Biomaterials 34 (2013) 9201–9209

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Enhancing microvascular formation and vessel maturation through temporal control over multiple pro-angiogenic and pro-maturation factors

Yevgeny Brudno [a,b], Alessandra B. Ennett-Shepard [a,c], Ruth R. Chen [a,c], Michael Aizenberg [b], David J. Mooney [a,b,*]

[a] School of Engineering and Applied Sciences, Harvard University, Cambridge, MA 02138, USA
[b] Wyss Institute for Biological Inspired Engineering, Harvard University, Boston, MA 02115, USA
[c] Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI 48109, USA

ARTICLE INFO

Article history:
Received 10 June 2013
Accepted 2 August 2013
Available online 22 August 2013

Keywords:
Controlled drug release
Angiogenesis
Growth factors
Drug delivery
Endothelial cell
Smooth muscle cell

ABSTRACT

Therapeutic stimulation of angiogenesis to re-establish blood flow in ischemic tissues offers great promise as a treatment for patients suffering from cardiovascular disease or trauma. Since angiogenesis is a complex, multi-step process, different signals may need to be delivered at appropriate times in order to promote a robust and mature vasculature. The effects of temporally regulated presentation of pro-angiogenic and pro-maturation factors were investigated *in vitro* and *in vivo* in this study. Pro-angiogenic factors vascular endothelial growth factor (VEGF) and angiopoietin 2 (Ang2) cooperatively promoted endothelial sprouting and pericyte detachment in a three-dimensional *in vitro* EC-pericyte co-culture model. Pro-maturation factors platelet-derived growth factor B (PDGF) and angiopoietin 1 (Ang1) inhibited the early stages of VEGF- and Ang2-mediated angiogenesis if present simultaneously with VEGF and Ang2, but promoted these behaviors if added subsequently to the pro-angiogenesis factors. VEGF and Ang2 were also found to additively enhance microvessel density in a subcutaneous model of blood vessel formation, while simultaneously administered PDGF/Ang1 inhibited microvessel formation. However, a temporally controlled scaffold that released PDGF and Ang1 at a delay relative to VEGF/Ang2 promoted both vessel maturation and vascular remodeling without inhibiting sprouting angiogenesis. Our results demonstrate the importance of temporal control over signaling in promoting vascular growth, vessel maturation and vascular remodeling. Delivering multiple growth factors in combination and sequence could aid in creating tissue engineered constructs and therapies aimed at promoting healing after acute wounds and in chronic conditions such as diabetic ulcers and peripheral artery disease.

1. Introduction

Therapeutic angiogenesis, the promotion of new blood vessel formation to re-establish adequate perfusion in ischemic tissues, offers great promise as a treatment for patients suffering from cardiovascular disease and acute injuries [1–3]. Many recent strategies have concentrated on delivering single factors involved in the initial stages of blood vessel formation, such as vascular endothelial growth factor (VEGF) [4,5]. However, blood vessels that sprout during the initial stages of angiogenesis must be stabilized in order to prevent regression and promote maturation of the nascent microvascular network into therapeutically functional vasculature [6,7]. Despite significant progress [8], promoting a robust angiogenic response and creating mature vasculature remain goals of vascular medicine and, more broadly, of regenerative medicine and tissue engineering.

Sprouting angiogenesis is a remodeling process in which blood vessels form via sprouting from pre-existing vessels. This normal, physiological event occurs in the embryo during development as well as in adults during wound healing, reproductive cycling, and inflammation. In response to physiologic stress due to injury, ischemic tissues secrete signaling factors, which (1) activate endothelial cells (EC) and pericytes to degrade the mural wall as well as cause pericyte detachment from the endothelium; (2) promote sprouting of endothelial cells toward ischemic areas

* Corresponding author. School of Engineering and Applied Sciences, Pierce Hall, Room 319, Harvard University, Cambridge, MA 02138, USA. Tel.: +1 617 384 9424.
E-mail address: mooneyd@seas.harvard.edu (D.J. Mooney).

0142-9612/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biomaterials.2013.08.007

guided by growth factor gradients; (3) lead to the anastomosis of immature endothelial sprouts to form immature vasculature and (4) guide the maturation of vessels through recruitment of mural cells and deposition of extracellular matrix around the now maturing blood vessels (Fig. 1). The complex, multi-step nature of this process suggests that the presentation of multiple signals at appropriate times is necessary to promote a robust, mature, and functional blood vessel network [7,9,10].

Previous studies have taken advantage of *in vitro* models of early angiogenesis to study the mechanisms of angiogenesis, and as a tool to predict the efficacy of therapeutic intervention [11]. These models generally involve culture of ECs under conditions that promote EC sprouting or tubule formation on 2D surfaces or inside of 3D matrices. These models have illuminated the roles of pro-angiogenic factors [4,5,12–16] and angiogenesis inhibitors [17,18] as well as other angiogenesis-related signaling pathways [13,19]. However, *in vitro* models rarely include mural cells and those that do [20,21] generally do not take into account mural cell behavior and their response to therapeutic growth factors. In addition to inhibiting the angiogenic response of endothelial cells [22,23], pericytes are suggested to control vessel contractility, tone and diameter [23–27] and to secrete factors necessary for endothelial survival and proliferation [28,29]. Pericytes and endothelial cells act as a functional and physical unit through the establishment of cell–cell heterotypic contacts, and synthesis and secretion of growth factors that promote their mutual survival [29–31].

Multiple factors that promote the initial and maturation phases of angiogenesis have been identified, and the co-administration of both types of factors enhances blood vessel regeneration [32–37]. VEGF is a potent mitogen for endothelial cells and initiates their sprouting and proliferation to form new, immature vessel sprouts [38,39]. While VEGF can initiate angiogenesis, additional factors are required to promote vessel maturation [34,40,41]. Platelet-derived growth factor B (PDGF) encourages maturation of the nascent vessels by activating and recruiting pericytes and smooth muscle cells that associate with endothelial sprouts, stabilizing them, and preventing regression [42–45]. The angiopoietins, -1 (Ang1) and -2 (Ang2), have opposing functions and compete for the same Tie-2 receptor [46]. Ang1 is a stabilizing factor that strengthens the interactions between pericytes/smooth muscle cells and ECs. In contrast, Ang2 weakens those same interactions, destabilizing blood vessels [46–48]. Importantly, factors that promote later stages of vascularization have been demonstrated to inhibit the early angiogenic stage. Specifically, PDGF inhibits VEGF-mediated angiogenesis in matrigel plugs and in chorioallantoic membranes, and inhibits VEGF-mediated pericyte migration *in vitro* [49]. The pro-maturation factor Ang1 serves to antagonize the interaction of the pro-angiogenesis factor Ang2 and its cognate receptor, Tie-2 [48].

The need to sustain angiogenic factor signaling for extended periods of time has motivated the development of a number of polymer systems that provide a sustained and localized release of VEGF and other factors [50–56]. These systems have demonstrated significant enhancement in the extent of angiogenesis, as compared to those utilizing bolus factor delivery, and improved function of the new vasculature in a number of different animal models and wound types [1,5,13]. Further, polymer systems that provide sustained and temporally controlled release of pro-angiogenic and pro-maturation growth factors [15,36,40,57,58] have been demonstrated. In particular, polymeric scaffolds capable of delivering VEGF and PFGF with distinct kinetics from a single, structural polymer scaffold led to a substantial increase in both vascular density and vessel maturation [12,15,36].

This report addresses the hypothesis that proper temporal presentation of Ang1 and Ang2 can enhance VEGF-mediated vascular network growth and PDGF-mediated vessel maturation to collectively improve vascularization. The administration of Ang2 during the early stages of angiogenesis was anticipated to enhance angiogenesis by destabilizing existing vessels and promoting endothelial sprouting. Release of Ang1 was anticipated to improve vessel maturation by stabilizing newly formed vessels via enhanced EC-pericyte cell adhesion and antagonizing Ang2 activity. The effects of these factors in combination with VEGF and PDGF were first studied in an *in vitro* system, capable of capturing both angiogenic sprouting and pericyte detachment, two indicators of early angiogenesis. *In vitro* insights were then applied to a mouse *in vivo* model of vascularization and vessel maturation using a macroporous polymer system designed to ensure the sustained and localized release of multiple growth factors with temporal control [36,58].

2. Materials and methods

2.1. Cell culture

Pooled Human Umbilical Vascular Endothelial Cells (HUVECs) (Lonza CC-2519) (passage 3) were used for all experiments and cultured in 2% FBS EGM-2 (Lonza, CC-3162). Newborn, placental pericytes (Promocell C-12980) were grown in 2% FBS Pericyte Medium (ScienCell 1201) and used at passage 6.

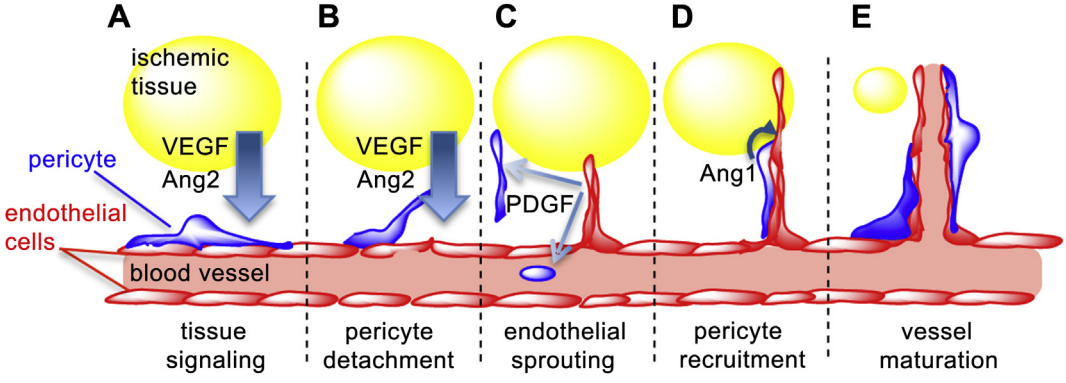

Fig. 1. Model of select growth factor signaling during angiogenesis. (A) Ischemic tissues (yellow) release pro-angiogenic factors such as VEGF and Ang2, creating growth factor gradients that signal blood vessels to increase capacity. (B) Pro-angiogenic factor signaling destabilizes EC-pericyte interactions, promoting pericyte detachment from the endothelium and EC sprouting away from existing vessels. (C) As sprouts grow and penetrate hypoxic tissues, they release PDGF, which activates and recruits pericytes from surrounding tissue and progenitor cells from the blood stream to nascent vessels. (D) Pericyte-derived Ang1 antagonizes the Ang2 receptor (Tie2) and serves as a stabilizing factor that strengthens pericyte-EC interactions and promotes vessel maturation and lumen formation (E). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)