14.011 - Sodium alginate and nanocellulose hydrogel as scaffold to in vitro 3D prostate cancer irradiated model.

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Introdução:

Recently, traditional cell culture systems structured in 2 dimensions using monolayers of cells in culture media are being replaced by 3D structures, in which cells can be organized in spheroids. To obtain these structures, hydrogels can be used as permeable to gas and nutrients scaffolds, also providing physical support to cells. This work aimed to produce a double network hydrogel containing sodium alginate (SA) and nanocellulose (NC), obtained by irradiation of microcrystalline cellulose, and its ability to maintain in culture of human prostate adenocarcinoma.

Objetivos:

To analyze whether SA+NC gels can keep viable 3D LNCap (prostatic carcinoma) in vitro, with and without exposure to radiation (0 and 2Gy gamma).

Métodos:

0.75g of microcrystalline cellulose (Sigma-Aldrich, 435236) dispersed in water were irradiated (300 kGy) (25 mm column height) in an electron beam source (Dynamitron® Job 188 ,RDI- Radiation Dynamics Inc.). The precipitated powder was washed in water by centrifugation. Nanocellulose pellet was added to a sodium alginate (2.5%) dissolved in PBS. LNCaP cells were maintained in RPMI 1640 medium in monolayers in culture flasks and controlled atmosphere (37º, 5% CO2). 24-well plates were used, pre-treated with Pluronic® F-127 solution (0.5g/mL in 2-propanol). The hydrophobic portions of Pluronic molecules were directed towards the center of the well, thus preventing cell adhesion to the culture plastic. In each well 1x105 cells were added, forming clusters of cells after 3 days. Clusters were removed and added to the hydrogel seeded in 96-well plates. Crosslinking was achieved using 100 µL of 2mM CaCl2 solution on top of the gels. After gelation, the saline solution was removed and the wells received 100µL of culture medium and were submitted to gamma irradiation with doses of 0 and 2 Gy (GammaCell, Canada), and further kept in incubator for 24h. Medium was replaced by fresh medium with Hoescht 33342 (10mg/mL) and SYTOX? Green (5mM) and kept in an incubator for 30 minutes. Plates were imaged in an INCell Analyzer 2500HS and images were obtained to determine the dead cell count.

Resultados:

Visual evidence of spheroids enclosed in gels showed increased cell viability in SA+NC comparing to SA gels only. No visual differences were observed in irradiated (2Gy) spheroids.

Conclusão:

SA+NC gels can sustain cell viability and cause no changes in cell radioresistance, being a suitable model to in vitro studies.

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