Mechanisms of Endothelial Cell Attachment, Proliferation, and Differentiation on 4 Types of Platinum-Based Endovascular Coils

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Key words
- Cerebral aneurysms
- Coils
- Endothelial cells
- Subarachnoid hemorrhage
- Tissue engineering

Abbreviations and Acronyms
cDNA: Complementary DNA
DEG: Differentially expressed gene
EC: Endothelial cell
ECM: Extracellular matrix
GDC: Guglielmi detachable coil
HUVEC: Human umbilical vein endothelial cell
IPA: Ingenuity Pathway Analysis
ISAT: International Subarachnoid Aneurysm Trial
PGLA: Polyglycolic acid

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OBJECTIVE: **A subarachnoid hemorrhage is neurologically devastating, with 50% of patients becoming disabled or deceased. Advent of Guglielmi detachable coils in 1995 permitted endovascular treatment of cerebral aneurysms. Coiling is efficacious and safe, but durability needs improvement, as nearly 20% of patients require further invasive intervention secondary to aneurysm recurrence. The aim of this study is to develop an in vitro model of endothelial cell (EC) proliferation and differentiation on four types of platinum-based coils, using gene expression profiling to understand EC biology as they colonize and differentiate on coils.**

METHODS: **Human umbilical vein ECs were grown in vitro on platinum coil segments. Growth patterns were assessed as a function of coil type. Gene expression profiles for coil attached versus coil unattached ECs were determined using immunohistochemistry and gene array analysis.**

RESULTS: **ECs showed rapid, robust attachment to all coil types. Some detachment occurred within 24–48 hours. Significant growth of remaining attached cells occurred during the next week, creating a confluence on coils and within coil grooves. Similar growth curve results were obtained with human brain ECs on platinum-based coil surfaces. Differentiation markers in attached cells (α_1, α_2, β_1 integrins) were expressed on immunostaining, whereas microarray gene expression revealed 48 up-regulated and 68 down-regulated genes after 24-hour growth on coils. Major pathways affected as a function of time of colonization on coils and coil type included those involved in regulation of cell cycle and cell signaling.**

CONCLUSIONS: **We developed an in vitro model for evaluating endothelialization of platinum coils to optimize coil design to support robust EC colonization and differentiation.**

INTRODUCTION

Thirty-five thousand patients suffer from aneurysmal subarachnoid hemorrhage yearly within the United States. The International Subarachnoid Aneurysm Trial (ISAT) reported a lower probability of death and disability for patients undergoing coiling of cerebral aneurysms as opposed to microsurgery (15). Although ISAT built the platform for launching endovascular treatment of cerebral aneurysms as a mainstay therapy, the durability of this treatment method remains inferior to that of microsurgery. Based on follow-up cerebral angiography, 10%–30% of all coiled aneurysms will show recurrence (1, 18). Recurrence of coiled aneurysms leads to increased morbidity secondary to retreatments, angiographic follow-up, and the real risk of rerupture.

The goal of endovascular occlusion of aneurysms is to introduce platinum-based coils into the cerebral aneurysm until the entire aneurysm volume is filled with thrombus and coils. Volumetric analysis shows that most aneurysms are only filled 20%–40% with coils, and the rest represents acute thrombus (1). Histopathologic analyses based on autopsy of patients who harbored coiled aneurysms show that within the first 4 weeks, the aneurysm houses organized thrombus, which then develops into minimal fibrous tissue. Endothelial proliferation seems to occur at 3 months after embolization (3, 4, 21). At 12 months after the procedure, coils seem to be embedded in fibrous tissue with endothelialization occurring over the neck of the aneurysm (3, 4, 21). Recurrence takes place when the endothelialization process does not occur across the neck of the aneurysm and thus, the pulsatility is transmitted to the coil/thrombus mass leading to coil compaction as the thrombus dissolves. Our goal is to understand the interaction of platinum coils with endothelial cells (ECs), with the future aim of creating an intra-aneurysmal environment for EC

proliferation across the neck of the aneurysm.

MATERIALS AND METHODS

Endovascular Coils

To facilitate growth curves, we purchased the following coils: Guglielmi detachable coils (GDCs) and Matrix coils from Boston Scientific, Natick, Massachusetts, USA; Cerecyte coils from Micrus Endovascular, San Jose, California, USA; and HydroCoils from MicroVention, Tustin, California, USA. Coil fragments measured 1 cm (each was weighed using an analytical balance), and were used for cellular attachment, growth analysis, immunohistochemistry, and gene array analysis.

Human Umbilical Vein EC Isolation

Umbilical cords were obtained from the Labor and Delivery Department, Thomas Jefferson University Hospital, Philadelphia, Pennsylvania, USA. Human umbilical vein ECs (HUVEC) were isolated from the cords using a collagenase digestion protocol, as described previously (9).

Cell Culture

HUVEC were cultured on tissue culture flasks coated with 0.2% gelatin. Cells were fed a complete media consisting of Media 199 (GIBCO, Carlsbad, California, USA), 10% fetal bovine serum (FBS; HyClone, Logan, Utah, USA), 50 μg/mL EC growth supplement, 50 μg/mL heparin sodium salt from porcine intestinal mucosa (Grade I-A; Sigma-Aldrich, St. Louis, Missouri, USA), 1% penicillin-streptomycin (GIBCO), and 0.1% Fungizone (GIBCO). EC growth supplement was isolated from bovine hypothalami, as described previously (12). HUVECs were used through passage 6. Similar growth curve experiments were also performed with human cerebral microvascular ECs (hCMEC/D3) at less than 10 passages (27). These cells were cultured for 2 weeks to reach confluency, at which time they were trypsinized and diluted to 1,000,000 cells/mL for experimentation. Similar cellular media, warming tray, and techniques were used for the growth curve assessment.

EC Seeding of Endovascular Coils

EC were trypsinized from confluent cultures and 1.0 mL of a 1×10^6 cells/mL suspension was placed in a sterile Eppendorf tube. One coil segment (~1 cm) was added to each tube and the weight measured. Samples were then placed in a tissue culture incubator on a rocking platform for 4–6 hours, and were gently rotated to ensure cell contact with the coil. Coils were then removed from the cell suspension and rinsed by dipping them once into a well of a 12-well tissue culture plate containing Hanks balanced salt solution at 2 mL/well. Coils were then placed into complete media in a 12-well tissue culture plate and returned to the tissue culture incubator. Culture medium was replaced every 2 days thereafter.

Measurement of EC Growth on Coils

At various times during culture (typically at days 1, 3, 7, 14, and 28), cells were removed from the coils by trypsinization and counted. Coils were placed in 0.5 mL trypsin/coil for 5–10 minutes; the resultant cell suspension was mixed 1:1 with complete media to neutralize the trypsin. Cells released from the coils were counted using a hemacytometer, and expressed as cells bound/milligram of coil. Micrographs of cell-populated coils were also taken before and after trypsinization using inverted microscopy.

Immunohistochemical Staining of EC-populated Coils

After ECs proliferated on GDCs for 7 days, coil segments and unattached cells that had become confluent within the wells were fixed using 10% buffered formalin. These two populations (attached and unattached cells) were incubated with primary antibodies to α_1 integrin, α_2 integrin, β_1 integrin, or platelet EC adhesion molecule overnight at 4°C. Samples were labeled with fluorescein isothiocyanate-conjugated secondary antibodies and imaged using fluorescent microscopy.

Examination of EC Gene Expression by Gene Array Analysis

Total RNA Isolation. DNA-free total RNA of cultured cells was isolated using the RNeasy Micro Kit (Qiagen, Valencia, California, USA) according to manufacturer's instructions. In brief, 1×10^5 cells from duplicate cultures (control and experimental) were pelleted, lysed in RNA lysis tissue buffer containing 1% (vol/vol) β-mercaptoethanol. DNase-treated RNA was ethanol precipitated and quantified on a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA), followed by RNA quality assessment by analysis on an Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, California, USA).

Microarray Methods. Ribo-single-primer-isothermal-amplification-based RNA amplifications and target preparations were performed according to the manufacturer's instructions (Ovation Biotin System; NuGEN Technologies, San Carlos, California, USA). Briefly, first-strand complementary DNA (cDNA) was synthesized from 50 ng of total RNA using reverse transcription with a unique oligo (dT)/RNA chimeric primer. In the second step, DNA/RNA heteroduplex double-strand cDNA was generated with DNA polymerase. In the third step, SPIA linear isothermal DNA amplification process was performed using DNA/RNA chimeric primer, DNA polymerase, and RNase H in a homogenous isothermal assay that provides efficient amplification of DNA sequence.

Fragmentation and Biotin Labeling. In the first step, DNA amplification products were fragmented by chemical and enzymatic fragmentation that yields single-stranded cDNA products in the 50- to 100-base range. In the second step, fragmented product is labeled by enzymatic attachment of a biotin-labeled nucleotide to the 3-hydroxyl end of the fragmented cDNA.

Hybridization and Bioinformatic Analysis of Messenger RNA Expression Profiling. Fragmented and biotin-labeled target (3.75 μg) in 200 μL of hybridization cocktail was used for each Affymetrix HG U133 Plus 2.0 array (Affymetrix, Santa Clara, California, USA), which contains 56,000 probe sets representing 34,000 well-characterized human genes. Target denaturation was done at 99°C for 2 minutes, and hybridization was performed for 18 hours. Arrays were washed and stained using GeneChip Fluidic Station 450, and hybridization signals were amplified using antibody amplification with goat IgG (Sigma-Aldrich) and anti-streptavidin biotinylated antibody (Vector Laboratories, Burlingame, California, USA). Chips were scanned on an Affymetrix GeneChip Scanner 3000 using GeneChip Operating Software version 3.0. A flow-chart analysis is shown

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