Tuning scaffold pore features to accomplish biomimicry in liver and pancreas 3D tumor models: a study on cancer cell aggregation, migration and morphotype transition

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Introduction: Cancer cell/scaffold constructs have shown more reliable microenvironment regeneration than culture plates and xenografting. Poly(vinyl alcohol) (PVA) is a biocompatible synthetic polymer able to cross-link upon freezing, giving rise to soft porous hydrophilic mats, which may mimic glandular organs. Biosynthetic PVA matrices with diverse pore features can be obtained acting on processing parameters and biomolecule type/concentration[1]. The aim of this report is to define the morphological features of the scaffolds affecting cancer cell assembly, so as to obtain reliable 3D models to study invasion and metastasis mechanisms occurring in hepatocellular carcinoma (HCC) and pancreatic ductal adenocarcinoma (PDAC), which are the 3rd and 4th cause of death in USA, respectively.

Materials and Methods: We fabricated hydrogels or sponges of different PVA/Gelatin (G) weight compositions (100/0-50/50 w/w) via mixture or emulsion of a 10% (w/v) solution of PVA (99% hydrolyzed, 85,000–146,000 Mw) with G, followed by 8 or 1 freezing cycles, and lyophilization. The scaffolds were characterized via scanning electron microscopy and mercury intrusion porosimetry. Primary PDAC cells were isolated from human tumor resections (Ethical approval from local hospital #3909-2013). HepG2 cells and PDAC cells were cultured in the scaffolds for 24 and 15 days, respectively, to create HCC and PDAC in vitro models. Biological analyses included biochemical metabolic assays (AlamarBlue, Neutral Red), and histology. Cell migration and morphotype transition were investigated by correlating cell morphology (round versus elongated) with cell distribution (center towards periphery) at sequential time-points.

Results and Discussion: Unlike the PVA/G hydrogels investigated, PVA/G sponges showed pore size increasing with G content (Figure 1). Among hydrogels, only the 80/20 w/w composition showed lamellar structure and orientation similar to those of native liver parenchyma[2]. Due to large pore size, the sponges best sustained PDAC cell aggregation with duct-like epithelial morphostructures, not present elsewhere[3]. Differently, HepG2 cells preferred small pore containment of hydrogels to assembly with a biomimetic organization (Figure 2). PVA/G 70/30 w/w sponges were chosen to study PDAC cell migration. Interestingly, an epithelial-mesenchymal transition was observed concomitantly to cell migration across the scaffolds. This phenomenon was timely studied against migration of normal (non-cancerous) cells, as a background for nutrient gradient-induced cellular motility. In the hydrogels, the HepG2 cells self-assembled into large and metabolically active clusters, each one showing morphotype transition of the frontline cells at late time-points.

Conclusions: Porosity of PVA/G scaffold specifically affected cancer cell aggregation depending on the tumor type (liver or pancreas). PDAC cells preferred large spheric while HepG2 small nested pores. When culturing both cell types within appropriate PVA/G cryo-scaffolds, morphotype transition was observed together with increased metabolism and cell migration at late time-points. These 3D in vitro models may help disclosing tumor biology, and developing new therapies.

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