TITLE: THREE-DIMENSIONAL EUKARYOTIC CELL CULTURE IN ROTATING WALL VESSEL BIOREACTOR AS MODEL FOR STUDYING BACTERIAL ADHESION AND INVASION

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ABSTRACT:
Eukaryotic cell cultures in three-dimensional (3-D) systems allow the obtention of organotypic structures that accurate replicate what occurs *in vivo*, with wide cellular differentiation, in contrast to monolayers obtained in classic two-dimensional cultures. These 3-D structures represent a very useful model for interaction studies between host-bacteria. In this research, we report on the development of a three-dimensional (3-D) organotypic culture model of Caco-2 cells to evaluate bacterial adhesion / invasion. It was observed that Caco-2 cells grown in 3-D using the rotating wall vessel (RWV) bioreactor (Synthecon, USA), recapitulate properties of the intestinal epithelium. Caco-2 human epithelial cell line (ATCC HBT37) were obtained from the Rio de Janeiro Cell Bank (UFRJ, Rio de Janeiro, Brazil) was maintained at 37°C in a humidified 5% CO_2 atmosphere until confluent growth was achieved. After reaching confluent growth, cells were subcultured by trypsinization with 0.05% trypsin / 0.02% EDTA. At first, 10^7 Caco-2 cells were inoculated into RWV bioreactor fillep up with 55 mL of DMEM medium and 250 mg Cytodex® microcarrier beads (Sigma- Aldrich, USA). It was incubated at 37°C in a humidified 5% CO_2 atmosphere for 28 days. At the end of this period, the organoids (eukaryotic cells attached to the beads in suspension) were collected and the cells counted, with recovery of ca. 10^8 cells. The analysis and documentation of the results were performed by confocal laser scanning microscopy, to visualize differential growth of 2-D and 3-D Caco-2 cell. For immunophenotyping, UEA1-FITC (lectin from *Ulex europaeus* fluorescein isothyocyanate labelled, Sigma-Aldrich, USA) was used to detect mucin. It was found that this marker was more abundantly expressed in 3-D than in 2-D cultured Caco-2 cells. The fluorescent dye DAPI was used for staining nuclei and so it was possible to document the cells arrangement on the beads. This 3-D cell culture was then used for adhesion and invasion assays with *Listeria monocytogenes* ATCC 19115 and *Listeria innocua* ATCC 33090. Our preliminary results indicated the 3-D culture is a powerful model that more closely mimicks interactions of bacteria with intestinal cells.

KEYWORDS: 3-D culture cell, *Listeria monocytogenes*, *Listeria innocua*, adhesion and invasion

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