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Supplementary Information for

Biological matrix composites from cultured plant cells

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Plant cell wall composition. Plant cell walls are naturally secreted by the protoplast on the outer surface of the plasma membrane. They primarily consist of four building blocks, cellulose, hemicellulose, pectin and lignin, at varying concentrations and spatial organization depending on the plant species, the cell's stage of development and function they serve. Cellulose is the main load bearing component of the cell wall and is organized into semi-crystalline microfibrils with excellent mechanical properties, with Young's modulus up to 130 GPa, and tensile strength up to 1 GPa, depending on crystallinity and physical characteristics (1). In the cell wall, cellulose microfibrils are immersed in a matrix of amorphous polysaccharides, hemicelluloses and pectins (2). Hemicelluloses bind individual cellulose fibrils together, assisting in the load transfer within the cell wall. Pectins form gels around the cellulose-hemicellulose fibrils as well as in the middle lamella, the region between neighboring plant cells, and heavily influence the cell wall porosity, thickness and cell adhesion (2). Lignin, an amorphous phenolic polymer which is deposited within the carbohydrate matrix of the cell wall in the final stages of cell differentiation, provides structural integrity by enabling load transfer to the cellulose microfibrils, while also providing resistance to external pathogens.

FTIR Analysis. We conducted FTIR measurements on the hydrated and (completely) dried cells as well as in the final dried compressed biocomposite, and the results confirm the presence of the same compounds during our processing steps. We compare our FTIR spectra with spectra from cultured tobacco cells (3) and cultured carrot cells (4) as well as with pure biopolymers (see Table S1 for peak-to-peak assignment) and confirm the multiple peaks assigned to carbohydrates in the 960-1370 cm^{-1} region. Briefly, the cellulose- and hemicellulose-related peaks are found at 1373, 1365, 1317, and 1034 cm^{-1}. The peaks at 1151, 1107, 1057 cm^{-1} are primarily associated with cellulose glycosidic vibrations. The pectin-assigned vibrations of the carboxylic ester at 1735-1740 cm^{-1} and carboxylic acid at 1600, 1414 cm^{-1} can be seen in our spectra (the 1600 peak is superimposed with water, phenolic and protein group vibrations). The 1238 cm^{-1} peak is attributed to C-O stretches in pectin and/or hemicellulose as well as in phenolics. Proteins and amino acids give rise to weak peaks at 1650 and 1550 cm^{-1}. Vibrations from phenolic compounds result in peaks at 1620-1630, 1515, 1238 cm^{-1}. The latter two are seen in the obtained spectra while the 1620-1630 overlaps with water and pectin vibrations making it non distinguishable. The hydrated living cells and the dried biocomposite have the same degree of pectin esterification (indicated by the ratio of the 1735/1414 peak intensities (5)). Thus our processing does not alter the pectin esterification state, while oven dried cells show a 50% reduced esterification as a consequence of heating.

In addition, the dominant broad peak at 3700-3000 cm^{-1} corresponds to hydrogen bonding in the constituent biopolymers. This broad band can be deconvoluted to peaks centered in 3400 cm^{-1} or higher wavenumbers which in pure cellulose materials, are attributed to intra-molecular hydrogen bonds of the cellulose chains, while peaks centered at lower wavenumbers have been associated with inter-molecular hydrogen bonds (6, 7). In all our spectra this band is centered between 3300-3400 cm^{-1} indicating the presence of intra- as well as inter-molecular hydrogen bonding between the cell wall biopolymers. Moreover, the redshifted hydrogen bonding band in the dried biocomposite and dried cells spectra, compared to the hydrated cells' spectrum, shows that the captured inter-molecular hydrogen bonding interactions in the biocomposite are due to the interactions between the cell wall biopolymers and to a lesser extent due to bound water. We note that the main carbohydrate peaks and primarily those associated with cellulose (1373 and 1317, 11057, 1034 cm^{-1} respectively), in addition to the pectin/phenolics 1238 cm^{-1} peak, are all found to be stronger in the biocomposite compared to the dried cells. This may be attributed to their higher concentration in the compacted assembly compared to when tested in loose powder form.

Molecular Dynamics. ReaxFF is a reactive force field (8) molecular dynamics simulation technique that employs bond order concept to consider the instantaneous interaction between atoms and molecules, and has been used in modeling thermo-mechanical properties of polymers with varying chemistries (9). ReaxFF provides a smooth transition between the non-bonded states and bonded states. This strategy allows the investigation of chemical reactions where bond formation and bond dissociation are involved during the course of the simulation. In general, the total interaction energy in ReaxFF is described by several contributions and can be summed up as follows:

$$E_{system} = E_{bond} + E_{over} + E_{under} + E_{val} + E_{tors} + E_{vdW} + E_{Coulomb} + E_{lp} + E_{H-bond}$$

The total energy of the system (E_{system}) consists of bonded or covalent interactions (which are bond-order-dependent) and nonbonded interactions. Bond-order-dependent terms include the bond energy (E_{bond}), over-coordination (E_{over}), undercoordination (E_{under}), and hydrogen bond (E_{H-bond}) interactions. Energy penalty terms include torsion angle energy (E_{tor}), valence angle energy (E_{val}), and lone pair energy (E_{lp}). Nonbonded interactions include van der Waals (E_{vdW}) and Coulomb energy ($E_{Coulomb}$). Bond order is directly calculated between all pairs of atoms from the interatomic distance and is updated at every MD or energy minimization step. The ReaxFF simulations for this investigation were carried out using SCM (Amsterdam Modeling Suite) (9).

Three types of polymer chains, hemicellulose, cellulose, and pectin along with coniferyl alcohol (Fig. S1), were added in a ratio of $\sim$ 2.69:2.27:1.02:1, as indicated from our compositional analysis, to a unit cell which represents the outer part of a cell wall. Considering that the material fabrication process compacts plant cells, bringing cell walls of adjacent cells in contact, our simulations start from the compaction of the two neighboring cell walls and allow the system to equilibrate at 300 K for 2.5 ps (Fig. S2).

After this, NPT simulations were carried out at 300 K at 0.101 MPa pressure and a damping constant of 500 fs. Finally the system is annealed from 300 K to 600 K and back to 300 K by carrying out consecutive NPT and NVT simulations as detailed in (9). The annealed structure (Fig. 2j) represents the compacted cell wall structure as produced by the fabrication

process. The virtual sample was subjected to tensile testing which revealed that fibrillar interlocking and hydrogen bonding interactions are the main intercellular bonding mechanisms as discussed in the main text. We note that the fibrillar interlocking is documented by the energy associated with bond (E_{bond}) and the torsional energies (E_{tors}) of the system, and as shown in Fig. S3 those components represent a substantial amount of the total energy of the system during deformation. Whereas the H-bonding interactions (E_{H-bond}) provide constant resistance to deformation due to cascading hydrogen bond breaking and reformation events (as documented in cellulose-based material in literature (10, 11).

XRD Analysis. From XRD patterns of the biocomposites, we confirm the presence of semicrystalline cellulose (Fig. 2m). Analysis of the diffraction patterns reveals that cellulose exists in multiple crystal polymorphs (I, II and III, marked in Fig. 2m) (12–14). The main diffraction peaks from native cellulose, I_α and I_β, correspond to (110) and (200) crystallographic planes respectively, and contribute to the broad 22-23° peak. The shoulders at 15° and 17° respectively are assigned to I_α and I_β diffractions from the (100), (010) and (1-10), (110) planes (12). Distinct contributions from cellulose II include the (110) peak at 19.9o and the (1-10) peak at 12.2°. Cellulose III diffractions from the (1-10), (100) and (010) planes give rise to the peaks at 20.9°, 20.6° and 11.6° respectively (12). Additionally, polysaccharide derivatives of mannose and galactose, which are backbone constituents of hemicellulose, give rise to the peaks at 19.1° and 24.8° (15, 16).

Water uptake. We perform water uptake and thickness swelling tests to dry biocomposite samples (see Materials and Methods). We measure a water uptake of 191 ± 53% (quantified in weight % gain) after 2 hours of immersion. Similarly, the thickness swelling after 2 hours is 173 ± 48% (quantified in volume % increase). In this timeframe, the water sensitivity of our biocomposites is similar to other wood-based composite materials, such as the mycelium-wood composites reported by Sun et al. (17). The results demonstrate that the adhesion between the mechanically compressed cells is poor upon immersion in water. This is expected in our samples, since we did not use chemical treatments or hydrophobic binders to initiate water-proofing properties. When used in wet or humid environments, it is reasonable to assume that surface treatments or coating of the biocomposites could be employed to mitigate water infiltration and damage.

Fig. S1. Minimized configuration of individual chains of (a) cellulose, (b) pectin, (c) hemicellulose (xyloglucan), and (d) phenolics (coniferyl alcohol).

Fig. S2. Unit cell with two cell walls before (a) and after (b) being equilibrated at 300 K for 2.5 ps.

Fig. S3. (a) Change in total energy (E_{system}) (1), E_{bond} (2), E_{H-bond} (3), and E_{tors} (4) during the tensile testing simulations. (b) MD simulation snapshots at different time points of sample deformation upon tensile loading.

Fig. S4. (a) Flexural Modulus and (b) modulus of rupture, calculated from 3-point bending experiments. Samples notation: BC: pure biocomposites material; 1: pine; 2: poplar; 3: oak; 4: walnut; 5: plywood; 6: MDF; 7: PS; 8: PP; 9: LDPE. All measured at the same conditions and strain rates.

Fig. S5. Typical stress-strain curves obtained from the biocomposite samples in (a) tension and (b) 3-point bending experiments.

Fig. S6. The biocomposites samples were subjected to 3-point bending tests in two perpendicular directions: (a) in plane with the cells lamellar arrangement and (b) normal to the cells stacking plane.

Fig. S7. SEM tangential-section views of the microstructure of (a) the biocomposite, (b) plywood, (c) MDF and (d) pine at the same magnification. (d) reproduced with permission from (18).

Table S1. FTIR peak assignment

Wavenumber (cm^{-1})	Vibration	Biopolymer Source	Reference
3200-3700	Intramolecular and intermolecular O-H Stretch	All biopolymers	(7, 19)
1735	Carboxylic Ester (C=O stretch)	Pectin, Hemicellulose, Phenolics	(3, 4, 20–23)
1650	Amide I (C=O stretch, C-N stretch)	Protein	(3, 4, 23)
1630	Phenolic stretch	Phenolics	(4, 19, 23)
1630-1650	OH bend	Water	(19, 24)
1600	Carboxylic acid (COO- stretch)	Pectin	(3, 23)
1540-1557	Amide II (N-H stretch, C-N stretch)	Protein	(3, 24, 25)
1516	C=C aromatic ring	Phenolics	(21, 22, 26)
1414	Carboxylic acid bending	Pectin, and to a lesser extent Hemicellulose	(20, 23)
1373	C-H bending	All carbohydrates	(25, 27)
1317	C-H bending	Cellulose primarily, hemicellulose	(25, 27)
1238	Syringyl ring, C-O stretch, C-O stretch of ester	Phenolics, Pectin and to a lesser extent hemicellulose)	(23, 26, 28)
1151	C-O-C glycosidic ring stretch	Cellulose, hemicellulose	(20, 24, 25)
1107	Glycosidic C-O, C-C, ring stretch	Cellulose	(20, 21, 25)
1057	C-O, C-C asymm. stretch, O-C-H bend	Cellulose	(20, 21, 24, 25)
1034	C-OH, C-O, C-C, C-CO stretch	Cellulose	(7, 20, 21, 24, 25)

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