Project Summary
Ineffective mucociliary clearance (MCC) is a common pathophysiologic process that underlies airway inflammation and infection. Decreased transepithelial Cl^- transport secondary to an acquired Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) deficiency may contribute to respiratory epithelial dysfunction by abrogating MCC and increasing mucus viscosity. The central hypothesis of the current proposal is that persistent mucosal inflammation and infection in chronic rhinosinusitis (CRS) results from acquired (partial) CFTR deficiency, creating a localized environment that impairs MCC. This hypothesis will be tested with three Specific Aims. Specific Aim 1 will investigate CFTR deficiency in a well-characterized *in vitro* culture model of sinonasal epithelium. Our Preliminary Data indicate that lipopolysaccharide not only promotes inflammation, but leads to CFTR repression in sinonasal epithelium. CFTR transcription, maturational processing (protein biochemistry), and channel potentiation (patch clamp analysis) will be used to determine the mechanism underlying this observation. Specific Aim 2 will test the hypothesis that Cl^- secretagogues can offset acquired defects in CFTR-mediated ion transport. Compounds of this class, including VX-770, UC_{CF}-152, and bioflavonoids have received considerable recent attention in both the scientific and lay press for their emerging role in cystic fibrosis (CF) therapeutics. We will investigate whether Cl^- secretagogues 1) overcome acquired CFTR defects and 2) stimulate MCC (measured by ciliary beat frequency). Specific Aim 3 will determine the extent of acquired CFTR deficiency in human CRS *ex vivo* and *in vivo*. Transepithelial ion transport will be quantified in sinus mucosal explants in the Ussing chamber and *in vivo*, using a well established nasal potential difference assay. The proposal will therefore develop an innovative approach to better understand the pathogenic mechanisms of CRS, a disease understudied in the past, and develop an entirely novel treatment strategy for sinus and nasal airway disease predicated on activation of fluid and electrolyte secretion with leading edge Cl^- secretagogues.

2. CANDIDATE'S BACKGROUND

My interest in biomedical research originated during medical school when I investigated the expression of PH-20, a hyaluronidase, in human laryngeal cancer specimens. We postulated that PH-20 overexpression served as a means to enhance tumor invasion through the epithelial basement membrane into underlying soft tissues. My work resulted in a publication in the "Archives of Otolaryngology – Head and Neck Surgery". The experience was exciting and motivated me to pursue research as an important component of my future career.

During otolaryngology residency, my commitment to subspecialty rhinology led me to complete basic science and clinical projects that focused on chronic rhinosinusitis (CRS). I contributed to translational studies involving cellular biology, animal research, and human clinical trials. This resulted in 13 peer-reviewed publications upon completion of my residency and a series of novel findings regarding both innate and adaptive immunity in CRS. One of my projects was published in the journal "Otolaryngology – Head and Neck Surgery" and received the 2nd place research award nationally from the American Academy of Otolaryngology – Head and Neck Surgery.

Based on a growing dedication to both basic and clinical science, I was accepted into the rhinology fellowship program at the University of Pennsylvania. I acquired techniques for establishing and maintaining polarized ciliated sinonasal epithelial air liquid interface cultures, measuring ciliary beat frequency (CBF), quantifying *in vivo* and *in vitro* bacterial biofilms, and assessing transepithelial ion transport in cultured cells. I was given the task of developing a cell culture methodology that would allow assessment of biochemical and physiological properties of sinonasal epithelium. Our tissue harvest and cell culture protocols were published in the journal "Biotechniques" and constitute an emphasis of my current research. My fellowship research training resulted in 15 publications in peer-reviewed journals. I was able to accumulate enough data during fellowship to successfully compete for a Young Clinical Scientist Award from the Flight Attendants Medical Research Institute, resulting in 3 to 5 years of funding.

Currently, I am a surgeon-scientist at the University of Alabama – Birmingham (UAB). Although I have not yet received NIH funding, I was recently awarded the James Johnston Hicks Endowed Chair of Otolaryngology by the Department of Surgery at UAB. Resources for the chair are specifically intended to support my translational research program. As an associate scientist in the UAB Gregory Fleming James Cystic Fibrosis (CF) Research Center, my rhinology research laboratory is dedicated to the study of CRS and the mechanisms underlying this debilitating disease. The CF center at UAB is one of the leading programs of its kind in the U.S. Because the center emphasizes studies of ion transport, cell biology, and translational aspects of airway research, the environment has been an outstanding source of mentoring and research support for my laboratory, as described below.

3. CAREER GOALS AND OBJECTIVES

My research focuses on two broad areas of investigation. First, we are pursuing the mechanisms underlying tobacco related effects on mucociliary function in nasal septal epithelia. This includes human sinonasal epithelium harvested during surgical procedures that I perform. Our initial results were published in the journal "Laryngoscope", and demonstrated suppression of CFTR-mediated transepithelial chloride (Cl^-) transport and blunted CBF as a result of exposure to tobacco products. Recent studies in our laboratory have also indicated that tobacco-smoke potently suppresses alternative Cl^- channel pathways, such as the calcium activated Cl^- channel (TMEM16A), in addition to CFTR.

Our second area of emphasis involves therapeutic interventions that enhance mucociliary clearance in both CF (where CRS is virtually universal) and non-CF CRS. The nasal airways *in vivo* have served as a testing site for clinical interventions relevant to mucociliary clearance, yet adequate *in vitro* models of sinonasal epithelium are not widely available. Well characterized primary upper airway cultures developed by my group represent an excellent means to evaluate novel CFTR-activating molecules under investigation at our institution and elsewhere. The biology of mucus is profoundly influenced by activity of epithelial Cl^- secretion. While promoting CFTR Cl^- transport and improving mucus clearance is a potential avenue for therapy of CF lung disease (see references [1-10] of this application), the same strategy also represents an exciting new method for treating ineffective mucociliary clearance in CRS.

My research program is strongly focused on several long-term goals. Academically, I am on the tenure track at UAB where I hope to be promoted within the next 3 to 4 years. This will require NIH funding, an excellent academic track record (both teaching and service), and a positive reputation among my colleagues nationally. In years 3-4 of this award, I will pursue NIH grant mechanisms (R-01) to establish sustained funding for my rhinology research. Through this NIH K08 award I hope to hone my research and grant-writing skills, develop new techniques in airway cell biology, and pursue novel ideas with the collaborative help of outstanding scientists and mentors at UAB. Scientifically, I hope to make a significant contribution to treatment for patients suffering with CRS.

4. CAREER DEVELOPMENT/TRAINING

Support through the K08 will be crucial to advancement of my independent research career. Over the next five years, NIH funding will allow me to address several priorities relevant to the objectives of my Career Development Plan. These include the following:

1. A mentoring committee to formalize research and career guidance. Since arriving at UAB, I have benefitted from my interactions with a multi-disciplinary team of experienced scientists. The mentoring committee for this project consists of J.P. Clancy, MD, (Division Chief, Pediatric Pulmonology, UAB; an international authority regarding mucociliary clearance and ion transport); J. Collawn, PhD (Professor, Cell Biology, expertise concerning biochemistry of airway ion channels, including CFTR and ENaC); L. Schwiebert, PhD (Associate Dean, expertise in airway epithelial inflammatory and cytokine responsiveness); and E. Rosenthal, MD (NIH-funded ENT specialist in my surgical division). Chair of the committee is Eric Sorscher, MD director of the UAB CF Center and a highly respected authority regarding folding of ion transport proteins in the plasma membrane. Additional detail regarding qualifications of these advisors is provided below. The committee is already established and meets every 3-4 months. I meet with Dr. Sorscher on a weekly basis. Advice and support from this committee has been instrumental in my past research and designing the experimental plan for the present K08.

2. Further training in laboratory techniques necessary to interpret and advance my laboratory program. This will include ion channel patch clamp analysis, biochemical studies of CFTR maturation (pulse chase, maturational efficiency), and clinical research relevant to nasal electrophysiology. This training will occur under the guidance of my mentoring committee. My didactic curriculum over the next 5 years will include graduate courses in molecular and cellular pathology offered by the UAB Program for Integrative Biomedical Sciences (IBS):

IBS 700 - Biological Chemistry and Cellular Physiology: This course provides a comprehensive and rigorous background in the principles of biochemistry, molecular biology, and cellular physiology. The course is divided into eight blocks (cell and protein biochemistry, metabolism, molecular genetics, membrane and organelles, cell adhesion and motility, cell cycle regulation and signaling, pharmacology and therapeutic principles, and cutting edge technology) and designed to ensure mastery of basic principles and their application to research protocols and performance. (8 credit hours)

IBS 701 - Pathophysiology and Pharmacology of Disease: The class includes three modules (Ion channels in health and disease; physiology and pathophysiology of organ systems; signal transduction) designed to furnish a solid foundation in mechanistic and therapeutic approaches to disease mechanism. (8 credit hours)

IBS 708 – Modern Drug Design and Development: Lectures focus on understanding principles and practice of drug discovery, design, evaluation and development. This course has a special emphasis on high throughput screening techniques and drug delivery. (8 credit hours)

3. Accomplish didactic training in the conduct and statistical analysis of clinical research necessary to design and interpret clinical trials. This will include the following:

BST 611- Intermediate Statistical Analysis I. Provides a thorough understanding of basic analysis methods, statistical models and applications of probability, commonly used sampling distributions, parametric and non-parametric one and two sample tests, confidence intervals, applications of analysis of two-way contingency table data, simple linear regression, and simple analysis of variance. Students apply these methods using current software such as Statistical Analysis System (SAS).

BST 612 - Intermediate Statistical Analysis II. This course describes the principles of simple and multiple regression. A major goal is to establish a firm foundation in the discipline upon which the applications of statistical and epidemiologic inference are built. This course requires a mentored clinical research project (Specific Aim 3 of the present K08, Section 11, below).

BST 624 - Experimental Design. Covers Intermediate experimental design and variance models using the Matrix approach. Factorial and nested (hierarchical) designs, blocking, repeated measures designs, Latin squares, incomplete block designs, and fractional factorials are included.

4. Acquire scientific and administrative skills crucial to an independent academic career. Formal sessions that focus on preparing grant submissions, developing expertise in scientific reasoning, and training in ethical research are summarized below:

- **Airway Epithelial Workshops** presented twice each month and attended by 20-30 UAB investigators. The workshops allow UAB scientists and outside speakers an opportunity to present their work, develop new collaborations, trouble shoot, and obtain advice concerning active research efforts. I presented in the workshop conference twice last year, and received very helpful and constructive input during those sessions.
- **Programmatic Seminar Series** provides faculty from multiple departments a forum for discussion and interaction, including formal presentations by internationally respected authorities in areas relevant to lung and upper airway biology. Approximately 5-7 outside speakers visit UAB each year to participate in the seminar series.
- **Didactic training in scientific writing and presentations:** The UAB Office of Program Planning and Educational Research has developed a "Scientific Writing Seminar Series" to provide practical assistance to junior faculty. The Series was designed with three components, including 16 hours of instruction, with 5 hours devoted to organizing, writing, and critiquing scientific manuscripts; 9 hours concerning planning, writing, and submitting NIH grant applications; and 2 hours developing basic skills to prepare and deliver effective presentations.
- **Local, National, and International Conferences:** I will participate in national and international meetings such as the North American Cystic Fibrosis Annual Meeting, the American Academy of Otolaryngology – Head and Neck Surgery Meeting, and the American Rhinologic Society Meeting. I will also continue to attend local conferences including UAB Surgical Grand Rounds, Otolaryngology Grand Rounds, Research Journal Club, and Dr. Sorscher's laboratory group meetings.

Table1: Anticipated Timeline for Dr. Woodworth's Career Development Plan

Career Development Activity	Percentage Effort, by Year				
	2010-2011	2011-2012	2012-2013	2013-2014	2014-2015
Didactic Training	30%	25%	25%	15%	5%
BST 611: Statistical Analysis I					
BST 612: Statistical Analysis II					
BST 624: Experimental Design					
IBS 700: Biological Chemistry and Cellular Physiology					
IBS 701: Pathophysiology and Pharmacology of Disease					
IBS 708: Drug Discovery					
Ethical Training in Research					
GRD 717					
IRB Investigator Training					
Ethics Conference (annual)					
IACUC Training					
Scientific Writing Seminar					
CF Center Research Meetings					
Professional Development	10%	10%	10%	10%	10%
Meet with Dr. Sorscher weekly					
Meet every 3-4 months with mentoring committee					
CF Center Scientific Curricula					
Participate in local, national, and international meetings					
Research Plan	35%	40%	40%	50%	60%
Basic/Translational Projects					
Clinical NPD study					
Analyze and publish					
Develop future projects					
R01 funding					
Total Effort to K08	75%	75%	75%	75%	75%
Clinical Responsibilities	20%	20%	20%	20%	20%
Teaching Responsibilities	5%	5%	5%	5%	5%

5. TRAINING IN THE RESPONSIBLE CONDUCT OF RESEARCH

I will complete the following courses/activities:

IRB Investigator Training: This four-hour course, offered twice a year in the spring and fall, is required of all key personnel involved in human subjects research. The objectives of the course are: 1) provide an introduction to behavioral and biomedical research ethics (e.g. "The Common Rule," The Nuremberg Code, and The Belmont Report, and current federal regulations; distinguish and apply various federal regulations including international principles; and clearly define human subject protection and human subject research) and 2) provide up to date institutional policies regarding human subject research (UAB's Multiple Project Assurance and Principal Investigator's responsibilities, IRB responsibilities, Institutional responsibilities). In addition to on-campus sessions, I will receive on-line training regarding this topic through the University of Miami Collaborative IRB Training Initiative (CITI) Human Subjects Research Education Program. This consists of 13 Parts; each module focuses on different aspects of bio-ethics and human subjects research. The IRB Office also requires continuing education (1 to 1½ hours) throughout the year on a variety of topics associated with human subjects research.

Responsible Conduct of Research / Principles of Scientific Integrity (GRD 717): This course surveys ethical issues and principles in the practice of science. Topics include ideals of good science, the responsibilities of authorship and peer review; potential problems raised by commercialization of research; scientists as public policy advisors; ethical issues involved in animal experimentation and clinical trials; and the nature, extent, and causes of fraud in the sciences. Relevant cases from the history of science as well as fictional case studies are used to involve students in the discussion. (3.0 credit hours)

Annual Ethics Conference: Sessions during this conference have included clinical research ethics, conflict of interest in research, authorship and publishing, ethically managing data and data access, statistical power and the ethics of data gathering, defining misconduct, and consequences of fraud in bioresearch. (20 hours of didactic training each year)

Annual IACUC Training: This 2 hour course provides annual certification and updates regarding the care and ethical use of laboratory animals.