Biosynthesis of Bacterial Cellulose for *in-situ* Assembly of Intelligent Packaging with Natural Dyes

Pengfei Lv,[a,*] Xiaomin Lu,[b] Huimin Zhou [c] and Xiaohang Sun [b]

Natural materials such as wood, bone, and skin continue to command the respect and admiration of materials scientists. It is difficult to achieve comparable properties by the use conventional industrial manufacturing processes. In this essay we are proposing a radical approach to the preparation of future intelligent packaging materials. Rather than attempting to assemble the chemical components at a nano-scale to make an intelligent package, our proposal is to let life itself take care of much of the assembly. We propose that the natural growth of bacterial cellulose can be used as a way to prepare a well-integrated structure at the nano-scale. Additives such as natural dyes can be introduced already during biosynthesis and thus become well integrated with the packaging material from the start. For example, one can develop a smart label for pH monitoring based on bacterial cellulose doped with natural dyes extracted from natural byproducts by *in situ* biosynthesis of cellulose. The resulting film has potential to be used as a visual indicator of the pH variations during storage of packaged food.

Keywords: Bacterial cellulose; Natural dyes; Intelligent packaging

*Contact information: a: College of Materials Science and Engineering, Tianjin University, Tianjin, 300350, P. R. China; b: Department of Forest Biomaterials, North Carolina State University, Raleigh, NC 27695, USA; c: Key Laboratory of Eco-textiles, Ministry of Education, Jiangnan University, Wuxi, 214122, P. R. China; *Corresponding author: 18861800875@163.com*

Introduction

Plastics have dominated the market in the food packaging area since the mid-1900s. Such dominance is not surprising, given plastics' good processability, availability of petroleum feedstocks, and low cost to meet the needs for packaging of a specific food product (Rhim *et al*. 2013). However, synthetic polymers from fossil fuels cause widespread environmental problems because of their non-biodegradable and non-renewable nature. These challenges have become even more acute with the development of intelligent packaging, which has higher demands for information content, sensing ability, and other features (Engel *et al*. 2019).

Intelligent packaging typically contains two categories of features: a data carrier such as a radiofrequency identification (RFID) tags or a barcode label; and a package indicator such as a pH, gas, or a time-temperature indicator (Kerry *et al*. 2006). Recently, pH indicators have become widely used, because changes in pH often can be used to detect the spoilage of food. Normally, a pH indicator is composed of two important parts, namely a pH sensitive dye and a solid support. In contrast to synthetic dyes, natural dyes are advantageous due to their safety and eco-friendly feature. For example, anthocyanins, which are known as flavonoids, can be extracted from fruits. Anthocyanins are nontoxic, natural, easy extractable, and water-soluble pigments. Their use in place of synthetic dyes can avoid inflammation, diabetes, cancer, neuronal diseases, and many other diseases. Importantly, anthocyanin is sensitive to pH conditions due to the presence of phenolic or conjugated substances (Ma *et al*. 2017).

Recently, many researchers have focused on the use of biodegradable polymers (Lu 2019). Because cellulosic food packaging film has the characteristics of transparency, firmness and flexibility, while concomitantly having good strength and hydrophilicity, cellulose has been widely used in food packaging (Tirtashi *et al*. 2019).

Bacterial Cellulose

Bacterial cellulose (BC), which has a three-dimensional nanostructure, is produced by a wide variety of microorganisms of the *Gluconacetobacter genus*. BC in the form of a nano-fiber membrane and has attracted great attention due to its high bio-degradability, high purity, high crystallinity, ultrafine three-dimensional network architecture with a distinct tunnel and pore structure, high specific surface area, and remarkable mechanical resistance, *etc*. (Lv *et al*. 2018). BC has the capacity to act as a host biopolymer to receive guest molecules to be incorporated in its matrix (Lv *et al*. 2017). Moreover, BC film with high specific surface area and high porosity is an ideal candidate for the development of a gas sensor based on detection of pH changes based on color monitoring. Volatile vapor emissions during food spoilage can show up as subtle pH shifts at the surface of packaging materials. Compared with other biopolymers, the porous structure of BC can improve the absorption of pH-changing liquids and gases, promoting the color-changing reaction of pH-sensitive dyes in the BC network. Therefore, the BC membrane has a great potential for being used as a solid support material for fabrication of more sensitive and rapid response intelligent packages. BC has the advantages of good biocompatibility, remarkable tensile strength, high water retention, high hydrophilicity, high crystallinity, and so on, and it can be used as the support material for intelligent pH indicator packaging.

Intelligent Packaging Film Preparation

A proposed concept for biosynthesis-based manufacture of intelligent packaging film production is shown in Fig. 1, wherein the circles represent dye molecules and the curved lines represent fibrils of BC.

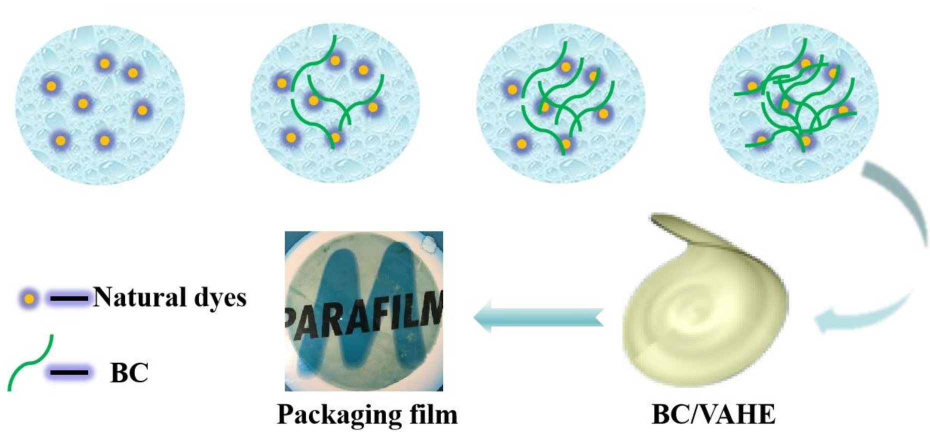

Fig. 1. Schematic diagram of the formation of intelligent packaging film

To begin, natural dyes can be extracted from natural by-products. Subsequently, the dyes are added into culture media and sterilized by the autoclave. For example, pre-cultured cells containing *Gluconacetobacter xylinus* can be inoculated into culture solution containing natural dyes. The flasks are incubated on a cultivation cabinet for some days. The synthesized BC-based nanocomposites are separated from the medium by filtration and purification to remove the microorganism and culture medium embedded in the cellulose material, and then repeatedly rinsed to neutral in pure water. Finally, the intelligent films are prepared in a vacuum-drying oven. The change of film color then can be measured after adjusting the pH.

Closing Thoughts

As in the example described above, there is potential to make effective use of the natural growth of cellulose-producing bacteria to create films and other suitable structures for use in packaging applications. Other types of packages that might be considered could include solid foams, barrier films, and different kinds of sensing devices. Many such packages also could be suitable for ink-jet application of bar codes and RFID devices. Though conventional technology, including 3D printing, has made great strides in the preparation of macroscopic structures, there has been a need for ways to organize materials better at the nanoscale. The use of life itself as part of the organizing system can open up new opportunities for high-performing and intelligent packaging materials.

Acknowledgements

This work is supported by China Postdoctoral Science Foundation funded project (No. 2019M661015).

References Cited

Engel, J. B., and Ambrosi, A., and Tessaro, I. C. (2019). "Development of biodegradable starch-based foams incorporated with grape stalks for food packaging," *Carbohydrate Polymers* 225, article no. 115234. DOI: 10.1016/j.carbpol.2019.115234

Kerry, J. P., O'grady, M. N., and Hogan, S. A. (2006). "Past, current and potential utilisation of active and intelligent packaging systems for meat and muscle-based products: A review," *Meat Science* 74(1), 113-130. DOI: 10.1016/j.meatsci.2006.04.024

Lu, X. (2019). "Cellulose nanocrystals for wrinkled fabric," *BioResources* 14(4), 7632-7635. DOI: 10.1002/adfm.200300002

Lv, P., Zhou, H., Mensah, A., Feng, Q., Wang, D., Hu, X., Cai, y., Lucia, L., Li, D., and Wei, Q. (2018). "A highly flexible self-powered biosensor for glucose detection by epitaxial deposition of gold nanoparticles on conductive bacterial cellulose," *Chemical Engineering Journal* 351, 177-188. DOI: 10.1016/j.cej.2018.06.098

Lv, P., Yao, Y., Li, D., Zhou, H., Naeem, M. A., Feng, Q., Huang, J., Cai, Y., and Wei, Q. (2017). "Self-assembly of nitrogen-doped carbon dots anchored on bacterial cellulose and their application in iron ion detection," *Carbohydrate Polymers* 172, 93-101. DOI: 10.1016/j.carbpol.2017.04.086

Ma, Q., Ren, Y., Gu, Z., and Wang, L. (2017). "Developing an intelligent film containing *Vitis amurensis* husk extracts: The effects of pH value of the film-forming solution," *Journal of Cleaner Production* 166, 851-859. DOI: 10.1016/j.jclepro.2017.08.099

Rhim, J. W., Park, H. M., and Ha, C. S. (2013). "Bio-nanocomposites for food packaging applications," *Progress in Polymer Science* 38(10-11), 1629-1652. DOI: 10.1016/j.progpolymsci.2013.05.008

Tirtashi, F. E., Moradi, M., Tajik, H., Forough, M., Ezati, P., and Kuswandi, B. (2019). "Cellulose/chitosan pH-responsive indicator incorporated with carrot anthocyanins for intelligent food packaging," *International Journal of Biological Macromolecules* 136, 920-926. DOI: 10.1016/j.ijbiomac.2019.06.148