CNC (s2) induces the greatest IL-1β production. When concentrations do not pose obvious cytotoxicity to THP-1 cells, 150 nm CNC (s1) induces the greatest IL-1β production.

The influence of material size and adjuvant types in THP-1 cells and J774 cells was examined in this research using a panel of CNCs. To expand the number of CNC length scales that could be compared, I employed acid hydrolysis. Experimental evidence has been gathered concerning the possible harmful effects of CNCs and CNFs, even though there are presently no published data indicating a safety risk for nanocellulose in the workplace or in consumer items. This research enriches the study of the potential cytotoxicity of nanocellulose with different size ranges and compares them to the most commercially used alum adjuvant. By presenting the cytotoxicity of CNCs in three sizes and three concentration levels, I considerably expand on current findings of nanocellulose biocompatibility and show how length is a crucial material property that supports pro-inflammatory and immunogenic actions in the innate immune system. Moreover, I demonstrate how this knowledge may be used to create novel immune-stimulating nanocellulose adjuvants with

significantly better biocompatibility since nanocellulose is competent to elicit an innate immune response but also possess better biocompatibility than alum and MSU adjuvants. Besides, because of either a deficiency in effective anti-tumor immunity or the generation of undesirable immunity, it has been shown that cancer vaccines require better adjuvants than those that are currently permitted, which include the most popular alum and incomplete Freund's adjuvant(*22*). Nanocellullose's potential as a novel vaccine adjuvant opens room for future research centered around its viability in avoiding undesirable immunity since its innate immune response is less than that of alum.

There may be some possible limitations in this study. First, the cytotoxicity and innate immune response estimates in the research are based on a small library of CNCs samples. There are, therefore, some considerations over the possible discrepancy in the performances of CNFs and BCs in similar testing. Since CNFs are made up of individual and aggregated nanofibrils formed of alternating crystalline and amorphous cellulose domains, giving it a soft and lengthy chain shape. Their physiological properties vary tremendously from CNCs, which will have some impacts on their cytotoxicity and innate immune stimulation. Second, to research the innate immune response of adjuvants, I utilized IL-1β production as a target. Other cytokines that participate in innate immunity were not researched in this research, including IL-2 and IFN-γ. Other cytokine production trends may vary with IL-1β production. Third, this research established a preliminary study of the ideal length and concentration ranges to produce the greatest adjuvant effects without cytotoxicity. The experiments are in vitro. The adjuvant effect tested in vivo may demonstrate adjuvant performances in mice models, which provide information regarding adjuvants' evaluations more accurately in immune systems.

Future research could, for instance, investigate the cytotoxicity and innate immune response of CNFs compared to CNCs, which provides more research in regard to their discrepancy in biocompatibility and innate immune stimulation. Such research can contribute to identifying the pattern of induced cytotoxicity and adjuvant effect in different nanocellulose types. Further examination of other cytokines production after adjuvant introduction could step further in terms of finalizing nanocellulose performances in innate immunity. Thereby providing a more comprehensive understanding of nanocellulose's size-dependent and concentration-dependent nature. Future in vivo experiments with respect to nanocellulose's impacts may delve deeper into other references of vaccination experiments, such as Ovalbumin (OVA). In vivo studies may extend current knowledge of nanocelluloses impacts in complex models and provide adjuvant evaluations more accurately.

References

[1] R. L. Coffman, A. Sher, R. A. Seder, Vaccine adjuvants: putting innate immunity to work. *Immunity* **33**, 492-503 (2010).

[2] M. Pizzuto *et al.*, Cationic lipids as one-component vaccine adjuvants: A promising alternative to alum. *J Control Release* **287**, 67-77 (2018).

[3] S. Awate, L. A. Babiuk, G. Mutwiri, Mechanisms of action of adjuvants. *Front Immunol* **4**, 114 (2013).

[4] L. Tomljenovic, C. A. Shaw, Aluminum vaccine adjuvants: are they safe? *Curr Med Chem* **18**, 2630-2637 (2011).

[5] L. Tomljenovic, C. A. Shaw, Do aluminum vaccine adjuvants contribute to the rising prevalence of autism? *J Inorg Biochem* **105**, 1489-1499 (2011).

[6] W. Hao *et al.*, Aluminum impairs cognitive function by activating DDX3X-NLRP3-mediated pyroptosis signaling pathway. *Food Chem Toxicol* **157**, 112591 (2021).

[7] M. Mold, E. Shardlow, C. Exley, Insight into the cellular fate and toxicity of aluminium adjuvants used in clinically approved human vaccinations. *Scientific Reports* **6**, 31578 (2016).

[8] R. Danielsson, H. Eriksson, Aluminium adjuvants in vaccines - A way to modulate the immune response. *Semin Cell Dev Biol* **115**, 3-9 (2021).

[9] M. Zhu, R. Wang, G. Nie, Applications of nanomaterials as vaccine adjuvants. *Hum Vaccin Immunother* **10**, 2761-2774 (2014).

[10] N. Lin, A. Dufresne, Nanocellulose in biomedicine: Current status and future prospect. *European Polymer Journal* **59**, 302-325 (2014).

[11] X. Wang *et al.*, The Crystallinity and Aspect Ratio of Cellulose Nanomaterials Determine Their Pro-Inflammatory and Immune Adjuvant Effects In Vitro and In Vivo. *Small* **15**, e1901642 (2019).

[12] D. Trache *et al.*, Nanocellulose: From Fundamentals to Advanced Applications. *Front Chem* **8**, 392 (2020).

[13] Z. Fang *et al.*, Critical Role of Degree of Polymerization of Cellulose in Super-Strong Nanocellulose Films. *Matter* **2**, 1000-1014 (2020).

[14] A. Chakrabarty, Y. Teramoto, Recent Advances in Nanocellulose Composites with Polymers: A Guide for Choosing Partners and How to Incorporate Them. *Polymers* **10**, 517 (2018).

[15] B. Pulendran, S. A. P, D. T. O'Hagan, Emerging concepts in the science of vaccine adjuvants. *Nat Rev Drug Discov* **20**, 454-475 (2021).

[16] B. J. Swartzwelter *et al.*, The Impact of Nanoparticles on Innate Immune Activation by Live Bacteria. *Int J Mol Sci* **21**, (2020).

[17] K. S. Korsholm *et al.*, The adjuvant mechanism of cationic dimethyldioctadecylammonium liposomes. *Immunology* **121**, 216-226 (2007).

[18] W. Lee, M. Suresh, Vaccine adjuvants to engage the cross-presentation pathway. *Front Immunol* **13**, 940047 (2022).

[19] K. Ivanov, E. Garanina, A. Rizvanov, S. Khaiboullina, Inflammasomes as Targets for Adjuvants. *Pathogens* **9**, (2020).

[20] A. Albanese, P. S. Tang, W. C. Chan, The effect of nanoparticle size, shape, and surface chemistry on biological systems. *Annu Rev Biomed Eng* **14**, 1-16 (2012).

[21] Y. Nakamura, A. Mochida, P. L. Choyke, H. Kobayashi, Nanodrug Delivery: Is the Enhanced Permeability and Retention Effect Sufficient for Curing Cancer? *Bioconjug Chem* **27**, 2225-2238 (2016).

[22] A. H. Banday, S. Jeelani, V. J. Hruby, Cancer vaccine adjuvants--recent clinical progress and future perspectives. *Immunopharmacol Immunotoxicol* **37**, 1-11 (2015).