Surface Functionalization of Spruce-Derived Cellulose Scaffold for Glycoprotein Separation

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Protein immobilization on a stationary phase, such as nanocelluloses, is widely used in biodiagnostic, biocatalytic, and bioseparation applications. With the top-down approach which utilizes the native hardwood honeycomb structure, mesoporous cellulose scaffolds can be fabricated without the need for energy-consuming production and bottom-up assembly of nanocelluloses. However, this approach is difficult for preparing softwood-based cellulose scaffolds due to the disintegration of wood cells after complete delignification. Herein, for the first time the use of spruce softwood with a homogenous cellular structure of longitudinally positioned and top-to-bottom joined tracheids is explored as a scaffold for protein immobilization. 1,4-butanediol diglycidyl ether is utilized to crosslink cell wall polysaccharides before the delignification step, thus improving the adhesion between tracheids. The native cellular structure of spruce is well preserved after the complete removal of lignin, enabling the successful production of a highly mesoporous and mechanically robust spruce-derived cellulose scaffold with exceptionally high specific surface area (219 m² g⁻¹). Further amination of the cellulose scaffold allows covalent immobilization of functional biomolecules, such as a lectin protein concanavalin A (Con A) and biotin, on the lumen surfaces and inside the porous cell wall. The Con A immobilized scaffold demonstrates native glycoprotein-binding activity and possible glycoprotein separation application.

1. Introduction

Cellulose nanomaterials are emerging host substrates and templates for immobilization of guest biomolecules such as enzymes and sensing molecules for diverse applications in biosensors, bioseparation, and biocatalysis.[1] Compared to solid support materials such as synthetic polymer resins, inorganic particles, and polymer/inorganic composites, cellulose nanomaterials or nanocelluloses offer unique properties including versatile surface chemistry, biocompatibility, biodegradability, optical and mechanical performance. Nanocellulose-based materials including nanopaper and aerogel from nanofibrillated cellulose,[2] membranes/felts from electropun cellulose nanofibers,[3] and cellulose nanocrystals (CNCs)[4] have been previously functionalized for point-of-care diagnosis, antibacterial medical textiles and filters, labeling and bioimaging applications. In addition, regenerated nanocellulose hydrogel,[5] bleached kraft wood pulp fibers,[6] filter paper,[7] and regenerated amorphous cellulose[8] have also been utilized for immobilization of functional enzymes, or for purification of cellulose-binding module-tagged protein. Furthermore, in order to obtain designed flow channels and pore structures instead of randomly packed columns for separation applications, regenerated cellulose columns have been prepared by using 3D printing and functionalized with ion exchange ligands for chromatographic purification of viral particles.[9] To avoid energy- and resource-intensive production methods for the bottom-up fabrication of cellulose structures with hierarchical order over different length scales using nanocellulose or regenerated cellulose, top-down structural engineering and novel chemical modification methods are more favorable in redesigning wood for different functional applications.[10]

Benefitting from the high amount of large, open-ended vessels in hardwood species (>30% of wood), enzymes have been immobilized on poplar wood by nanoparticle-mediated adsorption and used as a flow-through heterogeneous biocatalyst.[11] Hardwoods have two primary types of cells with very different geometries and functions: open-ended water-conducting vessels with a diameter of 50–800 µm, and load-bearing fibers of 1–2 mm length and 15 µm width.[12] On the other hand, tracheids with a width of ~30 µm and a length of 2–4 mm are the predominant wood cells in softwood species.[12a] Tracheids are close-ended and joined top-to-bottom via pits to allow the conduction of water upward. Compared to hardwoods, softwoods have a rather homogenous cellular structure with uniform-sized wood cells (Figure S1, Supporting Information). Such cellular
structure is an ideal scaffold as solid support for immobilization of functional biomolecules and has not been exploited previously. Native wood samples have a much lower specific surface area (SSA, $<1 \text{ m}^2 \text{ g}^{-1}$) compared to individualized wood pulp fibers and cellulose nanofibers. In order to extract cellulose scaffold from wood structure, pretreatment using alkaline sulfite pulping, sodium hypochlorite bleaching, or lignin-specific acidic bleaching should be employed to remove lignin. After the delignification, SSA values in the range of $13–207 \text{ m}^2 \text{ g}^{-1}$ could be obtained for hardwoods. However, softwoods are notoriously susceptible to delignification-induced disintegration, as after complete removal of the binder, lignin, their cellular structure cannot be preserved. Mainly hardwood species, such as balsa, bassa, birch, and poplar, have previously been delignified while keeping their structural integrity. One of the main reasons for the resistance to disintegration for hardwood during delignification as compared to softwood is their compositional difference. There are much more cellulose microfibrils in middle lamella, the joint region between adjacent cells, in hardwood than softwood, resulting in stronger adhesion between the adjacent cells even after removal of lignin. Another reason is the seasonal growth variation in softwood, resulting in early- and latewood with different lignin content and density. As the earlywood fractions contain less amount of lignin, an uneven delignification leads to the loss of structural integrity in these regions. Therefore, in order to preserve the native cellular structure of softwood after a complete delignification process, the adhesion between the tracheids must be enhanced.

Herein, we report a novel strategy for the fabrication of spruce-derived porous cellulose scaffolds that preserve the natural cellular structure of softwood. Such cellulose scaffold has been functionalized for immobilization of a lectin protein for subsequent glycoprotein separation. Biotin has also been conjugated to the scaffold to demonstrate its chemical versatility. To avoid disintegration of the softwood cellular structure, spruce wood samples were crosslinked with 1,4-butanediol diglycidyl ether (BDDE) or poly(ethylene) glycol diglycidyl ether (PEGDGE) before the delignification process (Figure 1a). The samples were pretreated by boiling in 1 wt.% sodium hydroxide (NaOH) to achieve a better penetration of BDDE and PEGDGE into the

![Figure 1.](image-url)

Figure 1. a) Schematic illustration of the three steps for the fabrication of cellulose scaffold that preserves the native spruce wood cellular structure. b) Covalent immobilization of concanavalin A (Con A) and biotin on the surface of spruce cellulose scaffold via the introduction of primary amine groups. The FE-SEM image shows the microstructure of the spruce-derived cellulose scaffold.
wood structure. The crosslinked spruce wood samples were subsequently delignified by using acidic sodium chlorite (NaClO₂). After lignin was removed, a highly mesoporous cellulose scaffold maintaining the natural wood cellular structure was successfully prepared. To further immobilize proteins, primary amine groups were introduced to the surface of the spruce cellulose scaffold that was decorated with epoxide functional groups via a reaction with epichlorohydrin (ECH) in potassium hydroxide (Figure 1b). The amount of reactive amine groups immobilized on the cellulose scaffold was quantified by labeling with fluorescein isothiocyanate (FITC). A lectin protein, concanavalin A (Con A), which selectively binds to mannose-containing glycoproteins, was covalently conjugated to the primary amine groups using glutaraldehyde. The application of Con A immobilized spruce cellulose scaffold for glycoprotein enrichment and separation was studied. Biotin was conjugated to the primary amine groups using the biotin N-hydroxysuccinimide ester (Biotin-NHS). The activity of conjugated biotin on the cellulose scaffold was demonstrated by using a fluorescence-labeled streptavidin.

2. Results and Discussions

In softwood spruce, the surface of cellulose microfibrils is covered with glucomannan inside the cell wall.[23] Alkaline extraction has been previously utilized to efficiently reduce the glucomannan content of spruce wood chips.[24] Thus in the extraction has been previously utilized to efficiently reduce the glucomannan content of spruce wood chips.[24] Thus in the extraction process, the spruce chips exhibited a typical alkaline darkening phenomenon (Figure 2a), due to the formation of coniferaldehyde, ortho-quinones, and other conjugated chemical structures.[25] After 3 h of alkaline treatment, a maximum weight loss of ≈20 wt.% was achieved (Figure 2b). The glucomannan content was significantly reduced from 16.7% to 3.4%, while the xylan content was reduced from 70% to 5.9%, as determined by the sugar analysis (Table S1, Supporting Information). The partial removal of glucomannan was also confirmed by Fourier transform infrared (FT-IR) spectroscopy (Figure S2, Supporting Information). The characteristic peak of carbonyl groups at 1730 cm⁻¹ disappeared due to decaylation of O-acetyl-galactoglucomannan.[20] In wood cell wall, lignin is associated with hemicellulose through lignin-carbohydrate linkages.[27] Therefore, the removal of glucomannan and xylan also resulted in a decrease in lignin content by 7%.

The cell wall (CW), middle lamella (ML), and cell corner (CC) structures of the alkaline treated spruce sample were characterized by using field emission scanning electron microscopy (FE-SEM). In the native spruce, the adjacent tracheids were tightly bonded together (Figure 3a). The CW, ML, and CC regions observed on cross-section perpendicular to the fiber axial direction (Figure 3b) and the cell lumen surface (Figure 3c) were smooth and dense. After the alkaline treatment, the adjacent tracheids were still tightly bonded (Figure 3d), and a large number of small voids were observed in the CW, CC, and ML regions (Figure 3e). Spruce glucomannan mainly exists in two forms, in close association with cellulose microfibrils along the fiber direction.[28] or as a matrix.[29] The removal of glucomannan led to the exposure of the microfibril bundles as observed on the lumen surface (Figure 3f). The alkaline treatment also generated pores with a double convex lens shape which could substantially ease the penetration of chemicals into the cell wall for the subsequent modification. The mesoporous feature of the alkali-extracted spruce sample was confirmed from an enlarged hysteresis loop in the nitrogen (N₂) adsorption/desorption isotherms (Figure 2e). The pore size distribution obtained by fitting the N₂ adsorption isotherm using the density function theory showed a significant increase of pore volume in the mesopore range of 2–10 nm (Figure 2f). The mesopore volume of the native spruce was 0.0005 cm³ g⁻¹, which increased to 0.08 cm³ g⁻¹ after the alkaline treatment (Table S2, Supporting Information). Correspondingly, the SSA value of the alkaline treated spruce was significantly increased to 88 m² g⁻¹, as compared to 0.3 m² g⁻¹ for the native spruce.

In order to improve the adhesion between the spruce tracheids and increase their resistance to disintegration during the lignin removal process, we used two linear homobifunctional crosslinking reagents, BDDE and PEGDGE, to crosslink the wood cell wall polymers after the alkaline treatment. BDDE has been used for crosslinking of the surface hydroxyl groups of polysaccharides.[30] PEGDGE with an average Mₙ of 500 has been reported to be able to crosslink the phenolic groups in lignin and produce gels with higher water retention.[31] The crosslinking reaction was performed in alkaline conditions at 80 °C. FT-IR analysis of the crosslinked spruce samples showed an increase in the absorption bands at 2917 and 2870 cm⁻¹, which were attributed to the asymmetric and symmetric vibrations of CH₂ derived from BDDE and PEGDGE, indicating successful crosslinking (Figure S2, Supporting Information). Subsequently, the crosslinked and control alkali-extracted samples were delignified using 1 wt.% NaClO₂ in 0.1 M sodium acetate buffer (pH 4.6) at 80 °C. After 10 h of delignification, the control alkali-extracted spruce sample (uncrosslinked) had a residual lignin content of 1% and the sample fell apart into several pieces, while the BDDE-crosslinked spruce sample had a residual lignin content of 0.4% and the sample was mechanically robust (Figure 2c,d). Much longer delignification time (20 h) was required for the PEGDGE crosslinked sample, and its final lignin content was 1.3%. This result indicates, that PEGDGE was preferable for crosslinking of lignin, while BDDE penetrated better into the cellulose and hemicellulose network and created crosslinks between polysaccharides owing to its lower molar mass. Further FE-SEM analysis of the BDDE crosslinked sample revealed, that there was a strong adhesion between tracheids both within earlywood and latewood regions and at their boundaries, and the adjacent tracheids were tightly bonded through cellulose microfibrils in ML and CC after lignin removal (Figure S3, Supporting Information). As a result, the structural integrity of the BDDE crosslinked spruce was well preserved even under magnetic stirring or orbital shaking, indicating the mechanical robustness, and the sample appeared white (Figure 2c). Similar results were found for the PEGDGE crosslinked sample, except for longer delignification time, and a slightly higher residual lignin content.

Successful removal of lignin was also confirmed by FT-IR analysis. The lignin characteristic peaks including conjugated C–O at 1658 cm⁻¹, skeletal vibration of aromatic ring
at 1509 cm\(^{-1}\), and C–O stretching of lignin guaiacyl unit at 1270 cm\(^{-1}\), were diminished after delignification (Figure S2, Supporting Information). The removal of lignin also resulted in stronger absorption at 3336 cm\(^{-1}\), which was attributed to increased amount of hydroxyl groups due to increased relative content of polysaccharides (Table S1, Supporting Information).

The characteristic peak at 1730 cm\(^{-1}\) corresponding to carbonyl groups was observed after delignification, which was possibly a result of oxidative degradation of lignin, observed before during an extended sodium chlorite delignification process.

The removal of lignin in the BDDE-crosslinked spruce generated even larger pores compared to the alkali-extracted sample.
These pores had slit-like structures deriving from the native bilayer organization of lignin and cellulose microfibrils in the cell wall.[33] The slit-like pores were observed both in inside the secondary cell wall (Figure 3h), and in the lumen surfaces (Figure 3i). These microstructure observations were confirmed by pore size distribution derived from the \( \text{N}_2 \) adsorption isotherm (Figure 2f). The volume of pores in the range of 2–10 nm was significantly increased, and a large volume of mesopores in the range of 10–50 nm was generated as compared to the alkali-extracted sample. Thus, the total mesopore volume after the delignification showed a remarkable increase from 0.08 to 0.4 \( \text{cm}^3 \text{ g}^{-1} \) (Table S2, Supporting Information). The highly mesoporous structure of the crosslinked, delignified spruce resulted in an extremely high SSA of 219 m\(^2\) g\(^{-1}\), more than five times higher than that of the delignified balsa wood (41 m\(^2\) g\(^{-1}\)).[34]

The delignified, BDDE-crosslinked spruce was used as a scaffold for further immobilization of biomolecules. To create a reactive handle for further functionalization, primary amine groups were grafted onto the scaffold through a two-step procedure as shown in Figure 1b. First, epichlorohydrin was used to react with the surface hydroxyls under alkaline conditions to graft epoxide groups. Then, pentaethylenehexamine with a spacer arm was reacted with the epoxide groups to introduce the primary amine group, and the successful grafting was confirmed by FT-IR. The FT-IR spectrum showed a broad and intense peak of symmetric N–H stretching band around 1600 cm\(^{-1}\) (Figure S4, Supporting Information), which was attributed to the primary amines. The amount of the reactive amine groups grafted on the wood scaffold were quantified by a labeling reaction with FITC. The amount of the reactive amine groups was 0.62 \( \mu \text{mol g}^{-1} \), as determined from the change of fluorescence intensity of FITC solution before and after the labeling reaction versus a standard curve of known FITC concentrations. This value corresponds to \( \approx \)1 FITC moiety per 587 nm\(^2\) inside the wood cell wall of the spruce cellulose scaffold which has an SSA of 219 m\(^2\) g\(^{-1}\). This value is lower than the amine-functionalized CNC, which has an amine group content of 0.03 mmol g\(^{-1}\) and 1 FITC moiety per 27 nm\(^2\).[35]

The amine-decorated spruce cellulose scaffold was used as a solid support for immobilization of lectin protein Con A, a natural affinity-tag for purification of mannose-containing biomolecules.[36] The Con A-immobilized spruce cellulose scaffold was further tested in affinity-based separation of glycosylated protein (ovalbumin) from non-glycosylated proteins (lysozyme), as illustrated in Figure 4a. Con A was covalently conjugated to the

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**Figure 3.** FE-SEM images of the cross-sections perpendicular to the fiber axial (longitudinal) direction, and corresponding higher magnifications showing the cell wall, cell corner, and middle lamella, and cross-sections parallel to the fiber axial direction showing the tracheid lumen surfaces of a,b,c) native spruce, d,e,f) alkali-extracted spruce, and g,h,i) delignified BDDE crosslinked spruce, respectively.
scaffold by a glutaraldehyde-mediated imine bond through the
prior grafted primary amine groups (Figure 1b) in order to avoid
biomolecule leaching.\(^{37}\) The spruce cellulose scaffold laden
with primary amine groups was first reacted with glutaralde-
hyde, and then incubated with various loading amounts of Con
A. The concentration of the Con A in the impregnation solu-
tion before and after the reaction was quantified by using the
Bradford method. The optimum loading capacity of the spruce
cellulose scaffold was determined to be 2.7 ± 0.3 mg of immo-
ibilized Con A per g of scaffold, as 22.5% of the initial loaded
Con A (12 mg g\(^{-1}\)) was immobilized (Figure S5, Supporting
Information). Further increasing the initial loading amount of
Con A did not significantly increase the immobilized amount of
Con A. This result indicated that most of the immobilized Con
A molecules were located inside the mesopores generated by
alkaline extraction and delignification, as the amount of protein
needed to form a single layer covering the lumen surface was
0.33 mg g\(^{-1}\). This value was estimated from the hydrodynamic
diameter of Con A (12 nm)\(^{38}\) and the lumen surface area of
the spruce cellulose scaffold (0.11 m\(^{2}\) g\(^{-1}\)), which was calculated
from the cell and lumen dimensions of earlywood and lateward
in spruce wood\(^{39}\) and the measured density (0.36 g cm\(^{-3}\)) of
the spruce cellulose scaffold. The herein immobilized amount of
Con A is 5 times higher than that of the enzymes immobilized
on the poplar hardwood via a gold nanoparticle-mediated pro-
tein adsorption method (0.56 mg g\(^{-1}\)).\(^{11}\) Owing to the high meso-
porosity of the spruce cellulose scaffold that allows the penetra-
tion of biomolecules inside the cell walls. Also, the immobilized
amount of Con A on this aminated spruce cellulose scaffold is
comparable to the commercial surface-activated magnetic beads
(Invitrogen Dynabeads M-270 Amine), which typically bind 5 µg
Immunoglobulin G per mg beads.

The Con A immobilized spruce cellulose scaffold was incu-
bated with Texas Red conjugated ovalbumin followed by thorough
washing to remove any unbound ovalbumin, using the aminated
spruce cellulose scaffold as the control. The sample was sectioned
by using a microtome blade and analyzed by confocal laser scan-
ing microscopy (Figure 4b). In the tangential cross-section, Con
A was successfully immobilized throughout the lumen surface of
the tracheids. In the longitudinal cross-section, Con A was indeed
localized both on the lumen surfaces and inside the porous cell
wall, as revealed from the amount of immobilized protein. The
mesopores generated by delignification, with widths ranging from
10 to 50 nm, were large enough to accommodate Con A with a
hydrodynamic diameter of 12 nm\(^{38}\) inside the wood cell wall.

To utilize the native glycoprotein-binding function of the
immobilized Con A, a binding test was performed, in which
the aminated and Con A immobilized spruce cellulose scaf-
dolf samples were incubated in a protein mixture containing
ovalbumin and lysozyme. The proteins were collected before
and after incubation with the spruce samples and separated
in sodium dodecyl sulfate-polyacrylamide gel electrophoresis
(SDS-PAGE) (Figure 4c). The results showed that ovalbumin,
with a molecular weight of 45 kDa, disappeared after incuba-
tion with the Con A immobilized spruce cellulose scaffold as
compared to the aminated spruce cellulose scaffold, indicating
selective binding of ovalbumin to the immobilized Con A. Some
nonspecific bindings of the lysozyme molecules were observed
for both spruce cellulose scaffolds, which was probably related
to the small molecular weight of lysozyme (14 kDa), making its
penetration easier into the porous wood structures. The wood
samples were washed thoroughly with buffer to remove the
nonspecifically bound lysozyme after incubation. Nonspecific
binding was not observed for the larger-sized ovalbumin, and
its content remained unchanged after incubated with the ami-
nated spruce cellulose scaffold as determined by fluorescence
spectroscopy using Texas Red conjugated ovalbumin. With 1 g

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**Figure 4.** a) Schematic illustration for the glycoprotein-binding function of
Con A in enrichment of glycosylated ovalbumin from ovalbumin-lysozyme
mixture. b) Confocal images of longitudinal and tangential cross-sections
of Con A immobilized spruce wood cell wall after capturing the Texas
Red conjugated ovalbumin, scale bars = 50 µm. c) SDS-PAGE showing
selective binding of ovalbumin from ovalbumin-lysozyme mixture onto
the Con A immobilized spruce. Lane 1: protein marker, lane 2: ovalbumin,
lane 3: lysozyme, lane 4: mixture of ovalbumin and lysozyme, lane 5: after
incubation with aminated spruce cellulose scaffold, lane 6: after incuba-
tion with Con A immobilized spruce cellulose scaffold.
of Con A immobilized spruce cellulose scaffold, 0.28 mg of ovalbumin could be enriched inside the scaffold. After washing the scaffold with mannose, 73% (0.21 mg) of the bound ovalbumin could be subsequently released. The obtained 73% recovery of the ovalbumin after the washing and elution steps is comparable to that obtained in protein purification by using commercial Immobilized Metal Ion Affinity Chromatography resin (75–80%).[40] as well as that previously reported for lectin affinity chromatography (69–77%).[41]

To further demonstrate the chemical versatility of the amminated spruce cellulose scaffold, biotin was coupled to the grafted primary amine groups by using biotin-NHS (Figure 1b)). Biotin serves as a ligand for streptavidin, and their interaction is exceptionally strong albeit non-covalent. The successful conjugation of biotin onto the amminated spruce cellulose scaffold was proved by the binding of Alexa Fluor 633 conjugated streptavidin. The amount of immobilized streptavidin was estimated to be 4.4 mg g⁻¹, which was in the same binding capacity range compared to the imine-immobilized Con A. The streptavidin with a radius of 5 nm[42] was also found to be localized both on the cell lumen surfaces, in the cell corner and middle lamella, and inside the cell walls (Figure S6, Supporting Information).

3. Conclusion

In summary, we have developed a new route for the fabrication of spruce softwood-derived hierarchical cellulose scaffolds with crosslinked polysaccharide-matrix and the subsequent immobilization of biomolecules via a useful amine-based reactive handle. First, we successfully produced amine-functionalized, highly porous cellulose scaffold which preserved the native cellular structure of spruce softwood and showed a remarkably high specific surface area (219 m² g⁻¹) and very low residual lignin content (0.4%). The key step for preparing the cellulose scaffold was BBDE crosslinking of the cell wall polysaccharides, which improved the adhesion of tracheids in both earlywood and latewood regions and allowed the complete removal of lignin without losing the structural integrity. Further decoration of primary amines on the cellulose scaffold enabled glutaraldehyde-mediated covalent immobilization of glycoprotein-binding Con A and direct covalent conjugation of biotin-NHS, which were shown to retain their native functionalities after immobilization, thereby facilitating affinity-based separation of ovalbumin from lysozyme and binding of streptavidin, respectively. The immobilized proteins were located not only on the lumen surfaces but also inside the cell wall, benefiting from a large volume of mesopores (0.4 cm³ g⁻¹) in the range of 10 to 50 nm in the cell wall that was generated by the alkaline and delignification treatments. This new route opens up new possibilities for biodiagnostic, biocatalytic, and bioseparation applications by using spruce-derived cellulose scaffold.

4. Experimental Section

Materials: Norway spruce (Picea abies) was purchased from Material Co. Ltd, Sweden and cut into chips with dimensions of 2 × 10 × 10 mm (longitudinal × radial × tangential) using a table-top circular saw. Acetic acid, Bradford Reagent, bromophenol blue, 1,4-butanediol diglycidyl ether (BDDE), poly(ethylene glycol) diglycidyl ether (PEGDGE, average Mn: 500), calcium chloride (CaCl₂), dimethyl sulfoxide (DMSO), epichlorohydrin (ECH), fluorescein isothiocyanate (FITC), glutaraldehyde, glycerol, manganese(II) chloride tetrahydrate (MnCl₂ · 4H₂O), magnesium chloride (MgCl₂), β-mercaptoethanol, Mini-protein TGX 4–20% gels (Bio-Rad), pentaethylenehexamine (PEHA), potassium hydroxide (KOH), sodium carbonate, sodium hydrogen carbonate, sodium acetate, sodium chloride (NaClO₃), sodium phosphate, and tris(hydroxymethyl)aminomethane hydrochloride (Tris·HCl) were purchased from Sigma-Aldrich, Germany. Canavalia ensiformis (Canavalia ensiformis) meal was purchased from Megazyme, Ireland. Ovalbumin Texas Red conjugate, Streptavidin Alexa Fluor 633 conjugate, PageBlue protein staining solution, and PageRuler Prestained protein ladder were purchased from Thermo Fisher Scientific, USA. Ultrapure water with a resistivity of 18.2 MΩ cm was used in all experiments.

Preparation of Spruce Cellulose Scaffold: In order to prepare extractive-free starting materials, spruce chips were extracted with a mixture of toluene and aceton (4:1 v/v) using a Soxhlet apparatus. After 10 cycles, the solvent was switched to ultrapure water for another 10 cycles to saturate the sample with water. The pre-extracted spruce was denoted as the native spruce. Alkaline extraction was performed by boiling the extractive-free spruce wood chips in 1 wt.% NaOH for 6 h. Subsequently, alkali-treated spruce (1 g) was crosslinked with BBDE or PEGDGE in a solution containing a) 5 mL of BBDE, 2 mL of 6.5 M NaOH and 15 mL of ultrapure water or b) 12.3 mL of PEGDGE, 2 mL of 6.5 M NaOH, and 7.7 mL of ultrapure water, respectively. The reaction time was 2 h and it was performed at 80 °C under mild magnetic stirring. The alkali-treated spruce wood chips with or without crosslinking were subsequently delignified with 1 wt.% NaClO₃ in 0.1 M sodium acetate buffer, pH 4.6 at 80 °C under mild magnetic stirring. The reaction time was up to 10 h for the uncrosslinked and BBDE crosslinked spruce samples, and 20 h for PEGDGE crosslinked spruce sample. After the reaction, the wood chips were thoroughly rinsed with ultrapure water to remove any residual chemicals. The primary amine groups were obtained by a further reaction between the epoxide groups onto the spruce cellulose scaffold, that is, delignified BBDE crosslinked spruce, the sample (1 g) was immersed into a solution containing 10 mL of DMSO and 4 mL of 1 M KOH, and the mixture was agitated at RT for 5 h on a see-saw rocker (SSL4, Stuart). To initiate the reaction, 5 mL of ECH was added. After 5 h, the reaction was ended and the sample was washed thoroughly with ultrapure water to remove any residual chemicals. The primary amine groups were obtained by a further reaction between the epoxide groups and PEHA, in 15 mL of PEHA and 10 mL of 0.6 M Na₂CO₃. The reaction and washing were performed in the same condition as described above. The primary amine groups that were introduced to the scaffold were quantified by fluorescent labeling with FITC, according to a previously reported method.[35] Briefly, the labeling was performed overnight at RT under mild agitation. The reaction was kept strictly in dark. The reaction contained 5 mL of FITC and 2 mL of 0.1 M NaHCO₃ (pH 9.0). The absorbance of the labeling solution was measured at a wavelength of 490 nm before and after it was reacted with the scaffold, and the amount of the amine-bound FITC was calculated based on a standard curve (Figure S4, Supporting Information).

Con A Immobilization: The amine-functionalized spruce cellulose scaffold (1 g) was first reacted with 10 mL of 10 wt.% glutaraldehyde under mild agitation. The reaction was ended after 2 h, and the sample was washed with 0.1 M sodium acetate buffer (pH 6.8) to remove any residual chemicals. Con A was immobilized to the cellulose scaffold by a covalent bond through the grafted glutaraldehyde in a reaction containing 12 mg of Con A in 2.5 mL 0.1 M sodium acetate buffer, pH 6.8. The immobilization reaction was carried out at 4 °C for 24 h, under mild agitation. To remove any unbound Con A molecules, the cellulose scaffold was thoroughly washed with 0.1 M sodium acetate buffer, pH 6.8. Bradford assay was used to quantify the amount of Con A that was immobilized on the cellulose scaffold. The assay was performed according to the manufacturer’s instructions. Briefly, the absorbance of the impregnation solution was measured at a wavelength of 595 nm before and after it was reacted with the scaffold sample, and the amount of the covalently-bound Con A was calculated based on a standard curve (Figure S5, Supporting Information).

Binding of Glycoprotein and Separation: Ovalbumin (2 mg) and lysozyme (2 mg) were dispersed in 40 mL of Tris·HCl buffer, pH 7.4 containing 5 mM MnCl₂, 5 mM MgCl₂, and 5 mM CaCl₂. The Con
A immobilized and the control aminated spruce cellulose scaffolds (1 g each) were immersed in the protein solution, gently agitated for 24 h at 4 °C, and then removed. Samples of 20 mL were collected from the protein solution before and after being reacted with the wood scaffold or the control wood, and subsequently concentrated into 1 mL using a centrifugal filter unit (AmiconUltra-15) with a 3 kDa cut-off membrane. For SDS-PAGE, the samples were diluted in a sample buffer containing 200 mM β-mercaptoethanol, 0.05% bromophenol blue, 5% SDS, and 50% glycerol in 225 mM Tris-HCl pH 6.8, and the proteins were denatured by boiling for 5 min before loading on Mini-protein TGX 4-20% gels. PAGEruler prestained protein ladder was used as a molecular mass marker, and the gels were stained using a PageBlue protein staining solution. To test the ovalbumin binding capacity of the immobilized Con A, Texas Red conjugated ovalbumin (1 mg) in 20 mL of the Tris-HCl buffer was reacted with the spruce wood scaffold (1 g) containing the immobilized Con A as described above. The scaffold was washed with the buffer to remove any nonspecifically bound ovalbumin, and the Con A-bound ovalbumin was subsequently eluted with 0.5 M mannose. The fluorescence intensities of the original ovalbumin solution, and the mannose eluate were measured on a CLARIOstar microplate reader with an excitation wavelength of 570 nm (bandwidth 20 nm) and an emission wavelength of 630 nm (bandwidth 40 nm). The amount of bound and eluted ovalbumin was calculated based on a standard curve (Figure S7, Supporting Information).

**Biotinylation and Binding of Streptavidin:** Amine-functionalized cellulose scaffold sample (0.1 g) was reacted with Biotin-NHS (2 mg) in 4 mL DMF. After stirring at 20 °C for 5 h, the sample was washed with DMF to remove the unreacted Biotin-NHS and then washed with water extensively. The biotin-functionalized cellulose scaffold was incubated in 2 mL PBS (pH = 6.8) buffer containing 1 mg Alexa Fluor 633 streptavidin for 6 h. The fluorescence intensity of streptavidin in PBS (pH = 6.8) solution was measured before and after reaction to quantify the bound streptavidin.

**Characterizations:** Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FT-IR) spectra were recorded at ambient temperature on a Spectrum System 2000 FT-IR spectrometer (PerkinElmer, USA) in a wavelength range of 4000 to 600 cm\(^{-1}\). Infrared Spectroscopy (ATR-FT-IR) spectra were recorded at ambient temperature on a Spectrum System 2000 FT-IR spectrometer (PerkinElmer, USA) in a wavelength range of 4000 to 600 cm\(^{-1}\). The temperature on a Spectrum System 2000 FT-IR spectrometer (PerkinElmer, USA) in a wavelength range of 4000 to 600 cm\(^{-1}\). The amount of bound and eluted ovalbumin was calculated based on a standard curve (Figure S7, Supporting Information).

**Supporting Information**

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

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**Conflict of Interest**

The authors declare no conflict of interest.
