428k Optimization of Tissue Disaggregation

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During the last two decades, phenomenal progress has been made in technologies for the detection/amplification/analysis of large molecules (like RNA, DNA) of biological interest (i.e. PCR and the various array systems). However, a common question/complaint of studies using these technologies is the homogeneity of the original sample. The lack of it can affect the desired final outcome dramatically. Despite knowing the importance of the role of the microenvironment in tumor development, genetic studies of solid tumors, whether sporadic or hereditary, to date, have typically treated them as single amorphous entities until recently. One of the collaborators on this project (C. Eng) has demonstrated that genetic alterations can differentially occur in the neoplastic epithelial compartment as well as the surrounding stromal compartment in sporadic human breast cancer as well as head and neck squamous cell carcinomas (HNSCC) (Kurose K 2001; Kurose K 2002). Therefore, the overall goal/aim of this proposal is to: Development/optimization of a process to separate and/or fractionate solid breast tumor samples into homogeneous cell factions for further molecular analysis. A variety of technologies/methodologies will be used to achieve this goal. They will include various types of enzymatic extra-cellular matrix (ECM) digestion techniques, cell separation based on physical properties (size, density, etc) as well as cell surface marker and internal marker techniques (flow cytometry, magnetic cell separation, magenetapheresis). This study puts emphasis on enzyme digestion of tissue using bacterial collagenase. Breast tissue predominantly consists of collagen I and III. Under malignant condition collagen I is overexpressed, assisting growth of tumor and making difficult to separate the cells of interest. Our aim is to develop understanding for enzyme digestion technique based on enzyme kinetics of collagenase and diffusion of collagenase through a porous tissue matrix. These parameters are important in predicting the time of tissue dissociation, which directly affects the final yield of the cells of interest.

Reference: Kurose K, Hoshaw-Woodard S, Adeyinka A, Lemeshow S, Watson PH, Eng C; Genetic model of multi-step breast carcinogenesis involving the epithelium and stroma: clues to tumour-microenvironment interactions; Hum Mol Genet. 2001 Sep 1;10(18):1907-13

Kurose K, Gilley K, Matsumoto S, Watson PH, Zhou XP, Eng C; Frequent somatic mutations in PTEN and TP53 are mutually exclusive in the stroma of breast carcinomas; Nat Genet. 2002 Nov;32(3):355-7. Epub 2002 Oct 15