Digital Quantification of Cluster Features for Salivary Tissue Engineering

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Objectives: Patients with head and neck cancer are prescribed radiation therapy which induces permanent salivary gland damage, resulting in hyposalivation and a reduced quality of life. Tissue engineering is a potential route towards de novo regeneration. Human salivary stem/progenitor cells (hS/PCs) encapsulated within hydrogels proliferate in vitro, but do not branch spontaneously as observed in vivo. Pre-clustering may provide 3-D building blocks with greater survival potential and adaptation toward the development of larger tissues. Digital imaging tools with supervised machine learning (ML) software were used to quantitatively evaluate cell clustering methods, toward a goal of automated cluster optimization.

Experimental Methods: hS/PCs derived from 6 patient samples were seeded onto low-adhesion clustering plates, and imaged over time. To assess differences in clustering behavior, Ilastik, an open-source supervised ML software for image segmentation and analysis, was applied to quantify cluster features, using a random forest classifier. Pixel classification was conducted to differentiate between cells and the wells in which they were contained, and control images, identified subjectively, were annotated to train the model. Batch processing then was used to analyze all images. Object classification identified cluster regions, from which features were quantified including object area, number of branches, total counts, kurtosis, and variance.

Results: For each feature, averages were calculated for a single well, and for 6 replicates in each plate. Cluster perimeters were identified by ML algorithms, and separated from loosely-adherent neighboring cells, and poor clustering specimens were identified by deviations in multiple parameters. However, high cluster quality could not be correlated readily between single quantitative measures and standard subjective assessments.

Conclusion: ML-based image analysis with Ilastik was useful in excluding patient samples with poor clustering potential, based on multiple individual measures. However, positive clustering behavior was more complex to confirm, and may require layered decision strategies to match subjective assessments.