Carbohydrate Polymers 114 (2014) 553–557

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Short communication

Creation of macropores in three-dimensional bacterial cellulose scaffold for potential cancer cell culture

Guangyao Xiong [a], Honglin Luo [a,b], Yong Zhu [b], Sudha Raman [c], Yizao Wan [a,*]

[a] School of Mechanical and Electrical Engineering, East China Jiaotong University, Nanchang 330013, China
[b] School of Materials Science and Engineering, Tianjin University, Tianjin 300072, China
[c] Department of Community and Family Medicine, Duke University, Durham, NC, USA

ARTICLE INFO

Article history:
Received 11 June 2014
Received in revised form 16 August 2014
Accepted 16 August 2014
Available online 2 September 2014

Keywords:
Biomaterials
Bacterial cellulose
Porous materials
Scaffold, Cancer cell

ABSTRACT

There is an increasing need for an effective *in vitro* model that can resemble the 3-D nature of tumor microenvironments. In this work, a 3-D bacterial cellulose (BC) scaffold with macropores was fabricated by a facile freeze drying method for potential culture of cancer cells. This *in vitro* study reported, for the first time, the role of macropores in the adjustment of cancer cell behavior when compared with previous results cultured in BC scaffolds without macropores. The scaffold was characterized by SEM and mercury intrusion porosimeter. A human breast cancer cell line (MDA-MB-231) cultured in the macroporous BC scaffold was examined *via* cell proliferation, histological and SEM analyses. The results demonstrated that the macroporous scaffold provided a good environment for cell viability, adhesion, proliferation, and infiltration. These findings suggested that the macroporous BC scaffold might have great potential for use in the *in vitro* culture of cancer cells.

1. Introduction

The standard *in vitro* approaches for cancer research and evaluation of anticancer drugs primarily involve the culture of tumor cells in Petri dishes. However, it is clear now that these two-dimensional (2-D) culture systems are incapable of accurately reflecting the microenvironment of tumors *in vivo*. Recently, three-dimensional (3-D) culture, as compared to 2-D culture, has been suggested as a much better *in vitro* cancer model to study the complex biological processes (Hutmacher, 2010; Hutmacher et al., 2010; Prewitz, Seib, Pompe, & Werner, 2012; Verbridge, Chandler, & Fischbach, 2010; Wang et al., 2014). 3-D scaffolds are preferable in tissue engineering. Similarly, 3-D *in vitro* model systems that are able to mimic the *in vivo* microenvironment are highly sought after in tumor engineering (defined as the construction of complex culture models that recapitulate aspects of the *in vivo* tumor microenvironment to study the dynamics of tumor development, progression, and therapy on multiple scales (Hutmacher et al., 2010). Numerous 3-D tumor engineering scaffolds made from collagen (Chen et al., 2012; Liu et al., 2014; Szot, Buchanan, Freeman, & Rylander, 2011a), chitosan-alginate (Kievit et al., 2010), poly(lactic acid) (PLA) (Sahoo, Panda, & Labhasetwar, 2005), poly(lactic-*co*-glycolide) (PLGA) (Sahoo et al., 2005), hyaluronan (Rhodes, Srivastava, Smith, & Longinotti, 2004), and silk fibroin protein (Talukdar et al., 2010) have been reported. Compared with aforementioned biomaterials, bacterial cellulose (BC), a natural nanofibrous polymer, shows some distinct properties. BC is synthesized extracellularly by the bacterium *Acetobacter xylinum*. In addition to such appealing properties as ultrahigh mechanical strength and modulus, high water holding capacity and porosity, and good biocompatibility (Pertile et al., 2012; Shi et al., 2012), BC displays intrinsic 3D network structure, and, in particular, BC fibers are at the nanometer scale which is the low limit of natural ECM fibers. Therefore, BC has been believed to be a promising material for tissue engineering scaffolds (Klemm et al., 2011; Petersen & Gatenholm, 2011). As suggested by a previous study (Hutmacher et al., 2010), the strategies developed originally for tissue engineering could be used in tumor engineering to enhance cancer research. It is thus reasonable to speculate that BC might be a promising material for tumor engineering scaffolds. However, investigation on BC scaffolds for tumor engineering has been very limited while extensive research has been carried out to develop BC scaffolds for tissue engineering. The only pioneering study on the *in vitro* culture of cancer cells on BC scaffolds demonstrated that these cancer cells cultured on BC did not have observable protrusions indicating undesirable cancer cell responses due to the absence of manufactured large porosity (Szot, Buchanan, Gatenholm, Rylander, & Freeman, 2011b). Furthermore, it has been accepted that pore structure is an

* Corresponding author. Tel.: +86 791 8704 612; fax: +86 791 8704 6122.
E-mail addresses: yzwantju@126.com, yzwan@tju.edu.cn (Y. Wan).

http://dx.doi.org/10.1016/j.carbpol.2014.08.073
0144-8617/

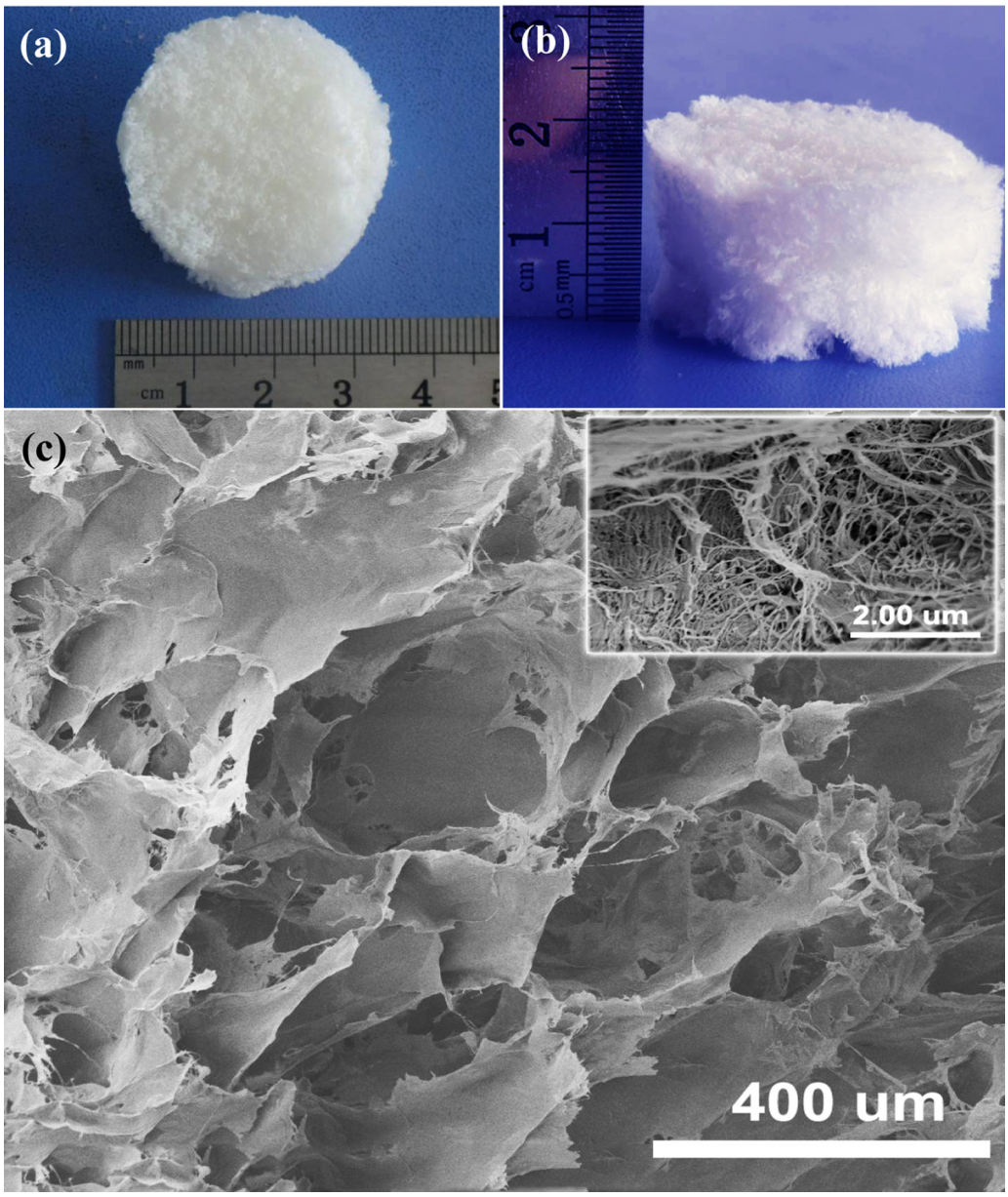

Fig. 1. Photos ((a) and (b)) and SEM image (c) of 3-D macroporous BC scaffold (inset presents a typical image of pore wall).

essential consideration in the development of scaffolds for tissue engineering and pores must be large enough to allow cell growth, migration and nutrient flow, vascularization, and new tissue formation and remodeling so as to facilitate host tissue integration upon implantation. Our previous work indicated that synovium-derived mesenchymal stem cells (MSCs) could proliferate deep (150 μm) into a BC scaffold with macropores (Gao et al., 2011). However, how the macropores affect cancer cell behavior has not been reported.

The present study, for the first time, examined whether a BC scaffold with macropores (defined as a pore diameter larger than 100 μm (Le Huec, Schaeverbeke, Clement, Faber, & Le Rebeller, 1995; Taboas, Maddox, Krebsbach, & Hollister, 2003) could be a good *in vitro* cancer model. These findings could then support the notion that alteration of scaffold pore structure might be able to modify the behavior of cancer cells and thus offer an effective strategy for the design and fabrication of good *in vitro* models for the study of cancer biology and the development of cancer therapeutics.

To this end, a novel BC scaffold with macropores was fabricated by a facile freeze drying process. A human breast cancer cell line (MDA-MB-231) was cultured in the macroporous BC scaffold and cell viability, adhesion, proliferation and infiltration were evaluated.

2. Materials and methods

2.1. Preparation of 3-D macroporous BC scaffold

BC pellicles were prepared according to the procedures described previously (Hong et al., 2006). The freeze–drying technique (Gao et al., 2011) with minor modifications was used to prepare the 3-D BC scaffold with macropores in this work. Briefly, the BC pellicles were crushed and mixed with deionized water using a home-made blender at 4000 rpm for 5 min to prepare an optimized 0.25 wt% BC emulsion. The BC emulsion was then poured into appropriate molds and freeze-dried at −50 °C for 1 day and the scaffold with a dimension of Φ10 × 1 mm was obtained.