New Therapeutic Window of Regenerative Opportunity in Diabetic Retinopathy by VESGEN Analysis

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Glenn Research Center
VESGEN Patent Pending at Lewis Field
Multi-Scale mapping of vascular pattern for development of regenerative and preventive therapies targeting diseases dependent on microvascular remodeling

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Vascular Alterations, Visual Impairments (VIIP) & Increased Intracranial Pressure (ICP), Immunosuppression & Bone Loss: NASA-defined risk categories for human space exploration and ISS Utilization
Abstract

Vascular pattern may serve as a useful new biomarker principle of complex, multi-scale signaling in pathological, physiological angiogenesis and microvascular remodeling. Each angiogenesis stimulator or inhibitor we have analyzed, including VEGF, bFGF, TGF-beta1, angiostatin and triamcinolone acetonide, has induced a novel ‘fingerprint’ or ‘signature’ biomarker vascular pattern that is spatio-temporally unique. Remodeling vasculature thereby provides an informative read-out of dominant molecular signaling, when analyzed by innovative, fractal-based VESsel GENeration (VESGEN) Analysis software. Using VESGEN to analyze ophthalmic clinical vascular images, we recently introduced a potential paradigm shift to the understanding of early-stage progression that suggests new regenerative opportunities for human diabetic retinopathy (DR), the major blinding disease for working-aged adults. In a pilot study, we discovered that angiogenesis oscillates as a surprising, homeostatic-like regeneration of retinal vessels during early progression of DR (IOVS 51(1):498). Results suggest that the term ‘non-proliferative DR’ may be a misnomer. In new studies, normalization of the vasculature will be determined from the response of vascular pattern to therapeutic monitoring and treatment. We have mapped and quantified in vivo experimental models of angiogenesis, lymphangiogenesis and intravital blood flow from cellular/molecular to higher systems levels that include a murine model of infant retinopathy of prematurity (ROP); developing and pathological coronary and placental-like vessel models; progressive intestinal inflammation, growing murine tumors, and other pathological, physiological and therapeutically treated tissues of transgenic mice and avian embryos.
Motivation for Microvascular Quantification and Mapping by VESGEN

1. Molecules in Vitro
2. Avian Eggs
3. Mouse
4. Humans

NASA IR&D to NIH
VESGEN
Mapping and Quantification of Branching Vascular Pattern

Vascular Trees
Diabetic Human Retina
Avian CAM, Yolksac and Mouse/Avian Coronary Vessels

Vascular Networks
Mouse Intestinal Inflammation, CAM Lymphatic Vessels, Abnormal Mouse Corneal Angiogenesis

Vascular Tree-Network Composites
Mouse Postnatal Retina
Early Embryonic Coronary Vessels, Juvenile and Adult Leaf Venation

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Panel to specify vessel type

Main panel
- Image specification
- Algorithm selection
- Process initiation
Dynamic Balance Hypothesis

Long-Term Translational and Basic Research Hypothesis

Vascular patterning provides integrative, insightful read-out of dominant molecular regulators in complex signaling pathways of angiogenesis and microvascular remodeling

Fractal-Based VESsel GENeration Analysis (VESGEN) Software

Fractal Dimension, $D_f$
Vessel Number Density, $N_v$
Vessel Length Density, $L_v$
Vessel Diameter, $D_v$
Branchpoint + Endpoint Densities, $Br_v + E_v$
VESGEN Hypothesis: ‘Fingerprint’ or ‘Signature’ Vascular Pattern As Integrative Readout of Complex Signaling

bFGF as Simple Stimulator

VEGF as Complexity Factor
Microvascular Research 72 (2006)

TGF-β1 as Simple Inhibitor but Complex Potentiator
Microvascular Research 59 (2000)

The form of an object is a ‘diagram of forces’
- D’Arcy Thompson
Progression of Diabetic Retinopathy by Clinical Fluorescein Angiography

**EARLY Vascular** Nonproliferative DR (NPDR)

**LATE Vascular** Proliferative DR (PDR)
Fractal Dimension ($D_f$) by Box-Counting

\[ p = 512 \quad p/2 = 256 \quad p/4 = 128 \quad p/8 = 64 \]
Vascular Pattern in the Human Retina Is Altered in Early-Stage Diabetes

![Diagram showing vascular patterns in normal and nonproliferative stages of diabetes](image)

- Normal
- Nonproliferative

![Bar graph showing fractal dimensions](image)

- Normal
- NPDR

Fractal Dimension

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<th></th>
<th>Center</th>
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<th>Center</th>
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Mapping of Progressive Diabetic Retinopathy by VESGEN

Mild NPDR  Moderate NPDR  Severe NPDR  PDR

Vascular Trees

Arteries

Veins

Angiogenesis Oscillates with Vascular Dropout during Progression of Diabetic Retinopathy

New Regeneration Opportunity

Vascular Remodeling Status (VRS)

Slight Trend toward Increasing Diameter of Larger Vessels during Progression of Diabetic Retinopathy

Grouping by Vascular Remodeling Status (VRS)

Conclusions on Novel Vascular Disease Biomarkers during Progression of Diabetic Retinopathy

New, surprising discovery on early-stage angiogenesis during moderate NPDR: *Does the retina retain the capacity to regenerate itself?*

**VESGEN as Research Discovery Tool**
Are results important for early-stage regeneration in other inflammatory diseases such as diabetic nephropathy and tumors?
VESGEN

Vascular Pattern as Informative Biomarker and Integrative Readout of Complex Signaling Pathways for Angiogenesis, Lymphangiogenesis and Other Microvascular Remodeling

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VESGEN mapping of vascular networks with GI inflammatory progression in experimental mouse DSS model

P Parsons and H-C Reinecker, accepted to *Grav Space Biology*

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Triamcinolone Acetonide (TA) Steroid Treatment in CAM Vascular Tree

Vascular Networks in Transgenic Mouse Retina

Fluorescence Microscopy

VESGEN Network Output
Distance Mapping
Colorized Skeleton

Mouse Retina
Control (P15)

D

E

F

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Coronary Vessel Network-to-Tree Transitions

Vickerman et al, VESGEN Review, Anatomical Record A 292(3), 2009
Lymphangiogenic Sprouting: By Filopodial Guidance?
Fig. 7 Parsons-Wingerter et al.
e5_a2
Taxonomic/Phylogenetic Identifiers

Botanical rules for leaf vascular patterning by branching order

Acer argutum

\[a\] Ellis, Daly, Hickey et al, Manual of Leaf Architecture, 2009

\[b\] Roth-Nebelsick, Uhl, Mosbrugger, Kerp, Annals of Botany 887:553-566, 2001
New VESGEN analysis of leaf venation for *Arabidopsis* with first bioinformatic dimensional analysis

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Novel Angiogenesis and Vascular Dropout Biomarkers by VESGEN

**Potential New Window of Therapeutic Opportunity for Early-Stage Regenerative Treatment**

**Surprising Oscillation of Angiogenesis with Vascular Dropout during DR Progression**

- First demonstration of angiogenesis during Moderate NPDR
- New longitudinal studies with Maria Grant
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