Fabrication of 3-Dimensional Tissue Models to Study Head and Neck Squamous Cell Carcinoma

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Introduction: Current approaches for the treatment of head and neck squamous cell carcinoma (HNSCC) have had limited success in reducing its occurrence or improving its prognosis. The 5 year survival for HNSCC (40-59%)[1] has not improved significantly in the last several decades. Given this, the discovery of novel and more predictive biomarkers[2-3] and sophisticated human tissue platforms[4] that mimic these cancers are needed to provide insights into factors that trigger disease progression[5-6] in order to guide the development of new therapies to treat these cancers.

Methods: Primary HNSCC tissue was obtained from patients at biopsy or resection, transported to the lab in culture medium (Fig. 1) on ice, then rinsed in 70% alcohol (Fig. 2) and three washes of PBS (Fig. 3). Each specimen was then cut in less than 5mm^3 segments (Fig. 4), placing half the specimen in 2D monolayer culture (Fig. 5) and banking some of the cells in liquid nitrogen while the other half was formalin fixed for routine pathologic analysis. After reaching confluence (Fig. 7), the 2D tumor associated fibroblasts were trypsinized (Fig. 8) and then transferred to a contracted collagen matrix in order to generate 3D tissues by growing cells at an air-liquid interface. Once grown, the tissues are being tested for cytokines/biomarkers that are believed to play a role in the pathogenesis of HNSCC.

Figure 1

Figure 2

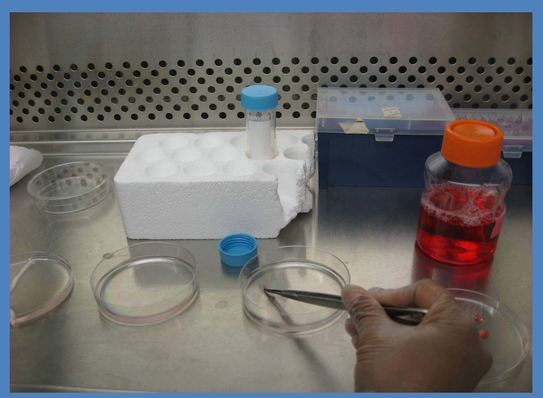

Figure 3

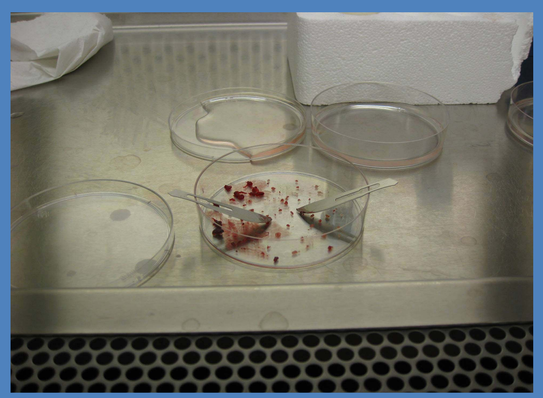

Figure 4

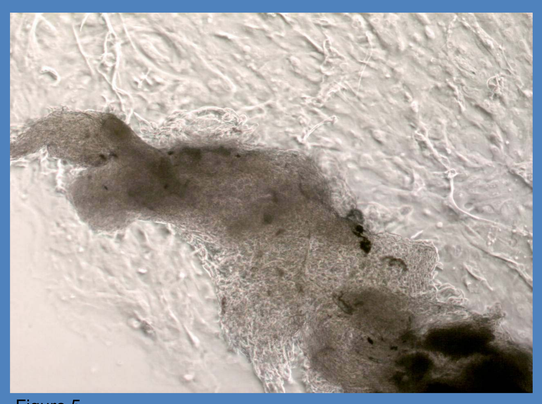

Figure 5

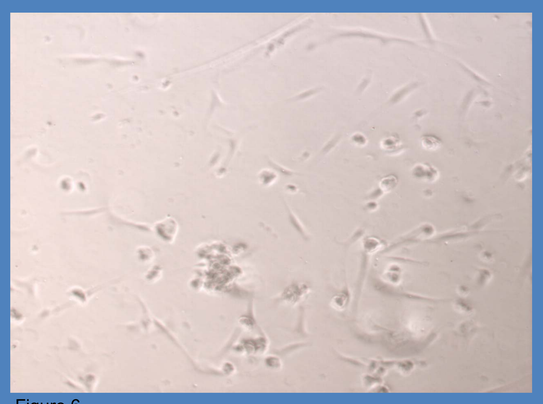

Figure 6

Figure 7

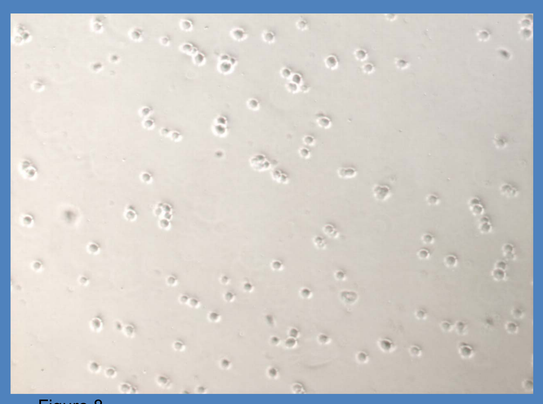

Figure 8

Results: Primary HNSCC tissue samples of less than 0.5cm were successfully transported to the lab and grown in 2D monolayer cultures (Fig. 5, 6). However in several of the early samples (3/4), cellular growth was overtaken by bacterial contamination as upper aerodigestive tumor sites are difficult to sterilize. Consequently, the transport media was then altered to include streptomycin and penicillin. Once a sample reached confluence (Fig. 7), it was trypsinized (Fig. 8) and then transferred to the collagen matrix. One of the initial four samples was successfully grown into a 3D tissue model on an air-liquid interface with three other samples in the process of being cultured.

The initial 3D culture grown in our lab from a temporal bone squamous cell carcinoma is hepatocyte growth factor/scatter factor (HGF/SF) negative. HGF/SF is an unique growth factor capable of inducing a number of biological responses in a wide variety of normal and neoplastic cells, including invasion, cell spreading, scattering, motility and shedding of cell-cell adhesion molecules.[7] HGF/SF has been shown to influence expression of angiogenic factors in HNSCC lines but not normal keratinocytes. As the line is currently negative for this factor, further testing will be done to see how this has influenced the levels of other factors, including vascular endothelial growth factor (VEGF), that induce angiogenesis in tumors. These 3D cultures will also be tested for the presence/absence of other factors believed to affect HNSCC such as E-cadherin and IL-6 to confirm that these 3D cultures replicate in vitro the behavior of HNSCC in vivo.

Conclusion: It is feasible to construct 3D tissues from primary human HNSCC samples in the lab. As further studies are completed, they demonstrate that these tissues will likely recapitulate their in vivo phenotype and gene expression patterns. This will subsequently provide the ability to work with other investigators to accelerate the design, screening and translation of new therapeutics to the clinical sphere.

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