CHALLENGES IN THREE-DIMENSIONAL (3D) PRINTING OF WOOD-DERIVED BIOPOLYMERS TOWARDS BIOMEDICAL APPLICATIONS

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ABSTRACT

The well-separated three main components from wood, i.e. cellulose, hemicelluloses, and lignin, are considered as promising candidates for replacing and improving the properties of oil-based and animal-derived biomaterials. To date, three-dimensional (3D) printing technology is expected to revolutionize the utilization of wood-derived biopolymers to tailor them obtaining advanced materials towards high-value applications, such as bioplastics and biomedical treatments. Here, we present an overview of our recent works on utilizing wood-derived biopolymers, especially nanocellulose and hemicelluloses, with different 3D printing techniques and elaborate the key challenges, which arose during those approaches. Spruce galactoglucomannan was found to be a promising candidate to partially replace natural bioplastic i.e. polylactic acid. Different nanocellulose-based inks have been successfully formulated and fabricated to scaffolds targeting at biomedical applications, in particular, wound healing application.

Keywords: Wood-derived biopolymers, 3D printing, biomedical applications, fused modeling deposing, direct ink writing

INTRODUCTION

Tissue engineering is coined as the tissue regeneration process combining artificial porous scaffolds, nutrients, and growth factors with cells from patients. The artificial porous scaffolds are generally mimicking the extracellular matrix (ECM), which is biologically secreted by cells for structural and biomedical support in the form of three-dimensional networks. In scaffold manufacturing, the need for comprehensive development of scalable methods for tailoring biomaterials with complex 3D structures is one of the most critical issues.[1] 3D printing has shown promising potential in complex tissue fabrication with a structural control from micro- to macro-scale meeting the requirements of tissue engineering.

Typically, three groups of biomaterials including ceramics, synthetic polymers, and natural polymers are mostly used in the fabrication of scaffolds for tissue engineering.[2] Ceramic scaffolds such as hydroxyapatite, tri-calcium phosphate, and bioactive glasses with typical high mechanical stiffness are mostly used in bone regeneration, dental, and orthopedic surgeries. However, the brittleness and difficulty of being shaped has limited its application in scaffold fabrication. Synthetic polymers such as polylactic acid (PLA), polystyrene, and polycaprolactone (PCL) have been used in the attempt to fabricate biological scaffolds due to easily being fabricated with tailored architectures and degradation characteristics. Natural polymers such as collagen and its derivatives, alginate-, and chitosan-derived biomacromolecules, as well as decellularized extracellular matrix (dECM) attracted great attention as the scaffold precursors owing to their inherent biocompatibility, bioactivity, and biodegradability. However, the heterogeneous sources for producing these natural materials and poor mechanical properties of the fabricated scaffolds have limited their use to specific biomedical applications. The use of composite scaffolds comprised of different phases has been increasingly receiving tendency with exploiting the advantages of each individual biomaterial group.

Recently, wood-derived biopolymers have been tremendously studied as biomaterials in tissue engineering.[3] Firstly, wood-derived biopolymers including cellulose, hemicelluloses, and lignin are recognized as biorenewable materials, which could be supplied from abundant sources. In particular, nanocellulose in the form of cellulose nanofibrils prepared from cellulose emerged as biomaterials to fabricate various tissue mimics owing to their hydrogel form, superior mechanical strength, and structure similarity to ECM. Hemicelluloses with properties of good water-solubility and ease to be functionalized have been investigated as hydrogel precursors in the applications of pharmaceuticals and tissue engineering. Nevertheless, the intrinsic affinity of hemicelluloses to cellulose indicates a promising methodology for the development of biomimetic materials in the course of biomedical applications.[4] Lignin in different forms has also been intensively studied for biomedical applications and as a drug delivery carrier, particularly due to its antioxidant activity. In short, the properties of the abundant and widely applicable wood-derived biopolymers, including their high thermal degradation stability, intrinsic gel formation, and easily being chemical modification, promote them as the feedstock materials for 3D printing techniques in different forms of solid powders, synthetic photo-curable resins,

thermal bioplastics, nanomaterial hydrogels, etc.

3D PRINTING TECHNIQUES AND CHALLENGES

3D printing, also known as three-dimensional printing additive manufacturing, offers the ability to rapidly, reproducibly, and precisely manufacture a wide range of object geometries via a computer-controlled layer-by-layer process.[5] Among different 3D techniques, fused modeling deposition (FDM), direct ink writing (DIW), inkjet printing, and lithography are commonly used for scaffold fabrications. In tissue engineering, the above-mentioned 3D printing strategies are often used to print complex scaffolds followed by a cell seeding process. Furthermore, 3D printing that involves the controlled deposition of cell-laden bioinks for tissue engineering is commonly referred as 3D bioprinting. In our recent studies, FDM and DIW 3D printing were used for the development of wood-derived inks in the forms of thermoplastics and hydrogels.

FDM 3D printing (**Fig. 1.a**) is one type of extrusion-based printing technique, which combines the molten-flow of the filament and solidification after cooling. It is a simple and cost-effective approach to manufacture scaffolds compared with other 3D printing techniques. As an essential requirement, the feedstock material must be in the filament form with a specific diameter. The hot melt extrusion technique is required to shape the filament through consequent processing materials with melting, extrusion to accurate diameter, cooling, and filament winding. Similarly, the well-shaped filament passes through the feeding roller, heater, and nozzle and the molten paste is printed layer-by-layer on a printing platform. The successive layer is deposited on the top of the previous layer. The two layers are fused together when the material cools and solidifies. In general, the FDM printing technique is commonly used for manufacturing thermoplastic-based materials such as PLA, PCL, ethylene-vinyl acetate, and acrylonitrile butadiene styrene (ABS).

DIW 3D printing is another type of extrusion-based technique, which shapes viscose hydrogels or slurries into 3D constructs, as shown in **Fig. 1.b**. Most of the time, the inks are stored in a syringe-like reservoir connected to a dispensing nozzle. Further, the flow of the inks is formed from the displacement of the syringe piston by either pneumatic or mechanical forces. After printing, the deposited inks keeps the shape fidelity combing different cross-linking strategies including chemical, ionic, enzymatic, as well as UV- and thermo-induced cross-linking. Chemical cross-linking is the most direct way to fix the shape of the printed objects and requires extra cross-linking reagents and specific reaction conditions. Ionic cross-linking is a simple and effective way to cross-link the manufactured scaffolds. However, the cross-linking rapidly initiates on the skirt and surface of the complex scaffolds resulting in the formation of shell on the skirt and surface, and further inhibits the transfer of ions to the inner structure of the 3D constructs. Moreover, the 3D constructs could not well keep their shape when the scaffolds are transferred into a cell culture medium, which usually is highly ionic. Enzymatic cross-linking is a mild way to achieve shape fidelity. However, the gelation window takes as slowly as a few hours, limiting its applicability in biofabrication. UV- and thermal induced cross-linking offers a very short gelation time window, which is required for *in situ* gelation during biofabrication.

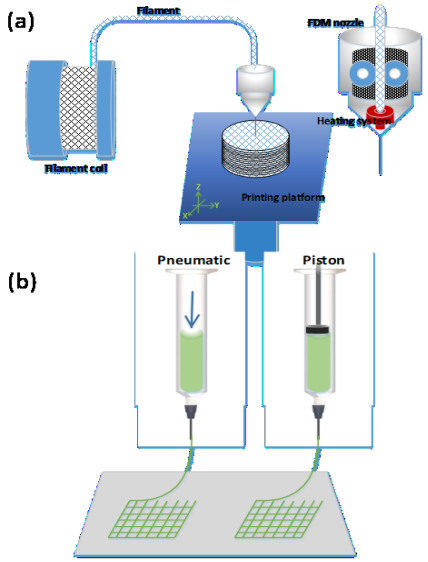

Fig. 1. Schematic illustration of the different printing techniques: (a) FDM, and (b) DIW.

WOOD BIOPOLYMERS IN 3D PRINTING

Cellulose and its derivatives such as microcrystalline cellulose, cellulose esters, and cellulose ethers have been applied as 3D printing ink feedstock. At the early stage, the cellulosic materials were mostly used for FDM 3D printing as a filler to the drug carrier materials in the study of controlled and personalized drug delivery. Carboxymethylated cellulose (CMC) is commonly used as viscosity-enhancing polymer together with other biopolymers for 3D printable ink formulation. Recently, the inks integrated with cellulose nanomaterials have attracted a great deal of attention due to their outstanding mechanical properties and biocompatibility. In 2011, cellulose nanofibrils (CNFs) were first proposed for fabricating implants and scaffolds for tissue engineering applications using inkjet printing technique by Gatenholm et al. [6] In the following studies, the CNF-based bioink formulations were studied for different applications including cartilage regeneration, human chondrocytes re-differentiation, and adipose tissue engineering. [7] In general, for the ink formulation, an auxiliary material is mostly required to meet the viscoelastic properties and to

offer the possibility to keep the printed structure via different cross-linking strategies. More comprehensive discussions could be referred to the review article on the overview of the subject. [3]

There are still very few studies on 3D printing using the side-stream biopolymers of hemicelluloses and lignin compared to other biopolymers. Still, hemicelluloses and lignin hold promising potential as scalable and high-value 3D printable feedstock materials. Recently, one of our studies successfully applied bulk spruce galactoglucomannan (GGM) in FDM printing. As the vast possibility of being functionalized, hemicelluloses e.g. xylan with grafted tyramine groups were utilized by Markstedt et al. acting as both a fiber surface modifier and a biodegradable cross-linker in the formulation of inks followed by enzymatic cross-linking. Similarly, lignin has attracted great attention in thermoplastics, which offers the possibility of utilizing lignin-based bioplastics in FDM printing. Nevertheless, lignin has been introduced into printing inks as a performance enhancer. For example, lignin-coated CNC was recently applied to both FDM and SLA 3D printing in combination with ABS and methacrylate resin, respectively. [8-9] Notably, lignin with tolerant concentrations and in different forms has been intensively studied for biomedical applications and as a drug delivery carrier, particularly due to its antioxidant activity. [10]

CASE STUDIES OF THE USE OF WOOD BIOPOLYMERS IN PRINTING

Challenges in formulation of wood biopolymers for FDM-based printing

One of our studies demonstrated the possibility of utilizing spruce GGM for partially replacing PLA in the FDM printing filaments. [11] It is a challenge to evenly mix PLA with GGM, since PLA and GGM have different polarity and GGM does not have a distinct melting temperature. Then, the uneven mixed composites could further cause a failure to the mechanical properties of the filament and the printed objects. To address this issue, a co-solvent blending method was established. In the study, GGM was firstly dissolved in DMSO. DCM was then added to obtain a DMSO:DCM ratio of 20:80. PLA pellets were then added. Ultrasonification was applied to ensure homogeneous mixing. Afterwards, the blends were precipitated by adding them dropwise into cold ethanol. All the precipitates were collected and dried. Filaments were further manufactured with NOZTEK pro extruder (UK) at 165 °C with a nozzle diameter of 1.7 mm after screening at different temperatures ranging from 150 °C to 185 °C. It has been demonstrated that up to 20 wt.% of PLA could be replaced by GGM without causing the failure of the mechanical properties. Examples of filament and printed scaffolds with good (**Fig. 2.a&b**) and poor (**Fig. 2.c&d**) surface qualities could be observed in **Fig. 2**.

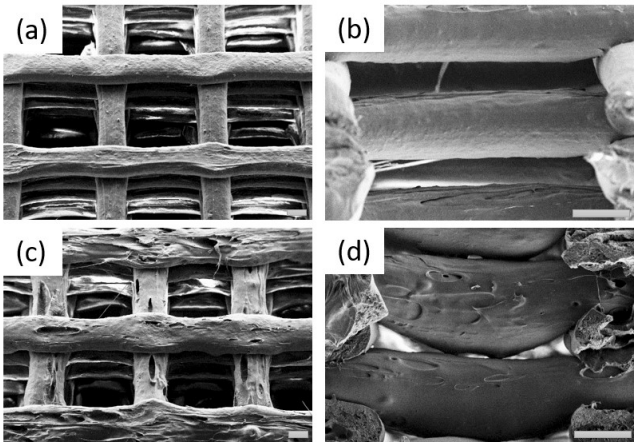

Fig. 2. Image of the printed scaffolds with 15 wt.% of GGM (a&b) and 25 wt.% of GGM (c&d) replacing PLA. Scale bar is 200 µm.

Challenges in formulation of wood biopolymers for DIW-based printing

In this section, our works regarding 3D printing of CNF-based inks are summarized. CNFs with the intrinsic gel status and their structure similarity to ECM act as promising substrates for cell culture and tissue engineering. The earlier findings of our study suggested that low-charged CNFs are cyto-compatible and support fibroblast cell growth.[4] Thus, in the following discussions, all the used CNF hydrogel was prepared from the controlled TEMPO-mediated oxidation with surface carboxylic density of 1.1±0.1mmol/g or close.

To formulate a suitable ink for DIW printing, the ink must show shear-thinning behavior, i.e. being easy to yield-flow through the nozzle with low resistance. Firstly, the CNF itself with different consistencies was demonstrated for printing due to its well-known shear-thinning property. As shown in **Fig. 3**, both the CNF dry content of 1 wt.% and even down to 0.7 wt.% showed gel-like property and obvious yield points. Yet, the printed scaffolds from 0.7 wt% could not keep shape stable in water for long (Inserted images, **Fig. 3**). This facilitated the development of low-concentration inks for tissue engineering, which could supply the relative loose polymer network encouraging cell-cell interaction, migration and more efficient metabolism, however, a critical requirement in improving scaffold's stability needs to be considered. Furthermore, the printability of both inks was demonstrated by printing scaffolds followed by 1,4-butanediol diglycidyl ether (BDDE) cross-linking in wet-state. The mechanical property could be well tuned via controlling the degree of cross-linking. [12] The representative scaffold manufactured by this method is shown in **Fig. 4a and b**. However, the cross-linking

accessibility is still low, which could not supply high mechanical strength for the scaffolds.

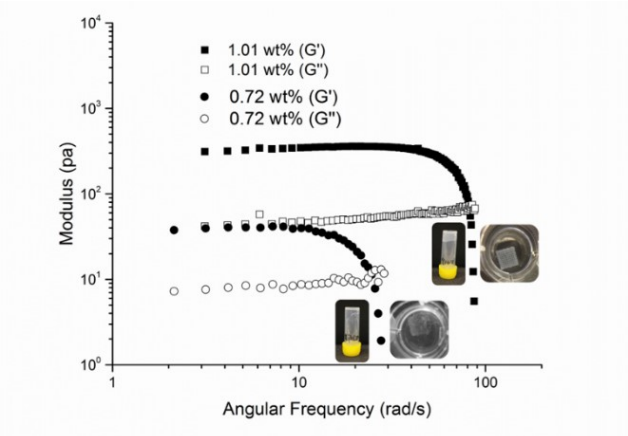

Fig. 3. Rheology of CNF hydrogels with different concentrations (0.72 wt.% and 1.01 wt.%). Two images of scaffolds: successful printing with 1.01 wt.%, and unsuccessful printing with 0.72 wt.%.

Next, CNF-based composite inks were proposed to yield a better mechanical property by cross-linking the auxiliary material. Less than 1 w/v % of gelatin methacrylate (GelMA) was formulated with CNFs to enhance shape fidelity by UV cross-linking. [13] However, during ink formulation, phase separation happened owing to the strong physical interaction between CNF and GelMA. It further clogged the printing needle resulting in the failure of scaffold manufacturing. Thus, the interaction between CNFs and the auxiliary material should be well studied before and after ink formulation to obtain homogeneous inks avoiding the nozzle clogging. After printing, UV cross-linking of GelMA was utilized to maintain the printed structure thanks to the introduction of methacrylate groups on GelMA polymer chain. As studied, a rapid cross-linking happens in less than 3 min. The printed scaffold with this method can be seen in **Fig. 4c**.

Furthermore, homogeneous inks were formulated by methacrylated GGM (GGMMA) as an auxiliary material with CNF attributed to the biomimetic affinity between hemicelluloses and cellulose. [14] The rapid gelation window of the formulated inks facilities the utilization of these all-wood-based biopolymers as the ink for DIW printing. The good resolution and printability of the ink is demonstrated in **Fig. 4d**. Most importantly, a wide and tunable spectrum of Young's moduli, ranging from 2.5 to 22.5 kPa, of different hydrogels could be achieved by varying the substitution degree in GGMMA and the compositional ratio between GGMMA and CNF.

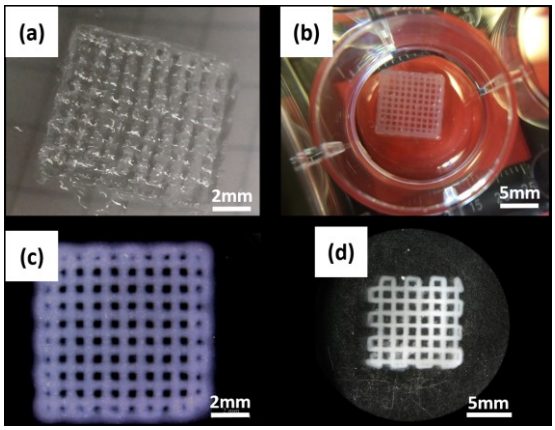

Fig. 4. (a) An illustration of poor shape fidelity of the CNF scaffold, (b) CNF scaffold after BDDE cross-linking, (c) scaffold manufactured from CNF/GelMA ink, and (d) high resolution scaffold from GGMMA/CNF ink.

CHALLENGES IN BIOCOMPATIBILITY

Biocompatibility of the formulated inks and printed scaffold is the prerequisite to determine the suitability of material for tissue engineering. Therefore, it is critical to avoid at best hazardous components from the preparation of ink precursors. In other words, the used single component such as CNFs or auxiliary materials must be cyto-compatible to cells. The synthesis of suitable CNFs and auxiliary materials still requires more attention in practice. Next, to certain extent, the formulated inks are better to have a sufficiently low dry content to supply relatively loose network and space for cell-cell interaction, migration and more efficient metabolism. However, the physical property of the scaffolds must properly meet the needs of cell behavior. The shape fidelity of the complex scaffolds via different cross-linking strategies also needs to be concerned, especially for bioprinting. For example, UV cross-linking is mostly an applicable method in clinical areas. However, the needs of photo-initiator, certain time of UV irradiation, and high intensity of UV light still need to be investigated and balanced for specific applications.

CONCLUSIONS

The application of wood-derived biopolymers to 3D printing techniques for biomedical applications is an emerging field. It offers unexploited potential to seek advanced functional materials from the most abundant and sustainable resources on earth, as well as to respond to the global trends towards personalized medicine and therapy. In particular, cellulose nanomaterials with their intrinsic gel-formability satisfy the application criteria for DIW printing. Hemicelluloses, which are easy to modify, hold a great promise as promoting agents, acting as both fiber surface modifiers and cross-linkable auxiliary materials in

the formulation of CNF-based inks for 3D printing. As an extensively interdisciplinary field, more researches still need to be performed before obtaining a clearer view regarding 3D (bio-) printing of wood-derived biopolymers.

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REFERENCES

1. Hollister, S. J. Scaffold design and manufacturing: from concept to clinic. *Adv. Mater.* 2009, 21 (32-33): 3330-3342.
2. O'brien, F. J. Biomaterials & scaffolds for tissue engineering. *Mater. Today* 2011, 14 (3): 88-95.
3. Xu, W.; Wang, X.; Sandler, N.; Willför, S.; Xu, C. Three-dimensional printing of wood-derived biopolymers: A review focused on biomedical applications. *ACS Sus. Chem. & Eng.* 2018, 6 (5): 5663-5680.
4. Liu, J.; Chinga-Carrasco, G.; Cheng, F.; Xu, W.; Willför, S.; Syverud, K.; Xu, C. Hemicellulose-reinforced nanocellulose hydrogels for wound healing application. *Cellulose* 2016, 23 (5): 3129-3143.
5. Van Wijk, A.; van Wijk, I. *3D printing with biomaterials: Towards a sustainable and circular economy*. IOS press: 2015.
6. Gatenholm, P.; Backdahl, H.; Tzavaras, T. J.; Davalos, R. V.; Sano, M. B., Three-dimensional bioprinting of biosynthetic cellulose (BC) implants and scaffolds for tissue engineering. Google Patents: 2014.
7. Gatenholm, P.; Martinez, H.; Karabulut, E.; Amoroso, M.; Kölby, L.; Markstedt, K.; Gatenholm, E.; Henriksson, I. Development of nanocellulose-based bioinks for 3d bioprinting of soft tissue. *3D Printing and Biofab.* 2018, 331-352.
8. Feng, X.; Yang, Z.; Chmely, S.; Wang, Q.; Wang, S.; Xie, Y. Lignin-coated cellulose nanocrystal filled methacrylate composites prepared via 3D stereolithography printing: Mechanical reinforcement and thermal stabilization. *Carbohydr. Polym.* 2017, 169: 272-281.
9. Feng, X.; Yang, Z.; Rostom, S. S.; Dadmun, M.; Xie, Y.; Wang, S. Structural, mechanical, and thermal properties of 3D printed L‐CNC/acrylonitrile butadiene styrene nanocomposites. *J. Appl. Polym. Sci.* 2017, *134* (31): 45082.
10. Figueiredo, P.; Lintinen, K.; Kiriazis, A.; Hynninen, V.; Liu, Z.; Bauleth-Ramos, T.; Rahikkala, A.; Correia, A.; Kohout, T.; Sarmento, B. In vitro evaluation of biodegradable lignin-based nanoparticles for drug delivery and enhanced antiproliferation effect in cancer cells. *Biomaterials* 2017, 121: 97-108.
11. Xu, W.; Pranovich, A.; Uppstu, P.; Wang, X.; Kronlund, D.; Hemming, J.; Öblom, H.; Moritz, N.; Preis, M.; Sandler, N. Novel biorenewable composite of wood polysaccharide and polylactic acid for three dimensional printing. *Carbohydr. Polym.* 2018, 187: 51-58.
12. Xu, C.; Zhang, B.; Wang, X.; Cheng, F.; Xu, W.; Molino, P.; Bacher, M.; Rosenau, T.; Willför, S.; Wallace, G. 3D printing of nanocellulose hydrogel scaffold with tunable mechanical strength towards wound healing application. *J.. Mater.Chem. B* 2018, Accepted.
13. Xu, W.; Molino Zhang, B.; Cheng, F.; Molino, J. P.; Yue, Z.; Su, D.; Wang, X.; Willför, S.; Xu, C.; Wallace, G. G. 3D printing of scaffolds with low-concentration inks formulated by nanocellulose assisted with gelatin methacrylate (GelMA) for wound healing application. *ACS Appl. Mater. & Inter.* 2018, Under submission.
14. Xu, W.; Zhang, X.; Långvik, O.; Wang, X.; Zhang, Y.; Österberg, M.; Willför, S.; Xu, C., Surface engineered biomimetic inks based on UV cross-linkable wood biopolymers for 3D printing. *ACS Appl. Mater. & Inter.* 2018, Under revision.