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Cell Tissue Science 2018_ A chemically-defined, and xeno-free cell culture medium for clinical manufacturing _ Ronald A. Nelson _ Wake Forest Institute for Regenerative Medicine_USA

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Background: Animal-derived biological extracts, including animal serum, are commonly used in tissue medium to supply bio-separable factors that support the support of cell viability and promote cell proliferation. These biological extracts are not chemically defined, are inconsistent in many other, and pose a risk of disease transmission. As such, these media supplements are not optimal materials to be used in clinical manufacturing processes. Our group has developed a chemically defined media well-characterized biological extract known within human platelet lysate. This media has been shown to provide exceptional support for many cell types derived from mesodermal embryonic embryonic tissue. Methods: Human citrus or recombinant versions of the main bioregulatory factors present in human platelet lyses were added to the modified DMEM-F12 minimal medium. Paired factors include platelet-derived growth factor, conversion factor-ins, insulin-like growth factor, vascular endothelial growth factor, fibroblast growth factor, hepatocyte growth factor, epidermal growth factor, and many other factors. Growth curves for a depth panel of commercially available human primary cells were generated using an IncuCyte S3 live cell imager. Conservation of cell phenotypes was confirmed by immunofluorescent determination of functional proteins specific to the cell type. Results: The proliferation rates for most mesoderm derived cell types in a chemically defined medium were equal to or superior to the measured proliferation rates in the cell supplier's recommended, chemically undefined medium. Conservation of functional biomarker expression indicated that the cell phenotype was maintained in multiple cell pathways for each cell type. However, two endothelial cell types, human umbilical vascular endothelial cells and human dermal microvascular cells, did not proliferate in a chemically defined medium. Conclusions: A chemically defined cell medium supported known components of biological extracts, human platelet lysate, was prepared using human sour and recombinant protein bioregulatory factors. This media formulation was shown to support proliferation and protection for the mesodermal origin of most cell types. Since this medium is chemically defined and xeno-free, it represents an optimal reagent used in clinical manufacturing procedures. Specific formulas for MSC media now exist to deal with these various applications, including serum-containing, serum-free, Zeno-free, and GMP-grade media. Rigorous internal control tests ensure reliable and consistent product quality. This allows for the elimination of fetal bovine serum, the most commonly used serum type for the culture of mammalian cells. Xeno-free media may contain human serum-derived components, but no components from animals different from humans. Xeno-free also does not guarantee animal-free, as most scientists define humans as animals. Xeno-free does not mean blood component-free because human blood components are often involved. Xeno-free media typically uses human serum or platelet lysates to exchange bovine-sourced constituents. Removing fetal bovine serum can be a big deal, and while X-free also means animal-free, the actual animal-free formulations are maligned. Xeno-free and animal-free are very different terms, and therefore to make the simplest decisions about cell culture media. While this eliminates (in theory) many potential contaminants, some human contaminants that cause problems in cell culture and downstream production.

Biography

Ronald A. Nelson, Jr. earned his Ph.D in Chemistry from Wake Forest University where he designed, synthesized and evaluated the effectiveness of phosphatidylinositol 3 kinase inhibitor prodrugs in treating androgen - independent prostate cancer. Serving in over 11 different leadership roles during his graduate career, Ronald's commitment to excellence led to 4 national and 15 local awards. Passionate about research, medicine, technology, his move to the Wake Forest Institute for Regenerative Medicine was a natural fit. As a postdoctoral research fellow at WFIRM, Ronald has been the research lead for several animal surgical, microsurgical, and treatment procedures. Ronald is also the research manager for the Regen Med Development Organization (ReMDO) where he utilizes his background in analytical chemistry, cancer biology, organic synthesis, and engineering to develop universal cell culture media that is chemically-defined and xeno-free. Ronald plans to enhance this media to recapitulate the regenerative potential of cells observed in embryogenesis.

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