Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

Beyond Static Pipes: Mechanisms and In Vitro Models of Vascular Aging

Martin W. Hetzer[1] and Simone Bersini[2,3]

[1]Molecular and Cell Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, California 92037, USA
[2]Regenerative Medicine Technologies Laboratory, Ente Ospedaliero Cantonale, 6900 Lugano, Switzerland
[3]Faculty of Biomedical Sciences, Università della Svizzera Italiana, 6900 Lugano, Switzerland

Correspondence: hetzer@salk.edu; simone.bersini@eoc.ch

The vascular system is a key player for the maintenance of healthy tissues, suggesting how the physiological decline of blood vessel functionality during aging could be a major contributor of organ degeneration. While basic research studies have begun to pinpoint potential mechanisms of vascular aging, it is now critical to translate them into therapeutically relevant options. Microphysiological systems represent a powerful tool to precisely control which combinations of stimuli are provided to in vitro reconstructed blood vessels and to analyze their functional consequences. After highlighting key aspects of vascular aging, this review discusses in vitro models that are able to recapitulate relevant features of blood vessel damage during aging. Strategies to improve current in vitro systems so that they will more faithfully recapitulate vascular aging are proposed, emphasizing the importance of combining in vivo models with microphysiological systems for an effective translation of vascular aging biomarkers and therapies to the clinical level.

The World is aging.

More than 90 million people in the European Union (EU) are aged 65 and older according to the last data published by the European Commission in 2019. Even more dramatically, the share of people aged 80 years or older in the EU population is projected to have a 2.5-fold increase by 2100, reaching 60.8 million (ec.europa.eu/eurostat/statistics-explained/index.php?title=Population_structure_and_ageing). Similarly, the number of people aged 65 or older in the United States is projected to rise from 12% to 22% of the population in just the next 30 years (Ungvari et al. 2018). These data pose critical socioeconomic challenges since the annual costs of caring for the elderly is growing exponentially, while the ratio between working-age persons actively contributing to the maintenance of an efficient welfare system and old adults is decreasing.

Cardiovascular diseases (CVDs) are the main cause of death among older people accounting for 1.68 million deaths each year in the EU, or 37.1% of all deaths (ec.europa.eu/eurostat/statistics-explained/index.php?title=Cardiovascular_diseases_statistics). Similarly, CVDs are responsible for about 33% of all deaths in the United States for people aged 65 (Ungvari et al. 2018).

Editors: Diane R. Bielenberg and Patricia A. D'Amore
Additional Perspectives on Angiogenesis: Biology and Pathology available at www.perspectivesinmedicine.org

 doi: 10.1101/cshperspect.a041180
Cite this article as *Cold Spring Harb Perspect Med* 2022;12:a041180

These numbers are considerably higher than the second most prevalent cause of death (i.e., cancer), which accounts for 25.8% of all deaths. Together with the huge societal costs of CVDs, the healthcare system is estimated to pay 210 billion euros each year in the EU (www.ehnheart.org/cvd-statistics.html), and more than 800 billion dollars in the United States by 2030 (Heidenreich et al. 2011).

Traditionally, research on CVDs has been focused on cardiac pathologies while underestimating the role of the microcirculation (Augustin and Koh 2017). This trend has been partially reversed during the last decade with the emergence of data pointing to a key role of blood vessels during development, tissue homeostasis, and disease (Potente and Mäkinen 2017), and pointing toward the important contribution of vascular networks for the maintenance of a functional cardiovascular system and for preventing age-related CVDs.

Given the essential role of a functional vascular system in the maintenance of a healthy organism, it is not surprising that the physiological aging of blood vessels is tightly connected with the functional decline of tissues and organs. Moreover, blood vessel loss of functionality is also associated with a wide range of age-related disorders that were not traditionally linked with the vasculature, including neurodegeneration, sarcopenia, osteoporosis, and kidney failure (Ungvari et al. 2018). For instance, the appearance of vascular dysfunction in the blood–brain barrier (BBB) occurs before the onset of cognitive impairment and the detection of amyloid β plaques, suggesting a causative role for BBB damage in the onset of Alzheimer disease (AD) or age-related dementia (Nation et al. 2019). Similarly, pericyte degeneration disrupts the microcirculation of the white matter due to the toxic accumulation of blood-derived fibrinogen, leading to reduced blood flow and functional deficits before the appearance of neuronal loss (Fig. 1A; Montagne et al. 2018). In the musculoskeletal system, aging vascular endothelial cells (VECs) in the bone marrow microcirculation produce high amounts of reactive oxygen species (ROS), which are responsible for the declining survival and proliferation of hematopoietic stem cells (HSCs) (Fig. 1B; Poulos et al. 2017). Aged skeletal muscle tissues are characterized by reduced capillary density, blood flow, and VEC mobility leading to muscle loss in older individuals (Fig. 1C; Das et al. 2018; Longchamp et al. 2018). Finally, the age-related decline in a specialized subset of VECs in the bone tissue (i.e., H-type VECs) is related to the disruption of physiological bone turnover, leading to bone loss and osteoporosis (Ramasamy et al. 2014), as well as to damage of the HSC niche (Fig. 1D; Kusumbe et al. 2016). Overall, instead of targeting the consequences of CVDs and other age-related diseases, interventions that aim at restoring the functionality of blood vessels have the potential to prevent or delay all vascular-related pathologies.

Our current knowledge of the biological mechanisms and signaling pathways related to blood vessel aging has significantly expanded during the last decade. Most studies were performed in animals (Poulos et al. 2017), whereas a few used clinical data to analyze the vascular-related, functional decline of specific tissues in humans (Nation et al. 2019).

It is now critical to translate the identified biomarkers and biological mechanisms into potentially relevant targets for future therapeutics. The design of advanced in vitro models coupling microphysiological systems, biofabrication, and tissue engineering would allow analysis of specific features of vascular aging with a level of control over the experimental parameters that cannot be achieved in vivo. This approach could promote the preclinical screening of novel targets and drugs before their translation into animals or humans. Indeed, microphysiological systems allow to provide mechanical (e.g., controlled wall shear stress, cyclic stretching/contraction) as well as biochemical (e.g., growth factor gradients) stimuli that are critical to promote the formation and maintenance of biofabricated blood vessels. At the same time, these systems are compatible with multiple analytical techniques (e.g., high-resolution 3D imaging, RNA-seq, flow cytometry, multiplex secretome analyses) that allow to extract heterogeneous data sets.

Recent review papers have discussed the design, characterization, and application of hu-

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

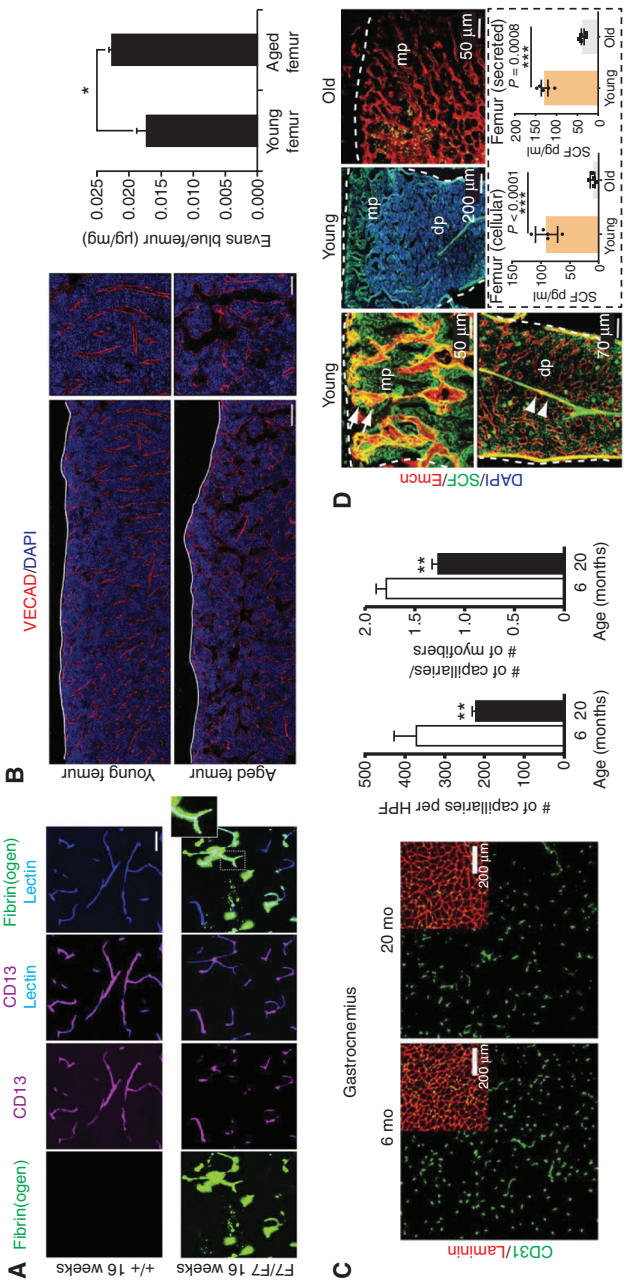

Figure 1. Blood vessel architectural and functional changes during aging in vivo. (*A*) Impairing the pericyte coverage of blood vessels in mouse brains leads to blood protein leakage. Formation of fibrinogen extravascular deposits (green), CD13-positive pericytes (magenta), and lectin-positive endothelial cells (blue) in the corpus callosum of 16-wk-old pericyte-deficient platelet-derived growth factor receptor β (Pdgfrb)$^{F7/F7}$ (F7/F7) and control (+/+) mice. Scale bar, 40 µm. (Panel *A* from Montagne et al. 2018; reprinted, with permission, from Springer Nature © 2014.) (*B*) Representative images of mouse femurs labeled with vascular-specific vascular endothelial cadherin (VECAD) antibody (red), showing morphological alterations in aged vasculature (white lines indicate cortical bone). Scale bars, 100 µm (longitudinal sections); 50 µm (*insets*). Analysis of vascular leakiness in young and aged femurs quantified through Evans blue dye extravasation. (Panel *B* from Poulos et al. 2017; reprinted, with permission, from the American Society for Clinical Investigation © 2017.) (*C*) Sections of mouse gastrocnemius showing CD31 (green) and laminin (red) staining. Quantification of number of capillaries and capillary/fiber ratio per high power field (HPF). (Panel *C* from Das et al. 2018; reprinted, with permission, from Elsevier © 2018.) (*D*) Confocal images showing stem cell factor ([SCF], green) staining in arteries (arrowheads) and endomucin (Emcn)$^{+}$ (red) metaphyseal (mp)-type H vessels (arrow) of young (3-wk-old) but not old (65-wk-old) mouse tibiae. Quantification of cellular and secreted SCF in young and old femur. (Panel *D* from Kusumbe et al. 2016; reprinted, with permission, from Springer Nature © 2016.)

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

man 3D vascular models (Bersini et al. 2016; Cochrane et al. 2019). Importantly, the complexity achieved by recent 3D in vitro models incorporating human-derived cells from healthy or pathologic donor tissues from different ages, genders, and ethnicities would provide results independent of the contributions of the remainder of the organism and allow focus on a specific vascular bed (e.g., microcirculation, postcapillary venules), cells (e.g., VECs, pericytes, perivascular fibroblasts), or tissues. This approach would be particularly relevant in light of the emerging idea of organ-specific vascular aging (Chen et al. 2021), which suggests that organ-specific biochemical and mechanical stimuli affect vascular aging.

Rather than focusing on classical concepts of large vessel aging or diseases, including atherosclerosis, for which the reader is encouraged to refer to already published review articles (Glass and Witztum 2001; Najjar et al. 2005), this review highlights key aspects of vascular aging, including the emerging concept of organ-specific blood vessel aging, and examples of in vitro modeling, which capture relevant features of blood vessel damage during physiological aging. Finally, a roadmap of the conceptual elements that should be integrated into current in vitro systems to better reproduce vascular aging and its consequences is proposed.

THE VASCULAR MICROENVIRONMENT DURING AGING

López-Otín and colleagues (2013) have categorized the cellular and molecular hallmarks of aging. Although these hallmarks apply to all cell types/tissues in the human body, specific features characterize the vascular compartment. Aging induces a wide spectrum of molecular and cellular alterations that result in profound structural and functional changes of small-size blood vessels and capillaries (Ungvari et al. 2018), which are responsible for the majority of mass transport across tissues (Jakab and Augustin 2020). Importantly, these vessels contain both VECs and supporting mural cells, including pericytes, fibroblasts, and smooth muscle cells, which partially cover the abluminal surface of the endothelium. This microenvironment also undergoes molecular changes during aging, which include oxidative stress, mitochondrial damage, inflammation, senescence, extracellular matrix (ECM) remodeling, glycocalyx damage, loss of cell identity, impaired protein homeostasis (also often referred to as proteostasis) and nutrient sensing, genomic instability, and epigenetic alterations (Ungvari et al. 2018).

While all these features are equally relevant to vascular aging, this review will primarily focus on those whose study can directly benefit from their implementation within in vitro models. These models will be discussed in the section Modeling Vascular Aging In Vitro.

Oxidative Stress and Inflammation

Oxidative stress, mitochondrial damage, and inflammation often converge toward the local activation of the endothelium and surrounding mural cells, triggering events that gradually induce recruitment of immune cells, deposition of provisional matrix, and cell identity loss (Schwartz et al. 2018). This unresolved vascular remodeling can be defined as a persistent instability and is typical of vascular damage during both physiological aging and disease. Oxidative stress has been observed in coronary arterioles comparing aged to young mice where both VECs and smooth muscle cells produce higher levels of ROS (Fig. 2A). In addition, oxidative stress is associated with increased inducible nitric oxide synthase (iNOS) and decreased endothelial nitric oxide synthase (eNOS) expression by VECs (Csiszar et al. 2002). However, other reports found no clear changes in eNOS expression by VECs during aging, and rather highlighted increased endothelin-1 as a key player in the reduced endothelial-dependent dilation observed in older adults (Donato et al. 2009).

Another source of ROS is represented by dysfunctional mitochondria. Physiological aging induces a decline in mitochondrial mass and biogenesis in VECs and is associated with altered expression of several components of the electron transport chain, leading to increased ROS production in aged blood vessels (Ungvari et al. 2018). This decline in mitochondrial function

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

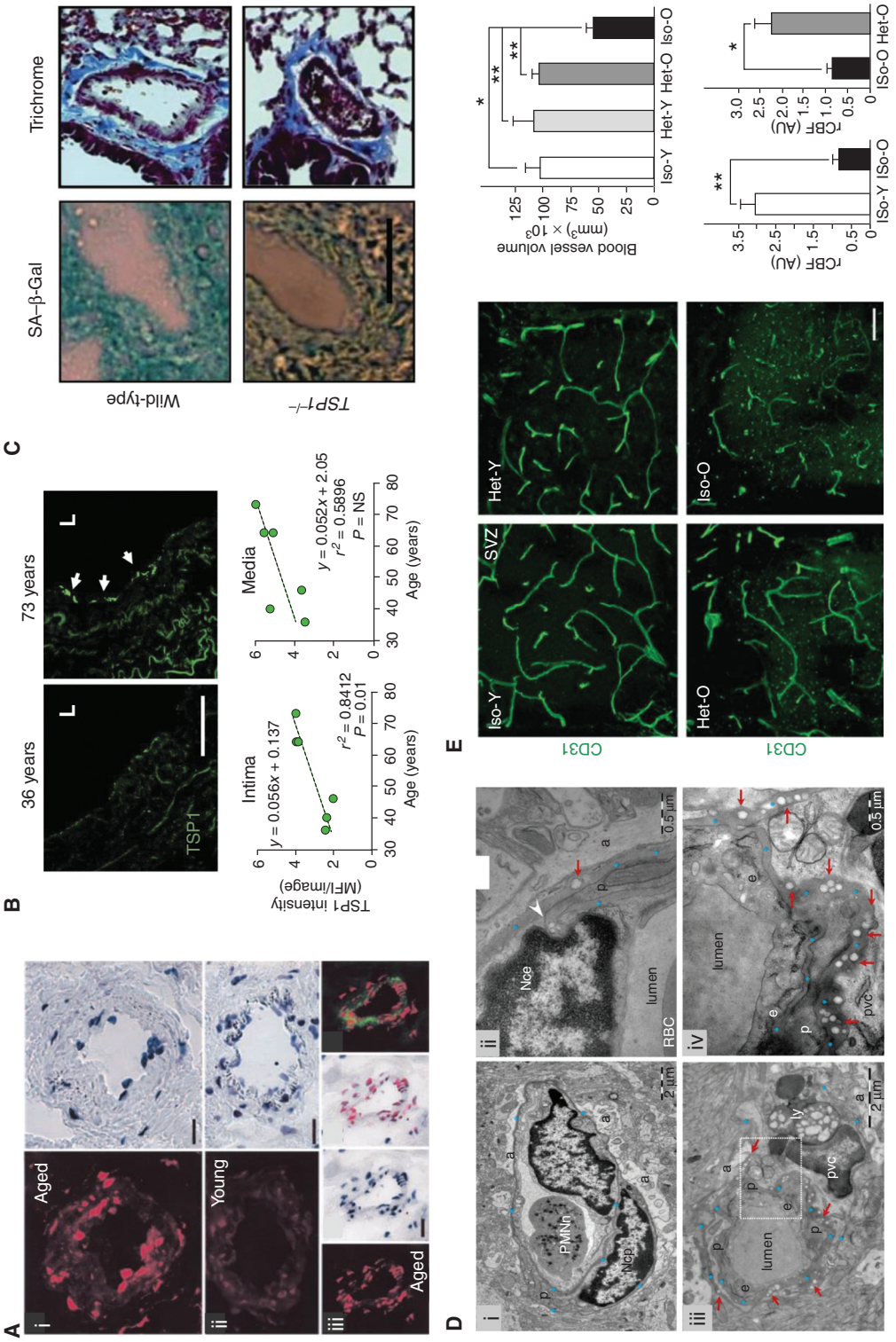

Figure 2. *(See following page for legend.)*

seems to be related to a compromised communication with the cell nucleus (Gomes et al. 2013). Importantly, mitochondrial DNA (mtDNA) is close to ROS production sites in mitochondria. The accumulation of ROS can induce mtDNA mutations, compromising the energy production system (Wallace 2005). Both oxidative stress and mitochondrial damage during aging converge toward the activation of nuclear factor κ light-chain enhancer of activated B cells (NF-κB) signaling in both VECs and smooth muscle cells (Csiszar et al. 2008; Ungvari et al. 2008). Activation of the NF-κB signaling leads to the expression of proinflammatory cytokines including interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor (TNF)-α (Zhang 2008; Song et al. 2012; Donato et al. 2015), which can induce compromised barrier function in blood vessels, an effect observed both in physiological aging and under pathological conditions (Farrall and Wardlaw 2009; Oakley and Tharakan 2014; Poulos et al. 2017; Banks et al. 2021). Oxidative stress and mitochondrial damage combine to the process of so-called inflammaging. This concept refers to a condition characterized by the presence of elevated levels of inflammatory markers and is connected with immune cell dysregulation, inflammasome activation, changes to microbiota composition, chronic infections, and cellular senescence (Ferrucci and Fabbri 2018). Features of oxidative stress, mitochondrial damage, and inflammation can be highly controlled in in vitro models of vascular aging and age-related diseases. Importantly, the use of donor-derived primary cells could help to clarify the mechanisms underlying the persistent instability that characterizes aged or compromised blood vessels and their interaction with the surrounding microenvironment. For instance, primary cells collected from transgenic small vessel disease models have allowed to analyze the bidirectional interaction between diseased VECs and other brain cell populations including astrocytes (Quick et al. 2021). Primary human cells would represent the ideal source, but the available material is limited and the operating procedures to

Figure 2. Aging of the vascular microenvironment. (*A*) Representative fluorescence and brightfield images of the same vessel sections from aged (*i*) and young (*ii*) coronary vessels labeled with dihydroethidium (red in presence of O_2·) and hematoxylin. Enlargement of an aged vessel section (*iii*): from *left* to *right*, labeling with dihydroethidium (red), hematoxylin staining, overlay of dihydroethidium (red) and hematoxylin, and overlay of dihydroethidium (red) and α smooth muscle actin (green). Scale bars, 10 µm. (Panel *A* from Csiszar et al. 2002; reprinted, with permission, from Wolters Kluwer Health © 2002.) (*B*) Fluorescence images of thrombospondin 1 (TSP1) abundance in intimal and media layers of pulmonary arteries of 36- and 73-yr-old subjects (arrows indicate intima). Scale bar, 100 µm. (L) Vascular lumen. Quantification of the mean fluorescence intensity (MFI) of TSP1 in the intima and media plotted as linear regression. (*C*) Images of lung blood vessel sections from middle-aged wild-type and $TSP1^{-/-}$ mice stained with senescence-associated β-galactosidase (SA-b-Gal, green signal) and Masson's trichrome (quantification of collagen deposition, blue signal). Scale bar, 100 µm. (Panels *C* and *D* from Meijles et al. 2017; reprinted, with permission, from the American Association for the Advancement of Science © 2017.) (*D*) Transmission electron microscopy of brain capillaries in a 6-mo-old mouse (*i*, *ii*) and a 24-mo-old mouse (*iii*, *iv*). Capillary from the 6-mo-old mouse (*i*) with uniform basement membrane (BM) adjacent to endothelial cell (nucleus of the endothelial cell [NCe], endothelial cell [e]) and enclosing a pericyte (p). (PMNn) Polymorphonuclear neutrophil, (a) end feet of astrocytes. (*ii*) Rare droplets (arrow) can be observed in the BM (*) of capillaries from young mouse brain. Arrowhead indicates a direct contact between pericyte and endothelial cell. (*iii*) The BM (*) of brain capillaries from the aged mouse is thicker, uneven, and with electron-lucent, single, or grouped droplets (arrows). Large lipid-containing lysosomes (ly) are present in a perivascular cell (pvc). (p) Pericyte, (a) end foot of astrocyte. (*iv*) Higher magnification of marked area in *iii*. (Panel *D* from Ceafalan et al. 2019; reprinted under the terms of the Creative Commons CC BY license, which permits unrestricted use, distribution, and reproduction in any medium.) (*E*) Confocal images of subventricular zone showing changes in vascularization following heterochronic parabiosis. (Iso-Y) Isochronic young, (Het-Y) heterochronic young, (Het-O) heterochronic old, (Iso-O) isochronic old. Quantification of blood vessel volume and cerebral blood flow. Scale bar, 50 µm. (Panel *E* from Katsimpardi et al. 2014; reprinted, with permission, from the American Association for the Advancement of Science © 2014.)

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

efficiently isolate cells postmortem or from surgical biopsies need to be further improved to avoid any alterations to the cell function.

Senescence

Another relevant feature of vascular aging whose study would greatly benefit from the highly controlled microenvironment available in complex vascular models is cellular senescence. This phenomenon is associated with the secretion of a wide set of inflammatory cytokines, including IL-6 and TNF-α, that define the senescence-associated secretory phenotype (SASP) (Bhayadia et al. 2016). Complex signaling pathways can lead to the activation of the senescence program. Senescence can be induced by activation of the DNA damage response and accumulation of the transcription factor GATA4 in aging tissues (Kang et al. 2015) or by telomere uncapping (Morgan et al. 2013). In addition, the accumulation of the matrix protein thrombospondin can induce VEC senescence by activating the production of ROS and triggering the DNA damage response (Fig. 2B,C; Meijles et al. 2017). Recently, significant interest has been captured by a class of drugs called senolytics, which have shown promising results in selectively removing senescent cells from several organs, as well as from the vascular compartment. For instance, chronic treatment with senolytics was shown to lessen signs of vascular aging including reducing calcifications and increasing bioavailability of nitric oxide (Roos et al. 2016). While these results are encouraging, several concerns regarding the efficacy of senolytics still remain. One biotech company active in the anti-aging field recently announced that the first novel senolytic drug to reach the clinical trial level failed to beat a placebo in the treatment of knee osteoarthritis (Dolgin 2020). Among the possible explanations for this failure is the fact that a single senolytic drug may be unlikely able to address all age-related conditions because senescent cells are not the same across aging tissues. Thus, more effective biomarkers should be selected to identity different types of senescent cells and to track their selective elimination by novel candidate senolytics (Dolgin 2020). In this regard, testing the performance of senolytics using in vitro models of the human vasculature could significantly aid in clarifying the mechanisms of senescent cell removal and its consequences on the surrounding cells.

ECM Remodeling, Perivascular Fibrosis, and Glycocalyx Damage

Vascular aging is characterized by significant changes at the level of ECM and basement membrane (BM), the thin layer of collagen type IV and laminin surrounding VECs. Early studies demonstrated that the thickness of the human BM increases with age (Xi et al. 1982). Apart from structural changes, the composition of the BM is also altered during aging. For instance, ultrastructural studies on mouse brain capillaries reported accumulation of lipid droplets during aging and highlighted that lipid-rich BM regions appeared located in pockets formed by astrocyte end feet (Fig. 2D; Ceafalan et al. 2019).

With respect to the perivascular ECM, it is important to highlight that senescence can alter the secretion of matrix metalloproteases (MMPs), which then modify the perivascular ECM and activate TGF-β signaling leading to altered collagen synthesis and perivascular fibrosis. While changes in the ECM surrounding large arteries can lead to increased systolic pressure and ventricular remodeling, compromised ECM of small size blood vessels during aging can affect microvascular transport and barrier function (Jacob 2003). For instance, the increased MMP-9 expression in aged mice has been linked with inflammation and vascular leakage, which in turn compromises cardiac functionality (Yabluchanskiy et al. 2014). Remodeling of the ECM and inflammation can also contribute to the progression of the endothelial-to-mesenchymal transition, leading to loss of cell identity (Fleenor et al. 2012; Dejana and Lampugnani 2018). Activation of endothelial-to-mesenchymal transition is associated with organ fibrosis (Ghosh et al. 2010).

Moving from the abluminal to the luminal space of blood vessels, the glycoprotein- and glycolipid-rich layer associated with the VEC surface (i.e., glycocalyx) undergoes changes not only during disease but in the course of physiological aging (Machin et al. 2019). Indeed, the

glycocalyx thickness decreases during aging in mice and humans, making the glycocalyx more permeable (Machin et al. 2018).

Investigating the extremely dynamic and progressive changes that occur at the level of perivascular ECM and glycocalyx represents a challenge in vivo. The combination of in vitro human vascular models with the end-point observations obtained from animal studies might offer a new perspective in the identification of novel targets and in the screening of compounds preventing or delaying the progression of the perivascular remodeling.

Circulating Factors

Early experiments of heterochronic parabiosis demonstrated that it is possible to partially restore the functionality of aged tissues (e.g., brain, heart) by surgically connecting the blood flow of older animals and younger animals (Fig. 2E; Loffredo et al. 2013; Katsimpardi et al. 2014; Ozek et al. 2018). Noteworthy, the circulating factor GDF-11 was identified as a potential candidate promoting vascular and tissue rejuvenation, although other reports suggested that further studies would be required to clarify the role of this molecule (Egerman et al. 2015). Single-cell sequencing of mouse brain VECs highlighted that blood vessels experience significant changes during aging including activation of oxidative stress pathways, inflammation, and innate immunity. Importantly, exposure to plasma from young animals partially rejuvenated brain VECs of older mice (Chen et al. 2020). Specifically, brain VECs from old mice express vascular cell adhesion molecule 1 (VCAM-1), which is then shed into the plasma. Treating young animals with soluble VCAM-1-rich plasma increased the expression of VCAM-1 in their brain VECs, whereas exposure to blocking antibodies against VCAM-1 counteracted the detrimental effect of plasma from old animals on younger mice (Yousef et al. 2019). Strikingly, blocking VCAM-1 was associated with reduced microglia activation and cognitive decline in old animals (Yousef et al. 2019). Overall, it is now clear that plasma from young donors contains factors that prevent or delay the onset of tissue/organ damage. However, mechanistic explanations and relevant targets in a human context are still unknown. It is envisioned that the high-throughput screening of these factors in in vitro models of the human vasculature would greatly enhance the possibility of identifying candidates for a future clinical translation.

Other Vascular Aging Hallmarks

The complexity of vascular aging involves other metabolic and (epi)genetic alterations. The reader is encouraged to refer to these review papers for detailed discussions regarding proteostasis (Kaushik and Cuervo 2015), deregulated nutrient sensing (Barzilai et al. 2012; Efeyan et al. 2015), genetic (Melzer et al. 2020), and epigenetic (Sen et al. 2016) changes during aging.

ORGAN SPECIFICITY AND VASCULAR AGING

Vascular beds have been traditionally classified into arteries, veins, capillaries, and intermediate vessels (e.g., arterioles and venules). In addition, three major subtypes have been identified among capillaries, namely, continuous, fenestrated, and sinusoidal vessels. During the last decade, exciting new studies have highlighted that in addition to this traditional classification, both VECs and supporting mural cells are not equal throughout the body, being characterized by organ-specific features that influence the functionality of the surrounding tissues during homeostasis and disease, as well as the reverse (Augustin and Koh 2017; Potente and Mäkinen 2017).

In an early attempt to classify organ-specific VECs, the Rafii group showed that cells from different organs in mice express a unique combination of transcription factors, cytokines, and adhesion molecules. Interestingly, transplantation of VECs derived from embryonic stem cells demonstrated that these cells can acquire organ-specific features based on the tissue where they engraft (Nolan et al. 2013). Organ-specific VECs generate specialized vascular niches secreting molecules called angiocrine factors that actively contribute to the maintenance of organ metabolism and participate in the regeneration of dam-

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

aged tissues (Rafii et al. 2016; Rafii and Gomez-Salinero 2018). Importantly, the communication with the surrounding microenvironment is bidirectional. For instance, local cues in the heart maintain a specific chromatin state in cardiac VECs inducing the expression of genes that are not expressed when the cell are isolated in culture (Yucel et al. 2020). VECs have been successfully isolated from fetal heart, lung, liver, and kidneys, and then cultured in vitro where they showed distinct barrier properties that mimicked organ-specific features observed in vivo (Marcu et al. 2018). More recently, advances in single-cell biology have allowed dissection of the organ specificity of VECs with an unprecedented level of resolution (Qiu and Hirschi 2019; Jakab and Augustin 2020; Paik et al. 2020). Similar to VECs, several studies highlighted that mural cells are characterized by a high level of diversity and plasticity (Holm et al. 2018; Liu and Gomez 2019), as well as G-protein-coupled receptor (GPCR) expression (Kaur et al. 2017) during homeostasis and disease (e.g., acute and chronic inflammation).

Although vascular cells show organ-specific features, the molecular and cellular mechanisms underlying vascular aging have been traditionally considered similar across vascular beds. However, it is emerging that the pattern of vascular aging is organ-specific. Using single-cell-resolution 3D imaging of vascular beds from young (i.e., less than 15-wk-old) and old (i.e., 55- to 70-wk-old) mouse tissues, it was demonstrated that vessel density and pericyte coverage decrease during aging, although the density of blood vessels and pericytes is partially preserved in highly remodeling organs such a skin, gut, bone, and uterus when compared with heart, brain, or kidney (Chen et al. 2021). In addition, pericytes were shown to transdifferentiate into fibroblasts during aging and degeneration of the vascular compartment was sufficient to drive senescence, which can then contribute to loss of organ functionality.

Taken together, the discovery of organ specificity of vascular cells and their interaction with the local microenvironment has contributed to a paradigm shift, whereby VECs and supporting mural cells are now active players during development and regeneration, as well as in a wide set of diseases including neurodegeneration, cancer, and fibrosis. It is expected that upcoming studies in the context of organ-specific vascular aging might shed light on the molecular mechanisms underlying the decline in organ functionality that occurs during physiological aging. The identification of these mechanisms is expected to foster the design of novel therapeutic approaches aimed at preventing or delaying vascular damage to preserve the organ function.

MODELING VASCULAR AGING IN VITRO

In this section, we will focus on the main features and findings of relevant in vitro 3D models that have been employed to analyze specific aspects of vascular aging over the last 5 years. In addition, relevant results obtained through simple 2D assays complementing animal models will be briefly described. The selection of manuscripts was based upon a PubMed search performed on May 5, 2021 using the key words "vascular, aging OR ageing, vitro OR microfluidic, model" reported in the title or abstract of each research product. Importantly, this section will not focus on in vitro models that were mainly used to study specific diseases, including those associated with aging (e.g., AD) (Maoz et al. 2018; Shin et al. 2019; Vatine et al. 2019).

The majority of simplified 2D in vitro models focused on one of the most studied aspects of aging, namely, cellular senescence. For instance, Cirilli and colleagues (2020) treated young human umbilical vein endothelial cells (HUVECs) with cigarette smoke extracts and observed signatures of cellular senescence, which were then partially reverted by treatment with ubiquinol and menaquinone-7 (Cirilli et al. 2020). Low concentrations of the chemotherapeutic drug doxorubicin were shown to induce senescence of immortalized VECs, which then expressed typical components of the SASP. Interestingly, treatment with SASP-containing conditioned medium induced platelet activation and aggregation (Venturini et al. 2020). It is important to highlight that senescent cells or their products can be in principle cleared, as demonstrated in animal models through different methods (e.g.,

genetic clearing using the INK-ATTAC model, treatment with dasatinib and quercetin, treatment with the Janus kinase (JAK1/2) inhibitor ruxolitinib) (Xu et al. 2015, 2018). Exosomes obtained from human embryonic stem cells were able to rejuvenate D-galactose-induced senescent HUVECs by partially restoring their ability to proliferate, migrate, and form tubular structures in vitro (Chen et al. 2019). This rejuvenation seemed to be associated with miR-200a, which was enriched in exosomes from embryonic stem cells (Chen et al. 2019). Aging induces endoplasmic reticulum (ER)-mitochondria tethering in VECs, which is associated with an increased Ca^{2+} uptake by mitochondria. This phenomenon makes senescent cells more vulnerable to cell death induced by mitochondrial Ca^{2+} overload (Madreiter-Sokolowski et al. 2019), as demonstrated by the selective death of senescent cells following treatment with the polyphenol resveratrol (Madreiter-Sokolowski et al. 2019). VECs are not the only vascular cells that have been studied in the context of senescence. Hyperhomocysteinemia is known to induce vascular aging, although the underlying mechanisms are largely unknown. Using vascular smooth muscle cells treated with homocysteine, Yan and colleagues observed increased expression of p16, p21, and p53, all markers of senescence. In parallel, impaired autophagy was detected following homocysteine treatment, whereas rapamycin partially restored autophagy and reverted the senescence phenotype (Yan et al. 2021).

Vascular senescence is associated with inflammation and oxidative stress. Using simple cell culture systems, Yan and colleagues engineered human vascular cells expressing an active form of the transcription factor Forkhead box O3 (FOXO3), which has been linked to longevity. These cells showed increased resistance to oxidative stress in vitro and promoted vascular regeneration in an animal model of ischemic injury (Yan et al. 2019). Similarly, treatment of HUVECs with melatonin was able to reduce oxidative stress, mitochondrial damage, and inflammation (NF-κB signaling) (Lee et al. 2018).

Another key feature of vascular aging is calcification, which often occurs at the level of supporting mural cells. In vitro models based on traditional 2D cell cultures of vascular smooth muscle cells represent a useful tool to study the mechanisms of calcification and test potential treatments (Herrmann et al. 2020). Huang and colleagues demonstrated that carnosine was able to inhibit the osteoblastic transdifferentiation of smooth muscle cells (reduced Runt-related transcription factor 2 [Runx2] and bone morphogenetic protein 2 [BMP-2] protein expression), and to reduce the production of ROS (Huang et al. 2020). Similar results were obtained by treating vascular smooth muscle cells with magnesium (Villa-Bellosta 2020). Calcification is also associated with blood vessel stiffening. Among the different causes of increased cellular stiffness is an abnormal level of intracellular sodium, which increases during aging. Using atomic force microscopy, Mewes and colleagues detected abnormally high levels of cellular stiffness in VECs exposed to increasing sodium concentrations. Through a simple 2D culture, the authors observed a decrease in cellular stiffness following inhibition of soluble adenylyl cyclase (Mewes et al. 2017).

Overall, monocultures of VECs or smooth muscle cells have proven to be useful to simplistically analyze specific features of vascular aging, including senescence and calcification. However, these models did not recapitulate the complexity of vascular niches, both from an architectural and functional point of view. For instance, 2D monoculture assays cannot be employed to study changes in blood vessel functionality, including vascular permeability and vessel dilation, or to analyze the contribution of specific treatments aimed at restoring this functionality during aging. To overcome this limitation and to complement animal models, a few 3D systems recapitulating the aging vasculature have been developed. For instance, modeling the BBB in vitro allowed an understanding of key mechanisms of vascular aging. Using a coculture of human brain microvascular VECs and astrocytes separated by a porous membrane, it was demonstrated that the complement component C3a, which is up-regulated in astrocytes during aging, can induce VEC activation, compromise adherens junction integrity, and induce barrier

loss (Propson et al. 2021). Although brain VECs and astrocytes were separated by a semipermeable membrane, coculture was sufficient to reveal a signaling axis between two different cell types, demonstrating that recapitulating a minimum level of complexity of the neurovascular unit was sufficient to understand an important mechanism of vascular damage during aging. A more complex system was designed by Yamazaki and colleagues, who employed brain VECs and pericytes derived from young (5- to 8-wk-old) and old (40- to 50-wk old) mice and cultured them on the opposite sides of a porous membrane placed on top of a well containing astrocytes. The authors observed that the integrity of endothelial tight junctions was compromised using cells from old mice. This effect was coupled with a reduced barrier functionality quantified through transendothelial electrical resistance (Fig. 3A; Yamazaki et al. 2016). While use of animal-derived cells might not fully recapitulate the biological mechanisms underlying human vascular aging, these experiments clearly showed that the integrity of the BBB is compromised during aging and that this effect is due to interactions within the neurovascular unit.

Moving toward increased complexity, a blood-vessel-on-a-chip containing human-induced pluripotent stem (iPS) cell–derived VECs was employed to analyze the toxicity of a library of nanoparticle (NP) formulations on the vasculature. The study of these interactions is particularly relevant for understanding the consequences that specific treatments using NPs can have on the vascular system. Surprisingly, it was demonstrated that NP toxicity was dependent on the source of vascular cells (i.e., iPS cell lines derived from fetal cells or from Hutchinson–Gilford Progeria Syndrome (HGPS) donors), suggesting that NP-based treatments should be optimized based on the patient age and pathological conditions. For instance, HGPS cells were more vulnerable to Ag-based NPs. In addition, the presence of flow within the blood-vessel-on-a-chip attenuated NP-mediated toxicity (Estronca et al. 2021). These results highlight the importance of recapitulating physiological biophysical stimuli to properly model the response of the vasculature. Salmon and co-authors cocultured VECs and mural cells in a 3D tissue engineered blood vessel that was exposed to chronic oxidative stress using H_2O_2 for 1 week. The treatment induced signs of senescence (e.g., increased p21 expression) in both cell types. However, the functional consequences of senescence were only observed in VECs, which were characterized by increased expression of the inflammatory molecule VCAM-1. Moreover, endothelium-dependent vasodilation was compromised, while vasoconstriction and endothelium-independent vasodilation were preserved (Salmon et al. 2020).

Using cells from donors affected by HGPS, a disease caused by a point mutation in the *LMNA* gene, which leads to an impaired architecture of the nuclear membrane and accelerated aging, Ribas and colleagues employed organ-on-a-chip technology to analyze the effect of biomechanical stimuli on iPS cell–derived smooth muscle cells. Interestingly, only cells from HGPS patients and not from healthy donors showed activation of an inflammatory response and signs of DNA damage when exposed to mechanical stimulation (Ribas et al. 2017). These observations might be compatible with perivascular fibrosis commonly observed in HGPS patients. In agreement with these findings, a simplified in vitro model coupled with in vivo observations also demonstrated that VECs expressing mutated *LMNA* showed an altered response to shear stress and decreased the expression of eNOS and nitric oxide, which might trigger a profibrotic response in mural cells (Osmanagic-Myers et al. 2019).

Current in vitro systems often lack a detailed characterization of vascular changes occurring from the gene expression to the functional level. Using a multianalytical approach combining microfluidic organ-on-a-chip, RNA sequencing, and secretome analyses validated within human tissue biopsies, Bersini and coauthors demonstrated that coculturing human dermal fibroblasts obtained from young donors can partially restore the functionality of old human dermal VECs. This functional restoration was analyzed in terms of BM integrity, secretion of inflammatory cytokines, activation of endothelial-specific gene-expression programs, and vascular permeability (Fig. 3B). Interestingly, it was further

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

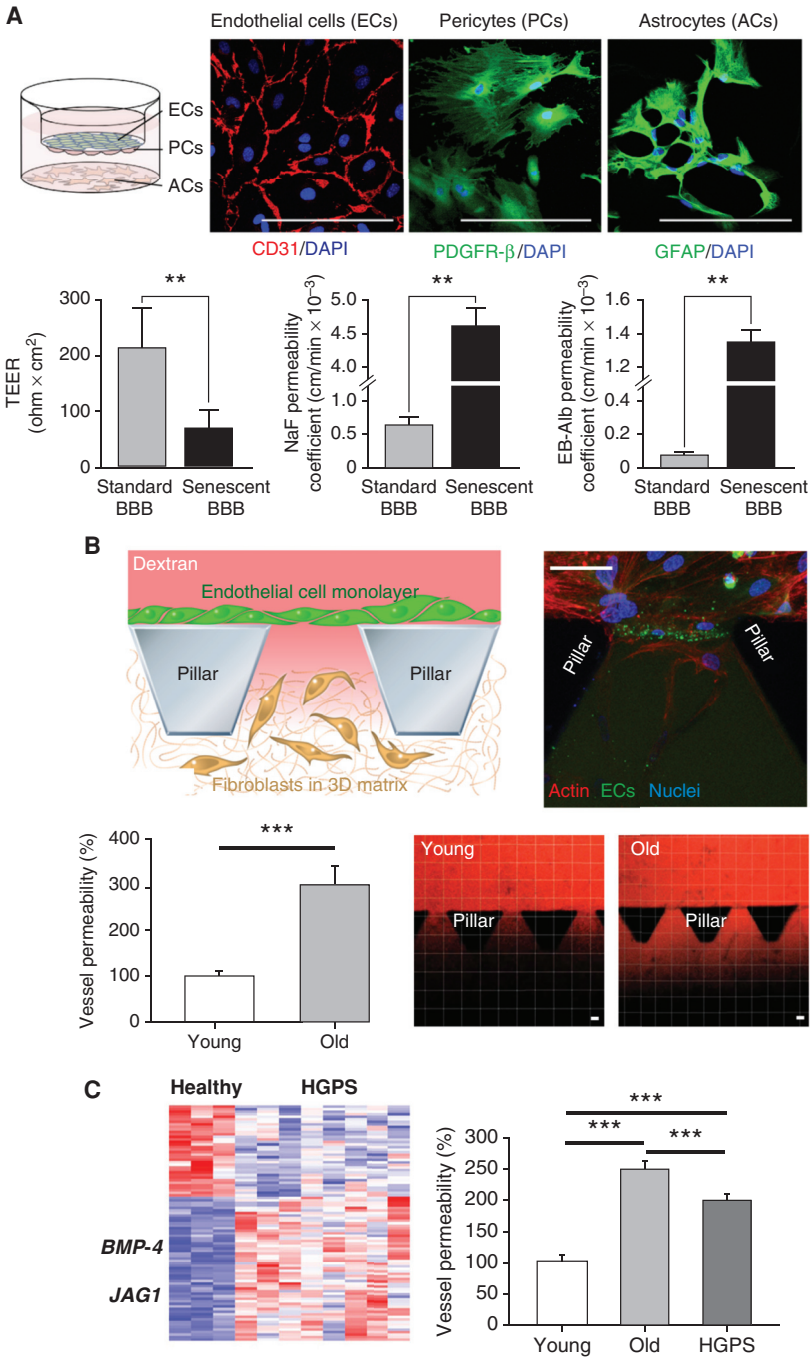

Figure 3. (*See following page for legend.*)

demonstrated that treating these rejuvenated cells with blood serum obtained from old human donors was sufficient to reinduce signatures of vascular aging. This approach allowed the identification of potential novel vascular aging biomarkers that were validated using human tissue biopsies from donors of different ages (Bersini et al. 2020a). Using a similar approach combined with the direct reprogramming of dermal fibroblasts from donors of different ages into VECs and smooth muscle cells, the same group analyzed vascular aging in the context of HGPS. Since the main consequence of this accelerated aging syndrome is vascular damage, the group used reprogrammed cells from HGPS patients and healthy, age-matched controls to analyze the contribution of dysfunctional smooth muscle cells. HGPS-derived cells induced vascular leakage, an effect partially due to the overexpression of BMP-4, which is associated with abnormal vascular permeability and smooth muscle cell calcification (Fig. 3C; Bersini et al. 2020b).

Overall, the combination of novel biofabrication strategies with advanced biological analyses has been demonstrated to be instrumental for the identification of features of vascular aging that were previously unknown.

CONCLUDING REMARKS

Aging is the major risk factor for a large set of diseases including CVDs and cancer. However, our understanding of the biological mechanisms of aging is still limited. In this context, the length of the cardiovascular system and its capillary distribution have traditionally represented a major obstacle for their comparative study. In addition, the complexity of blood vessels lies not only in the classical artery-capillary-vein distinction, but also in the emerging role of organ-specific vascular differentiation, which has consequences during development, physiological aging, and disease.

Figure 3. Advanced in vitro models of vascular aging. (*A*) In vitro coculture models of the blood–brain barrier. Mouse endothelial cells ($CD31^+$, red) and pericytes (platelet-derived growth factor receptor (PDGFR)-β^+, green) were cultured on a semipermeable membrane insert, whereas astrocytes (glial fibrillary acidic protein $(GFAP)^+$, green) were cultured into the bottom of the culture well. Cells were obtained from middle-aged or young mice. The barrier integrity was quantified using transendothelial electric resistance (TEER) measurements and the permeability coefficient for sodium fluorescein (NaF) and Evans blue (EB) albumin. The barrier was compromised in the presence of cells from middle-aged mice. (Panel *A* from Yamazaki et al. 2016; reprinted, with permission, from Wolters Kluwer Health © 2016.) (*B*) 3D in vitro models of biomechanical stimulation of Hutchinson–Gilford progeria syndrome (HGPS) blood vessels. Blood vessels experience cyclic strain due to pulsatile blood flow. Hence, a microfluidic vascular model containing two overlapping channels was designed. A cross-sectional view of the microfluidic device shows the smooth muscle cell layer cultured on top of the membrane and a view during vacuum stimulation shows the downward membrane deformation. The expression of inflammatory markers (i.e., interleukin [IL]-6 and 1β) was significantly higher in systems embedding smooth muscle cells derived from HGPS donors compared to healthy controls when exposed to biomechanical stimulation (strain level up to 16%). (*C*) Microfluidic model of human endothelial cell aging. Schematic and confocal image of the model that embedded in separate compartments human dermal fibroblasts obtained from young and old healthy donors and endothelial cells (green). The model revealed that the integrity of the vascular barrier was restored when endothelial cells from an old donor were cocultured with dermal fibroblasts derived from young donors (fluorescent 70 kDa dextran was used for permeability measurements). Actin (red) stained both endothelial cells and fibroblasts. Scale bars, 50 μm. (Panel *C* from Bersini et al. 2020a; reprinted, with permission, from the authors © 2020.) (*D*) Transcriptional and functional analysis of smooth muscle cells directly reprogrammed from HGPS and healthy, age-matched dermal fibroblasts donors revealed that potential markers involved in vascular permeability (i.e., bone morphogenetic protein 4 [*BMP-4*] and jagged 1 [*JAG1*]) were upregulated in HGPS-derived cells. Analysis of vascular permeability within a microfluidic model demonstrated that the integrity of the endothelial barrier was compromised in the presence of smooth muscle cells reprogrammed from HGPS and old donors compared to young healthy donors. (Panel *D* from Bersini et al. 2020b; reprinted under the terms of the Creative Commons Attribution License, which permits unrestricted use and redistribution provided that the original author and source are credited.)

Simplified in vitro models have been so far developed to analyze specific features of vascular aging, while the majority of experimental approaches have been performed in rodents. Only a few more complex systems have attempted to bridge the gap with in vivo studies.

How can in vitro models be improved to more faithfully mimic the aging vasculature of a specific organ? The first element to restore is complexity. Although a few models have explored cocultures and attempted to reproduce the 3D architecture of small tissue units, more effort needs to be directed to the design of organ-specific culture conditions. For instance, organ-on-a-chip technologies allow compartmentalization of different cell populations into specific regions of microfluidic devices. However, it is still not possible to spatially organize the multiple niches that form a tissue (e.g., perisinusoidal niche, periarteriolar niche, osteoblastic niche in the bone) and at the same time promote proper maturation, which often requires different biochemical (e.g., growth factor gradients) and biophysical (e.g., luminal and interstitial flow) stimuli, occurring at different time scales for each niche. In addition, the ECM used to embed cells and tissues is generally undercharacterized. Oversimplified natural or synthetic matrices are employed to maintain cell viability, but the matrices should be better optimized to allow cells to better mimic their physiological behavior. Similarly, the composition of the cell culture medium should be properly tuned over time to allow the correct maturation of the tissue and the interaction among different niches, and the media formulation should be carefully modified toward animal-free recipes for those systems that aim to study the contribution of human circulating proteins/lipids/genetic materials. Overall, the optimization of these features would go a long way to allowing the full investigation of the complexity of interactions driving vascular aging.

Another key aspect to be considered is the use of human cells. Although basic mechanisms are conserved among humans and rodents, the functionality of the immune system is different. Since inflammaging is a key aspect of aging, the interactions between vascular cells and immune cells are fundamental to understand the mechanisms of human vascular aging. However, obtaining human primary cells is often challenging for specific tissues, especially for the brain. Direct reprogramming of human dermal fibroblasts into organ-specific cells, including VECs and smooth muscle cells, is a valuable alternative to primary cells. Recently, different studies have highlighted the relevance of directly reprogrammed cells (Van Tuyn et al. 2005; Han et al. 2014), particularly in the context of aging (Mertens et al. 2015; Bersini et al. 2020b). Indeed, reprogrammed cells do not lose the aging signature of the cells of origin compared to iPS cells.

Regarding potential applications of in vitro vascular aging models, the patient-specific longitudinal monitoring of individual vascular health could represent a clinical breakthrough. Indeed, the vascular system is not easily accessible for tissue biopsies and the possibility of combining routine blood works with personalized models of the patient vasculature starting from a simple skin biopsy would increase the understanding of how the vasculature is performing during physiological aging, diseases, or treatment (e.g., vascular changes due to chemotherapeutic drugs). Importantly, in light of the fact that the cells could be obtained from people of different age, gender, ethnicity, body mass index, and other relevant features, these systems would also allow more effective drug screening assays.

Concluding, the combination of biofabrication with patient-derived cells and advanced analytical techniques (e.g., single-cell genomics and proteomics) will allow the recapitulation of the complexity of human, organ-specific vasculatures during aging and the analysis of cell–cell and cell–matrix interactions that are challenging to observe in vivo. Most importantly, this approach will allow more effective screenings of drugs before moving to animal studies; hence, fostering an efficient translation of biological discoveries into therapies for a healthier aging.

ACKNOWLEDGMENTS

The authors greatly acknowledge the Paul F. Glenn Center for Biology of Aging Research at The Salk Institute. The authors were supported by an AHA/Allen Initiative in Brain Health and

Cognitive Impairment award made jointly through the American Heart Association and the Paul G. Allen Frontiers Group: 19PABH 134610000.

REFERENCES

Augustin HG, Koh GY. 2017. Organotypic vasculature: from descriptive heterogeneity to functional pathophysiology. *Science* **357:** eaal2379. doi:10.1126/science.aal2379

Banks WA, Reed MJ, Logsdon AF, Rhea EM, Erickson MA. 2021. Healthy aging and the blood–brain barrier. *Nat Aging* **1:** 243–254. doi:10.1038/s43587-021-00043-5

Barzilai N, Huffman DM, Muzumdar RH, Bartke A. 2012. The critical role of metabolic pathways in aging. *Diabetes* **61:** 1315–1322. doi:10.2337/db11-1300

Bersini S, Yazdi IK, Talò G, Shin SR, Moretti M, Khademhosseini A. 2016. Cell–microenvironment interactions and architectures in microvascular systems. *Biotechnol Adv* **34:** 1113–1130. doi:10.1016/j.biotechadv.2016.07.002

Bersini S, Arrojo e Drigo R, Huang L, Shokhirev MN, Hetzer MW. 2020a. Transcriptional and functional changes of the human microvasculature during physiological aging and Alzheimer disease. *Adv Biosyst* **4:** 2000044. doi:10.1002/adbi.202000044

Bersini S, Schulte R, Huang L, Tsai H, Hetzer MW. 2020b. Direct reprogramming of human smooth muscle and vascular endothelial cells reveals defects associated with aging and Hutchinson–Gilford progeria syndrome. *eLife* **9:** e54383. doi:10.7554/eLife.54383

Bhayadia R, Schmidt BMW, Melk A, Hömme M. 2016. Senescence-induced oxidative stress causes endothelial dysfunction. *J Gerontol Ser A Biol Sci Med Sci* **71:** 161–169. doi:10.1093/gerona/glv008

Ceafalan LC, Fertig TE, Gheorghe TC, Hinescu ME, Popescu BO, Pahnke J, Gherghiceanu M. 2019. Age-related ultrastructural changes of the basement membrane in the mouse blood–brain barrier. *J Cell Mol Med* **23:** 819–827. doi:10.1111/jcmm.13980

Chen B, Sun Y, Zhang J, Zhu Q, Yang Y, Niu X, Deng Z, Li Q, Wang Y. 2019. Human embryonic stem cell-derived exosomes promote pressure ulcer healing in aged mice by rejuvenating senescent endothelial cells. *Stem Cell Res Ther* **10:** 142. doi:10.1186/s13287-019-1253-6

Chen MB, Yang AC, Yousef H, Lee D, Chen W, Schaum N, Lehallier B, Quake SR, Wyss-Coray T. 2020. Brain endothelial cells are exquisite sensors of age-related circulatory cues. *Cell Rep* **30:** 4418–4432.e4. doi:10.1016/j.celrep.2020.03.012

Chen J, Sivan U, Tan SL, Lippo L, de Angelis J, Labella R, Singh A, Chatzis A, Cheuk S, Medhghalchi M, et al. 2021. High-resolution 3D imaging uncovers organ-specific vascular control of tissue aging. *Sci Adv* **7:** eabd7819. doi:10.1126/sciadv.abd7819

Cirilli I, Orlando P, Marcheggiani F, Dludla PV, Silvestri S, Damiani E, Tiano L. 2020. The protective role of bioactive quinones in stress-induced senescence phenotype of endothelial cells exposed to cigarette smoke extract. *Antioxidants* **9:** 1008. doi:10.3390/antiox9101008

Cochrane A, Albers HJ, Passier R, Mummery CL, van den Berg A, Orlova VV, van der Meer AD. 2019. Advanced in vitro models of vascular biology: human induced pluripotent stem cells and organ-on-chip technology. *Adv Drug Deliv Rev* **140:** 68–77. doi:10.1016/j.addr.2018.06.007

Csiszar A, Ungvari Z, Edwards JG, Kaminski P, Wolin MS, Koller A, Kaley G. 2002. Aging-induced phenotypic changes and oxidative stress impair coronary arteriolar function. *Circ Res* **90:** 1159–1166. doi:10.1161/01.RES.0000020401.61826.EA

Csiszar A, Wang M, Lakatta EG, Ungvari Z. 2008. Inflammation and endothelial dysfunction during aging: role of NF-κB. *J Appl Physiol* **105:** 1333–1341. doi:10.1152/japplphysiol.90470.2008

Das A, Huang GX, Bonkowski MS, Longchamp A, Li C, Schultz MB, Kim LJ, Osborne B, Joshi S, Lu Y, et al. 2018. Impairment of an endothelial NAD^+-H2S signaling network is a reversible cause of vascular aging. *Cell* **173:** 74–89.e20. doi:10.1016/j.cell.2018.02.008

Dejana E, Lampugnani MG. 2018. Endothelial cell transitions. *Science* **362:** 746–747. doi:10.1126/science.aas9432

Dolgin E. 2020. Send in the senolytics. *Nat Biotechnol* **38:** 1371–1377. doi:10.1038/s41587-020-00750-1

Donato AJ, Gano LB, Eskurza I, Silver AE, Gates PE, Jablonski K, Seals DR. 2009. Vascular endothelial dysfunction with aging: endothelin-1 and endothelial nitric oxide synthase. *Am J Physiol Hear Circ Physiol* **297:** H425–H432. doi:10.1152/ajpheart.00689.2008

Donato AJ, Morgan RG, Walker AE, Lesniewski LA. 2015. Cellular and molecular biology of aging endothelial cells. *J Mol Cell Cardiol* **89:** 122–135. doi:10.1016/j.yjmcc.2015.01.021

Efeyan A, Comb WC, Sabatini DM. 2015. Nutrient-sensing mechanisms and pathways. *Nature* **517:** 302–310. doi:10.1038/nature14190

Egerman MA, Cadena SM, Gilbert JA, Meyer A, Nelson HN, Swalley SE, Mallozzi C, Jacobi C, Jennings LL, Clay I, et al. 2015. GDF11 increases with age and inhibits skeletal muscle regeneration. *Cell Metab* **22:** 164–174. doi:10.1016/j.cmet.2015.05.010

Estronca L, Francisco V, Pitrez P, Honório I, Carvalho L, Vazão H, Blersch J, Rai A, Nissan X, Simon U, et al. 2021. Induced pluripotent stem cell-derived vascular networks to screen nano–bio interactions. *Nanoscale Horizons* **6:** 245–259. doi:10.1039/D0NH00550A

Farrall AJ, Wardlaw JM. 2009. Blood–brain barrier: ageing and microvascular disease—systematic review and meta-analysis. *Neurobiol Aging* **30:** 337–352. doi:10.1016/j.neurobiolaging.2007.07.015

Ferrucci L, Fabbri E. 2018. Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty. *Nat Rev Cardiol* **15:** 505–522. doi:10.1038/s41569-018-0064-2

Fleenor BS, Marshall KD, Rippe C, Seals DR. 2012. Replicative aging induces endothelial to mesenchymal transition in human aortic endothelial cells: potential role of inflammation. *J Vasc Res* **49:** 59–64. doi:10.1159/000329681

Ghosh AK, Bradham WS, Gleaves LA, De Taeye B, Murphy SB, Covington JW, Vaughan DE. 2010. Genetic deficiency of plasminogen activator inhibitor-1 promotes cardiac fibrosis in aged mice: involvement of constitutive transforming growth factor-β signaling and endothelial-to-

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

mesenchymal transition. *Circulation* **122:** 1200–1209. doi:10.1161/CIRCULATIONAHA.110.955245

Glass CK, Witztum JL. 2001. Atherosclerosis: the road ahead. *Cell* **104:** 503–516. doi:10.1016/S0092-8674(01)00238-0

Gomes AP, Price NL, Ling AJY, Moslehi JJ, Montgomery MK, Rajman L, White JP, Teodoro JS, Wrann CD, Hubbard BP, et al. 2013. Declining NAD^+ induces a pseudohypoxic state disrupting nuclear-mitochondrial communication during aging. *Cell* **155:** 1624–1638. doi:10.1016/j.cell.2013.11.037

Han JK, Chang SH, Cho HJ, Choi SB, Ahn HS, Lee J, Jeong H, Youn SW, Lee HJ, Kwon YW, et al. 2014. Direct conversion of adult skin fibroblasts to endothelial cells by defined factors. *Circulation* **130:** 1168–1178. doi:10.1161/CIRCULATIONAHA.113.007727

Heidenreich PA, Trogdon JG, Khavjou OA, Butler J, Dracup K, Ezekowitz MD, Finkelstein EA, Hong Y, Johnston SC, Khera A, et al. 2011. Forecasting the future of cardiovascular disease in the United States: a policy statement from the American heart association. *Circulation* **123:** 933–944. doi:10.1161/CIR.0b013e31820a55f5

Herrmann J, Babic M, Tölle M, Eckardt KU, van der Giet M, Schuchardt M. 2020. A novel protocol for detection of senescence and calcification markers by fluorescence microscopy. *Int J Mol Sci* **21:** 3475. doi:10.3390/ijms21103475

Holm A, Heumann T, Augustin HG. 2018. Microvascular mural cell organotypic heterogeneity and functional plasticity. *Trends Cell Biol* **28:** 302–316. doi:10.1016/j.tcb.2017.12.002

Huang Y, Wang J, Luo M, Yan D, Zhang C. 2020. Carnosine attenuates vascular smooth muscle cells calcification through mTOR signaling pathway. *AGING Med* **3:** 153–158. doi:10.1002/agm2.12125

Jacob MP. 2003. Extracellular matrix remodeling and matrix metalloproteinases in the vascular wall during aging and in pathological conditions. *Biomed Pharmacother* **57:** 195–202. doi:10.1016/S0753-3322(03)00065-9

Jakab M, Augustin HG. 2020. Understanding angiodiversity: insights from single cell biology. *Development* **147:** dev 146621. doi:10.1242/dev.146621

Kang C, Xu Q, Martin TD, Li MZ, Demaria M, Aron L, Lu T, Yankner BA, Campisi J, Elledge SJ. 2015. The DNA damage response induces inflammation and senescence by inhibiting autophagy of GATA4. *Science* **349:** aaa5612. doi:10.1126/science.aaa5612

Katsimpardi L, Litterman NK, Schein PA, Miller CM, Loffredo FS, Wojtkiewicz GR, Chen JW, Lee RT, Wagers AJ, Rubin LL. 2014. Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors. *Science* **344:** 630–634. doi:10.1126/science.1251141

Kaur H, Carvalho J, Looso M, Singh P, Chennupati R, Preussner J, Günther S, Albarrán-Juárez J, Tischner D, Classen S, et al. 2017. Single-cell profiling reveals heterogeneity and functional patterning of GPCR expression in the vascular system. *Nat Commun* **8:** 15700. doi:10.1038/ncomms15700

Kaushik S, Cuervo AM. 2015. Proteostasis and aging. *Nat Med* **21:** 1406–1415. doi:10.1038/nm.4001

Kusumbe AP, Ramasamy SK, Itkin T, Mäe MA, Langen UH, Betsholtz C, Lapidot T, Adams RH. 2016. Age-dependent modulation of vascular niches for haematopoietic stem cells. *Nature* **532:** 380–384. doi:10.1038/nature17638

Lee FY, Sun CK, Sung PH, Chen KH, Chua S, Sheu JJ, Chung SY, Chai HT, Chen YL, Huang TH, et al. 2018. Daily melatonin protects the endothelial lineage and functional integrity against the aging process, oxidative stress, and toxic environment and restores blood flow in critical limb ischemia area in mice. *J Pineal Res* **65:** e12489. doi:10.1111/jpi.12489

Liu M, Gomez D. 2019. Smooth muscle cell phenotypic diversity. *Arterioscler Thromb Vasc Biol* **39:** 1715–1723. doi:10.1161/ATVBAHA.119.312131

Loffredo FS, Steinhauser ML, Jay SM, Gannon J, Pancoast JR, Yalamanchi P, Sinha M, Dall'Osso C, Khong D, Shadrach JL, et al. 2013. Growth differentiation factor 11 is a circulating factor that reverses age-related cardiac hypertrophy. *Cell* **153:** 828–839. doi:10.1016/j.cell.2013.04.015

Longchamp A, Mirabella T, Arduini A, MacArthur MR, Das A, Treviño-Villarreal JH, Hine C, Ben-Sahra I, Knudsen NH, Brace LE, et al. 2018. Amino acid restriction triggers angiogenesis via GCN2/ATF4 regulation of VEGF and H2S production. *Cell* **173:** 117–129.e14. doi:10.1016/j.cell.2018.03.001

López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. 2013. The hallmarks of aging. *Cell* **153:** 1194–1217. doi:10.1016/j.cell.2013.05.039

Machin DR, Bloom SI, Campbell RA, Phuong TTT, Gates PE, Lesniewski LA, Rondina MT, Donato AJ. 2018. Advanced age results in a diminished endothelial glycocalyx. *Am J Physiol Hear Circ Physiol* **315:** H531–H539. doi:10.1152/ajpheart.00104.2018

Machin DR, Phuong TT, Donato AJ. 2019. The role of the endothelial glycocalyx in advanced age and cardiovascular disease. *Curr Opin Pharmacol* **45:** 66–71. doi:10.1016/j.coph.2019.04.011

Madreiter-Sokolowski CT, Waldeck-Weiermair M, Bourguignon MP, Villeneuve N, Gottschalk B, Klec C, Stryeck S, Radulovic S, Parichatikanond W, Frank S, et al. 2019. Enhanced inter-compartmental Ca^{2+} flux modulates mitochondrial metabolism and apoptotic threshold during aging. *Redox Biol* **20:** 458–466. doi:10.1016/j.redox.2018.11.003

Maoz BM, Herland A, Fitzgerald EA, Grevesse T, Vidoudez C, Pacheco AR, Sheehy SP, Park TE, Dauth S, Mannix R, et al. 2018. A linked organ-on-chip model of the human neurovascular unit reveals the metabolic coupling of endothelial and neuronal cells. *Nat Biotechnol* **36:** 865–874. doi:10.1038/nbt.4226

Marcu R, Choi YJ, Xue J, Fortin CL, Wang Y, Nagao RJ, Xu J, MacDonald JW, Bammler TK, Murry CE, et al. 2018. Human organ-specific endothelial cell heterogeneity. *iScience* **4:** 20–35. doi:10.1016/j.isci.2018.05.003

Meijles DN, Sahoo S, Al Ghouleh I, Amaral JH, Bienes-Martinez R, Knupp HE, Attaran S, Sembrat JC, Nouraie SM, Rojas MM, et al. 2017. The matricellular protein TSP1 promotes human and mouse endothelial cell senescence through CD47 and Nox1. *Sci Signal* **10:** eaaj1784. doi:10.1126/scisignal.aaj1784

Melzer D, Pilling LC, Ferrucci L. 2020. The genetics of human ageing. *Nat Rev Genet* **21:** 88–101. doi:10.1038/s41576-019-0183-6

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

Mertens J, Paquola ACM, Ku M, Hatch E, Böhnke L, Ladjevardi S, McGrath S, Campbell B, Lee H, Herdy JR, et al. 2015. Directly reprogrammed human neurons retain aging-associated transcriptomic signatures and reveal age-related nucleocytoplasmic defects. *Cell Stem Cell* **17:** 705–718. doi:10.1016/j.stem.2015.09.001

Mewes M, Nedele J, Schelleckes K, Bondareva O, Lenders M, Kusche-Vihrog K, Schnittler HJ, Brand SM, Schmitz B, Brand E. 2017. Salt-induced Na^+/K^+-ATPase-α/β expression involves soluble adenylyl cyclase in endothelial cells. *Pflugers Arch Eur J Physiol* **469:** 1401–1412. doi:10.1007/s00424-017-1999-6

Montagne A, Nikolakopoulou AM, Zhao Z, Sagare AP, Si G, Lazic D, Barnes SR, Daianu M, Ramanathan A, Go A, et al. 2018. Pericyte degeneration causes white matter dysfunction in the mouse central nervous system. *Nat Med* **24:** 326–337. doi:10.1038/nm.4482

Morgan RG, Ives SJ, Lesniewski LA, Cawthon RM, Andtbacka RHI, Dirk Noyes R, Richardson RS, Donato AJ. 2013. Age-related telomere uncapping is associated with cellular senescence and inflammation independent of telomere shortening in human arteries. *Am J Physiol Hear Circ Physiol* **305:** H251–H258. doi:10.1152/ajpheart.00197.2013

Najjar SS, Scuteri A, Lakatta EG. 2005. Arterial aging: is it an immutable cardiovascular risk factor? *Hypertension* **46:** 454–462. doi:10.1161/01.HYP.0000177474.06749.98

Nation DA, Sweeney MD, Montagne A, Sagare AP, D'Orazio LM, Pachicano M, Sepehrband F, Nelson AR, Buennagel DP, Harrington MG, et al. 2019. Blood–brain barrier breakdown is an early biomarker of human cognitive dysfunction. *Nat Med* **25:** 270–276. doi:10.1038/s41591-018-0297-y

Nolan DJ, Ginsberg M, Israely E, Palikuqi B, Poulos MG, James D, Ding B-S, Schachterle W, Liu Y, Rosenwaks Z, et al. 2013. Molecular signatures of tissue-specific microvascular endothelial cell heterogeneity in organ maintenance and regeneration. *Dev Cell* **26:** 204–219. doi:10.1016/j.devcel.2013.06.017

Oakley R, Tharakan B. 2014. Vascular hyperpermeability and aging. *Aging Dis* **5:** 114–125.

Osmanagic-Myers S, Kiss A, Manakanatas C, Hamza O, Sedlmayer F, Szabo PL, Fischer I, Fichtinger P, Podesser BK, Eriksson M, et al. 2019. Endothelial progerin expression causes cardiovascular pathology through an impaired mechanoresponse. *J Clin Invest* **129:** 531–545. doi:10.1172/JCI121297

Ozek C, Krolewski RC, Buchanan SM, Rubin LL. 2018. Growth differentiation factor 11 treatment leads to neuronal and vascular improvements in the hippocampus of aged mice. *Sci Rep* **8:** 17293. doi:10.1038/s41598-018-35716-6

Paik DT, Tian L, Williams IM, Rhee S, Zhang H, Liu C, Mishra R, Wu SM, Red-Horse K, Wu JC. 2020. Single-cell RNA sequencing unveils unique transcriptomic signatures of organ-specific endothelial cells. *Circulation* **142:** 1848–1862. doi:10.1161/CIRCULATIONAHA.119.041433

Potente M, Mäkinen T. 2017. Vascular heterogeneity and specialization in development and disease. *Nat Rev Mol Cell Biol* **18:** 477–494. doi:10.1038/nrm.2017.36

Poulos MG, Ramalingam P, Gutkin MC, Llanos P, Gilleran K, Rabbany SY, Butler JM. 2017. Endothelial transplantation rejuvenates aged hematopoietic stem cell function. *J Clin Invest* **127:** 4163–4178. doi:10.1172/JCI93940

Propson NE, Roy ER, Litvinchuk A, Köhl J, Zheng H. 2021. Endothelial C3a receptor mediates vascular inflammation and blood–brain barrier permeability during aging. *J Clin Invest* **131**. doi:10.1172/JCI140966

Qiu J, Hirschi KK. 2019. Endothelial cell development and its application to regenerative medicine. *Circ Res* **125:** 489–501. doi:10.1161/CIRCRESAHA.119.311405

Quick S, Moss J, Rajani RM, Williams A. 2021. A vessel for change: endothelial dysfunction in cerebral small vessel disease. *Trends Neurosci* **44:** 289–305. doi:10.1016/j.tins.2020.11.003

Rafii S, Butler JM, Ding B-S. 2016. Angiocrine functions of organ-specific endothelial cells. *Nature* **529:** 316–325. doi:10.1038/nature17040

Rafii S, Gomez-Salinero JM. 2018. Endothelial cell adaptation in regeneration. *Science* **362:** 1116–1117. doi:10.1126/science.aar4800

Ramasamy SK, Kusumbe AP, Wang L, Adams RH. 2014. Endothelial notch activity promotes angiogenesis and osteogenesis in bone. *Nature* **507:** 376–380. doi:10.1038/nature13146

Ribas J, Zhang YS, Pitrez PR, Leijten J, Miscuglio M, Rouwkema J, Dokmeci MR, Nissan X, Ferreira L, Khademhosseini A. 2017. Biomechanical strain exacerbates inflammation on a progeria-on-a-chip model. *Small* **13:** 1603737.

Roos CM, Zhang B, Palmer AK, Ogrodnik MB, Pirtskhalava T, Thalji NM, Hagler M, Jurk D, Smith LA, Casaclang-Verzosa G, et al. 2016. Chronic senolytic treatment alleviates established vasomotor dysfunction in aged or atherosclerotic mice. *Aging Cell* **15:** 973–977. doi:10.1111/acel.12458

Salmon EE, Breithaupt JJ, Truskey GA. 2020. Application of oxidative stress to a tissue-engineered vascular aging model induces endothelial cell senescence and activation. *Cells* **9:** 1292. doi:10.3390/cells9051292

Schwartz MA, Vestweber D, Simons M. 2018. A unifying concept in vascular health and disease. *Science* **360:** 270–271. doi:10.1126/science.aat3470

Sen P, Shah PP, Nativio R, Berger SL. 2016. Epigenetic mechanisms of longevity and aging. *Cell* **166:** 822–839. doi:10.1016/j.cell.2016.07.050

Shin Y, Choi SH, Kim E, Bylykbashi E, Kim JA, Chung S, Kim DY, Kamm RD, Tanzi RE. 2019. Blood–brain barrier dysfunction in a 3D in vitro model of Alzheimer's disease. *Adv Sci* **6:** 1900962.

Song Y, Shen H, Schenten D, Shan P, Lee PJ, Goldstein DR. 2012. Aging enhances the basal production of IL-6 and CCL2 in vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* **32:** 103–109. doi:10.1161/ATVBAHA.111.236349

Ungvari Z, Labinskyy N, Gupte S, Chander PN, Edwards JG, Csiszar A. 2008. Dysregulation of mitochondrial biogenesis in vascular endothelial and smooth muscle cells of aged rats. *Am J Physiol Hear Circ Physiol* **294:** H2121–H2128. doi:10.1152/ajpheart.00012.2008

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

Ungvari Z, Tarantini S, Donato AJ, Galvan V, Csiszar A. 2018. Mechanisms of vascular aging. *Circ Res* **123:** 849–867. doi:10.1161/CIRCRESAHA.118.311378

Van Tuyn J, Knaän-Shanzer S, Van De Watering MJM, De Graaf M, Van Der Laarse A, Schalij MJ, Van Der Wall EE, De Vries AAF, Atsma DE. 2005. Activation of cardiac and smooth muscle-specific genes in primary human cells after forced expression of human myocardin. *Cardiovasc Res* **67:** 245–255. doi:10.1016/j.cardiores.2005.04.013

Vatine GD, Barrile R, Workman MJ, Sances S, Barriga BK, Rahnama M, Barthakur S, Kasendra M, Lucchesi C, Kerns J, et al. 2019. Human iPSC-derived blood–brain barrier chips enable disease modeling and personalized medicine applications. *Cell Stem Cell* **24:** 995–1005.e6. doi:10.1016/j.stem.2019.05.011

Venturini W, Olate-Briones A, Valenzuela C, Méndez D, Fuentes E, Cayo A, Mancilla D, Segovia R, Brown NE, Moore-Carrasco R. 2020. Platelet activation is triggered by factors secreted by senescent endothelial HMEC-1 cells in vitro. *Int J Mol Sci* **21:** 3287. doi:10.3390/ijms21093287

Villa-Bellosta R. 2020. Dietary magnesium supplementation improves lifespan in a mouse model of progeria. *EMBO Mol Med* **12:** e12423. doi:10.15252/emmm.202012423

Wallace DC. 2005. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu Rev Genet* **39:** 359–407. doi:10.1146/annurev.genet.39.110304.095751

Xi YP, Gerda Nette E, West King D, Rosen M. 1982. Age-related changes in normal human basement membrane. *Mech Ageing Dev* **19:** 315–324. doi:10.1016/0047-6374(82)90015-X

Xu M, Palmer AK, Ding H, Weivoda MM, Pirtskhalava T, White TA, Sepe A, Johnson KO, Stout MB, Giorgadze N, et al. 2015. Targeting senescent cells enhances adipogenesis and metabolic function in old age. *eLife* **4:** e12997. doi:10.7554/eLife.12997

Xu M, Pirtskhalava T, Farr JN, Weigand BM, Palmer AK, Weivoda MM, Inman CL, Ogrodnik MB, Hachfeld CM, Fraser DG, et al. 2018. Senolytics improve physical function and increase lifespan in old age. *Nat Med* **24:** 1246–1256. doi:10.1038/s41591-018-0092-9

Yabluchanskiy A, Ma Y, Chiao YA, Lopez EF, Voorhees AP, Toba H, Hall ME, Han HC, Lindsey ML, Jin YF. 2014. Cardiac aging is initiated by matrix metalloproteinase-9-mediated endothelial dysfunction. *Am J Physiol Hear Circ Physiol* **306:** H1398–H1407. doi:10.1152/ajpheart.00090.2014

Yamazaki Y, Baker DJ, Tachibana M, Liu CC, Van Deursen JM, Brott TG, Bu G, Kanekiyo T. 2016. Vascular cell senescence contributes to blood–brain barrier breakdown. *Stroke* **47:** 1068–1077. doi:10.1161/STROKEAHA.115.010835

Yan P, Li Q, Wang L, Lu P, Suzuki K, Liu Z, Lei J, Li W, He X, Wang S, et al. 2019. FOXO3-engineered human ESC-derived vascular cells promote vascular protection and regeneration. *Cell Stem Cell* **24:** 447–461.e8. doi:10.1016/j.stem.2018.12.002

Yan W, Cao Y, Zhen P, Ji D, Chai J, Xue K, Dai H, Wang W. 2021. Decreased autophagy of vascular smooth muscle cells was involved in hyperhomocysteinemia-induced vascular ageing. *Clin Exp Pharmacol Physiol* **48:** 524–533. doi:10.1111/1440-1681.13442

Yousef H, Czupalla CJ, Lee D, Chen MB, Burke AN, Zera KA, Zandstra J, Berber E, Lehallier B, Mathur V, et al. 2019. Aged blood impairs hippocampal neural precursor activity and activates microglia via brain endothelial cell VCAM1. *Nat Med* **25:** 988–1000. doi:10.1038/s41591-019-0440-4

Yucel N, Axsom J, Yang Y, Li L, Rhoades JH, Arany Z. 2020. Cardiac endothelial cells maintain open chromatin and expression of cardiomyocyte myofibrillar genes. *eLife* **9:** e55730. doi:10.7554/eLife.55730

Zhang C. 2008. The role of inflammatory cytokines in endothelial dysfunction. *Basic Res Cardiol* **103:** 398–406. doi:10.1007/s00395-008-0733-0

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

Cold Spring Harbor Perspectives in Medicine

Beyond Static Pipes: Mechanisms and In Vitro Models of Vascular Aging

Martin W. Hetzer and Simone Bersini

Cold Spring Harb Perspect Med 2022; doi: 10.1101/cshperspect.a041180 originally published online January 31, 2022