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Cell-injected bacterial cellulose scaffolds for guided tissue regeneration

F.V. Berti[(1)*], D.O.S. Recouvreux[(1)], C.R. Rambo[(1)], R.M. Ribeiro-do-Valle [(2)], P.F. Dias[(3)] and L.M. Porto[(1)]

(1) InteLAB, Chemical and Food Engineering Department, UFSC, email: fernanda@intelab.ufsc.br
(2) LFPN, Pharmacology Department, UFSC
(3) LEBIMA, Cell Biology, Embryology and Genetics Department, UFSC
* Corresponding author.

Abstract – This work reports on the production of scaffolds based on bacterial cellulose (BC) hydrogels that can be used for guided tissue regeneration. Fibroblasts of the L929 strain were injected into BC hydrogels to migrate and proliferate. Confocal microscopy images revealed that the cells are distributed within the hydrogel along its thickness. Cell-injected BC scaffolds exhibit properties that are suitable for applications in tissue engineering, such as guided tissue regeneration.

Tissue engineering is defined as an interdisciplinary field that applies the principles of engineering and life sciences for the development of biological substitutes designed to maintain, restore or improve tissue functions. Recently, different methods by which autologous cells are cultured *in vitro* and transplanted back into the patient through the injection of cell suspensions haves been developed [1]. Scaffolds serve as mechanical and physical supports that allow surrounding cells to migrate into them, regenerating the damaged tissue. Reports suggest that in addition to the chemical nature the architecture of a scaffold has a decisive effect on the developing tissue during culture [2]. Therefore, a challenge in tissue engineering is to develop scaffolds with adequate porosity and specific properties for cell adhesion and new tissue formation [1]. This work reports on the production of scaffolds based on bacterial cellulose (BC) hydrogels that can be used for guided tissue regeneration. Bacteria *G. hansenii* ATCC 23769 were used for BC synthesis. Thick BC scaffolds were produced after three days in culture, according to [3]. Fibroblasts (L929 strain) were used to evaluate cell adhesion, migration and distribution inside the BC hydrogels. Interaction of cells with the BC was studied in a 24 well plates, where 8×10^5 cells were injected into the samples and cultured for 7 days in the presence of 10% fetal bovine serum. The substrates were then harvested, washed with 1×phosphate-buffered saline solution and fixed with 2.5% glutaraldehyde for 1h. The cell-injected BC hydrogels were stained with DAPI (4',6-diamidino-2-phenylindole) [4]. Cell distribution was evaluated by confocal microscopy (Leica DMI6000HB) by scanning the cell-injected scaffolds from center to periphery. Confocal images were processed using ImageJ®. BC thick membranes are composed of a gelatinous structure with thickness of approximately 2 mm and a denser membrane, formed at the interface liquid/air (Figure 1). Fibroblasts were injected in the center of the gelatinous part of the scaffolds, where the cellulose fibers are arranged in an open fibrous net structure with higher porosity, which allow cell migration. Cells are distributed in the cellulose fibrous network of the BC gelatinous body (Figure 2). In the color scale red represents cells that are closer to the surface and blue are cells located in deeper planes. The fibroblast cells grew horizontally and migrated vertically suggesting penetration inside the BC hydrogels. Cell-injected BC scaffolds exhibit properties that are suitable for applications in tissue engineering, such as guided tissue regeneration. These structures can be directly implanted in tissue deficient regions as scaffolds containing the appropriate cultured cells.

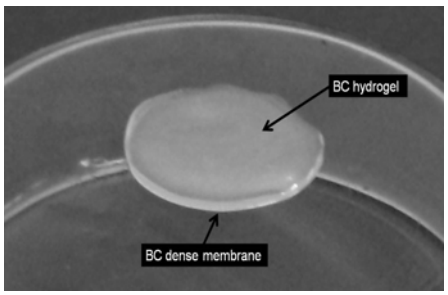

Figure 1: Photograph of a thick BC hydrogel.

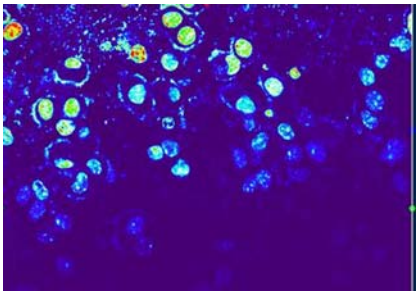

Figure 2: Confocal image of a cell-injected BC hydrogel.

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