ANGIOCRINE
BIOSCIENCE

VeraVec™ PLATFORM

Novel avenues of discovery in endothelial cell biology and stem cell expansion

ENDOTHELIAL HETEROGENEITY AND ANGIOCRINE FACTORS:

New Paradigms Reveal the Complexity of Vascular Physiology

Endothelial cells (ECs) were once thought to be a simple cellophane coating that prevents the contents of the blood from leaking into the surrounding tissues. Over time, however, additional details regarding their function have been uncovered. The capacity of ECs to regulate coagulation, trans-cellular migration of inflammatory cells, matrix remodeling, and thromboregulatory functions have all been discovered over the past several decades [1]. The EC layer is now appreciated as a master regulator of tissue homeostasis, regeneration, and healing. Most recently, ECs have been found to be essential in the regulation of stem cell homeostasis and proliferation via production of various growth factors, extra-cellular matrix (ECM) components, and other microenvironment modifiers, termed angiocrine factors. Developmental biologists continue to uncover EC-derived angiocrine factors that dictate the formation and maturation of various tissues, such as the bone marrow, liver, kidney, spleen, and testis, to name a few [2-6]. Additionally, the capacity to influence organ function extends to tissue regeneration during periods of both acute and chronic injury [7-9]. Thus, to model these regenerative scenarios in vitro would lead to profound revelations in both vascular and stem cell biology. Unfortunately, the understanding of EC biology has been hampered due to the difficulty of establishing stable and physiologically relevant in vitro EC culture systems.

OVERCOMING THE OBSTACLES:

The Generation of *VeraVec* ECs, Highly Stable and Proliferative ECs for in vitro systems

Previous efforts to derive and culture both mouse and human ECs have met with two major hurdles: a reliance on irreducibly complex media additives indispensable for their propagation, and the subsequent maintenance of the EC phenotype over successive passages in vitro. Angiocrine Bioscience has overcome both of these crippling limitations in EC research with a novel technology. The *VeraVec*™ series of ECs utilizes a single protein from Adenovirus (serotype 5), E4ORF1, to adapt ECs to culture for multiple passages, but does NOT confer immortalization, alteration of the EC fate, or saturation of any signaling cascade[10]. *VeraVec* ECs are indistinguishable from early-passage naïve ECs in angiogenic assays, mimicking primary cell behavior, and they are fully competent to respond to angiogenic stimulants, such as VEGF and FGF, with proper activation of downstream targets [11, 12]. As *VeraVec* cells adapt to culture conditions, the E4ORF1 maintains the phenotypic identity of ECs, which can be cultured for 3-5 times as many passages as naïve counterparts without phenotypic drift **(Figure 1)**.

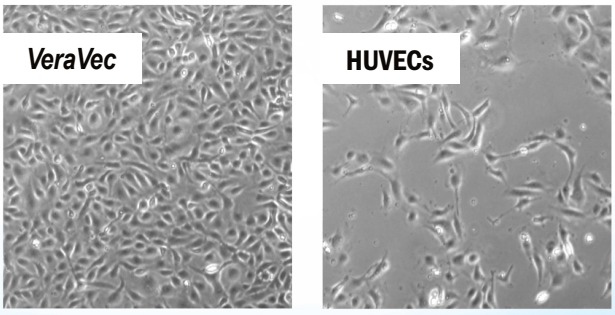

Figure 1. The same harvest of HUVECs was divided and either handled in a classical manner or with the *VeraVec* technology for 9 passages.

DEFINE YOUR CONDITIONS:

Human *VeraVec* ECs are compatible with multiple media formulations, including SERUM-FREE conditions

Naïve ECs, like most primary cells cultured in vitro, require the use of irreducibly complex media additives (ie. serum, endothelial cell growth mitogen (ECGM), etc) that cloud biological processes. Additionally, many of the constituents of these compounds are deleterious to long-term EC culture growth, decreasing their proliferation capacity and eventually leading to phenotypic drift [13]. Fortunately, *VeraVec* ECs (both mouse and human) are resistant to the damaging effects of prolonged serum-dependence, resulting in a highly stable and proliferative EC population compared to naïve cells. In fact, human *VeraVec* ECs can be cultured in media that is completely absent of these irreducibly complex media additives without negatively affecting their viability or stability.* This allows for the examination of ECs without the clouding influence of serum or the need to bring them to the brink of apoptosis to study a particular phenomenon. Angiocrine Bioscience offers two tiers of human *VeraVec* ECs (originally isolated from the umbilical cord vein – HUVECs). The first, our "classic" human *VeraVec* ECs (hvera101) have been cultured in typical serum-containing media, but is resistant to the negative effects of serum over long-term passaging. The second, our "serum-free" human *VeraVec* ECs (hVera110) have been derived in media that has NEVER contained any type of irreducibly complex media additive. The only required cytokines for the growth of these "serum-free" *VeraVec* ECs are VEGF and FGF, which affords the user an EC population that has never been influenced by the effects of serum or ECGM. **(Figure 2)**

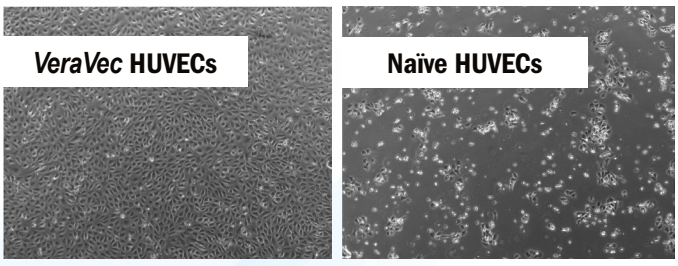

Figure 2. *VeraVec* ECs and HUVECs were cultured in serum free, stimulant free media for 3 days. Attrition was evident within 24 hours for Naïve HUVECs. *VeraVec* HUVECs demonstrated no deleterious reaction to the lack of serum and stimulation.

*Serum independence tolerance will differ among the *VeraVec* ECs ranging from viability in a monolayer to slowed cytokine-dependent proliferation.

In vitro functionality of our serum-independent *VeraVec* ECs is confirmed by their capacity to form tube-like structures in various protein matrices. While informative, the formation of these 3D structures from naïve ECs are notoriously unstable and typically degrade within just one day of formation. Contrarily, *VeraVec* ECs form the intricate lattice of tubes with two substantial advantages: *a)* the ability to form 3D structures in the absence of serum; *b)* the remarkable stability of the matrices, which can be maintained far longer than naïve cells **(Figure 3)**. The removal of serum from the media had no effect on the formation or maintenance of 3D structure formation. Thus, the researcher can use highly defined co-culture mechanisms to study their particular cell system or vascular process of interest.

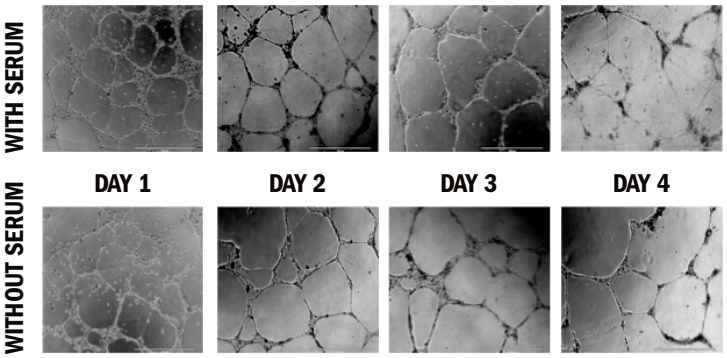

Figure 3. *VeraVec* ECs are fully competent to perform vascular functions, yet without the need for extreme metabolic stimulation or oncogenes. Note that the presence of serum has no effect on the *VeraVec* ECs ability for form vessel networks on BD Matrige. Also note that the tubules last for days, whereas naïve HUVECs often only last for 12-18 hours.

MOUSE SPECIFICITY:

Tissue-Specific Mouse *VeraVec* ECs can be Used to Establish Novel in vitro Model Systems

The animal system of choice for many researchers is the mouse. We have identified numerous distinctions between the vasculature of different microvascular beds [14]. This ground-breaking research has revealed that each vascular bed is distinct at both a structural and a molecular level **(Figure 4)**. Cell surface receptors within each tissue uniquely sensitize the endothelial cells to their environment. Likewise, ECM deposition and angiocrine factor secretion are also highly variable in ECs associated with different organs. This combination of factors and micro-environmental cues creates the vascular stem cell niche, which is imperative for the proper function of the tissues with which they associate.

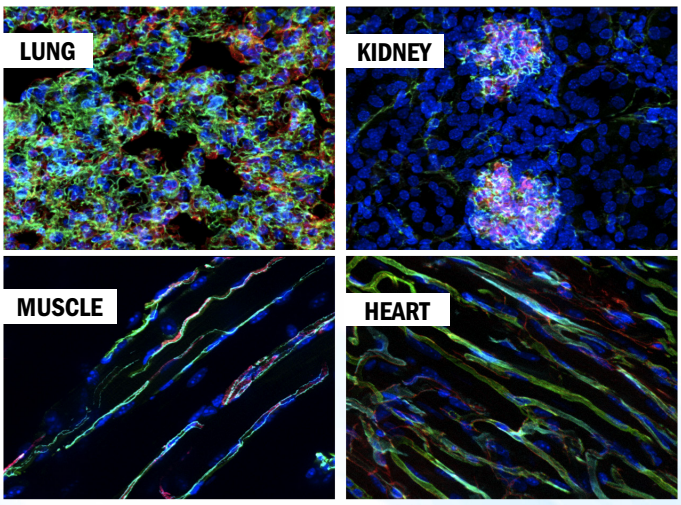

Figure 4. The endothelial cells of each tissue has profoundly distinct morphologies and functions.

Thus, an ability to study ECs from each individual vascular bed would be a great benefit to the vascular community. Sadly, in vitro culture of ECs from the majority of mouse tissues has been a particular challenge to researchers for decades. The introduction of primary ECs to culture conditions results in a rapid loss of EC identity and massive cell death. However, the combination of the *VeraVec* technology platform plus our patented intravital labeling and isolation techniques has led to the generation of robust and stable mouse EC populations from multiple organs **(Figure 5)**.

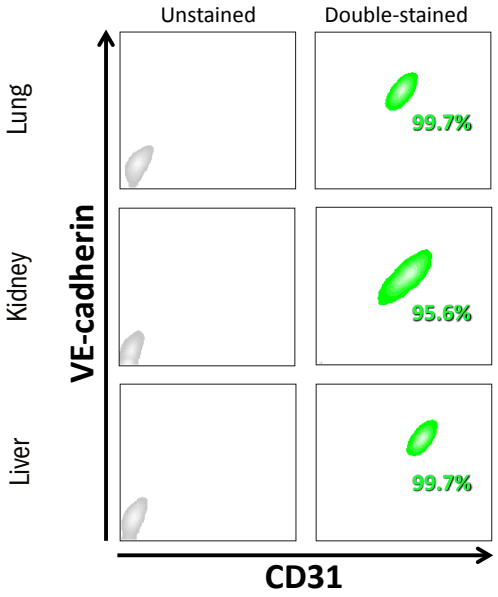

Figure 5. Mouse *VeraVec* endothelial cells from lung, liver and kidney maintain the endothelial cell phenotype after numerous passages.

The distinctions noted in vivo are substantially maintained in vitro. ECs isolated from different tissues have divergent morphologies, growth rates, cell surface receptors, and form unique structures in 3D. Notably, our proprietary technology ensures that only ECs from the microvasculature are obtained – macrovascular and lymphatic ECs are excluded during the isolation/purification process. For the first time, vascular and stem cell biologists have the capacity to study primary endothelial cells from both mouse and human in identical settings for the advancement of their research.

STABILITY EQUALS REPRODUCIBILITY

Global Transcriptome Profiling Confirms *VeraVec*™ ECs are Stable Over Time

The inability to reproduce experimental data leads to frustration and diminished confidence in results, not to mention a waste in

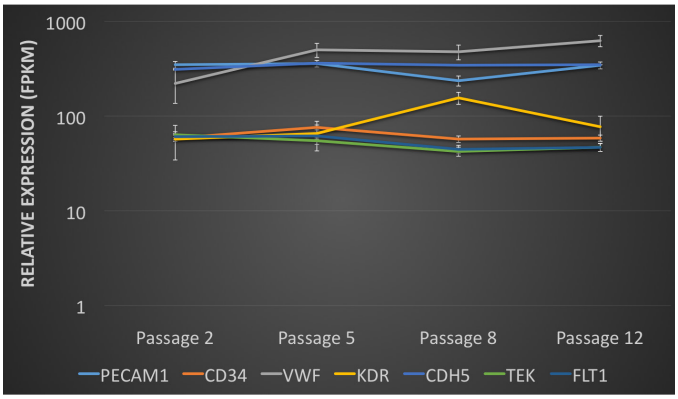

Figure 6. *VeraVec* HUVECs maintain endothelial cell surface markers after serial passages at consistent and reproducible levels.

both time and expenses. To demonstrate the pronounced improvement in stability and reproducibility of *VeraVec* ECs, three independent isolations of naïve ECs were isolated and enhanced with the *VeraVec*™ technology. All samples were cultured for multiple passages, and RNA was isolated at various stages to assess the transcriptome profile **(Figure 6)**. *VeraVec* ECs maintained consistent expression of numerous EC genes over successive passages. Thus, studies utilizing *VeraVec* ECs will be significantly enhanced by affording the researcher a greater number of cell passages with reproducible results.

EXPANDING YOUR CELLS:

VeraVec™ ECs can serve as a Stem Cell Expansion Platform for the expansion of multiple cell types

Recent advances in vascular biology research have revealed the role of ECs in modulating the vascular stem cell niche. Throughout both development and adulthood, as well as during healing processes, ECs have been found to produce indispensable angiocrine factors for regulating stem cell quiescence and expansion. The concept that blood vessel components are essential in regulating stem cell function is a radical departure from previously existing paradigms. More specifically, the observation of ECs expanding stem cell populations in vitro eluded scientists for decades, as researchers were beholden to the use of serum and other complex media additives to maintain EC viability. Without the need for these additives, *VeraVec* cells were able to recreate the vascular stem cell niche in vitro. Using hematopoietic stem cells derived from mouse bone marrow and human umbilical cord blood, *VeraVec* ECs proved to be a viable platform for the expansion of bonafide stem cells. Unlike other technologies for stem cell expansion, the expanded cells were not myeloid biased, nor did they lose any of their potency subsequent to expansion. Following *VeraVec*-mediated expansion, transplanted HSPCs maintained multi-lineage engraftment and serial reconstitution ability. Notably, the rate of engraftment and frequency of long term repopulating stem cells were higher after *VeraVec* expansion [15]. The utility of the in vitro vascular stem cell niche is also highlighted by their ability to enhance embryonic stem cell cultures [16]. From fetal to adult, and quiescence to tissue regeneration, the angiocrine factors secreted by the endothelial cells in each tissue dictate the behaviors of stem cell populations. This level of control for stem cells from adult tissues, induced pluripotent stem cells, embryonic, and malignant sources is now available in vitro from the *VeraVec*™ series of endothelial cells.

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