Comparison of E Block and X Block Scaffold Systems for 3D Spheroid Formation and Apoptosis Regulation in HepG2 Cells

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Introduction: Hepatocellular carcinoma (HCC) is the most common primary liver cancer and is associated with high mortality due to its resistance to treatment. Conventional two-dimensional (2D) cell culture systems fail to replicate the in vivo microenvironment. Therefore, three-dimensional (3D) scaffold-based models offer a more physiologically relevant platform. This study aimed to compare two biodegradable scaffolds, E Block and X Block, in terms of their ability to support 3D spheroid formation and modulate apoptosis markers in HepG2 cells at different cell densities.

Methods: This experimental study was conducted at Maltepe University Cancer and Stem Cell Research Center. HepG2 cells were seeded onto E Block and X Block scaffolds at densities of 100,000; 500,000; and 1,000,000 cells. Cultures were maintained for 15 days in DMEM supplemented with 10% FBS. Morphological evaluations were performed via inverted microscopy and hematoxylin-eosin staining. Immunofluorescence analysis of apoptotic markers (p53 and BCL-2) was performed using confocal microscopy. Data were analyzed using GraphPad Prism software with ANOVA ($p<0.02$). Ethical committee approval was not required for this in vitro study.

Results: Both scaffold types supported 3D spheroid formation. The 500,000-cell group produced the most compact and well-formed spheroids in both scaffold systems. BCL-2 expression decreased significantly at 500,000 cells and increased at 1,000,000 cells, suggesting a cell density-dependent mitochondrial apoptotic response. No significant difference in p53 expression was observed among the groups.

Discussion: E Block and X Block scaffolds effectively supported 3D organization and allowed for the analysis of apoptotic responses in HepG2 cells. A cell density of 500,000 was identified as optimal in terms of spheroid formation and apoptosis regulation. These findings

indicate the potential of scaffold-based systems in liver cancer modeling and drug screening applications.

Keywords: hepatocellular carcinoma, 3D culture, apoptosis, HepG2, biodegradable scaffold