Preparation of Degradable Biological Carrier With LCC and its Application in Culture of Hepatocytes

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Abstract. The purpose of this article is to extract lignin-carbohydrate complexes (LCC) with poplar as raw material which was used to prepare bio-carrier by freeze-drying method. The, chemical properties and morphological of LCC porous biological carriers were analyzed by GPC, FT-IR, scanning electron microscopy (SEM) and optical microscopy. The FT-IR spectrum results indicated that LCC which are composed of lignin and polysaccharide, with a typical LCC structure. Galactose have a specific ability to recognize liver cells owing to the presence of receptors on hepatocytes. Cell counting results showed that the cells increases fastest while the proliferation rate of the liver cell in LCC is obviously higher than that of control group. These results indicated that poplar LCC is very biocompatible which it might be a great potential biological carrier material for human hepatocyte culture.

1. Introduction

As a kind of organic substance, Lignin has large amount in nature, just less than cellulose[1] and chitin, and mostly founded in woody plants and herbaceous plants[2-4]. The lignin-carbohydrate complex (LCC) is composed of lignin and polyglycans (polygalactose, xylose and glucomanan) hrough a firm covalent bond formed by heterogeneous polymers which is a good biological material. Many researchers [5-8] extracted LCC from plants by various methods and further studied their structure and properties. LCC has good biocompatibility and mechanical strength owing to LCC contains both hydrophobic lignin rigid blocks and hydrophilic polysaccharide flexible blocks. In recent years, Erakovic [9], Mansur [10] and Martinez [11] have studied the biocompatibility of lignin/hydroxyapatite bio-scaffolds. These results showed that the combination of lignin and polyhydroxy compounds bulk material has good biocompatibility. Previous studies have shown that the polysaccharide portion of LCC not only has good biocompatibility, but also lignin molecules dose which has aroused widespread concern about the biocompatibility of LCC-based materials.

Porous carrier have large specific surface area which increased the cell growth area, and improve cell culture density [12]. LCC is rich in galactose and mannose which has capability of recognizing by hepatocyte [13-14].In order to study the application of LCC in human hepatocytes culture, the LCC porous biological carrier materials were prepared by freeze drying method. The molecular structure, lignin and monosaccharide contents, and morphologies of porous carriers were characterized by GPC, FT-IR, scanning electron microscopy (SEM) and optical microscopy. These results indicated that LCC has good biocompatibility which can be used as carrier material for human hepatocyte culture.
2. Experimental section

2.1 Materials
Poplar tree was obtained from Wuhan nursery garden. Human hepatocytes was provided by Pricells company (Wuhan, China).

2.2 Preparation of poplar LCC
Poplar wood meal extracted with benzene/ethyl alcohol (2/1, V/V) following hot water, and then dried in vacuum drying oven for 7 days. The extractives-free wood mill was further ground for 72h in a vibration ball mill with water cooling. The Poplar LCC was then purified with previous method [15], which was shown in Fig1.

2.3 Preparation of LCC based Hydrogels porous carriers.
Poplar LCC (100mg) from ball milling of poplar wood were placed in different test tubes. The NaOH solution (200μL, 3.3mol/L) was then added to each tube. Subsequently, the mixtures were then stirred in an ice bath for 4h until LCC completely dissolved. To the above solutions, polyethylene glycol glycidyl ether (50μL) was added and further stirred until all polyethylene glycol glycidyl ether was dissolved. The mixture was stirred at 50 °C for 24 h to obtain hydrogel porous biological carrier. This was subsequently added to Erlemeyer flask and then washed with distilled water to until the water become clear and pH values become 7. Then the sample was freeze-drying for 24h.

Figure 1. The isolation and purification of LCC from poplar wood.
3. Results and discussions

The peak of 3417 cm\(^{-1}\) are attributed to the hydroxyl group of poplar LCC which was shown in figure 2. The absorption of alcohol based C-O at 1045 cm\(^{-1}\) indicated the presence of polysaccharide. The peaks at 1509 cm\(^{-1}\) and 1421 cm\(^{-1}\) are assigned to the absorption of the aromatic structures in lignin moieties. The FT-IR spectrum results indicated that LCC which are composed of lignin and polysaccharide, with a typical of lignin-carbohydrate complexes structure.

![FT-IR spectroscopy of the LCC.](image)

Table 1. Chemical composition of poplar LCC.

| Compositional | lignin | Glucose | Xylose | Galactose | Arabinose | Mannose | Total sugar |
|---------------|--------|---------|--------|-----------|-----------|---------|-------------|
| content/%     | 27.49  | 4.83    | 33.15  | 5.67      | 4.25      | 13.52   | 61.42       |

The average molecular weight (Mw), number average (Mn) and polydispersity (Mw/Mn) of poplar LCC were shown in Table 3. The molecular weight of The LCC sample is relative low for bio-carrier. Therefore, it should be cross-linked during the preparation of bio-carrier in order to increase the strength and stability.

Table 2. Results of weight-average (Mw), number-average (Mn) molecular weights and polydispersity indexes (Mw/Mn) of LCC.

| Sample | Mw | Mn | Mw/Mn |
|--------|----|----|-------|

Galactose can be recognized by the receptors on liver cells with high physiological activity to hepatocytes [16]. Galactose has been used to enhance the selective interaction between biological bio-carrier and hepatocytes. As shown in Table 1, the galactose content in LCC macromolecules is 5.67%. The contents of lignin and total sugar are 23.9% and 64.62% respectively, which will lead to good physical strength of LCC-based porous biological carrier had.
Human hepatocytes (L-02) were stably cultured in the presence of a biological carriers. As shown in Figure 3, a large number of hepatocytes are found to adhere to the spherical biological carriers. These results showed that LCC is nonpoisonous, biocompatible and suitable for the hepatocyte culture. The cell growth experiment with bio-carriers and the control group at 1–7th is shown by cell counting. As shown in Figure 4, the cells of cultured on the bio-carriers and the control group showed a slow increase trend in the first three days. On the 5th day, the cell proliferation reached a maximum. The proliferation rate of the experimental group was significantly higher than that of the control group.

![Figure 3](image1.png)

**Figure 3.** The inverted microscope image (A) and SEM image (B) of human hepatocytes L-02 cultured on the carriers.

![Figure 4](image2.png)

**Figure 4.** Hepatocytes growth curve

4. **Summary**

Lignin-carbohydrate complexes (LCC) were isolated from poplar wood by ball milling method. The FT-IR analysis showed that the LCC spherical carrier consisted of lignin and polysaccharide with a typical of LCC structure. Chemical analysis showed that the contents
of galactose, lignin and total sugar were 5.67%, 27.49% and 61.42%, respectively. The spherical carrier was prepared with the LCC through cross-linking by polyethylene glycol glycidylic ether. The results indicated that the spherical biological carrier has good compatible with the liver cells owing to galactose which can be recognized by receptors of liver cells. A large number of hepatocytes were found to adhere to the spherical biological carrier. The results of cell counting show that the cell proliferation is the fastest on the 5th day. The proliferation rate of hepatocytes with LCC-based bio-carrier is significantly higher than that of control experiment. These results indicated that LCC is non-toxic, biocompatible and suitable for hepatocyte culture.

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