

DESIGN CONSIDERATIONS TO ENSURE ACCURACY WHEN USING THE RESAZURIN REDUCTION ASSAY TO NONINVASIVELY QUANTIFY CELL EXPANSION WITHIN PERFUSED EXTRACELLULAR MATRIX SCAFFOLDS

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Analysis of perfusion-based bioreactors for organ engineering and a detailed evaluation of dynamic changes within maturing cell-laden scaffolds are critical components of ex vivo tissue development that remain understudied topics in the tissue and organ engineering literature. Precise measurement of cell numbers within bioartificial tissues and extracellular matrix scaffolds is necessary to provide measurement assurance and rigorous characterization of cell behavior within three-dimensional (3D) scaffolds. Accurate benchmarking of tissue function and biosynthetic activity to cell number facilitates comparison of data across experiments and between laboratories to increase rigor and reproducibility in tissue engineering and biofabrication. Soluble, fluorescent indicators of metabolic activity are valuable, noninvasive tools for estimating viable cell number. We investigated experimental conditions in which resazurin is a reliable indicator of cell content within 3D extracellular matrix kidney and liver scaffolds, and we present recommendations on experimental methodology for its optimal use. Resazurin is reduced to resorufin in proportion to metabolic activity of viable cells. Using three renal cell lines and one hepatic cell line, we show that correlation of viable cell number with the rate of resorufin generation may deviate from linearity at higher cell density, low resazurin working volumes, and/or longer incubation times – all of which contribute to depleting the working pool of resazurin. Importantly, we also show that the resazurin reduction rate in cell-conditioned medium is about double that in fresh culture medium. This finding has the potential to increase assay sensitivity, while saving expensive media. In conclusion, while the resazurin reduction assay provides a powerful, noninvasive readout for cell growth within extracellular matrix scaffolds, assay conditions may strongly influence its applicability for accurate quantification of cell number. The approach and recommendations developed in this study to maintain the pool of reducible resazurin may be used as a guide for application-specific optimization of the resazurin reduction assay to obtain accurate measurements of cell content in bioengineered tissues.