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FIRST THINGS FIRST: PRIMARY CELLS IN RESEARCH

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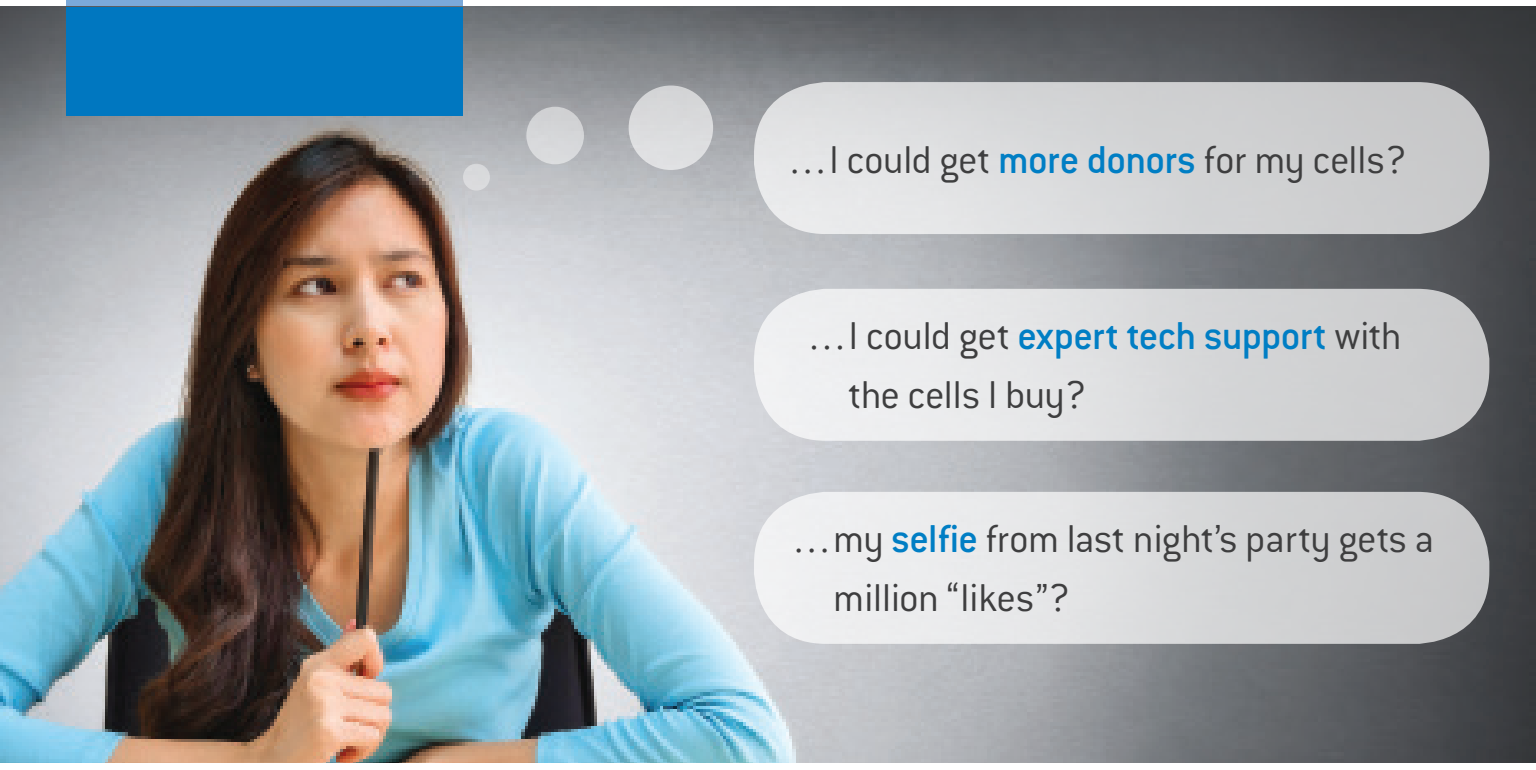

Solving Today's Primary Cell Culture Challenges

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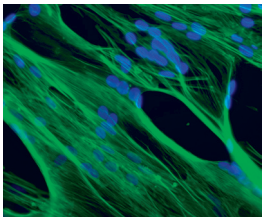

Skeletal Muscle Cells and Media

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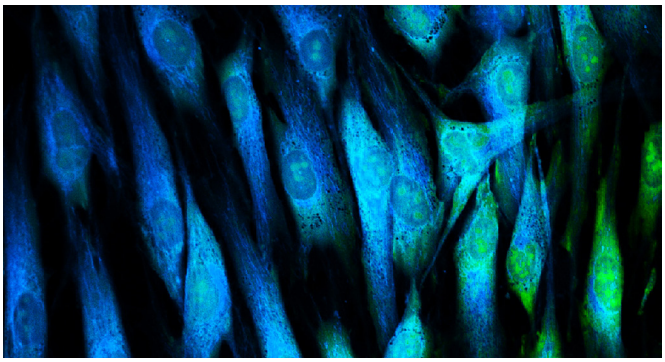
A Primer on Primary Cells



Primary cells are collected directly from the living tissue of a donor, either living or recently deceased. Primary cells are dissociated using mechanical and enzymatic methods, for which protocols vary depending on the species of origin (e.g., human, mouse) and type of tissue involved. The procedure generally includes the following steps: tissue cutting and mincing, enzyme incubation, a series of washes, trituration and micro-filtering fractionation, and finally re-suspension and seeding of your collected cell population.[1] For tissues that are more-loosely associated with the surrounding fibrous connective tissue, mechanical homogenization may be sufficient for dissociation. If your tissue source is the peripheral blood, differential centrifugation will typically isolate your population of interest.

Primary cells have a limited lifespan due to the shortening of chromosomal telomeres associated with each cell division. After about 20 to 60 doublings, telomeres become too short to withstand another cycle, resulting in halted cell division. This phenomenon is known as the Hayflick limit.[2] For a cell line to have an infinite doubling capacity, it must be immortalized via transformation using either viral or chemical induction methods. Viral genetic manipulation introduces a viral gene (e.g., SV-40, E6, E7) that efficiently promotes proliferation, allowing for the cell to undergo endless cell division.[1] Similarly, chemical induction methods rely on a carcinogen (e.g., ionizing radiation, nickel chloride, benzopyrene) to alter the genetic makeup of primary cells to favor unlimited proliferation.

After just one passage, your primary-cell culture becomes a secondary-cell culture, also known as a cell strain. However, although termed a cell line, its lifespan is finite (unless immortalized, as previously mentioned). This limited ability to divide recapitulates a cell's life in vivo, where once fully differentiated to perform its specialized function, a cell will stop proliferating—known as our intrinsic cancer-protective aging process. Moreover, some primary cells are post-mitotic and do not proliferate at all—in vivo or in culture (e.g., neurons, skeletal muscle cells, cardiomyocytes, pericytes, terminally differentiated hepatocytes). Post-mitotic primary-cell culture therefore requires fresh tissue for cell dissociation with every experiment performed.

Immortalized cell lines typically have a much faster doubling time and are valuable because they will continue to divide, providing a consistent supply of working material. However, such cell lines drift genetically with each division, weakening their biologic relevance. The advantage to their use is especially apparent when an identical genetic background is desired, because an entire clonal population can be generated from one cell line to share the same genotype. Mammalian primary cells, however, are a valuable tool in life science research because they physiologically resemble the donor tissue from whence they came and retain its naturally heterogeneous genetic makeup. They are defined and highly pure in their in vivo tissue-specific characteristics, and, as such, are increasingly being used to more reliably study various biological processes such as cellular metabolism, signaling, cancer, and aging, in addition to applications for drug screening and development on a larger scale.[3]

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1. Alberts B, "Isolating cells and growing them in culture," *Molecular Biology of The Cell*, ed 4, Garland Science, New York, NY, 2002.
2. Hayflick L, "The limited in vitro lifetime of human diploid cell strains," *Exp Cell Res* 37:614-636, 1965.
3. ASCB Newsletter, "Advantages and difficulties of working with primary cells," November 2015.

Primary Planning:
A Workflow of Primary Cell Culture Processes

The key to primary-cell culture success is developing a standardized protocol based on the right combination of dissociation methods for your cell type of interest. Adaptation to an in vitro environment requires the optimization of growth conditions, which are tissue- and cell-specific. Which workflow will you choose?

The Multifaceted Workflow: Do It Yourself (DIY)

VS

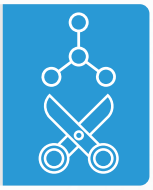
Timesaver Workflow: Rapid and Simple

1. **Literature Review & Protocol Development**
   Review the scientific literature, gather proven advice that will generate a protocol built for maximum cell yield and viability

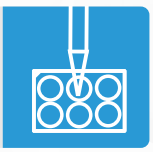
2. **Application for Tissue Procurement**
   Federal and state legislation may be complex and stringent, but must be followed

3. **Source Raw Materials**
   Procure the specialized raw materials and reagents necessary, and identify your tissue source

4. **Primary Cell Isolation**
   Enzymatic: A predetermined combination of enzymes dissociates cells from connective tissue and the associated fibrous network
   Mechanical: A dounce homogenizer dissociates cells from sections loosely associated with connective tissue



5. **Culture Establishment**
   Serum media requirements and concentrations of various cytokines, growth factors, nutrients, and hormones must be considered



6. **Cryopreservation**
   Primary cells are very delicate! The chosen cryoprotectant must be added to the storage media in a precise amount, at the right temperature. Cells should always be frozen at a controlled rate

7. **Quality Control (QC)**
   QC does not just refer to the authentication, characterization, and viability of your final cell population—all media and reagents should be subjected to testing as a quality checkpoint

8. **Scale Up**
   With standardized isolation and QC protocols, batched and pooled donation paves the way to scale

1. **Select Your Cells**
   Review the scientific literature, select a well-validated cell type from a company you trust

2. **Culture & Go**
   Benefit from reduced regulatory paperwork and pre-optimized protocols for culture and cryopreservation

Going In Vitro: Your Biological Model in a Dish

Primary cells naturally grow as either anchorage-independent or anchorage-dependent, and this is entirely determined by their originating in vivo tissue source: either the peripheral blood (anchorage-independent) or a solid-tissue organ (anchorage-dependent). Once isolated and given 24 to 48 hours to adhere and/or initiate, maintenance begins.

Broadly speaking, all dissociated mammalian cells, regardless of type or origin, have the requirement of being grown under conditions closely mimicking human physiological conditions (i.e., 37° C, 5% CO_2). Additionally, they require a pH-regulated growth environment, including cell-culture medium supplemented with cell type-specific nutrients, growth factors, glucose, and/or hormones. Research efforts into identifying the minimal conditions required for a particular cell type to thrive and function properly in low to serum-free media have uncovered the essential components that a growth medium must contain, including insulin, transferrin, glucose, and various salts, vitamins, and amino acids.[1] However, in addition to these, the culture media can also contain tissue- and cell-specific cytokines and growth factors required for proliferation and survival. For example, both primary endothelial and vascular smooth muscle cells will require added L-glutamine and fibroblast growth factor (FGF), but additional tissue-specific factors should be added to prevent fibroblast outgrowth—a common confounding contamination factor.[2] If tinkering with media recipes is something you'd rather leave to the experts, rest assured that you can always reduce media optimization efforts by purchasing primary cell culture media that has been pre-optimized for tissue of origin and culture format.

Establishing a primary-cell culture can be a finicky process due to both the sample-handling process and culture-condition optimization. Using antibiotics during the isolation and dissociation procedure can help prevent contamination from both the host tissue and improper practices. Nevertheless, time and devotion to your technique and reagents are the most important factors contributing to your success. It is essential that you are mindful throughout of potential contamination sources, the optimal cell confluence (60%–80%) for passaging, and possible cell clumping or improper cell adherence.

Advances in our understanding of cell and molecular biology, combined with technological improvements, have put human primary-cell culture at the forefront of cell-based model systems designed to study different disease pathologies and develop novel strategies for tissue regeneration. Primary cells retain their tissue-specific biochemical signaling pathways ex vivo, and therefore in your dish, providing valuable insight into the behavior such cells exhibit in vivo. Technological advances have made it possible for researchers to further expand on the benefits of using human primary cells through the development of 3-dimensional (3-D) scaffold platforms to recapitulate the in vivo microenvironment in which cells reside. In this way, you can re-create not only the microscopic cell behavior you wish to observe but also your cells' relationship with their surrounding structural environment as would be experienced in the originating organ tissue. As our understanding of the complexity with which cell–cell and cell–extracellular matrix (ECM) communication occurs continues to grow, so does the need for more sophisticated and truly tissue-representative model systems.

References

1. Alberts B, "Isolating cells and growing them in culture," *Molecular Biology of The Cell*, ed 4, Garland Science, New York, NY, 2002.
2. Pan C et al., "Comparative proteomic phenotyping of cell lines and primary cells to assess preservation of cell type-specific functions," *Mol Cell Proteomics* 8(3):443-450, 2009.

Physiologically Relevant In Vitro Models

Acellular 3D Cultures in RAFT™ 3D Cell Culture System

Cells are embedded inside high-density collagen scaffold for tissue modeling and long-term culture.

Now that you have successfully established your culture, how can you best apply the native properties primary cells possess to create a functional in vivo model for your research interests?

Unlike infinitely proliferating cell lines, primary cell culture models have the advantage of maintaining the physiological and biochemical properties of the tissue from which they arose, thereby providing unmodified and high-quality results. Maintaining primary cell cultures in a 2-dimensional (2-D) growth environment is the most common approach to studying various disease processes. This method has been used since the start of the twentieth century, and is therefore well established with a plethora of comparative results available in the scientific literature. 2-D cell culture can be manipulated without much difficulty, generating swift experimental data due to comfort with analysis. With much due credit to the advances made using this approach, it is hard to conceptualize human biology in 2-D, as our tissue and cellular organization is far more complex. With this system, cells are typically grown as a monolayer and maintained in a flat, unyielding tissue culture-coated plastic flask or dish. This type of housing thus fosters a flat, sheet-like, and over-extended cell morphology not typical of their in vivo equivalents.

Advancing your primary cell culture to better represent the in vivo microenvironment via 3-D culture is a powerful way to reproduce the cell–cell and cell–ECM interactions present within a complex organism. 3-D cell culture is typically made up of a scaffold or matrix in which cells naturally form aggregates or spheroids as they divide, allowing for continuous interaction with each other and their surrounding microenvironment.[1] Culturing primary cells in a 3-D environment overcomes the atypical cell morphology seen in 2-D cell culture and is a more accurate spatial representation facilitating the many cell signaling receptor interactions that influence downstream pathways, cell behavior, and even drug toxicity and response.[2,3] Furthermore, a 3-D matrix of primary cells positions their growth in such a way that those cells largely exposed to medium (top) are viable and proliferating, whereas those more interior are dormant and hypoxic due to a limited supply of oxygen and nutrients.[1] This type of heterogeneity is more closely representative of in vivo conditions and is particularly important for generating authentic data in cancer research and drug efficacy studies.

Although 3-D cell culture began to gain traction in the 1980s, only in the last 10 or so years has its significance been recognized.[1] 3-D cell culture is now preferred by life science researchers performing a vast range of molecular biology applications because of its ability to not only accurately reproduce the cell phenotype observed within the living tissue, but also the surrounding structural microenvironment–essential to generating predictive data prior to animal testing and clinical trials. The advantages of data generated with such native tissue characteristics span across the spectrum of precision medicine, metabolic disease etiology, drug toxicology and development, and regenerative science, among others.

References

1. Edmondson R, et al., "Three-dimensional cell systems and their applications in drug discovery and cell-based biosensors," *Assay Drug Dev Tech* 12(4):207-218, 2014.
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